CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: Trimethyl borate

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

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

Index Number: **005-005-00-1**

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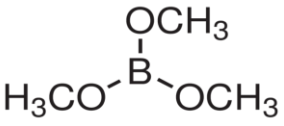
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Trimethyl borate
Other names (usual name, trade name, abbreviation)	Trimethyl borate Azeotrope/Pure Trimethyl borate Pure Borester O Boric acid, trimethyl ester Methyl borate Trimethoxy borane Trimethoxy borine Trimethoxy boron
ISO common name (if available and appropriate)	Unknown
EC number (if available and appropriate)	204-468-9
EC name (if available and appropriate)	Trimethyl borate
CAS number (if available)	121-43-7
Other identity code (if available)	Unknown
Molecular formula	C3H9BO3
Structural formula	
SMILES notation (if available)	COB(OC)OC
Molecular weight or molecular weight range	103.9
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

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
Trimethyl borate (CAS: 121-43-7)	Mono-constituent substance, purity typically >98%	Flam. Liq. 3: H226 Acute Tox. 4*: H312	Flam. Liq. 2: H225 Flam. Liq. 3: H226 Acute Tox. 4: H302, H312 Eye Irrit. 2: H319 Repr. 1B: H360 Repr. 2: H361 STOT SE1: H370, H372

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Methanol (CAS 67-56-1)	Confidential, see Annex.	Flam. Liq. 2: H225 Acute Tox. 3: H331 Acute Tox. 3: H311 Acute Tox. 3: H301 STOT SE 1: H370	Not relevant	No

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

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

No additives relevant for classification.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling

	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	005-005-00-1	Trimethyl borate	204-468-9	121-43-7	Flam. Liq. 3 Acute Tox. 4*	H226 H312	GHS02 GHS07 Wng	H226 H312			
Dossier submitters proposal	005-005-00-1	Trimethyl borate	204-468-9	121-43-7	**Add** Repr. 1B	**Add** H360FD	**Add** GHS08 **Modify** Dgr	**Add** H360FD			
Resulting Annex VI entry if agreed by RAC and COM	005-005-00-1	Trimethyl borate	204-468-9	121-43-7	Flam. Liq. 3 Acute Tox. 4* Repr. 1B	H226 H312 H360FD	GHS02 GHS07 GHS08 Dgr	H226 H312 H360FD			

The generic concentration limit of 0.3% will apply for toxicity to reproduction.

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

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	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	Harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no harmonised classification and labelling for trimethyl borate for reproductive toxicity.

The substance is classified as Repr. 1B by multiple REACH registrants. The registration dossier of trimethyl borate does not contain any data on reproductive or repeated dose toxicity of the substance itself. Instead read-across was applied to boric acid and methanol. Under physiological conditions trimethyl borate is rapidly hydrolysed into boric acid (CAS 10043-35-3) and methanol (CAS 67-56-1), see reaction formula below (Steinberg *et al.*, 1957). Therefore, classification for reproductive toxicity based on read-across is proposed as earlier applied for borates.

$B(OCH_3)_3 + 3\ H_2O \rightarrow B(OH)_3 + 3\ HOCH_3$

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required: Not required as the proposal is for CMR properties.

5 IDENTIFIED USES

Trimethyl borate is used in the following products: welding & soldering products, and laboratory chemicals in building & construction and scientific research & development. This substance is also used by professional workers in the production of metal products, formulation of mixtures (welding & soldering products) and as intermediate in the manufacturing of chemicals (welding & soldering products) at industrial sites.

6 DATA SOURCES

Publicly disseminated ECHA reports or registrant's dossiers have been used to summarise data for trimethyl borate, boric acid and methanol:

- The Health Council of the Netherlands dossier on methanol (2006)[1]
- ECHA online registration dossier for methanol, boric acid and trimethyl borate
- ECHA SVHC support document for boric acid (2010)[2]
- ECHA CLH report for disodiumoctaborate anhydrate (2013)[3]
- ECHA CLH report for disodiumoctaborate tetrahydrate (2013)[4]
- ECHA CLH report for methanol (2013)[5]

[1] https://www.gezondheidsraad.nl/documenten/adviezen/2006/06/13/methanol

[2] https://echa.europa.eu/documents/10162/d51fd473-40ec-4831-bc2d-6f53bdf9cbbe

[3] https://echa.europa.eu/documents/10162/13626/clh_report_disodiumoctaborate_anhydrate_df007652_59.pdf

[4] https://echa.europa.eu/documents/10162/13626/clh_report_disodiumoctaborate_tetrahydrate_DF007892_51.pdf

[5] https://echa.europa.eu/documents/10162/a0a4cc5a-f736-5f35-facc-999a4505feae

- ECHA CLH report for boric acid and borates (2018)[6]
- RAC Opinion on new scientific evidence on the use of boric acid and borates in photographic applications by consumers (2010)[7]
- RAC Opinion on proposing harmonised classification and labelling at EU level of methanol (2014)[8]
- RAC Opinion proposing harmonised classification and labelling at EU level of boric acid (2014)[9]
- RAC Opinion proposing harmonised classification and labelling of Disodium octaborate anhydrate (2014)[10]
- RAC Opinion proposing harmonised classification and labelling of Disodium octaborate tetrahydrate (2014)[11]
- RAC Opinion on proposed harmonised classification and labelling of boric acid, diboron trioxide, tetraboron disodium heptaoxide hydrate, disodium tetraborate anhydrous, orthoboric acid sodium salt, disodium tetraborate decahydrate and disodium tetraborate pentahydrate (2019)[12]

7 PHYSICOCHEMICAL PROPERTIES

Information on physicochemistry is cited from the publicly disseminated REACH registration dossier on trimethyl borate.

Table 7: Summary of physicochemical properties for trimethyl borate

Property	Value	Reference[1]	Comment (*e.g.* measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid	Study report 2012	Measured
Melting/freezing point	-31 °C	Study report 2004	Measured
Boiling point	68.2 - 68.6 °C	Study report 2004	Measured
Relative density	0.91	Study reports 1998, 2000, 2006	Measured
Vapour pressure	1.48×10^4 Pa (at 20 °C)	Study report 2004	Measured
Surface tension	Data waived	-	-
Water solubility	Data waived	-	-
Partition coefficient n-octanol/water	Data waived	-	-

[6] https://echa.europa.eu/documents/10162/74c34c0b-2129-abf5-a0f4-2c5b21d7d926

[7] https://echa.europa.eu/documents/10162/13641/rac_opinion_borates_20100429_en.pdf/2cee4acb-1f38-4578-9f30-e1e68e527b50

[8] https://echa.europa.eu/documents/10162/ff8fac92-706d-8612-f6be-db051f0cdbc5

[9] https://echa.europa.eu/documents/10162/4db9bc68-844e-c557-8914-ab491743d471

[10] https://echa.europa.eu/documents/10162/7d740d8c-5cd5-872b-5da2-e549983a9ff9

[11] https://echa.europa.eu/documents/10162/658b802c-1ca3-663e-4bd4-437369d715de

[12] https://echa.europa.eu/documents/10162/584263da-199c-f86f-9b73-422a4f22f1c3

Property	Value	Reference[1]	Comment (*e.g.* measured or estimated)
Flash point	-8 °C	Study report 2013	Measured
Flammability	Data waived	-	-
Explosive properties	Non explosive	Study report 2013	Estimated
Self-ignition temperature	308 °C	Study report 2013	Measured
Oxidising properties	Data waived	-	-
Granulometry	Not applicable	-	-
Stability in organic solvents and identity of relevant degradation products	Data waived	-	-
Dissociation constant	Data waived	-	-
Viscosity	Data waived	-	-

[1]As cited in the publicly disseminated REACH registration dossier for trimethyl borate (https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14170/4/2)

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

According to the REACH registration dossier, trimethyl borate is rapidly (too fast to measure) hydrolysed into boric acid and methanol in water under physiological conditions (Steinberg *et al.*, 1957). Therefore, trimethyl borate in humans will predominantly be present as boric acid and methanol.

As stated in the REACH registration dossier for boric acid: absorption of boric acid via oral and inhalation route is close to 100%. Absorption via skin is neglectable and estimated to be 0.5% in worst case scenario. Boric acid is not further metabolised due to the strong B-O bond and is distributed rapidly followed by excretion via urine. Half-life of boric acid via oral route is approximately 21 h in humans, but shorter half-lives have been reported for mouse and rat; 1 h and 3 h, respectively. Higher renal clearance of boric acid in rats and mouse are responsible for these faster excretion rate observed. Potency of accumulation of boric acid is low. In bones, relative amounts of boric acid were found to be 2-3 higher as compared to other tissues. Different clearance rates of boric acid in humans are linked to differences in glomerular filtration rate, *e.g.* variable rates of glomerular filtration rate (GFR) during pregnancy.

Absorption of methanol is readily through oral, inhalation and dermal routes and is distributed uniformly to all organs and tissues according to the water content, as stated in the REACH registration dossier for methanol. Up to 98% of the total dose is excreted from the human body through metabolism. Route of unchanged excretion of methanol is 2% and 1% in exhaled air and urine, respectively. Half-life time of methanol in humans depends on dose; ~3 hours at <100 mg/kg bw and 24 h at >1000 mg/kg bw. Toxicokinetic and metabolism is found to be different in species, *e.g.* faster metabolic conversion rates in rodents have been measured as compared to human. Methanol metabolism occurs mainly in the liver. Methanol is converted in formaldehyde through alcohol dehydrogenase oxidation in humans, but through catalase/peroxidase in rats. In humans, formaldehyde is conjugated with glutathione and then hydrolysed to formic acid and subsequently transformed into formate. Formate is then oxidised in carbon dioxide and water. The metabolic conversion of

formate is considerably lower (2.5 times) in humans than in rats. Accumulation of formate results in metabolic acidosis leading to ocular toxicity in humans.

Registrants have proposed read-across for trimethyl borates for classification using data on methanol and boric acid. Furthermore, read-across for borates has previously been applied for various other borates, including disodium octaborate anhydrate and disodium octaborate tetrahydrate and justified by RAC (RAC, 2010, RAC, 2014b, RAC, 2014c, RAC, 2014d, RAC, 2019, ECHA, 2013a, ECHA, 2013b). Furthermore, the European Court of Justice concluded that read-across for borates was justified (Court of Justice of the European Union, 2011[13]). Accordingly, read-across for trimethyl borate to data on boric acid- and/or methanol-induced reproductive and developmental toxicity is proposed in this dossier.

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

Trimethyl borate is predominantly present as undissociated boric acid and methanol in aqueous conditions and at physiological pH, like other borates.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated in this dossier.

10.2 Acute toxicity - dermal route

Not evaluated in this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated in this dossier.

10.4 Skin corrosion/irritation

Not evaluated in this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated in this dossier.

10.6 Respiratory sensitisation

Not evaluated in this dossier.

10.7 Skin sensitisation

Not evaluated in this dossier.

[13] https://op.europa.eu/en/publication-detail/-/publication/c8d55d22-0b78-41e3-9477-6b038f7a3bdf/language-en

10.8 Germ cell mutagenicity

Not evaluated in this dossier.

10.9 Carcinogenicity

Not evaluated in this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Information on trimethyl borate

There is no information on the reproductive toxicity of trimethyl borate itself. The registrants have applied read-across from the data on boric acid and on methanol because trimethyl borate is quickly hydrolysed into these two substances. After oral or inhalation exposure, complete hydrolysis is expected in the body. As a result, no differences in uptake and toxicity after oral exposure is expected for trimethyl borate compared to boric acid and methanol alone. Therefore, information on the reproductive toxicity of both hydrolytic products was provided below.

Information on boric acid and other borates

Scientific data relevant for boron, boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate regarding reproductive toxicity have been reviewed by RAC (RAC, 2014b, RAC, 2014d, RAC, 2014c, RAC, 2019). Currently, a harmonised classification for borates of Repr. 1B applies. However, a specific concentration limit (SCL) is currently used for some borates and recently a generic concentration limit (GCL) has been suggested (ECHA, 2018). This report has been discussed and adopted by RAC (RAC, 2019).

Information on boron and its effects on sexual function and fertility up to 2018 has been thoroughly reviewed in the CLP report for boric acid and borates, and has been adopted in this report (ECHA, 2010, ECHA, 2013a, ECHA, 2013b, ECHA, 2018). In addition, new studies on effects of boric acid on fertility published in 2019 and 2020 have been included.

Table 8: Summary table of animal studies on adverse effects on sexual function and fertility of boric acid

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Oral			
Sub-chronic toxicity study No TG study Feed Rat Sprague-Dawley M/F 10 per group Pre-dates GLP Klimisch score 2	Boric acid (purity not stated), 0, 300 (52.5), 1000 (175), 3000 (525), 10000 (1750), 30000 (5250) ppm boric acid (B) equivalent to 0, 15 (2.6), 50 (8.8), 149 (26), 503 (88), 1486 (260) mg boric acid (B)/kg bw/day	Reduction of bw, clinical signs of toxicity and testicular atrophy at ≥503 (88) mg boric acid (B)/kg bw/day. Partial testicular atrophy observed in one male at 26 mg B/kg bw/day. NOAEL is 50 (8.8) mg boric acid (B)/kg bw/day. LOAEL is 149 (26) mg boric acid (B)/kg bw/day.	(Study report, 1962)
Three-generation reproductive toxicity study No TG study Feed Rat Sprague-Dawley M/F 8(M)+16(F) per group Pre-dates GLP	Boric acid (purity not stated), 0, 670 (117), 2000 (350), 6700 (1170) ppm boric acid (B) equivalent to 0, 34 (5.9), 100 (17.5), 336 (58.5) mg boric acid (B)/kg bw/day 14 weeks + 3 generation	Highest dose level resulted in testes atrophy before first mating, no litters were produced. Testes atrophy at 24 months: 0, 34, 100, 336 mg boric acid/kg bw/day; 3/10, 1/10, 4/10, 10/10, respectively. Infertility observed in rats (M+F) dosed at highest dose when mated with untreated rats. No adverse effects in mid and low dose in any generation. NOAEL (F0, F1, F2) is 2000 (350) ppm boric acid (B) equivalent to 100 (17.5) mg boric acid (B)/kg bw/day. LOAEL (F0) is 6700 ppm (1170) ppm boric acid (B) equivalent to 336 (58.5) mg boric acid (B)/kg bw/day.	(Study report 1966) (Weir *et al.*, 1972)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Klimisch score 2	follow up		
Two-generation reproductive toxicity study NTP's reproductive assessment Feed Mice Swiss CD1 M/F 40(M)+40(F) in control group 20(M)+(20)F in dosed groups GLP Klimisch score 2	Boric acid (>99% pure), 0, 1000 (175), 4500 (787.5), 9000 (1575) ppm boric acid (B) equivalent to 0, 152 (26.6), 636 (111.3), 1262 (220.9) mg boric acid (B)/kg bw/day	F0: no litters produced upon exposure to 1262 (220.9) mg boric acid (B)/kg bw/day. Significantly (p<0.05) reduced weight of testis at two highest doses compared to control group of 51% and 86%, respectively. Concentration and percentage motile of spermatozoa significantly (p<0.05) reduced in two highest dose groups. F1: oestrous cycles significantly (p<0.05) shorter at 152 (26.6) mg boric acid (B)/kg bw/day. NOAEL (F0, F1) is <152 (26.6) mg boric acid (B)/kg bw/day. LOAEL (F0, F1) is 152 (26.6) mg boric acid (B)/kg bw/day.	(NTP (National Toxicology Program), 1990, Fail *et al.*, 1991)[1]
Sub-acute study Experimental study Gavage Mice Swiss Albino M 10 per dose GLP not specified Klimisch score 2	Boric acid (≥99.5% pure), 0, 115 (20.1), 250 (43.8), 450 (78.8) mg boric acid (B)/kg bw/day[2] 4-6 weeks	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.	(Aktas *et al.*, 2020)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		≥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.	

