Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Disodium octaborate tetrahydrate

EC number: 234-541-0
CAS number: 12280-03-4

CLH-O-0000003655-70-03/F

Adopted

14 March 2014

14 March 2014

CLH-O-0000003655-70-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonized classification and labelling (CLH) of:

Chemicals name: Disodium octaborate tetrahydrate
EC number: 234-541-0
CAS number: 12280-03-4

The proposal was submitted by **The Netherlands** and received by the RAC on **5 April 2013.**

All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonized System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD), is no longer given.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **30 April 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **14 June 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Bert-Ove Lund**

To ensure the consistency of the opinions for disodium octaborate tetrahydrate and boric acid (dossier submitter: Poland), the (co-)Rapporteurs appointed for boric acid, **Normunds Kadikis** and **Paola Di Prospero Fanghella**, collaborated closely in support of the current opinion.

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonized classification and labelling was reached on **14 March 2014** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion on **Disodium octaborate tetrahydrate** that should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	No current annex VI entry									
Dossier submitters proposal	005-021-00-9	disodium octaborate tetrahydrate	234-541-0	12280-03-4	Repr. 1B	H360FD	GHS08 Dgr			Repr. 1B; H360FD: C ≥ 4,5%
RAC opinion					Repr. 1B	H360FD	GHS08 Dgr			*
Resulting Annex VI entry if agreed by COM					Repr. 1B	H360FD	GHS08 Dgr			

* The RAC opinion includes the derivation of the generic concentration limit (GCL) based on the new Guidance (Version 4.0 – November 2013, section 3.7.2.5. Setting of specific concentration limits). Using the new guidance, the GCL of 0.3% w/w should apply and there is thus no need for an SCL.

SCIENTIFIC GROUNDS FOR THE OPINION

PHYSICAL HAZARD ASSESSMENT

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

The Dossier Submitter (DS) proposed no classification for physical hazards. No studies were performed on disodium octaborate tetrahydrate (DOT). However, according to the molecular structure, no hazardous effects are expected. The Dossier Submitter (DS) concluded that DOT does not need to be classified for physico-chemical properties according to Regulation EC 1272/2008 (CLP).

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

DOT is a non-volatile, non-flammable inorganic solid. It is not expected to have explosive or oxidizing properties either. Based on the molecular structure of the substance, RAC agreed with the DS that no classification for physico-chemical properties is justified according to CLP.

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The DS summarised acute toxicity results for DOT with LD_{50} oral rat = 2550 mg/kg, LD_{50} dermal rat > 2000 mg/kg and LC_{50} inhalation rat > 2010 mg/m^3 and determined that boric acid and other borates are of low acute toxicity. The DS concluded that DOT does not meet the criteria to be classified for acute oral, dermal and inhalation toxicity according to CLP.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Oral acute toxicity
Accidental or intentional poisoning incidents with borates have been reported in the scientific literature. However, lethal doses are not well documented. The potential lethal oral dose of boric acid is reported to be 3 – 6 g for children and 15 - 20 g for adults.

With respect to animal data, one key study on acute oral toxicity on rats was provided in the dossier (Doyle, 1988). The oral LD_{50} value was 2250 mg/kg bw. Additional rat studies on boric acid provide LD_{50} values of 3450 mg/kg bw and >2000 mg/kg bw (US EPA, 2006). Additionally, the studies in rats with disodium tetraborate anhydrous, disodium tetraborate pentahydrate and boric oxide revealed LD_{50} of >2000, 3305, and >2600 mg/kg bw, respectively.

The CLP criterion for acute oral toxicity category 4, H302 is $300 < LD_{50} \leq 2000$ mg/kg bw. Based on this comparison, RAC agreed with the DS that no classification for acute oral toxicity is justified.

Inhalation acute toxicity
No human data are available.

With respect to animal data, one key acute inhalation toxicity study was conducted in Sprague-Dawley rats exposed to DOT (5 males, 5 females, duration of exposure 4h) (Wnorowski, 1994d). The inhalation LD_{50} value was > 2.01 mg/l. According to CAR (2006), Doc IIIA the sample was ground in a ball mill for 24 hours achieving particles of MMAD 2.8µm + GSD 2.15µm. It is reported that the inhalation study used the highest attainable dose, and that this dose of 2 mg/l caused no mortality.

Additionally, in an inhalation study in which rats were exposed to boric acid at actual concentrations of 2.12 mg/l for 4h no deaths were observed, and a study in rats with disodium tetraborate pentahydrate revealed an LC_{50} of >2.04 mg/l.

The CLP criterion for acute inhalation toxicity category 4, H332 is $1 < LC_{50} \leq 5$ mg/l for dusts and mists. RAC agreed with the DS that no classification for acute inhalation toxicity is justified taking into account the remark concerning the highest attainable dose.

Dermal acute toxicity

No human data are available.

With respect to animal data, one key acute dermal toxicity study was conducted in Sprague-Dawley rats exposed to DOT (Doyle, 1989a). The dermal LD_{50} value was > 2 000 mg/kg bw. In addition, other borates appear to have low acute dermal toxicity. In a study in rabbits, the dermal LD_{50} value for boric acid was >2000 mg/kg bw. Acute dermal toxicity studies with disodium tetraborate decahydrate and disodium tetraborate pentahydrate revealed no deaths at a limit dose of 2000 mg/kg bw.