[1]Adopted from CLH report for boric acid and borates (ECHA, 2018)

[2]Conversion factor of 0.175, based upon molecular weight, was used to calculate boron content in boric acid

Dose of boron (B) is indicated in between brackets. Bw; body weight; GSH: reduced glutathione; MDA: malondialdehyde

Table 9: Summary table of human data on adverse effects on sexual function and fertility of boron

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Publication	Boron, occupational and environmental exposure	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/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)	This study did not observe statistically significant differences in sperm quality parameters (concentration, morphology, motility) or reproductive hormone levels (LH, FSH and testosterone) between exposure groups.	(Duydu *et al.*, 2018a)[1]
Publication	Boron, occupational and environmental exposure	Retrospective study Male workers in Bandirma and Bigadic, Turkey n: 304 Control group: <50 ng/g	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	(Duydu *et al.*, 2019)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		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	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.	
Publication	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)

[1]Adopted from CLH report for boric acid and borates (ECHA, 2018)

DBE: daily boron exposure; FSH: follicle stimulating hormone; LH: luteinizing hormone

Information on methanol

Information on effects of methanol on sexual function and fertility has been thoroughly reviewed by the Health Council of the Netherlands in 2006 and has been adopted here (The Health Council of the Netherlands, 2006).

In addition, studies from the online registration dossier and the toxicological review of methanol of the United States Environmental Protection Agency (EPA) have been added (US EPA, 2013).

Table 10: Summary table of animal data on adverse effects on sexual function and fertility of methanol

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Oral			
Carcinogenicity study Similar to OECD TG 451 Drinking water Rats Sprague-Dawley 100(M)+100(F) GLP Klimisch score 1	Methanol (99.8% pure), 0, 50, 500, 2000 mg/kg bw/day 104 weeks	A slight increase in bw of male and female rats of the high dose was observed (not specified). A dose-related increase of total malignant tumours (*e.g.* carcinomas of the ear duct, osteosarcomas of the head and hemolymphoreticular neoplasia) in the males and female groups was observed. No treatment-related non-neoplastic changes were detected by gross inspection or histopathological examination. In the reproductive organs of the animals of the high dose group, a statistically significant increase of testicular interstitial hyperplasia, testicular adenomas and sarcomas of the uterus was observed. NOAEL is 500 mg/kg bw/day. LOAEL is 2000 mg/kg bw/day.	(Soffritti *et al.*, 2002)[1]
Mechanistic study Experimental study Gavage Mice B6C3F1 M 10 per group GLP not specified Klimisch score 2	Methanol (purity not stated), 0, 1000 mg/kg bw/day 5 days	Increase (n.s.) in sperm abnormalities: 1.86 ± 0.91% vs. 1.12 ± 0.39% in control.	(Ward *et al.*, 1984)[1]
Inhalation			
One-generation reproduction toxicity study Similar to OECD TG 415 Monkey	Methanol (purity not stated), 0, 200, 600, 1800 ppm (0, 262, 786,	No effects on bw gain and clinical observations. No effects on menstrual cycles, conception rate, live-birth index. Duration of gestation was decreased but still within normal range. Complications at delivery included vaginal bleeding without labour and long-term non-productive labour. These complications were not related to methanol treatment.	(Burbacher *et al.*, 1999)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Macaca fascicularis F 11-12 per group GLP not specified Klimisch score 2	2358 mg/m^3, whole body) 2.5 h/day 7d/week Exposure from pre-breeding to pregnancy		
Sub-acute study Experimental study Monkey *Macaca fascicularis* 3(M)+3(F) GLP unknown Klimisch score 2	Methanol (purity not stated), 0, 500, 2000, 5000 ppm (0, 655, 2620, 6550 mg/m^3, whole body) 6 h/day 5d/week 4 weeks	No effect on bw and clinical observations. No effects on weight and macroscopic observations of reproductive organs.	(Andrews *et al.*, 1987)[1]
Two-generation reproduction study Similar to OECD TG 416 Rats Sprague-Dawley 30(M)+30(F) GLP not specified Klimisch score 2	Methanol (reagent grade), 0, 10, 100, 1000 ppm (0, 13.1, 131, 1310 mg/m^3, whole body) 20 h/day F0: 8 weeks old to mating	F0: 1000 ppm (1310 mg/m^3): significantly (p<0.05) reduced bw starting at week 7 (males) and food consumption (males and females) F1: 1000 ppm (1310 mg/m^3): testis descent was completed within 16 through 20 post-natal days with the maximum at day 17 and 18 (32.4 and 38.9%, respectively), while in the respective control descent was completed from 15 through 21 days with the maximum at day 19 (31.9%), indicating an earlier descent related to treatment. Absolute and relative brain weights, pituitary and thymus were significantly (p<0.05) lowered in the high-dose groups of either 8 (males and females), 16 (males) and 24 weeks (females). Relative weights were not affected. No histopathological or abnormalities in movement, emotional and learning abilities. F2: 1000 ppm (1310 mg/m^3): testis decent was completed within	(New Energy Development Organization, 1987, Takeda, 1988)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	(M) or lactation (F) F1: birth to mating (M) or weaning of F2 pups 21 days post-delivery (F) F2: birth to 21 days old (M+F)	15 through 19 post-natal days with the maximum at day 16 and 17 (41.5 and 40.4%, respectively), while in the respective control descent was completed from 16 through 22 days with the maximum at day 17 and 18 (38.5 and 30.8%, respectively). Significantly (p<0.05) reduced weight of brain, pituitary and thymus at week 8 (males and females). Relative weights were not affected. No histopathological or abnormalities in movement, emotional and learning abilities. NOAEC (F1, F2) is 100 ppm equivalent to 131 mg/m^3. LOAEC (F1, F2) is 1000 ppm equivalent to 1310 mg/m^3.	
Combined chronic toxicity/carcinogenicity study Similar to OECD TG 453 Rats F344 M(52)+F(52) GLP unknown Klimisch score 2	Methanol (reagent grade), 0, 10, 100, 1000 ppm (0, 13.1, 131, 1310 mg/m^3, whole body) 19.5 h/day 7d/week 24 months	Testicular atrophy, cataract formation, exophthalmia, small eye ball, alopecia and paralysis of the hind leg, related to aging and not related to dose. 1000 ppm (1310 mg/m^3): decreased bw in females (-4%, n.s.) between 51-72 weeks. Significantly (p<0.05) reduced food consumption in males (day 210-365), but this did not correspond to reduced bw.	(New Energy Development Organization, 1985b, New Energy Development Organization, 1987)
Sub-acute study Similar to OECD TG 412 Rats Sprague-Dawley M/F 10-15 per group GLP unknown Klimisch score 2	Methanol (purity not stated), 0, 300, 3000 ppm (0, 393, 3930 mg/m^3, whole body) 6 h/day 5d/week 4 weeks	No effects on clinical observations, bw and food consumption. No histopathological effects on reproductive organs.	(Poon *et al.*, 1994)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Sub-acute study Similar to OECD TG 412 Rats Sprague-Dawley M/F 10-15 per group GLP unknown Klimisch score 2	Methanol (purity not stated), 0, 2500 ppm (0, 3275 mg/m^3, whole body) 6 h/day 5d/week 4 weeks	No effects on clinical observations, bw and food consumption. No histopathological effects on reproductive organs.	(Poon *et al.*, 1995)[1]
Sub-acute study Experimental study Rats CD 5(M)+5(F) GLP unknown Klimisch score 2	Methanol (purity not stated), 0, 500, 2000, 5000 ppm (0, 655, 2620, 6550 mg/m^3, whole body) 6 h/day 5d/week 4 weeks	No effects on bw, increased incidence of discharge around eyes and nose. No effects on weight and macroscopic observations of reproductive organs.	(Andrews *et al.*, 1987)[1]
Acute study Experimental study Rats Long-Evans M 10 per group GLP unknown Klimisch score 2	Methanol (purity not stated), 0, 200, 5000, 10000 ppm (0, 262, 6550, 13100 mg/m^3, whole body) 1-6 h,	No effect on bw. No effect on testis weight. Significant effects on hormone concentrations (LH, FSH, prolactin, testosterone) were observed but the direction and magnitude of these effects were strongly depended on whether or not the animals has been acclimated to the experimental conditions. No NOAEC can be derived due to different effects observed.	(Cooper *et al.*, 1992)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	treated once		
Sub-acute study Experimental study Rats Sprague-Dawley M 9-10 per group GLP unknown Klimisch score 2	Methanol (purity not stated), 0, 200 ppm (0, 262 mg/m^3, whole body) 8 h/day 5d/week 1-6 weeks	No effects on bw. No effect on weight and macroscopical examination of testis and seminal vesicles. No effect on serum levels of testosterone.	(Lee *et al.*, 1991)[1]
Sub-acute study Experimental study Rats Long-Evans M 8-13 per group GLP unknown Klimisch score 2	Methanol (purity not stated), 0, 50, 200, 800 ppm (0, 66, 262, 1048 mg/m^3, whole body) 20 h/day 13 weeks	No statistically significant effects on bw. No effects on testis weight, gross abnormalities and incidence of testicular lesions.	(Lee *et al.*, 1991)[1]
Sub-acute study Experimental study Rats Sprague-Dawley M 5 per group GLP unknown Klimisch score 2	Methanol (purity not stated), 0, 200, 2000, 10000 ppm (0, 262, 2620, 13100 mg/m^3, whole body) 8 h/day 5d/week	Effects measured on testosterone and LH concentrations were not dose dependent. 200 ppm: testosterone concentration decreased in week 2 (-45%) and 6 (-68%). 2000 ppm: testosterone concentration decreased in week 6 (-41%). 10000 ppm: LH concentration increased in week 6 (211%). No effect of methanol on rate of [^{14}C]-testosterone clearance from blood.	(Cameron *et al.*, 1984)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	6 weeks		
Acute study Experimental study Rats Sprague-Dawley M 5 per group GLP unknown Klimisch score 2	Methanol (purity not stated), 0, 200 ppm (0, 262 mg/m^3, whole body) 6 h/day 1 or 7 days	After 1 day, serum levels of testosterone were decreased (-59%) immediately after exposure and returned to control levels after 18 h. No effects after 7 days exposure.	(Cameron *et al.*, 1985)[1]
Combined chronic toxicity/carcinogenicity study Similar to OECD TG 453 Mice B6C3F1 52(M)+53(F) GLP unknown Klimisch score 2	Methanol (reagent grade), 0, 10, 100, 1000 ppm (0, 13.1, 131, 1310 mg/m^3, whole body) 19.5 h/day 7d/week 18 months	No dose-related effects on bw, food consumption, urinalysis, haematology or clinical chemistry parameters. 1000 ppm: significantly (p<0.05) decreased testis weight, one animal had severe testicular atrophy. Significantly (p<0.05) increased abs. kidney and spleen weight, swellings of spleen, preputial glands and uterus (females). NOAEC is 100 ppm equivalent to 131 mg/m^3. LOAEC is 1000 ppm equivalent to 1310 mg/m^3.	(New Energy Development Organization, 1985a, New Energy Development Organization, 1987)

[1]Adopted from dossier on methanol from the Health Council of the Netherlands (The Health Council of the Netherlands, 2006)

Abs: absolute, bw: body weight; FSH: follicle stimulating hormone; LH: luteinizing hormone; n.s.: not statistically significant

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

Information on trimethyl borate

There is no information on reproductive toxicity for trimethyl borate available. Trimethyl borate is quickly hydrolysed into boric acid and methanol. Therefore, read-across from data on boric acid and methanol is used here.

Information on boric acid and borates

Animal studies

RAC concluded that data from multiple animal studies clearly demonstrated boron-induced reproductive toxicity in repeated dose toxicity and mating studies conducted in mice, rats and dogs (RAC, 2019). Specifically, abnormalities in testes were observed in all studies and species which impairs fertility. Shorter oestrous cycles, reduced sperm motility and spermatozoa concentration are other indicators of impaired fertility described upon exposure to boric acid in animals. A new study (Aktas *et al.*, 2020), not included in the latest RAC opinion (RAC, 2019), confirms earlier observations for effects on the male reproductive organs.

Aktas *et al.* (2020) 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 11. 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. Furthermore, 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 this does suggest mutagenicity in sperm cells, the OECD has concluded that the alkaline comet assay should not be applied to assess DNA damage in germ cells because of high variable background levels in DNA damage (OECD, 2016). The ED10 (10% change as compared to control) based on membrane integrity, live cells and DNA damage in spermatozoa would be 115-450 (20.1-78.8) mg boric acid (B)/kg bw/day.

Table 11: DNA damage, cell viability and motility in sperm cells after a 6-week exposure to boric acid

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

Human studies

Duydu *et al.* (2018) studied effect on fertility upon environmental and occupational exposure to boron (Duydu *et al.*, 2018a). No adverse effects were found on sperm quality (motility, concentration, morphology) or on hormone levels (luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone). Highest exposure group was on average exposed to 47.17 mg B/day, which corresponds to 0.6739 mg B/kg bw/day based upon an average weight of 70 kg, corresponding to an average blood boron levels of 570.6 ng B/g blood (=571 ppb).

Duydu *et al.* (2019) described significantly ($p<0.05$) increased levels of boron in semen and urine in medium, high and extreme exposure groups in male residing in Turkey and exposed to boron through environment and/or occupation (Duydu *et al.*, 2019). However, no correlation between blood boron levels and Y:X ratio or semen boron levels and Y:X ratio was found. Also, no effect on sex ratio was described between exposure

groups. Blood boron levels of >400 ng B/g blood (maximum blood boron level of 1099.93 ng B/g blood) were measured in the extreme boron exposure group with an estimated daily boron exposure of 44.91 mg B/day. This corresponds to 0.6416 mg B/kg bw/day based upon an average weight of 70 kg. Daily intake of boron was estimated based on water and food samples, taken at home and work, and were thus not determined individually.

Basaran *et al.* (2019) investigated adverse effects of boron upon environmental and occupational exposures in male and females in two separate studies (Basaran *et al.*, 2019). Exposure groups were determined based upon blood boron levels; no daily boron exposures were provided to compare with animal studies. No reasons were found to assume genotoxicity resulting in impaired fertility under conditions of normal handling and use of boron compounds in males. Similar blood boron levels were found in the highest exposure group as compared to the highest exposure group in the study earlier published by Duydu *et al.* (2018).

No effects on sexual function and fertility were observed in the available epidemiological studies. However, the daily exposure levels to boron were well below LOAELs (26-58.5 mg B/kg bw/day) observed in animal studies. Therefore, these epidemiological results are not contradictive to the animal results. Furthermore, RAC previously concluded that the available human data on fertility and sexual function do not contradict the animal data (RAC, 2014b, RAC, 2014c, RAC, 2014d, RAC, 2019).

Information on methanol

Animal studies

In a carcinogenicity study (similar to OECD TG 451) of Soffritti *et al.*, methanol was administered in drinking water at concentrations of 0, 50, 500 and 2000 mg methanol/kg bw/day to groups of male and female Sprague-Dawley rats (n=100/sex/group) for 104 weeks (Soffritti *et al.*, 2002). Residual liquids were removed daily, and bottles were refilled daily with fresh solutions. The experiment ended after 153 weeks with the death of the last animal. Upon death, a wide range of tissues and organs were sampled for histopathological examinations, including the reproductive organs. No effects were observed in food consumption. Water consumption of female animals of the high dose group was decreased during the first 48 weeks. A slight increase in body weight of male and female rats of the high dose was observed (not specified). No substantial changes in survival or behavioural changes were observed among the groups. A dose-related increase of total malignant tumours (*e.g.* carcinomas of the ear duct, osteosarcomas of the head and hemolymphoreticular neoplasia) in the males and female groups was observed. No treatment-related non-neoplastic changes were detected by gross inspection or histopathological examination. In the reproductive organs of the animals of the high dose group, a significant ($p<0.05$) increase of testicular interstitial hyperplasia (data not shown), testicular adenomas and sarcomas of the uterus was observed (Table 12). The LOAEL is 2000 mg/kg bw/day.

Table 12: Adenomas and carcinomas in testis, uterus (Soffritti *et al.*, 2002)

	Testicular cell adenoma	Uterus & vagina (adenocarcinoma + malignant Schwannoma)
Control	12.0%	3.0%
50 mg/kg bw/day	9.0%	3.0%
500 mg/kg bw/day	13.0%	5.0%
2000 mg/kg bw/day	17.0%	5.0%

As part of a study into the effects of formalin, Ward *et al.* examined the effect of methanol on sperm morphology. Crl:B6C3F1 mice were exposed by gavage to 0 (n=5) or 1.0 (n=10) g/kg bw/day methanol for 5

days (Ward *et al.*, 1984). The percentage of abnormal sperm morphologies was not statistically significantly increased (1.86 ± 0.91 *vs.* 1.12 ± 0.39% in control).

In a one-generation reproduction toxicity study (similar to OECD TG 451), Burbacher *et al.* studied the reproductive and developmental effects of exposure to methanol via inhalation in two cohorts of female *Macaca fascicularis* monkeys (Burbacher *et al.*, 1999). 9-12 animals were exposed to methanol 0, 200, 600 and 1800 ppm (0, 262, 786, 2358 mg/m^3, whole body) for 2.5 h/day, 7 days/week during premating (about 120 days), mating (about 65 days) and gestation (about 163 days). Males were not exposed to methanol. Maternal body weights were weighed weekly and clinical observations were performed daily. Menstrual cycles were evaluated every day prior to and during exposure. Rate of conception, weight gain during pregnancy, pregnancy and delivery complications, pregnancy duration and live- and stillbirths were recorded. In general, females were allowed to deliver unless complications necessitate a Caesarean-section. No effect of methanol exposure was observed on female body weight gains (Table 13), clinical observations, menstrual cycles (Table 14), conception rate and live-birth index (Table 15). The duration of pregnancy (Table 13) and gestation (Table 16) was significantly (p<0.05) decreased in all treatment groups but still within the normal range for this strain of animals. No statistically differences were found in the measurements of birth size in the methanol exposure groups (Table 16). Some delivery complications were noted (vaginal bleeding with no signs of labour and unproductive labour for at least 3 nights) that required a Caesarean section but, most probably, these findings were not related to methanol exposure.

Table 13: Maternal weight gain during pregnancy and duration of pregnancy in *Macaca fascicularis*[a]

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

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

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

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

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

Table 14: Lengths of menstrual cycles for baseline and prebreeding exposure periods in *Macaca fascicularis*[a]

Exposure Group	Baseline[b]			Exposure[c]			
	Cycle 1	Cycle 2	Cycle 3	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Control (n = 11)	31 ± 1	30 ± 1	30 ± 1	31 ± 1	30 ± 1	30 ± 1	30 ± 1
200 ppm (n = 12)	29 ± 1	29 ± 1	28 ± 1	29 ± 1	29 ± 1	30 ± 1	29 ± 1
600 ppm (n = 11)	29 ± 1	29 ± 1[d]	30 ± 1	30 ± 1	29 ± 1	30 ± 1	29 ± 1
1,800 ppm (n = 12)	31 ± 1	29 ± 1	29 ± 1	29 ± 0	29 ± 1	29 ± 1	31 ± 1

[a] Values are presented as means ± SE in days.

[b] No statistically significant differences were found in the length of the menstrual cycle across the four methanol-exposure groups during the baseline period (ANOVA; $p < 0.12$, all tests).

[c] No statistically significant differences were found in the length of the menstrual cycle due to methanol exposure (ANOVA; $p = 0.45$).

[d] n = 10 due to abnormal cycle length (88 days) for one cycle in one animal.

Table 15: Results of timed-mating procedures in *Macaca fascicularis*[a,b]

Exposure Group	Conception Rate	Pregnancy Complication Rate	Live-Born Delivery Rate
Control	9/11 (82%)	2/9 (22%)	8/9 (89%)
200 ppm	9/12 (75%)	2/9 (22%)	9/9 (100%)
600 ppm	9/11 (82%)	3/9 (33%)	8/9 (89%)
1,800 ppm	10/12 (83%)	3/10 (30%)	9/10 (90%)

[a] No statistically significant differences were found in conception rates, complication rates or live-birth delivery rates (Fisher's exact test; $p = 1.0$, all tests).

[b] Conception Rate = number of conceptions/number of animals mated. Pregnancy Complication Rate = number of complications during pregnancy and delivery/number of animals pregnant. Live-Birth Delivery Rate = number of live-birth deliveries/number of animals pregnant.

Table 16: Birth characteristics of live-born offspring in *Macaca fascicularis*[a]

Exposure Group	Gestation Length (days)	Infant Characteristics[b]				
		Birthweight (g)	Crown–Rump Length (mm)	Head Circumference (mm)	Head Length (mm)	Head Width (mm)
Control (n = 8)	168 ± 2 (168–178)	369 ± 14 (295–425)	180 ± 3 (167–192)	182 ± 1 (177–186)	63 ± 1 (60–64)	49 ± 0 (47–50)
200 ppm (n = 9)	160 ± 2 (153–172)	344 ± 14 (290–420)	175 ± 3 (165–188)	179 ± 1 (194–183)	62 ± 1 (59–65)	48 ± 1 (45–50)
600 ppm (n = 8)	162 ± 2 (153–166)	368 ± 25 (280–475)	176 ± 4 (165–196)	180 ± 3 (170–191)	62 ± 1 (58–67)	48 ± 1 (59–65)
1,800 ppm (n = 9)	162 ± 2 (150–169)	369 ± 21 (260–465)	177 ± 3 (158–189)	181 ± 2 (170–188)	63 ± 1 (59–65)	48 ± 1 (45–51)

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

[b] Gestation lengths for the methanol-exposed groups were significantly shorter than that for the control group (ANOVA post hoc tests; $p < 0.04$, all tests). No statistically significant differences were found in the measurements of birth size across the four methanol-exposure groups (ANOVA; $p < 0.24$, all tests).

The sub-chronic effects after exposure to methanol via inhalation was evaluated in monkeys by Andrews *et al.* (Andrews *et al.*, 1987). Male and female cynomolgus monkeys (*Macaca fascicularis*) (n=3/sex/ group) were exposed for 4 weeks to 0, 500, 2000 or 5000 ppm (0, 655, 2620, 6550 mg/m^3, whole body) of methanol for 6

h/day, 5 days/week. At sacrifice, among other organs, the testes, epididymis and ovaries were weighed and macroscopically examined. No effects on body weights were observed and there were no treatment-related clinical observations. Macroscopic observations and reproductive organ weights revealed no effects of methanol among the groups.

In a two-generation reproduction study (similar to OECD TG 416), 30 male and 30 female Sprague-Dawley rats/group were exposed to 0, 10, 100 or 1000 ppm methanol (0, 13, 131, 1310 mg/m^3, whole body), 20 h/day via inhalation as a vapour (New Energy Development Organization, 1987, Takeda, 1988; only a summary report of this study is available). The F0 generation was exposed starting at 8 weeks old to end of mating (males) or lactation period (females). The F1 generation was exposed from birth to end of mating (males) or weaning of F2 pups 21 days post-delivery (females). The F2 generation were exposed from birth to 21 days old. No adverse effects on were observed in F0 except for a reduced bodyweight of the male rats at 1000 ppm being significant from week 7 and food consumption at 1000 ppm in both males and females. There were no effects on the fertility indices in the F0 and the F1. Testis descent was earlier as compared to control in F1 and F2 pups (Table 17). Descent was completed within 15 through 20 post-natal days with the maximum at day 17 and 18 in F1 pups and at day 16 and 17 in F2 pups at 1000 ppm, while in the respective control, descent was complete from 16 through 21 days with the maximum at day 19 in F1 pups and at day 17 and 18 in F2 pups. Furthermore, significantly (p<0.05) reduced weight of brain, pituitary and thymus in pups at 1000 ppm (1310 mg/m^3) at 8 (male and female), 16 (male) and 24 weeks (female) were observed in F1 pups (data not specified in original reports). Relative weights were not statistically significantly affected. In F2 pups significantly (p<0.05) decreased brain, pituitary and thymus weights were noted at the highest dose level in males and females at 8 weeks (data not specified in original report). Histopathological examination, however, revealed no changes suggesting treatment related effects in these organs. Furthermore, no abnormalities were observed between treatment groups in movement function, emotional and learning ability tests. The decreased brain weight upon treatment to methanol vapor was further investigated in a follow-up study (limited details on methods provided in original study report); 10-14 Sprague-Dawley rats/sex/group were exposed to 0, 500, 1000 or 2000 ppm (0, 655, 1310, 2620 mg/m^3) methanol at GD 0 throughout the F1 generation (duration of inhalation per day and/or week not specified). Brain weight was significantly and dose-related decreased (p<0.05) at ≥1000 ppm in rat pups as soon as 3 weeks after birth (Table 18). This was mainly because of decreased weights of cerebrum and cerebellum, which was noted in male and female pups mostly at 2000 ppm 8 weeks after birth. Thus, a toxic effect of methanol at ≥ 1000 ppm was noted but was considered to be slight.

Table 17: Testis descent in F1 and F2 rat pups upon exposure to methanol vapor

	F1		F2	
Post-natal day	Control	1000 ppm methanol	Control	1000 ppm methanol
15	0.9	0	0	1.1
16	6.2	7.4	9.9	41.5
17	18.6	32.4	38.5	40.4
18	22.1	38.9	30.8	14.9
19	31.9	14.8	14.3	2.1
20	17.7	6.5	4.4	0
21	2.7	0	1.1	0
22	n.a.	n.a.	1.1	0

Values stated as percentage of pups with downward migration of testes (final length of the gubernaculum reached) on stated post-natal day.

n.a.: no data available

Table 18: Brain weights in F1 rat pups upon exposure to methanol vapor

			Methanol levels (ppm)			
		Parameter	0	500	1000	2000
3-weeks old	Males	Brain weight (g)	1.45 ± 0.06	1.46 ± 0.08	1.39 ± 0.05*	1.27 ± 0.06***
	Females	Brain weight (g)	1.41 ± 0.06	1.41 ± 0.07	1.33 ± 0.07**	1.26 ± 0.09***
6-weeks old	Males	Brain weight (g)	1.78 ± 0.07	1.74 ± 0.09	1.69 ± 0.06**	1.52 ± 0.07***
	Females	Brain weight (g)	1.68 ± 0.08	1.71 ± 0.08	1.62 ± 0.07	1.55 ± 0.05***
8-weeks old	Males	Brain weight (g)	1.99 ± 0.06	1.98 ± 0.09	1.88 ± 0.08**	1.74 ± 0.05***
		Olfactory bulb (g)	0.083 ± 0.010	0.087 ± 0.009	0.084 ± 0.010	0.080 ± 0.009
		Cerebrum (g)	1.603 ± 0.045	1.604 ± 0.078	1.513 ± 0.063**	1.416 ± 0.040***
		Cerebellum (g)	0.295 ± 0.019	0.294 ± 0.014	0.283 ± 0.017	0.246 ± 0.019***
	Females	Brain weight (g)	1.85 ± 0.05	1.83 ± 0.07	1.80 ± 0.08	1.67 ± 0.06***
		Olfactory bulb (g)	0.077 ± 0.009	0.075 ± 0.009	0.078 ± 0.010	0.074 ± 0.011
		Cerebrum (g)	1.503 ± 0.043	1.484 ± 0.062	1.463 ± 0.061	1.369 ± 0.058**
		Cerebellum (g)	0.270 ± 0.013	0.271 ± 0.011	0.258 ± 0.019	0.227 ± 0.014***

Values presented as mean ± SD. *$p<0.05$, **$p<0.01$, ***$p<0.001$

In a combined chronic toxicity and carcinogenicity study (similar to OECD TG 453) 52 male 52 and female Fisher F344 rats per group were exposed to 0, 10, 100, 1000 ppm methanol (0, 13.1, 131, 1310 mg/m^3, whole body), 19.5 h per day, 7 days per week for 2 years (New Energy Development Organization, 1985a, New Energy Development Organization, 1987). No methanol-induced statistically significant differences in urinalysis, haematology, or clinical chemistry parameters were noted. Testicular atrophy, cataract formation, exophthalmia, small eye ball, alopecia and paralysis of the hind leg were observed upon methanol exposure, but these effects were described to be related to age. Decreased body weight (-4%, not statistically significant (n.s.)) was observed between 51-72 weeks in females, while food consumption was significantly ($p<0.05$) reduced at day 210-365 in males but did not correspond to reduced body weight.

In two sub-acute studies (similar to OECD TG 412) of Poon *et al.*, male and female Sprague-Dawley rats (10-15/sex/group) were exposed to methanol (0, 300, 3000 ppm (0, 393, 3930 mg/m^3, whole body) in the first study, and 0, 2500 ppm (0, 3275 mg/m^3, whole body) in the second study) by inhalation for 6 h/day, 5 days/week for 4 weeks (Poon *et al.*, 1994, Poon *et al.*, 1995) in the second study. No effects of methanol on clinical signs, body weights and food consumption were observed. Histopathological examination of the reproductive organs revealed no effects of methanol.

The sub-chronic effects after exposure to methanol via inhalation was evaluated in rats by Andrews *et al.* (Andrews *et al.*, 1987). Male and female CD rats (n= 5/sex/group) were exposed for 4 weeks to 0, 500, 2000 or 5000 ppm (0, 655, 2620, 6550 mg/m^3, whole body) of methanol for 6 h/day, 5 days/week. At sacrifice, among other organs, the testes, epididymis and ovaries were weighed and macroscopically examined. No effects on body weights were observed, but increased incidence of discharges around the eyes and nose were observed.

Cooper *et al.* performed two studies with Long-Evans rats (n=10/group) in which the acute effects of inhalation of methanol on male sex hormones (LH, FSH, testosterone, prolactin) were determined (Cooper *et al.*, 1992). In the first study, the concentration of methanol was 0, 200, 5000 and 10000 ppm (0, 262, 6550, 13100 mg/m^3,

whole body) for 6 h. In the second study, the concentration was 0, 5000 ppm (0, 6550 mg/m^3, whole body) for 1, 2 and 6 h. Hormone levels were determined just after exposure (study 1 and 2) and 18 h after the end of exposure (study 1). Furthermore, half of the animals were acclimated (2 weeks prior to handling) to the experimental conditions and the other half was not-acclimated. No effects on body weight and testis weights were observed. Statistically significant effects of methanol exposure were observed on serum levels of hormones in study 1 (Figure 1) and in study 2 (Figure 2), but the direction and magnitude of the effects were strongly dependent on whether or not the animals had been acclimated to the test situation.