The CLP criterion for acute dermal toxicity category 4, H312 is $1000 < LD_{50} \leq 2000$ mg/kg bw. Based on the comparison with this criterion, RAC agreed with the DS that classification for acute dermal toxicity is not justified; however, it is noted by the DS that in some of the studies the test material was not moistened, so an optimal contact with the skin was not ensured.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

The DS proposed no classification for specific target organ toxicity – single exposure (STOT SE). Accidental or intentional poisoning incidents with borates have been reported (CAR, 2006; section IIA). Acute effects may have included nausea, vomiting, gastric discomfort, skin flushing, excitation, convulsions, depression and vascular collapse. It is considered likely by the DS that the observed clinical signs reflect nonspecific toxicity rather than a specific target organ toxicity. Additionally, animal data were not giving indications that DOT induced a specific target organ toxicity following single oral, dermal or inhalation exposure. Details on the effects observed in the acute toxicity studies cannot be provided because these details were not included in the CAR and the DS had no access to these data. The DS concluded that DOT does not meet the criteria for STOT-SE classification according to CLP.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

According to CLP, a specific target organ toxicant causes adverse health effects produced by a single exposure. These effects should include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or should produce serious changes to the biochemistry or haematology of the organism. Category 3 for STOT SE is used only to address narcotic effects and respiratory tract irritation which are not reported for DOT.

RAC noted that detailed information on the effects on test animals during acute toxicity studies is not available. According to CLP, reliable and good evidence from human cases or epidemiological studies can be used as the basis to classify substances in Category 1 for STOT-SE. In exceptional cases, human evidence can also be used to place a substance in Category 2. Poisoning cases of

humans show only nonspecific clinical signs (nausea, vomiting, gastric discomfort, skin flushing, excitation, convulsions, depression and vascular collapse).

RAC agreed with the conclusion of the DS that DOT does not meet the criteria for classification as STOT SE according to CLP.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

The DS proposed no classification for skin corrosion/irritation. The DS reported studies conducted in rabbits with boric oxide, DOT, sodium tetraborate decahydrate and sodium tetraborate pentahydrate. None of these substances caused skin irritation relevant for classification at doses of 0.5 g. Similarly, boric acid does not cause skin irritation when applied to the intact or abraded skin of rabbits at a dose of 0.5 g. The DS concluded that DOT does not meet the criteria for classification as skin corrosion/irritation according to CLP.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

No human data are available.

One key skin irritation study conducted in rabbits was provided (Doyle, 1989b). 0.5 gram of test substance was moistened in physiological saline and applied on the skin of rabbits. Individual animal scores and averages were not available. However, the average erythema score for 3 male and 3 female animals at 24, 48 and 72 h was 0.22 (the irritant effect was reversible by 72h) but the average edema scores at 24, 48 and 72h was "0". In addition, other B compounds (boric acid, boric oxide, sodium tetraborate decahydrate and sodium tetraborate pentahydrate) did not cause skin irritation.

In conclusion, RAC agreed with the DS that DOT does not meet the criteria for classification as skin irritation according to CLP.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The DS proposed no classification for eye corrosion/irritation. Workers exposed occupationally to borax dust (disodium tetraborate decahydrate, average air concentration 4.1 mg/m^3) reported eye irritation, dry mouth, nose or throat, sore throat and productive cough (Garabrant *et al*., 1984 in CAR, 2006; section IIA). No data on eye irritation due to exposure of humans to DOT were available. In studies with animals on boric oxide and DOT, no eye irritation was observed. Disodium tetraborate decahydrate and sodium tetraborate pentahydrate did cause eye irritation, possibly due to the crystalline nature of these compounds. Boric acid induced conjunctivae redness and chemosis and minor effects on the iris. The effects were reversible within 7 days. According to the DS, DOT does not meet the criteria to be classified for eye irritation according to CLP.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

No data on eye irritation due to exposure of humans to DOT were available. Workers exposed occupationally to borax dust (disodium tetraborate decahydrate, average air concentration 4.1 mg/m^3) reported eye irritation, dry mouth, nose or throat, sore throat and productive cough (Garabrant *et al*., 1984 in CAR, 2006; section IIA).

One key eye irritation study using New Zealand White rabbits exposed to DOT was provided (Doyle, 1989d). Individual animal scores and averages were not available. However, according to CAR (2006), Doc IIIA, 0.1 ml volume (weight of the test substance 0.053 g) was instilled. Exposure period was 24h followed by rinsing with physiological saline. Post exposure period was 7 days. Average 24h, 48h, 72h scores for 3 male and 3 female rabbits were the following: Cornea: 0; Iris: 0.11; Conjunctiva redness: 0.94; Conjunctiva chemosis: 0.89. Minor effects on the iris and the conjunctivae redness and chemosis were reversed by day 10.