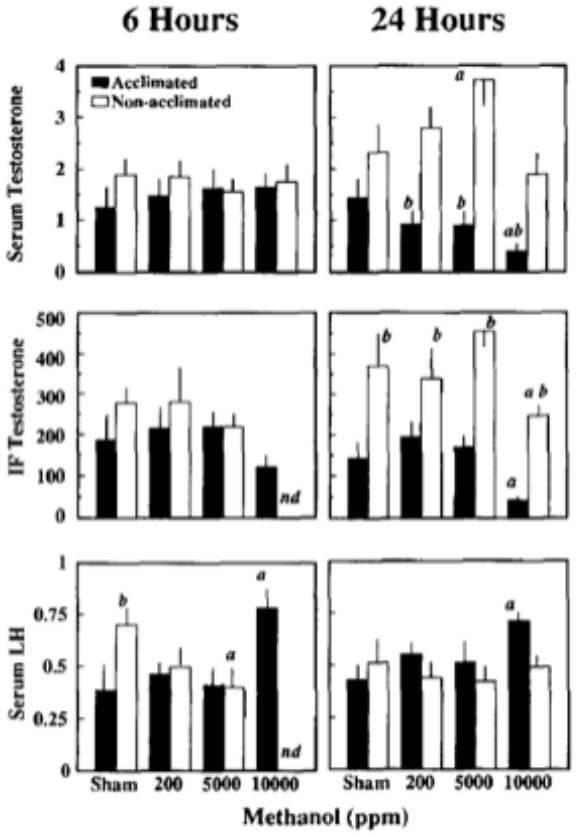


Figure 1: Serum and interstitial fluid testosterone and serum luteinizing hormone (LH) concentrations (ng/ml ± SEM) in rats killed 6 or 24 h after the initiation of exposure to methanol for 6 h in study 1 (n=10 rats per group; (a) versus sham-exposed control, under same handling condition, $p<0.05$; (b) acclimated versus non-acclimated, $p<0.05$; (nd) not determined).

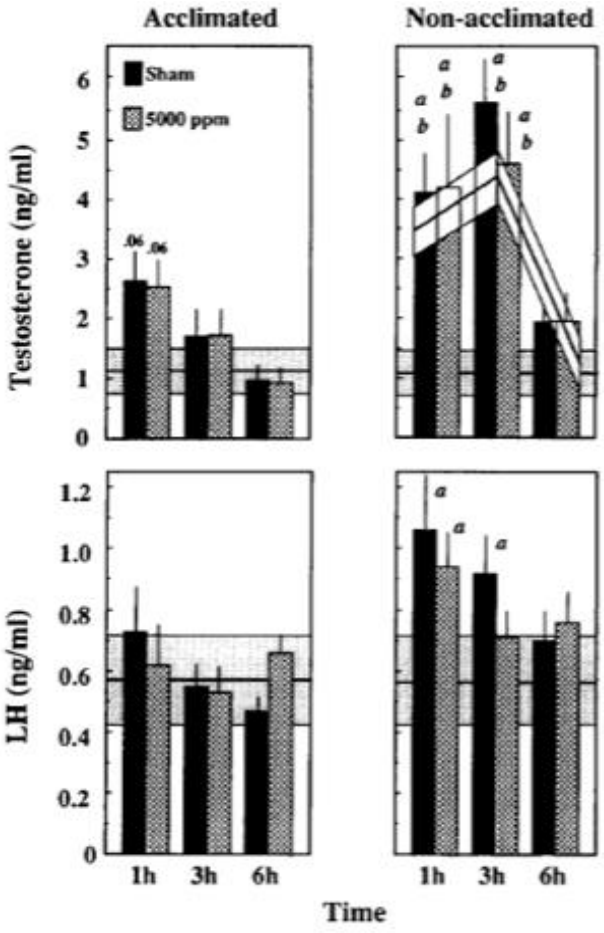


Figure 2: Serum testosterone and luteinizing hormone (LH) concentrations (ng/ml ± SEM) in rats killed after 1, 3 or 6 h exposure to 5000 ppm methanol in study 2. For each panel, mean values for cage-control, non-acclimated rats are depicted as a horizontal line bisecting a stippled line representing the SEM. Mean and SEM of testosterone concentrations observed in non-acclimated, etherized rats are shown in lightly stippled overlay on upper right panel (n=10 rats per group; (a) versus acclimated group under same exposure condition, $p<0.05$; (b) versus cage controls, $p<0.05$).

Lee *et al.* studied the effect of methanol on serum concentrations of testosterone and testis and seminal vesicle weights in male Sprague-Dawley rats (n=9-10/group) exposed to 200 ppm (262 mg/m^3, whole body) of methanol 8 h/day, 5 days/week for 1, 2, 4 or 6 weeks in study 1 (Lee *et al.*, 1991). Animals were sacrificed on the last day of exposure. No statistically significant changes in serum testosterone levels were measured upon exposure to methanol in study 1 (Table 19). In addition, no effects on body weights, macroscopic examination and weight of reproductive organs and testosterone concentrations were observed. In study 2, the age-dependent effect of methanol on testis morphology was studied in Long-Evans rats (n=8-13 group). The younger animals were 10 months old at the end of the exposure period (0, 50, 200 and 800 ppm (0, 65.5, 262, 1048 mg/m^3, whole body) for 20 h/day for 13 weeks), the older males were 18 months old at the end of the exposure period (0 and 800 ppm (0 and 1048 mg/m^3, whole body) for 20 h/day for 13 weeks). For both ages, no effects of methanol exposure were observed on body weights, testis weights, gross testicular abnormalities and incidence of testicular lesions.

Table 19: Serum testosterone levels of Sprague-Dawley rats exposed to 200 ppm methanol in study 1

		Testosterone concentration (ng/ml serum[a])	
Exposure period (weeks)	Age of rats (weeks)	Air exposed	200 ppm methanol
1	9	5.91 ± 4.09 (100%)	6.18 ± 2.12 (105%)
2	10	3.78 ± 3.45 (100%)	6.65 ± 5.81 (176%)
4	12	6.42 ± 2.78 (100%)	6.66 ± 5.18v (104%)
6	14	6.07 ± 2.45 (100%)	3.79 ± 2.37 (62%)

[a]Values are mean ± SD, values between brackets indicate percentage vs. corresponding control.

Cameron *et al.* studied the effect of methanol via inhalation (0, 200, 2000, 10000 ppm methanol (0, 262, 2620, 13100 mg/m^3, whole body) for 8 h/day, 5 days/week for 1, 2, 4 and 6 weeks) on reproductive hormones in groups of 5 male Sprague-Dawley rats (Cameron *et al.*, 1984). Animals were sacrificed 16 h after the last exposure to determine serum levels of testosterone, FSH and LH. Serum levels of testosterone were statistically significantly decreased in the low dose group at week 2 (-45%) and 6 (-68%) and in the mid dose group at week 6 (-41%; Table 20). A statistically significant increase in serum LH levels (211%) was observed in the animals of the high dose group at week 6 (LH was not measured at the other weeks; Table 20). No dose-dependent effects were observed on serum hormone levels upon exposure to methanol. In an additional study to determine the mechanism of decreased serum testosterone levels, male animals (5/group) were exposed to 200 ppm (262 mg/m^3, whole body) methanol for 6 weeks. Following the last exposure, the rats were given an i.v. injection with [^{14}C]testosterone. The authors concluded that methanol had no effect on the rate of testosterone removal from the blood, indicating that methanol might have a direct effect on testicular testosterone production.

Table 20: Effects of inhaled methanol on the serum concentrations of testosterone, LH and FSH in male mature rats

	Length of exposure			
Methanol (ppm)	1 week	2 weeks	4 weeks	6 weeks
	Testosterone			
0	100 ± 25	100 ± 22.3	100 ± 16	100 ± 23
200	98 ± 33	55 ± 17*	79.2 ± 22	32 ± 31*
2000	115 ± 38	74 ± 8.8	73 ± 16	59 ± 18*
10000	152 ± 43	111 ± 32	83 ± 20	119 ± 45
	LH			
0	100 ± 25	N.D.	N.D.	100 ± 20
200	70 ± 14	N.D.	N.D.	83 ± 60
2000	N.D.	N.D.	N.D.	120 ± 110
10000	80.3 ± 14	N.D.	N.D.	311 ± 107*
	FSH			

0	100 ± 17	N.D.	N.D.	100 ± 26
200	91 ± 7.4	N.D.	N.D.	106 ± 15.5
2000	N.D.	N.D.	N.D.	125 ± 24
10000	84.3 ± 8.3	N.D.	N.D.	105 ± 17

Mean concentrations of hormones ± SD are given in % of respective controls; *p<0.05, N.D.: not determined.

In a second study of Cameron *et al.*, Sprague-Dawley rats (5/group) were exposed by inhalation to 0 and 200 ppm methanol (0, 262 mg/m^3, whole body), 6 h/day for 1 or 7 days (Cameron *et al.*, 1985). Animals were sacrificed immediately or 18 h after the last exposure to measure serum levels of testosterone, LH and corticosterone. After the 1-day exposure, serum testosterone levels were significantly decreased (-59%, p<0.05) immediately after exposure and returned to control values after 18 h (Table 21). No effects were observed after the 7-days exposure.

Table 21: Mean serum levels of testosterone, LH and corticosterone (± SD) in male mature rats after inhalation of methanol.

	1-day exposure		7-day exposure	
Methanol (ppm)	End of exposure	18 h post exposure	End of exposure	18 h post exposure
	Testosterone			
0	100 ± 17	100 ± 20	100 ± 26	100 ± 17
200	41 ± 16*	98 ± 18	81 ± 22	82 ± 27
	LH			
0	100 ± 30	100 ± 35	100 ± 28	100 ± 36
200	86 ± 32	110 ± 40	78 ± 13	70 ± 14
	Corticosterone			
0	100 ± 20	N.D.	100 ± 21	N.D.
200	115 ± 18	N.D.	74 ± 26	N.D.

Mean concentrations of hormones ± SD are given in % of respective controls; *p<0.05, N.D.: not determined.

In a combined chronic toxicity and carcinogenicity study (similar to OECD TG 453) 52 male and 53 female B6C3F1 mice per group were exposed to 0, 10, 100 or 1000 ppm methanol vapour (0, 13.1, 131, 1310 mg/m3, whole body), 19.5 h per day, 7 days per week for 18 months (New Energy Development Organization, 1985a, New Energy Development Organization, 1987). No dose-related effects on survival, body weight, food consumption, urinalysis, haematology or clinical chemistry parameters were noted. At 1000 ppm (1310 mg/m^3), testicular atrophy was observed, relative and absolute weight of testis were significantly (p<0.05) reduced (data not specified in original report). One animal showed severe testicular atrophy with a relative weight of testis of 25% as compared to other animals at 1000 ppm (1310 mg/m^3). In females, significantly (p<0.05) increased absolute weights of kidney and spleen were observed at 1000 ppm (1310 mg/m^3), but relative weights were not statistically different. In addition, swellings of spleen, preputial glands and uterus were observed at necropsy. The LOAEC is 1000 ppm (1310 mg/m^3).

Methanol exposure did not result in major effects on fertility and sexual function in monkeys, rats or mice. The duration of pregnancy and gestation was statistically significantly reduced upon exposure to methanol vapor in monkeys but was still within the normal range (Burbacher *et al.*, 1999). In mice, statistically significantly decreased testes weight and severe testicular atrophy in one animal were observed at 1000 ppm (New Energy Development Organization, 1985a, New Energy Development Organization, 1987). In rats, reduced relative and absolute weight of testis or earlier testis descent were observed but was not dose-related (New Energy Development Organization, 1985b, New Energy Development Organization, 1987, Takeda, 1988). A carcinogenicity study demonstrated statistically significantly increased incidence of adenomas and sarcomas in the reproductive organs of rats (Soffritti *et al.*, 2002). Mechanistic studies indicated that methanol induced a decrease in serum levels of testosterone in rats (Cameron *et al.*, 1984, Cameron *et al.*, 1985, Lee *et al.*, 1991, Cooper *et al.*, 1992). No dose-response relationship was demonstrated and a decrease of serum testosterone levels was not always observed; duration of exposure, acclimation (acclimated vs. non-acclimated) and/or time of measurement of serum levels (direct after exposure vs. 18 h after exposure) could have influenced the degree of decrease in serum levels of testosterone (Cameron *et al.*, 1985, Cooper *et al.*, 1992).

Human studies

No studies were found demonstrating adverse effects on fertility or sexual function or absence of such effects upon methanol exposure in humans.

10.10.3 Comparison with the CLP criteria

Classification in category 1A is based on human data and is not applicable as there is no information on the effects of trimethyl borate on sexual function and fertility in humans. No clear evidence of adverse effects on sexual function and fertility by boron in humans have been found, based on human epidemiological studies focused on environmental and occupational exposure, as also discussed by RAC (RAC, 2019). For methanol, no epidemiologic information on sexual function and fertility in humans is available and thus classification in category 1A is not justified.

Classification in category 1B is based on sufficient data in animals showing clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. There is no information available on the reproductive toxicity of trimethyl borate itself. The registrants have applied read-across from the data on boric acid and other borates, and on methanol because trimethyl borate is quickly hydrolysed into these two substances. After oral and inhalation exposure, complete hydrolysis in the body is expected. As a result, no differences in uptake and toxicity is expected after exposure to trimethyl borate and to boric acid and methanol.

The available information on boric acid justifies classification in category 1B as previously advised by RAC for multiple borates (RAC, 2010, RAC, 2014b, RAC, 2014c, RAC, 2014d, RAC, 2019). The new information by Aktas *et al.* (2020) suggests that this occurs via oxidative stress in testicular tissue. No such effects were observed in the available human studies with borates. However, boron exposures in human studies are often far below LOAELs found in animal studies. Published animal studies are therefore relevant to assume comparable boric acid-induced adverse fertility effects in humans. Seen the fast and complete hydrolysis of trimethyl borate into methanol and boric acid such a classification would also be warranted for trimethyl borate. However, only if the toxicity of the other hydrolytic product methanol would not be so high that it would prevent such effects due to mortality or excessive toxicity.

Multiple studies have indicated some minor methanol-induced effects on sexual function and fertility in rodents via oral and inhalation, varying from altering hormone levels, earlier testis descent and increased incidence of tumour formation in testis and uterus in the absence of observed general toxicity. However, an

increase in tumours of the reproductive organs is not normally considered to justify classification for effects on sexual function and fertility as most tumours occur mainly late in life. Females are already in menopause at this age and the male reproductive function has also declined. No effects on the reproductive organs were observed in the repeated dose studies. In addition, this increase was only observed at 2000 mg/kg bw/day for 3 years which is clearly above the limit dose (Soffritti *et al.*, 2002). The earlier testis descent in the two-generation study by Takeda (1988) in rats does not seem to affect the fertility as there was no reduction in the fertility of the F1. Also, it is unclear whether the earlier testis descent is due to the in utero exposure indicating a developmental effect or due to the post-natal exposure indicating an effect on sexual function and fertility. No effects on fertility was observed in the available reproductive studies. Further, no adverse effects on sexual function and fertility upon exposure to methanol are observed in non-human primates. Adverse effects on sexual function and fertility were only noted upon exposure to high levels of methanol; 2000 mg/kg bw/day via the oral route or 1000 ppm (1310 mg/m^3) via the inhalation route, above or near the lethal dose (300-1000 mg/kg bw) of methanol in humans, according to IPCS (IPCS, Environmental Health Criteria 196, Methanol, WHO, 1997[14]). In addition, due to the difference in metabolism in rodents compared to humans resulting in differences in the concentration of the different metabolites, the results in rodents may not be relevant to humans. Therefore, the limited effects of methanol in rodents do not warrant classification in category 1B.

Upon hydrolysis of trimethyl borate, the simultaneous exposure to methanol at dose levels inducing an effect on sexual function and fertility may result in severe toxicity and or mortality that could prevent such reprotoxic effects from boric acid. These toxic effects cannot be compared in animals as the toxicity in animals for methanol is not representative for the effects in humans (RAC, 2014a). Therefore, this comparison has to be done for effects in humans. The ED10 for fertility and sexual function of boric acid is 103 mg/kg bw/day, as earlier reviewed by RAC (RAC, 2019). This ED10 for boric acid can be converted to an ED10 for trimethyl borate of 173 mg/kg bw/day (103 * 103.9 / 61.83 = 173 mg/kg bw/day) and extrapolated to humans considering the correction factor for allometric scaling (rat = 4; ECHA, 2012), resulting in a human ED10 of 173 / 4 = 43 mg/kg bw/day. The simultaneous exposure to methanol at the human ED10 would be (43 /103.9) * (3 * 32) = 40 mg/kg bw/day as 3 moles of methanol are formed from 1 mole of trimethyl borate, which is below the minimal lethal dose of methanol in humans (300 - 1000 mg/kg bw). This results in a clear margin between the ED10 for effects on sexual function and fertility in humans and the minimal lethal dose of methanol. Therefore, it is considered that there is insufficient evidence to conclude that the toxicity of the hydrolytic product methanol would prevent such reprotoxic effects being induced by boric acid after exposure to trimethyl borate. Classification of trimethyl borate in category 1B for effects on sexual function and fertility is warranted based on the expected effects of the hydrolytic product boric acid.

Classification in category 2 is based on limited data in animals. Category 2 is not considered relevant as there are clear effects on sexual function and fertility of the hydrolytic product boric acid in multiple good studies on reproduction.

In the RAC evaluation of the change of the SCL into GCL for a number of borates the ED10 for boric acid for effects on sexual function and fertility was determined at 103 mg/kg bw/day (RAC, 2019). As one mole of boric acid is formed from 1 mole of trimethyl borate, the ED10 for trimethyl borate is 103 * 103.9 / 61.83 = 173 mg/kg bw/day. According to section 3.7.2.6.3 of the CLP Guidance (2017), a substance with a 4 < ED10 < 400 mg/kg bw/day belongs to the **medium** potency group The ED10 for trimethyl borate is clearly within the potency limits for the medium potency group (GCL). Therefore, no SCL is justified.

There is no information on the effects of trimethyl borate on sexual function and fertility via the inhalation and the dermal route. It could be argued that the uptake via the skin is limited. However, after inhalation the same

[14] https://wedocs.unep.org/handle/20.500.11822/29474

rapid and complete hydrolysis can be expected and therefore also the same effects as via the oral route. Therefore, no route should be specified for the classification as **Repr. 1B, H360F**.

10.10.4 Adverse effects on development

Information on trimethyl borate

There is no information on reproductive toxicity for trimethyl borate available. Trimethyl borate is quickly hydrolysed into boric acid and methanol. Therefore, read-across from data on boric acid and methanol is used here.

Information on boric acid and borates

In Table 22 and Table 23, data earlier included in CLP report for borates and boric acid are cited (ECHA, 2018). These studies have been reviewed by RAC in 2019 (RAC, 2019). New animal and human studies published in 2018 and 2019 on boric acid or other borates have been added.

Table 22: Summary table of animal studies on adverse effects on development of boric acid

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Oral			
Prenatal developmental toxicity study OECD TG 414 Feed Rat Sprague-Dawley M/F 28-32 per dose group GLP Klimisch score 2	Boric acid (purity not stated), Doses: 0, 250, 500, 750, 1000, 2000 ppm, equivalent to 19 (3.3), 36 (6.3), 55 (9.6), 76 (13.3), 143 (25) mg boric acid (B)/kg bw/day GD 0-20	Dams: no toxicity NOAEL is 2000 ppm, equivalent to 143 (25) mg boric acid (B)/kg bw/day. Foetuses: 76 (13.3) mg boric acid (B)/kg bw/day: 1530 ng B/g blood (vs. 229 ng B/g blood in control), reduction in the mean foetal bw per litter (-4%*), short 13th rib (1.2%* vs. 0.7% foetuses/litter in control) and wavy rib (2.1%* vs. 0% foetuses/litter in control). 143 (25.0) mg boric acid (B)/kg bw/day: 2820 ng B/g blood (vs. 229 ng B/g blood in control), reduction in the mean foetal bw per litter (-12%*), short 13th rib (1.5%* vs. 0.7% foetuses/litter in control) and wavy rib (9.9%* vs. 0% foetuses/litter in control). NOAEL is 55 (9.6) mg boric acid (B)/kg bw/day. LOAEL is 76 (13.3) mg boric acid (B)/kg bw/day.	(Study report 1994, Price *et al.*, 1996, Price *et al.*, 1997)[1]
Prenatal developmental toxicity study Similar to OECD TG 414	Boric acid (>98% pure), 0, 78 (13.7), 163 (28.4), 330	Dams: altered food intake and significantly (p<0.05) increased relative weight of kidney (≥163 (28.4) mg boric acid (B)/kg bw/day). Significantly (p<0.05) decreased bw gain and gravid uterine weight (≥330 (57.8) mg boric acid (B)/kg bw/day). Foetuses: significant (p<0.05) reduction of bw (≥78 (13.7) mg boric acid (B)/kg bw/day), increased incidence of malformations of short rib XIII (≥78	(Heindel *et al.*, 1992)[2]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Feed Rat Sprague-Dawley M/F 26-28 per group GLP Klimisch score 2	(57.8), 539 (94.3) mg boric acid (B)/kg bw/day GD 6-15 for highest dose group GD 0-20 for other dose groups	(13.7) mg boric acid (B)/kg bw/day), significantly (p<0.05) increased prenatal mortality (≥539 (94.3) mg boric acid (B)/kg bw/day). NOAEL (maternal) is 78 (13.7) mg boric acid (B)/kw bw/day. LOAEL (maternal) is 163 (28.4) mg boric acid (B)/kg bw/day. NOAEL (teratogenicity/embryotoxicity) is <78 (13.7) mg boric acid (B)/kg bw/day (based on bw). LOAEL (teratogenicity/embryotoxicity) is 78 (13.7) mg boric acid (B)/kg bw/day (based on bw).	
Two-generation reproductive toxicity study NTP's reproductive assessment Feed Mice Swiss CD1 M/F 40(M)+40(F) in control group 20(M)+(20)F in dosed groups GLP Klimisch score 2	Boric acid (>99% pure), 0, 1000 (175), 4500 (787.5), 9000 (1575) ppm boric acid (B) equivalent to 0, 152 (26.6), 636 (111.3), 1262 (220.9) mg boric acid (B)/kg bw/day	F0: live pups per litter reduced (-51%* from control) at 636 (111.3) mg boric acid (B)/kg bw/day. No litters produced at highest dose. Reduction of 15% of adjusted bw of pups at 636 (111.3) mg boric acid (B)/kg bw/day. F1: oestrous cycles significantly (p<0.05) shorter at 152 (26.6) mg boric acid (B)/kg bw/day. NOAEL (F0, F1, F2) is <1000 (175) ppm boric acid (B) equivalent to <152 (26.6) mg boric acid (B)/kg bw/day. LOAEL (F0, F1, F2) is 1000 (175) ppm boric acid (B) equivalent to 152 (26.6) mg boric acid (B)/kg bw/day.	(NTP (National Toxicology Program), 1990, Fail *et al.*, 1991)
Prenatal developmental toxicity study GLP Feed	Boric acid (>98% pure), 0, 248 (43), 452 (79), 1003	Dams: significantly (p<0.05) decreased bw gain and gravid uterine weight (≥1003 (175) mg boric acid (B)/kg bw/day). Dose-related increase in incidence of renal tubular dilation. Foetuses: significant (p<0.05) reduction of average bw per litter (≥452 (79) mg boric acid (B)/kg bw/day), significantly (p<0.05) increased resorption per litter (≥1003 (175) mg boric acid (B)/kg bw/day).	(Heindel *et al.*, 1992)[2]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Mice Swiss-Albino CD-1 M/F 26-28 per group GLP Klimisch score 2	(175) mg boric acid (B)/kg bw/day GD 0-17	NOAEL (teratogenicity/embryotoxicity) is 248 (43) mg boric acid (B)/kg bw/day. LOAEL (teratogenicity/embryotoxicity) is 452 (79) mg boric acid (B)/kg bw/day.	
Inhalation			
Prenatal developmental toxicity study OECD TG 414 Rat Sprague-Dawley M/F 25 per dose GLP not specified Klimisch score 2	Boric acid (20% w/w, purity not stated) in cellulose insulation (CI) aerosols 0, 15, 90, 270 mg CI/m^3, nose only, equivalent to 0.65 (0.11), 4.0 (0.69) and 11 (2.0) mg boric acid (B)/kg bw/day 6 h/day equivalent exposure started GD 6-19	Dams: no difference in bw between exposure groups. However, 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). NOAEL (maternal) is 15 mg/m^3 CI (estimated delivered dose: 0.65 (0.11) mg boric acid (B)/kg bw/day). LOAEL (maternal) is 92 mg/m^3 CI (estimated delivered dose: 4.0 (0.69) mg boric acid (B)/kg bw/day). Foetuses: no adverse effects found on development 4.0 (0.69) mg boric acid (B)/kg bw/day: reduction (-6%*) bw females. 11.0 (2.0) mg boric acid (B)/kg bw/day: reduction bw males (-6%*) and females (-7%*). NOAEL: 265 mg/m^3 CI (estimated delivered dose: 11 (2.0) mg boric acid (B)/kg bw/day). Foetal toxicity is not evident, besides reduction in foetal bw, no statistically significant differences were found in development of foetuses.	(Pleus *et al.*, 2018)

[1]Adopted from CLH report for boric acid and borates (ECHA, 2018)

[2]Adopted from Annex XV transitional reports. Boric acid (ECHA, 2010)

* statistically significant different from control $p<0.05$

Dose of boron (B) is indicated in between brackets.

Table 23: Summary table of human data on adverse effects on development of boron

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Publication	Boron, environmental exposure	Prospective study Mother-child cohort in Northern 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 boron levels of >80 µg/l were found to be inversely associated with birth length (B-0.69 cm, 95% CI:-1.4, p = 0.04 per 100 µg/l serum B). No statistically significant associations between boron exposure and birth weight or head circumference were found.	(Igra *et al.*, 2016)[1]
Publication	Boron, environmental exposure	Retrospective study Females residing in Marmare, Turkey n: 190 Pregnancy outcomes (sex ratio, preterm birth, birth weight, congenital anomalies, abortion, 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 blood in the highest exposure group.	(Duydu *et al.*, 2018b)

[1]Adopted from CLH report for boric acid and borates (ECHA, 2018)

DBE: daily boron exposure

Information on methanol

Scientific data relevant for methanol regarding developmental toxicity have been reviewed by RAC in 2014 (RAC, 2014a). Information on methanol and its effects on development from the CLH report for methanol have been adopted in this report (ECHA, 2013c).

Table 24: Summary table of animal studies on adverse effects on development of methanol

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Oral, gavage, i.v., i.p.			
Prenatal development toxicity study Experimental study Gavage Rats Long-Evans M/F 10-13 per group GLP not specified Klimisch score 2	Methanol (≥99.9% pure), 0, 1.3, 2.6, 5.2 ml/kg bw (0, 1030, 2059, 4118 mg/kg bw) Treated once on GD 10	Dams: -4118 mg/kg bw: decreased bw gain (-23%*) and reduced food consumption (-36%*). Foetuses: Dose-related response observed in decrease of mean bw of pups or total foetuses with anomalies. -1030 mg/kg bw: reduction (-18%*) bw, increase foetuses with anomalies (91.6%* vs. 53.8% in control), -2059 mg/kg bw: (-15%*) bw, increase foetuses with anomalies (81.8%* vs. 53.8% in control) -4118 mg/kg bw: (-8%*) bw, increase foetuses with anomalies (100%* vs. 53.8% in control), increase incidence undescended testes (60%* vs. 0% in control), increase incidence eye anomalies (30%* vs. 0% in control) NOAEL (teratogenicity/embryotoxicity) is <1030 mg/kg methanol (based on bw and foetuses with anomalies). LOAEL (teratogenicity/embryotoxicity) is 1030 mg/kg methanol (based on bw and foetuses with anomalies).	(Youssef *et al.*, 1997)
Prenatal development toxicity study Experimental study Gavage Rats Wistar M/F 10-17 per group GLP not specified Klimisch	Methanol (purity not stated), 0 or 2500 mg/kg bw/day GD 6-15	Dams: No effects on maternal toxicity was reported Foetuses: Decreased bw (-7%*), increased incidence skeletal anomalies (45%* vs. 6% in control), rib anomalies (36% vs. 3% in control), cervical anomalies (35% vs. 1% in control).	(De-Carvalho *et al.*, 1994)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
score 2			
Prenatal development toxicity study Experimental study Drinking water Rats Long-Evans M/F 10 per group GLP not specified Klimisch score 2	Methanol (purity not stated), 0, 2% *v/v* (0, 2500 mg/kg bw) GD 15-17 or GD 17-19	Dams: No effects on maternal toxicity was reported. No effects were observed on litter size, pup mortality, birth weight, pup weight gain during lactation and the day of eye opening. Pups: The proportion of pups successfully attaching to nipples did not differ statistically significant across the treatment groups (F(2,27) = 2.35). The methanol groups were different from control group latencies (F,(2,27) = 7.57**). Prenatal exposure to methanol, therefore, produced a statistically significant impairment in suckling behaviour that was evident 24 h after birth. The proportion of pups successfully reaching the home area within 3 minutes did not differ across treatment groups, (F(2,27) = 2.16). On the other measures of homing behaviour, the methanol groups were similar, and both differed sharply from the control group. Of pups that successfully reached the home area, those exposed prenatally to methanol exhibited longer latencies than controls (F(2,27) = 23.01***). The methanol-exposed animals took about twice as long as control pups. Their increased latencies may have been due, in part, to the tendency for methanol-exposed pups to choose the wrong initial direction more often than controls. Further, pups in both methanol groups crossed more rectangles than controls to reach the home area (F(2,27) = 11.34**). In addition, the total number of rectangles crossed during the entire homing test was elevated over control levels (F(2,27) = 7.19**).	(Infurna *et al.*, 1986)[1]
Prenatal development study Experimental study i.v. injection Rats Sprague-Dawley M/F 4-6 per group GLP not	Methanol (>99% pure), 0, 100, 500 mg/kg bw GD 0-20, Treated once at GD 14 or 20	100 mg/kg: H_2O uptake rate decreased 30% (GD 14) or 31% (GD 20) 500 mg/kg: H_2O uptake rate decreased 57% (GD 14) or 45% (GD 20) Data indicated methanol decreased uteroplacental blood flow, decreasing methanol presentation to the conceptus and possibly producing conceptual hypoxia.	(Ward *et al.*, 1996)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
specified Klimisch score 2			
Prenatal development toxicity study Experimental study Gavage Mice CD-1 M/F 6-7 per group GLP not specified Klimisch score 2	Methanol (not stated), 0, 4000, 5000 mg/kg bw Treated once on GD 7	Dams: No effects Foetuses: Foetal weight and the incidences of live and dead foetuses were not affected. 4000 mg/kg bw: increased resorptions (4.3 vs. 1.3 in control) 5000 mg/kg bw: increased resorptions (6.0 vs. 1.3 in control) Skeletal examinations revealed that maternal MeOH exposure (4000 and 5000 mg/kg bw) can alter segment patterning in the developing mouse embryo, resulting in posteriorisation of cervical vertebrae. LOAEL (foetal) is 4000 mg/kg bw.	(Connelly *et al.*, 1997)[1]
Prenatal development toxicity study Experimental study Gavage Mice CD-1 F 4-8 per group GLP not specified Klimisch score 2	Methanol (>99% pure), 0 and 4000 mg/kg bw/day GD 6-15	Dams: Reduced bw at GD 15 (-14%, n.s.) and 17 (-22%), bw gain not affected. Foetuses: Reduced bw (-17%, $p<0.05$), decrease of live foetuses per litter (5.9 vs. 10.5 live/litter in control, n.s.), increased incidence cleft palate (43.5% per litter vs. 0% in control, $p<0.01$) and exencephaly (28.8% per litter vs. 0% in control, n.s.).	(Rogers *et al.*, 1993)
Prenatal development toxicity	Methanol (>99%	Net maternal bw gain was not affected by dietary folic acid or MeOH treatment.	(Fu *et al.*, 1996)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
study Experimental study Gavage Mice CD-1 M/F 21-24 per group GLP not specified Klimisch score 2	pure), 0 and 5000 mg/kg bw/day GD 6-10	Maternal bw were similar among the groups throughout gestation with the exception that on GD 18, dams fed adequate folic acid and treated with water had higher bw than the marginal folic acid-water group. Non-gravid maternal bw were similar among the groups. Implantation sites, live and dead foetuses, and resorptions were counted, foetuses were weighed individually and examined for cleft palate and exencephaly. The marginal folic acid dietary treatment resulted in low maternal liver (-50%) and red cell folate (-30%) concentrations, as well as low foetal tissue folate concentrations (-60% to -70%) relative to the adequate folic acid dietary groups. Marginal folic acid treatment alone resulted in cleft palate in 13% of the litters (vs. 0% in control). Marginal folic acid and MeOH treatment resulted in a further increase in the litters affected by cleft palate (72% of litters affected). The percent of litters affected by exencephaly was highest in the marginal folic acid and MeOH group. These results show that marginal folate deficiency in pregnant dams statistically significantly increased teratogenic potency of MeOH.	
Prenatal development toxicity study Experimental study Gavage Mice CD-1 M/F 13-29 per group GLP not specified Klimisch score 2	Methanol (purity not stated), 0, 4000, 5000 mg/kg bw/day GD 6-15	During gestation, maternal bw were significantly (p<0.05) affected by dietary folic acid treatment. Dams in the 400 nmol folic acid/kg group (low) had a significantly (p<0.05) lower bw compared to dams in the 600 (marginal) and 1200 (adequate) nmol folic acid/kg groups. MeOH significantly (p<0.05) reduced the gestational weight gain in dams fed the 600 and 1200 nmol folic acid/kg groups. Both of these parameters were affected by folate treatment; dams in the 400 nmol folic acid/kg folate group gained less weight compared to the 600 and 1200 nmol folic acid/kg groups. MeOH did not affect these parameters. Maternal haematocrit levels were not affected by either MeOH or folate treatment. Plasma folate concentrations were not statistically significant affected by folate or MeOH treatment. Maternal liver weight was increased with low dietary folate; MeOH treatment resulted in increased liver weight in the 600 and 1200 nmol folic acid/kg groups. However, when based on non-gravid bw, only folate treatment had an effect. Similarly, kidney weights were increased with the lower diet folate and MeOH treatment. Relative kidney weights based on non-gravid bw were affected only by folate treatment. There was no effect of either treatment on total or relative spleen weight. Gravid uterus weights were lowest in the low dietary folate	(Sakanashi *et al.*, 1996)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		and MeOH groups with the lowest value occurring in the 400 nmol folic acid/kg group treated with 5000 mg/kg bw methanol. This lower gravid uterus weight reflected an increased number of resorptions in the low folic acid and methanol treated groups. Foetuses were examined for external (cleft palate and exencephaly) and skeletal anomalies. Both MeOH and low dietary folic acid increased the incidence of cleft palate, with the highest number of affected litters in the low dietary folic acid group. These results support the concept that maternal folate status can modulate the developmental toxicity of methanol. In conclusion, both MeOH and low dietary folic acid increased the incidence of cleft palate, with the highest number of affected litters in the low dietary folic acid group. These results support the concept that the maternal folate status can modulate the developmental toxicity of MeOH.	
Prenatal development study Experimental study i.v. injection Mice CD-1 M/F 4-6 per group GLP not specified Klimisch score 2	Methanol (>99% pure), 0, 100, 500 mg/kg bw GD 0-18, Treated once at GD 18	100 mg/kg: H_2O uptake rate decreased 26% 500 mg/kg: H_2O uptake rate decreased 47% Data indicated methanol decreased uteroplacental blood flow, decreasing methanol presentation to the conceptus and possibly producing conceptual hypoxia.	(Ward *et al.*, 1996)[1]
Inhalation			
Prenatal development toxicity study Experimental study Monkey *Macaca*	Methanol (>99% pure), 0, 200, 600, 1800 ppm (0, 262, 786, 2358 mg/m^3, whole	Dams: Although not statistically significant, five MeOH-exposed females were Caesarean sectioned due to pregnancy complications such as uterine bleeding and prolonged unproductive labour. The mean length of pregnancy in the MeOH-exposed groups was significantly ($p<0.05$) decreased by 6 to 8 days when compared to controls. Pups:	(Burbacher *et al.*, 2004)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
fascicularis M/F 48 per group GLP not specified Klimisch score 2	body) 2.5 h/day 7d/week throughout pregnancy	There were no MeOH-related effects on offspring birth weight or new-born health status. A total of 34 live-born infants were delivered (control=8, 200 ppm=9, 600 ppm=8, 1800 ppm=9). One female each in the control and 600-ppm group delivered a stillborn infant and a Caesarean section was required to deliver a hydrocephalic infant who died in utero in the maternal 1800-ppm group. Overall results: for this non-human primate model, daily 2.5 h exposures to MeOH vapor from 200 to 1800 ppm for nearly 1 year do not cause overt maternal toxicity in *M. fascicularis* females. The menstrual cycle and the ability of females to conceive were unaffected by these exposures. The incidence of maternal complication during pregnancy and delivery was high in the MeOH-exposed females (28% (8/28), for the MeOH exposed females vs. 22% (2/9) for the control). The increase in complications, however, was not statistically significant when compared to controls. The health status of live-born offspring was unaffected by maternal MeOH exposure. MeOH exposures were associated, however, with a reduction in the length of pregnancy (168, 160, 162 and 162 days). The reduced pregnancy lengths of the MeOH-exposed females may reflect the premature activation of the foetal HPA axis that controls timing of birth. Whether this represents a direct (foetal) or indirect maternal treatment effect is unknown. Independent of the specific biological mechanism, the reduced pregnancy durations of MeOH-exposed dams suggest a systematic disturbance in the timing of labour and delivery	
One-generation reproduction toxicity study Similar to OECD TG 415 Monkey *Macaca fascicularis* M/F 11-12 per group GLP not specified	Methanol (purity not stated), 0, 200, 600, 1800 ppm (0, 262, 786, 2358 mg/m^3, whole body) 2.5 h/day 7d/week Exposure from pre-breeding to pregnancy	Maternal: No effects on maternal toxicity were reported. Pups: Weight and size: No effects were observed of the infants at birth and at 9 months of age (severe wasting, resulting in euthanasia, was observed in two female pups of the high dose group after 12 months of age). Neurobehavioral function tests did not show significant MeOH-related effects on most domains of early behavioural development. No effects on social and neuro/behavioural development. However, MeOH exposure was associated with a delay in early sensorimotor development for male infants of all dose groups and with deficits in visual recognition memory for all infants of all dose groups.	(Burbacher *et al.*, 1999)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Klimisch score 2			
Prenatal development toxicity study Similar to OECD TG 414 Rat Sprague-Dawley M/F 36 per group GLP not specified Klimisch score 2	Methanol (purity not stated), 0, 200, 1000, 5000 ppm (0, 270, 1330, 6650 mg/m^3, whole body) 22.7 h/day GD 7-17	Dams: 5000 ppm dose: decrease in body-weight gain, food and drinking water consumption. One dam died, another one had to be killed before delivery. After delivery: Gestation time was prolonged (0.7 days); food and drinking water consumption were reduced during lactation; Foetal: 5000 ppm dose: about 50% of the foetuses with ventricular septal defects (visceral malformation in 16/20 litters or 64/131 foetuses) vs. 0% or near 0% in all other groups, and residual thymus (variation in all 20 litter or 70/131 foetuses) vs. about 2.4% to 2.9% in 4 litters each of all other groups. Other changes included significantly (p<0.05) increased incidence of skeletal anomalies: atresia of cervical arch/vertebra foramen costotransversarium (45%), bifurcated vertebral centre (14%) and cervical rib (65%) as well as excessive sublingual neuropore (50%), all of which malformations having no or little relevance in the other group except of atresia foramen with about 25% in the control and about 4% to 8% in the other exposure groups. Neo-/postnatal findings: live foetuses showing poor vitality (ca. 17% = on average 2/12 pups per litter died as compared with overall mortality 1 to 2% in the other groups). Retardation of growth was significantly (p<0.05) up to at weaning. Water consumption was reduced, in particular for females. At 8 weeks, brain, thyroid (males), thymus and testis weights were significantly (p<0.05) lower, and pituitary-gland weight of males was significantly (p<0.05) higher; 16% of the offspring (15/91 in 8/12 litters) had hemilateral absence of thymus. NOAEC (maternal/foetal) is 1000 ppm (1330 mg/m^3). LOAEC (maternal/foetal) is 5000 ppm (6550 mg/m^3).	(New Energy Development Organization, 1987, Takeda, 1988)[1]
Two-generation reproduction study Similar to OECD TG 416	Methanol (reagent grade), 0, 10, 100, 1000 ppm (0, 13, 131, 1310 mg/m^3,	F0: no effects were observed. F1: 1000 ppm (1310 mg/m^3): testis descent was completed within 16 through 20 post-natal days with the maximum at day 17 and 18 (32 and 39%, respectively), while in the respective control, descent was complete from 16 through 21 days with the maximum at day 19 (32%), indicating an earlier descent related to treatment. Absolute and relative brain weights, pituitary and thymus were significantly (p<0.05) lowered in the high-dose	(New Energy Development Organization, 1987, Takeda, 1988)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Rats Sprague-Dawley 30(M)+30(F) GLP not specified Klimisch score 2	whole body) 20 h/day F0: 8 weeks old to mating (M) or lactation (F) F1: birth to mating (M) or weaning of F2 pups 21 days post-delivery (F) F2: birth to 21 days old (M+F)	groups of either 8 (male and female), 16 (male) and 24 weeks (female). F2: 1000 ppm (1310 mg/m^3): As in F1 males, earlier descent of testis was noted. Significantly (p<0.05) reduced weight of brain, pituitary and thymus at week 8 (male and female). NOAEC (F1, F2) is 100 ppm equivalent to 131 mg/m^3. LOAEC (F1, F2) is 1000 ppm equivalent to 1310 mg/m^3.	
Prenatal development toxicity study Similar to OECD TG 414 Rat Sprague-Dawley M/F 13-15 per group GLP not specified Klimisch score 2	Methanol (>99% pure), 0, 5000, 10000, 20000 ppm (0, 6550, 13100, 26200 mg/m^3, whole body) 7 h/day GD:1-19 at 0-10000 ppm GD:7-15 at 20000 ppm	Dams: Slight unsteady gait only during the first days of exposure no effects on the bw and food consumption. Foetal: No resorptions 5000 ppm dose: no adverse effects 10000 ppm dose: -Bw decrease (female/male: 2.93* ± 0.26/3.12* ± 0.30 g vs 3.15 ± 0.32 g/3.34 ± 0.36 g in control), this effect may be caused by the increased number of foetuses. -Increase in the incidence of skeletal malformations (2% vs. 0% control) in cranium, vertebrae and ribs and visceral malformations (2% vs. 0% control) in eye, brain-exencephaly and encephaloceles- and cardiovascular and urinary system even if not statistically significant. 20000 ppm: In total 93% of litters and 54% of foetuses were affected by: -Bw decrease (female/male: 2.76* ± 0.47/2.82* ± 0.56 g vs. 3.15 ± 0.32 g/3.34 ± 0.36 g in control). -Statistically significant increase in the incidence of skeletal malformations	(Nelson *et al.*, 1985)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		(72% vs. 0% in the control) in cranium, vertebrae and ribs and visceral malformations (15% vs. 0% in control). In conclusion, it was observed that the percentage of litter with abnormal foetuses for 0, 5000, 10000 and 20000 ppm was 0, 15, 47 and 93%*. NOAEC (foetal) is 5000 ppm (6550 mg/m^3). LOAEC (foetal) is 10000 ppm (13100 mg/m^3). NOAEC (maternal) is 10000 ppm (13100 mg/m^3 ;as noted by NPT Expert Panel). LOAEC (maternal) is 20000 ppm (262000 mg/m^3).	
Prenatal development toxicity study Similar to OECD TG 414 Rat Long-Evans M/F 12 per group GLP not specified Klimisch score 2	Methanol (>99% pure), 0, 4500 ppm (0, 5895 mg/m^3, whole body) 6 h/day GD 6 until PN day 21	Dams: No effects on bw. Subtle behavioural changes were observed. Pups: Subtle behavioural changes were observed. No effect on bw was observed.	(Stern *et al.*, 1997)[1]
Prenatal development toxicity study Experimental study Rat Long-Evans M/F 5-6 per group GLP not	Methanol (>99% pure), 0, 15000 ppm (0 or 19650 mg/m^3, whole body) 7 h/day GD 7-19	Dams: Bw decreased during the first days of exposure. Pups: No treatment related effects were observed on pup mortality (2 dead pups at birth in control group). Incidence of malformed pups increased (two malformed pups in one litter of MeOH-treated group showing anophthalmia and agenesis of optical nerve), litter size (10.2 vs. 10.8 in control) and implantation loss (11.8 vs. 13.8 in control) but on post-natal day 1 (6.4 g* vs. 7.1 g in control) and 35 (females/males 116/129** g vs. 122/139 g in control) pup weights were slightly, but statistically significantly, lower in the MeOH treated animals than in the control animals.	(Stanton *et al.*, 1995)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
specified Klimisch score 3		Except for a small delay in vaginal opening (31.4** day vs. 29.7 day in control), no effects were observed on any of the developmental parameters measured.	
Prenatal development toxicity study Similar to OECD TG 414 Mice Crl:CD-1 M/F 5-17 per group GLP not specified Klimisch score 2	Methanol (purity not stated), 0, 1000, 2000, 5000, 7500, 10000, 15000 ppm (0, 1310, 2620, 6550, 9825, 13100, 19650 mg/m^3, whole body) 7 h/day GD 6-15	Maternal: One dead animal in each of 7500, 10000 and 15000 ppm group. No treatment related effects on clinical observations and bw. Foetuses: ≥2000 ppm: dose-related increased incidence of cervical ribs (49.6%** vs. 26.0% in control) ≥5000 ppm: dose-related increased incidence of cleft palate and exencephaly (15.5%*** vs. 0.13% vs. in control) ≥7500 ppm: dose-related decreased number live foetuses/litter (8.6** vs. 10.3 in control) ≥10000 ppm: increased incidence of fully resorbed litters (5* vs. 0 in control) and decreased foetal bw (-11%***) NOAEL (foetal) is 1000 ppm (1310 mg/m^3). LOAEL (foetal) is 2000 ppm (2620 mg/m^3).	(Rogers *et al.*, 1993)
Prenatal development toxicity study Similar to OECD TG 414 Mice CD-1 ICR BR M/F 20-27 per group GLP not specified	Methanol (>99% pure), 0, 10000 ppm (0, 13100 mg/m^3, whole body) 6 h/day GD 6-15	Maternal: No effects on maternal toxicity was reported. Foetuses: GD at 6-15: reduced foetal bw (0.81±0.03* g vs. 0.93±0.02 g in control) and increased incidences of resorptions (32.2%* vs. 4.4% in control), neural tube defects (46%* vs. 0% in control), cleft palate (82% vs. 0% in control) and digit malformations were observed /(36%* vs. 0%). GD at 7-9: the incidence of resorptions (13.4%* vs. 1.1% in control), neural tube defects (33% vs. 0% in control) and cleft palate (33% vs. 0% in control), but not the incidence of digit malformations, was increased whereas the number of live foetuses was decreased (10.4±0.9%* vs. 12.8±0.5 in control). GD at 9-11: only cleft palate (24%* vs. 0% in control) and digit malformations (12% vs. 0% in control) but no neural tube defects were observed.	(Bolon *et al.*, 1993)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Klimisch score 2		Conducted as pilot study	
Prenatal development toxicity study Similar to OECD TG 414 Mice CD-1 ICR BR M/F 20-27 per group GLP not specified Klimisch score 2	Methanol (>99% pure), 0, 5000, 10000 and 15000 ppm (0, 6550, 13100 and 19650 mg/m^3, whole body) 6 h/day GD 7-11	Dams: -GD 7: no effects on maternal bw Neurological effects (ataxia, circling, tilted heads or depressed motor activity) were observed. Resorptions were increased at 15000 ppm (39%* vs. 2.7% in control) as consequence the number of live foetus was decreased. -GD 7-9: at 15000 ppm maternal bw gain during gestation was decreased and neurological symptoms (ataxia, circling, tilted heads or depressed motor activity) were observed on the first days of exposure. Increased resorptions (0/5000/10000/15000 ppm: 2.7/0.5/16.6/46.2%*). -GD: 9-11: the dams showed neurological symptoms but no effect on bw and resorptions was observed. Foetal: -GD 7-9: at 15000 ppm number of live foetuses (7.9±1.1%* vs. 12±0.4% in control), and foetal weight were significantly (p<0.05) decreased (0.82±0.02*% vs. 0.92±0.05% in control). Developmental effects at 0, 5000, 10000 and 15000 ppm; neural tube defects: 0, 0, 30 and 65%*; cleft palate: 9, 4, 50* and 88%; renal variations: 41, 100*, 90 and 75%; ocular defects: 0, 0, 10* and 53%; and tail anomalies: 0, 0, 40* and 65%. -GD: 9-11: no neural tube defects and ocular defects were observed while renal variations, cleft palate, and limb and tail anomalies were observed. LOAEC (foetal) is 5000 ppm (6550 mg/m^3). NOAEC (maternal) is 10000 ppm (13100 mg/m^3). LOAEL (maternal) is 5000 ppm (19650 mg/m^3).	(Bolon *et al.*, 1993)[1]
Prenatal development toxicity study Similar to OECD TG 414 Mice CD-1	Methanol (>99% pure), 0 or 10000 ppm (0 and 13100 mg/m^3, whole	Dams: Peak maternal blood MeOH concentration at the end of the exposure was about 4 mg/ml, MeOH was cleared from maternal blood within 24 h. Some fully resorbed litters were observed with 2-day MeOH exposure. Litters: GD 6-7: Foetal bw was decreased as compared to their controls (0.97 g vs 1.10 g in control). Number of dead and resorbed foetuses was increased (3.3% vs. 0.2% in control). GD 7-8: Number of dead and resorbed foetuses was increased (2.9%* vs.	(Rogers *et al.*, 1997)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
M/F 12-19 per group GLP not specified Klimisch score 2	body) 7 h/day 1- or 2-day exposures GD 5-13	0.8 in control). GD 10-11: Number of live foetuses per litter was decreased (8.1%* vs. 12.3% in control) Foetuses (two-days exposure): Significantly ($p<0.05$) increase of incidences compared to controls for 2-day exposure: cleft palate, exencephaly and skeletal defects were the foetal anomalies observed. -Cleft palate: occurred with 2-day exposures on GD 6-7 through GD 11-12 (peak on GD 7-8) and with 1-day exposures on GD 5 through 9 (peak on GD 7); -Exencephaly: occurred with 2-day exposures on GD 6-7 through GD 8-9 (peak on GD 6-7) and with 1-day exposure on GD 5 through 8 (peak on GD 7); -Skeletal elements malformed included the exoccipital (peak on GD 6-7 (22.5%); GD 5 (9.9%)), atlas (peak on GD 6-7 (72.3%); GD 5, 6 (55.5%, 55.3%)), axis (peak on GD 6-7(22.3%); GD 7 (28.8%)), cervical vertebra 7 with a rib (peak on GD 6-7 (73.7%); GD 7 (45.4%)) and lumbar vertebra 1 with a rib (peak on GD 7-8 (68.3%); GD 7 (39.4%). Foetuses (1-day exposure): An increased incidence of foetuses with 25 presacral vertebrae (normal 26) was observed with MeOH exposure on GD 5; whereas an increased incidence of foetuses with 27 presacral vertebrae was observed with methanol exposure on GD 7. According to the authors the results of this study indicate that gastrulation and early organogenesis represent the period of increased embryonic sensitivity to MeOH.	
Prenatal development toxicity study Experimental study Mice CD-1 M/F 12-14 litters per group	Methanol (>99% pure), 0 or 10000 ppm (0 and 13100 mg/m^3, whole body) 6 h/day Single	MeOH exposure induced signs of acute MeOH toxicosis (central nervous system depression and ataxia) which resolved within 1 h after the end of the exposure period. The incidence of open anterior neural tubes in GD 10 embryos (9.65 ± 3.13%* vs. 0% in control) was increased.	(Dorman *et al.*, 1995)[1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
GLP not specified Klimisch score 2	treatment GD 8		

[1]Adopted from CLH report for methanol (ECHA, 2013c)

* statistically significant different from control $p<0.05$

** statistically significant different from control $p<0.01$

*** statistically significant different from control $p<0.001$

HPA: hypothalamic-pituitary-adrenal

Table 25: Summary table of human data on adverse effects on development of methanol

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Publication	Cleaner product containing methanol, toluene and isopropanol	Human case report Inhalation of cleaning product	32-years old, gravid 7, para5 at 32 weeks gestation required Caesarean section. The course of her current pregnancy had been significant for eight hospital admission for inhalants overdose (primarily carbonator cleaner containing methanol, toluene and isopropanol). A 1570 g male foetus was delivered via Caesarean incision and no maternal and neonatal postoperative complications were reported.	(Kuczkowski *et al.*, 2004)[1]
Publication	Methanol	Human case report Ingestion	A 28-year-old woman, gravid 3, para 2, EGA 30 weeks, with HIV infection, asthma, and history of cocaine use and hospitalization, two months earlier for unexplained metabolic acidosis and lethargy and in respiratory distress. Due to the mother's altered	(Belson *et al.*, 2004)[1]

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			mental status the reason and time of her exposure remain unknown. The history of a previous hospitalization with an undiagnosed acidosis might have suggested a repetitive behaviour such as methanol ingestion The high anion gap metabolic acidosis in the new-born was likely due to several factors: 1) formic acid from the foetal metabolism of methanol, 2) prolonged maternal acidosis, 3) lactate produced from methanol metabolism and 4) poor tissue perfusion. Formic acid level was not measured on the new-born, therefore no comment on extent of the metabolic process was made.	
Publication	Carburettor cleaner containing methanol	Human case study Inhalation	A woman exposed repeatedly during pregnancy (16 and 27 weeks of gestation) was admitted to the hospital because of acute intoxication (severe anion gap hyperosmolar metabolic acidosis showing blood methanol levels of about 450 mg/l). At 31 weeks of gestation, she was found obtunded and given sodium bicarbonate, to correct acidosis, and ethanol, followed by an emergency Caesarean section for acute foetal distress. At birth, the infant was of appropriate weight but presented acute foetal distress with significant metabolic acidosis. Initial hypotonia was followed by generalised hypertonicity of lower extremities within a week after birth. Neurosonogram showed bifrontal cystic lesions in the frontal area. The frontal cysts measured 1 cm x 1 cm on the	(Bharti, 2003)[1]

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			right side and 0.8 cm x 0.9 cm on the left side. Magnetic resonant imaging performed on day 3 after birth showed extensive bifrontal cystic leukomalacia with some cortical atrophy and the areas of leukomalacia not communicating with the ventricles. Ventricular size was normal. There was no midline shift. The infant passed an initial hearing screen for both ears.	
Publication	Methanol	Human clinical case study Intentional exposure	56 patients with a diagnosis of solvent abuse (including MeOH) in pregnancy present to a Manitoba teaching hospital. 12 patients of 56 mothers with a diagnosis of solvent (including MeOH) abuse in pregnancy showed preterm birth (21.4%), nine infants had major anomalies (16.1%), seven infants had foetal alcohol syndrome-like facial features (12.5%) and six neonates had hearing loss (10.7%). Substance abuse in pregnancy is associated with severe maternal and neonatal sequelae. Physicians must be aware of this increasing problem in the obstetrical population and assistance should be offered to each woman, ideally before a woman becomes pregnant, but at least at the first contact a pregnant woman makes with the health care community.	(Scheeres *et al.*, 2002)[1]
Publication	Methanol Occupational exposure	Inhalation and cutaneous	Information about the occupational exposure of 851 women (100 mothers of babies with oral clefts and 751 mothers of healthy referents) who worked during the first trimester of pregnancy was obtained from an interview.	(Lorente *et al.*, 2000)[1]

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			This interview was blindly reviewed by industrial hygienists, who assessed the presence of chemicals and the probability of exposure. All women were part of a multicentre European case-referent study conducted using 6 congenital malformation registers between 1989 and 1992. The odds ratio (OR) for cleft lip (with or without cleft palate) was 3.61 (95% CI 0.91-14.4). Due to the limited number of subjects, the committee is of the opinion that this result must be interpreted with caution.	
Publication	Methanol	Human case report Ingestion: 250-500 ml methanol in 38th week of pregnancy	Five hours after methanol ingestion, the woman was slightly acidotic and had a serum methanol level of 2300 mg/l and a formic acid concentration of 336 mg/l. Treatment consisted of ethanol and bicarbonate administration together with haemodialysis. Six days later, the woman gave birth to an infant with no signs of distress. A 10-year follow-up of the child revealed no visual disturbances.	(Hantson *et al.*, 1997)[1]

[1]Adopted from CLH report for methanol (ECHA, 2013c)

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

Trimethyl borate

There is no information available on the reproductive toxicity of trimethyl borate itself. Read-across from data on boric acid and on methanol is used for trimethyl borate, which are both hydrolysis products of trimethyl borate.

Boric acid and borates

Animal studies

In multiple ECHA reports, boric acid-induced adverse effects on development in animal studies have been reviewed (ECHA, 2013a, ECHA, 2013b, ECHA, 2018). NOAELs and LOAELs were observed of 55 (9.6) mg

boric acid (B)/kg bw/day and 76 (13.3) mg boric acid (B)/kg bw/day, respectively (ECHA, 2010, ECHA, 2013a, ECHA, 2013b, ECHA, 2018). Developmental abnormalities included enlargement of lateral ventricles in the brain and agenesis and shortening of the 13th rib in the absence of maternal toxicity, as recently assessed by RAC (RAC, 2019).

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 (Table 26). 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).

Table 26: Maternal organ damage upon exposure to boric acid via inhalation

Dose mg boric acid (B)/kg bw/day)	Gross lesion in lung or liver, GD 20 (% dams of total)	Lungs pale, GD 20 (% dams of total)	Lungs mottled, GD 20 (% dams of total)
0	4	0	4
0.65 (0.11)	4	4	0
4.0 (0.69)	64*	40*	36*
11 (2.0)	76*	64*	8

* $p<0.05$ compared to control group as determined by Fisher's exact test

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 (Table 27). 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 should be regarded as supportive data but not as leading data. The LOAEL (maternal) is 92 mg/m^3 CI (estimated delivered dose: 4.0 (0.69) mg boric acid (B)/kg bw/day).

Table 27: Mean bw and malformations in foetuses upon exposure to boric acid via inhalation

Dose mg boric acid (B)/kg bw/day)	Mean foetal bw/litter, GD 20 (% vs. control)	Interparietal alterations, GD20 (no. of malformed foetuses per litter)	Sternebrae alterations, GD20 (no of malformed foetuses per litter)	Rib alterations, GD20 (no. of malformed foetuses per litter)
0	M: 100 F: 100 Total: 100	0.2	3.0	0.2
0.65 (0.11)	M: 103 F: 101 Total: 102	0.2	2.0	0.2

4.0 (0.69)	M: 97 F: 94* Total: 95*	0.4	3.3	0.2
11 (2.0)	M: 94* F: 93* Total: 93*	0.5	4.2	0.3

* $p<0.05$ compared to control group as tested by ANOVA/Kruskal-Wallis followed by a *post hoc* Dunnett's test

Human studies

Igra *et al.* (2016) demonstrated an inversely associated link between serum boron concentrations (>80 µg/l) with birth length (0.69 cm shorter, p = 0.043, per 100 µg/l increased serum boron concentrations) in a population in northern Argentina (Igra *et al.*, 2016). This correlation was more profound in the third trimester at higher serum concentrations of boron. Blood boron levels of 75 ng B/g blood corresponding to 80 µg B/l were found, as calculated earlier in the CLH dossier on boric acid and borates (ECHA, 2018). Boron was present in drinking water, besides lithium, arsenic and caesium. The presence of lithium could be a confounder, as lithium has previously been associated with decreased birth length (Harari *et al.*, 2015). Furthermore, other confounders have been raised in this study, such as effects of attitude on pregnancy and birth, as stated in the RAC opinion on boric acid and borates (RAC, 2019).

Duydu *et al.* (2018) observed no boron-induced adverse effects on pregnancy outcomes (based on various read-outs, *e.g.* birth weights, congenital anomalies etc.) in females residing in Turkey (Duydu *et al.*, 2018b). In this study it was assumed that exposure to boron in these women was consistent over time due to presence of high levels of boron in the environment. In the highest exposure group, blood boron levels ranged from 151.81 to 957.66 ng B/g blood.

It should be noted that blood boron levels and daily boron exposure described in human epidemiological studies were much lower as compared to blood boron levels and daily boron dose found at LOAEL in animal studies, corresponding to 1270 ng B/g blood and 13.3 mg B/kg bw/day, respectively (Price *et al.*, 1997). Data from human studies do not indicate a boron-induced adverse developmental effect found in animals but not relevant to humans.

Methanol

Animal studies

Effects of methanol on development in animal studies have been reviewed by RAC in 2014 (RAC, 2014a). Upon exposure to methanol, reduced body weight, skeletal and eye anomalies have been described in rat and mouse foetuses (Nelson *et al.*, 1985, New Energy Development Organization, 1987, Takeda, 1988, Bolon *et al.*, 1993, Rogers *et al.*, 1993, De-Carvalho *et al.*, 1994, Stanton *et al.*, 1995, Connelly *et al.*, 1997, Youssef *et al.*, 1997). In addition, delay in early foetal sensorimotor development have been described in non-human primates (Burbacher *et al.*, 1999). There are studies available where methanol was administered via intraperitoneal injection in pregnant mice and rabbit to study developmental effects, these studies will not be further discussed here as this administration route is considered not to be a relevant route of administration for classification (Degitz *et al.*, 2004, Rogers *et al.*, 2004, Sweeting *et al.*, 2011).

Youssef *et al.* (1997) treated 10-13 female Long-Evans rats to 0, 1030, 2059 or 4118 mg methanol/kg bw by gavage, once on GD 10. Decreased body weight gain (-23%, $p<0.05$) and reduced food consumption (-36%) was observed in dams at 4118 mg/kg bw. At ≥1030 mg/kg bw, statistically significantly reduced foetal body

weight (-18%) and increased incidence of anomalies (91.6% vs. 53.8% in control) were noted. At 4118 mg/kg bw, incidence of undescended testes (60% vs. 0% in control, $p<0.05$) and eye anomalies (30% vs. 0% in control, $p<0.05$) increased. The LOAEL (maternal/foetal) is 1030 mg/kg bw.

Rogers *et al.* (1993; similar to OECD TG 414) exposed 5-17 female Crl:CD-1 mice/group to 0, 1000, 2000, 5000, 7500, 10000 or 15000 ppm methanol (0, 1310, 2620, 6550, 9825, 13100, 19650 mg/m^3, whole body) via inhalation, 7 h/day, at GD 6 to 15 in study 1. No treatment related effects on clinical observations and body weight were noted in dams. Increased incidence of cervical ribs (49.6% vs. 26.0% in control, $p<0.01$) were observed in foetuses at 2000 ppm and was a clear dose response. Increased incidence of cleft palate and exencephaly (15.5% vs. 0.13% vs. in control, $p<0.001$) was noted at 5000 ppm. Number of live foetuses per litter (8.6 vs. 10.3 in control, $p<0.01$) significantly decreased at 7500 ppm and was also found to be statistically significantly reduced at higher doses. At 10000 ppm, incidence of fully resorbed litters (5 vs. 0 in control, $p<0.05$) increased and foetal bw decreased (-11%, $p<0.001$). The LOAEL (foetal) is 2000 ppm (2620 mg/m^3).

In study 2, Rogers *et al.* (1993) exposed 4 to 8 female Crl:CD-1 mice/group to 0 or 4000 mg methanol/kg bw/day via gavage at GD 6 to 15. Reduced body weight was noted in dams at GD 15 (-14%) and 17 (-22%) but was not statistically different from control and body weight gain was not changed (Table 28). Live foetuses per litter was decreased upon exposure to methanol (5.9 vs. 10.5 live/litter in control but was not statistically significant. In foetuses, body weight was reduced (-17%, $p<0.05$) and increased incidence of incidence cleft palate (43.5% per litter vs. 0% in control, $p<0.01$) and exencephaly (28.8% per litter vs. 0% in control, n.s.) were noted.

Table 28: Effects on outcome of pregnancy of methanol exposure by oral gavage on GD 6-15[1]

	Distilled water	4 g/kg methanol[2]
A. Dams		
No. pregnant	4	8
No. fully resorbed	0	3
No. dead	0	1
Dam weight (g)		
Gestation day 6	26.4	26.5
day 8	29.8	29.9
day 10	32.1	31.8
day 12	36.1	35.3
day 15	43.6	37.3
day 17	48.6	38.1
Net dam weight gain[3]	2.65	3.06
B. Litters[4]		
No. implants/litter	11.8	9.3
No. live/litter	10.5	5.9
No. dead/litter	1.3	3.4
Fetus weight (g)	1.21	1.00*
C. External fetal examinations		
No. fetuses examined	42	47
Cleft palate (CP)		
Fetuses/litters	0/0	20/8
Percent/litters		43.5**
Exencephaly (EX)		
Fetuses/litters	0/0	9/5
Percent/litter		28.8
CP or EX		
Fetuses/litters	0/0	29/8
Percent/litter		72.3*
CP and EX	0/0	0/0

[1]Animals which were not pregnant at term were excluded from this table; [2]Given in twice daily (0800 and 1500 h) doses of 2000 mg/kg bw each; [3]Maternal weight gain from GD 6 to 17 minus the gravid uterus weight; [4]Values do not include data from fully resorbed litters; *statistically significant different from vehicle control $p<0.05$; **statistically significant different from vehicle control $p<0.01$.

Developmental toxicity upon exposure to methanol in rats and mice have been demonstrated in multiple studies. The lowest LOAEL reported for the oral route is described by Youssef *et al.* (1997; 1030 mg/kg bw/day) and the lowest LOAEC reported for the inhalation route is described by Rogers *et al.* (1993; 2000 ppm or 2620 mg/m^3), as reviewed by RAC (RAC, 2014a).

Human studies

Most described cases of exposure to methanol during pregnancy include exposure to other substances besides methanol and are case reports. In the 38th week of pregnancy, a case describing ingestion of 250-500 ml of methanol only was reported (Hantson *et al.*, 1997). Serum levels of 2300 mg/l of methanol and 336 mg/l of formic acid were measured in the mother. The woman gave birth 6 days after ingestion of methanol, no adverse effects in the baby were noted. Ten years later, no visual disturbances were found in the child.

Upon exposure to a carburettor cleaning product containing methanol during pregnancy, a woman was hospitalised at week 16 and 27 of gestation due to acute intoxication (Bharti, 2003). Methanol serum levels of ~450 mg/l were measured. She gave birth, via an emergency Caesarean section at week 31, to a child presenting acute foetal distress but with an average body weight. Bifrontal cystic lesions in the frontal brain region and bifrontal cystic leukomalacia were noted in the baby. The child passed an initial hearing screen for both ears. A similar case of inhalation of a cleaning product containing methanol during pregnancy was described by Kuczkowski *et al.* (2004). Delivery of a baby of average body weight via Caesarean incision was described. No maternal or neonatal complications were described.

Occupational exposure to various substances, including methanol, during pregnancy and foetal development was described by Lorente *et al.* (2000); 100 mothers of babies with oral clefts and 751 mothers of healthy babies were interviewed. The odds ratio for cleft lip (with or without cleft palate) was 3.61 (95% confidence interval 0.91-14.4). Results of this study should be interpreted carefully, as the number of participants was limited.

10.10.6 Comparison with the CLP criteria

Classification in category 1A is based on sufficient data from human studies and is not applicable as there is no information on the effects of trimethyl borate on development in humans. No adverse effects upon exposure to boric acid or boron on development were found in human studies. For methanol, studies have provided limited evidence adverse effects on development, due to co-exposure of other substances or low number of participants, as earlier reviewed (RAC, 2014a). Therefore, a classification in category 1A is not justified.

Classification in category 1B is based on sufficient data in animals showing clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. There is no information on the reproductive toxicity of trimethyl borate itself. The registrants have applied read-across from the data on boric acid and other borates, and on methanol because trimethyl borate is quickly hydrolysed into these two substances, which is also expected to take place in the human body.

The available information on boric acid justifies classification in category 1B as previously advised by RAC for boric acid and multiple borates (RAC, 2010, RAC, 2014b, RAC, 2014c, RAC, 2014d, RAC, 2019). Lowest LOAEL for boric acid in regard to developmental toxicity is 76 mg boric acid/kg bw, equivalent to 13.3 mg B/kg bw/day (Price *et al.*, 1996). For trimethyl borate, this is equivalent to 128 mg/kg bw/day (76 * 103.9 / 61.83 = 128 mg/kg bw/day), since 1 mole of boric acid is formed from 1 mole of trimethyl borate.

Studies demonstrated developmental toxicity upon exposure to methanol in rats or mice, such as increased resorption, skeletal and eye anomalies (Nelson *et al.*, 1985, New Energy Development Organization, 1987, Takeda, 1988, Bolon *et al.*, 1993, Rogers *et al.*, 1993, De-Carvalho *et al.*, 1994, Stanton *et al.*, 1995, Connelly *et al.*, 1997, Youssef *et al.*, 1997). No adverse effects on development upon exposure to methanol are observed

in non-human primates (Burbacher *et al.*, 1999, Burbacher *et al.*, 2004), demonstrating a different mechanism responsible for methanol-induced developmental toxicity in rodents as compared to non-rodents. The LOAEL/LOAEC for methanol described by Youssef *et al.* (1997; 1030 mg/kg bw/day) and Rogers *et al.* (1993; 2000 ppm or 2620 mg/m^3) in rodents are the lowest reported dose levels in literature in regard to developmental toxicity for methanol via the oral and inhalation route (RAC, 2014a). RAC concluded that classification based on animal studies is not warranted because methanol will result in severe toxicity and mortality in humans at lower doses, before methanol-induced developmental toxicity is expected.

Developmental toxicity induced by boric acid is more relevant than methanol to use read-across for trimethyl borate, as methanol will result in severe toxicity and mortality in humans at lower doses, before methanol-induced developmental toxicity is expected. An ED10 for boric acid for developmental toxicity could not be determined, therefore the LOAEL of 76 mg/kg bw/day of boric acid for developmental toxicity was used, as reviewed by RAC (RAC, 2019). At 128 mg/kg bw/day trimethyl borate (equivalent to LOAEL of 76 mg/kg bw/day of boric acid for developmental toxicity), the relative dose of methanol formed would be 118 mg/kg bw/day ((128 /103.9) * (3*32) = 118 mg/kg bw/day), as 3 moles of methanol are formed from 1 mole of trimethyl borate. This is below the LOAEL for developmental toxicity found for methanol of 1030 mg/kg bw in rats, as reported by Youssef *et al.* (1997). Effect levels have to be determined in humans, as mechanisms of developmental toxicity of methanol in rodents are not relevant to human (RAC, 2014a). When applying an allometric scaling factor for rat to human of 4 (ECHA, 2012), the estimated LOAEL in humans is 128 / 4 = 32 mg/kg bw/day trimethyl borate, where an estimated amount of (32 / 103.9) * (3*32) = 30 mg/kg bw/day methanol is formed. Considering the acute toxic potency of methanol, the minimal (oral) dose of methanol considered acute toxic for humans is 300-1000 mg/kg bw. It is thus expected that in humans developmental toxicity induced by boric acid occurs at a lower dose than the lethal dose of methanol. Therefore, it is considered that there is insufficient evidence to conclude that the toxicity of the hydrolytic product methanol would prevent such reprotoxic effects being induced by boric acid after exposure to trimethyl borate. Classification of trimethyl borate in category 1B for effects on development is justified based on the expected effects of the hydrolytic product boric acid.

Classification in category 2 is based on limited data in animals. Category 2 is not considered relevant as there are clear effects on development of the hydrolytic product boric acid in multiple good studies.

Recently, RAC reviewed the change of the SCL into GCL for boric acid and other borates, where a LOAEL (76 mg/kg bw/day) for boric acid for developmental toxicity was used, as no ED10 could be derived (RAC, 2019). This is equivalent to 128 mg/kg bw/day of trimethyl borate (see calculations above). This is within the potency limits of 4 to 400 mg/kg bw/day for the GCL, and thus a SCL is not justified for trimethyl borate.

After oral intake or inhalation of trimethyl borate, rapid and complete hydrolysis can be expected. Both oral and inhalation routes have been demonstrated to result in development toxicity of boric acid. Uptake of trimethyl borate via the skin is limited but cannot be excluded to be a route of intake. Therefore, no route should be specified for the classification as **Repr. 1B, H360D**.

10.10.7 Adverse effects on or via lactation

Information on trimethyl borate

There is no information available on the effect of trimethyl borate itself on or via lactation. Trimethyl borate is quickly hydrolysed into boric acid and methanol. Therefore, read-across from data on boric acid and methanol is used here.

Information on boric acid and borates

Animal studies

Relatively small amounts of boric acid have been detected in milk, indicating a limited risk of adverse effects on or via lactation (Beyer *et al.*, 1983).

Human studies

No studies were found.

Information on methanol

Animal studies

Azziz *et al.* treated pups to methanol (>99% pure) via lactating 80 female Wistar rats/group to 0, 1, 2 and 4% (*v/v*) methanol (equivalent to approximately 450, 900 and 1800 mg methanol/kg bw/day, using: 20 ml/day, 0.35 kg bw and a density of methanol of 0.79 g/ml) in drinking water (*ab libitum*) on postnatal day 1-21, kept on a folic acid-deficient or -sufficient diet for 14-16 weeks (Aziz *et al.*, 2002). At 4% (*v/v*) methanol in rats with a folic acid-sufficient diet, decreased body weight gain of pups was described. Furthermore, neurodevelopment toxicity was noted (observed via spontaneous locomotor activity, conditioned avoidance response, dopaminergic and cholinergic receptors, striatal dopamine levels, expression of growth-associated protein in the hippocampal region). Exposure to methanol via lactation thus affected brain development in pups.

Human studies

There were no studies found regarding methanol-induced effects on human lactation.

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

Information on trimethyl borate

There is no information available on effects on or via lactation for trimethyl borate. Read-across from data on boric acid and on methanol is used for trimethyl borate, which are both hydrolysis products of trimethyl borate.

Information on boric acid and borates

There is currently not sufficient data available to propose classification for adverse effects on or via lactation based on a previous CLH report for Boric Acid and Borates (ECHA, 2018).

Information on methanol

There is one study available of methanol-induced adverse effects via lactation in rats at the top dose. It is, however, unknown whether this mechanism of adverse effects in rodents is relevant to humans, seen the difference in metabolism of methanol in rodents and humans.

10.10.9 Comparison with the CLP criteria

There are no animal data of adverse effects on or via lactation for trimethyl borate, and only limited data for boric acid and other borates. There is one study available of methanol-induced adverse effects via lactation in rats at 1800 mg methanol/kg bw/day. Mechanisms of developmental toxicity of methanol in rodents are not relevant to human, effect levels thus have to be determined in humans (RAC, 2014a). Applying an allometric scaling factor for rat to human of 4 (ECHA, 2012), this is 1800 / 4 = 450 mg/kg bw/day, which is clearly above the minimal lethal dose in humans for methanol. Therefore, it is considered unlikely that methanol-induced effects via lactation can occur in humans. Overall, the available information do not justify classification for adverse effects on or via lactation due to absence of data.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classification of trimethyl borate as **Repr. 1B, H360FD** is proposed, based on adverse effects on fertility and development caused by the hydrolytic product boric acid, without an indication of the exposure route and without a specific concentration limit.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this dossier.

10.13 Aspiration hazard

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier.

13 ADDITIONAL LABELLING

Not relevant.

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15 ANNEXES

See Annex for confidential information.