Besides, disodium tetraborate decahydrate and sodium tetraborate pentahydrate did cause eye irritation in animals, possibly due to the crystalline nature of these compounds. Boric acid induced conjunctivae redness and chemosis and minor effects on the iris. The effects were reversible within 7 days.

The CLP criteria for eye irritation for category 2 are: at least in 2 of 3 tested animals, a positive response of: corneal opacity ≥ 1 and/or iritis ≥ 1, and/or conjunctival redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2.

In conclusion, RAC agreed with the DS that DOT does not meet the criteria for classification as serious eye damage/eye irritation according to CLP.

RAC evaluation of respiratory sensitisation

Summary of the Dossier submitter's proposal

The DS proposed no classification for respiratory sensitisation. No human or animal data that indicate that DOT causes respiratory sensitization were found by the DS. The substance should not be classified for respiratory sensitization due to lack of data.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

RAC agreed with the DS that classification of DOT for respiratory sensitization is not justified due to lack of data.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The DS proposed no classification for skin sensitisation. No evidence of skin sensitisation in humans exposed occupationally to borates has been reported (CAR, 2006). With respect to animals, DOT was tested in a Buehler method skin sensitization test. DOT was applied at a concentration of 95% (powder moistened with water) during both the induction and challenge phase of the test. No signs of skin sensitisation were observed. However, because there was no sign of skin irritation in the induction phase, the test did not meet the guideline requirements. Nevertheless, no sensitising properties were reported for other borates either in animal studies. The DS concluded that DOT does not meet the criteria for skin sensitisation according to CLP.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

No evidence of skin sensitization in humans exposed occupationally to borates has been reported.

One key study on skin sensitisation of DOT in Guinea pigs is provided (Wnorowksi, 1994h). According to CAR (2006), Doc IIIA, 20 animals as a test group as well as 20 animals as a positive control group and 10 animals as a negative control group were used. For both induction and challenge treatments, the substance was moistened with distilled water (95% w/v). Very faint

erythema (score: 0.5) was noted at three test sites 24 hours after first induction dose. No other adverse effect observed. See detailed information in the supplemental information part below.

According to CLP, substances shall be classified as skin sensitisers if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or if there are positive results from an appropriate animal test.

In conclusion, RAC agreed with the DS that no classification of DOT as skin sensitiser is justified according to CLP.

Supplemental information - In depth analyses by RAC

Detailed information including induction/challenge/scoring schedule for skin sensitisation test of DOT (CAR (2006), Doc IIIA)

Treatments	**Buehler test**	**Observations/Remarks** *give information on irritation effects*
	day of treatment	
Induction 1	day 0	Very faint erythema (0.5) was noted at three test sites 24 hours after first induction dose. No other adverse effect observed
Induction 2	7	No irritation observed
Induction 3	14	No irritation observed
challenge	28	No irritation observed
(rechallenge)		
scoring 1	29	No irritation observed
scoring 2	30	No irritation observed

RAC evaluation of specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

The DS proposed no classification for specific target organ toxicity – repeated exposure (STOT RE). The DS reported that no information is available for DOT regarding repeated dose toxicity. Considering the fact that borates (including DOT) will predominantly exist as undissociated boric acid in physiological conditions, the toxicological properties of borates are expected to be similar. Therefore, read across to DOT is applied using data on boric acid and other borates, including disodium tetraborate decahydrate.

Accidental or intentional poisoning incidents with borates have been reported. Multiple exposures (high levels > 1g) result in various symptoms which may appear in isolation or together and include dermatitis, alopecia, loss of appetite, nausea, vomiting, diarrhea, and focal or generalised central nervous system irritation or convulsions. A 28-year-old woman who ingested around 0.5 g of boric acid (in baby powder) every day for two years suffered from anaemia, which reversed on ceasing ingestion. It is not clear from the study whether the observed effects are due to B exposure, to exposure to other substances or to nutritional deficiency. Infants aged from 6 to 19.5 weeks ingested borax (as a honey-borax mixture which had been applied to pacifiers) for periods of 4 to 12 weeks. The mean intake was 0.98 g boric acid/day (ranged from 0.55 g to 2 g) for a 10 kg child. The observed effects were convulsions, generalised seizures and focal seizures. There were no dermal effects. Minor occurrences of vomiting and loose stools were also described (CAR, 2006; Doc IIA).

With respect to animal investigations, 5 oral studies in mice, rats and dogs on boric acid as well as 5 oral studies in rats and dogs on disodium tetraborate decahydrate were provided. Effects on the

testes and on blood parameters were the main critical effects. Boric acid induced a decrease in testes weight, testicular atrophy, and haematological effects (extramedullary haematopoiesis, decreased hemoglobin (Hb) and cell volume, presence of haemosiderin in reticular cells of the liver and in proximal tubules of the kidney) indicative of increased red blood cell destruction. In the 90 days study in mice and in the 2-year study in rats, the animals appeared to be more sensitive to the effects on the haematopoietic system (LOAEL 17.5 mg B/kg bw/day) than on the testes. The dogs appeared to be more sensitive to the effects of boric acid on the testes. Similar results were obtained from studies on disodium tetraborate decyhadrate. The 90 days feeding study on boric acid in dogs yielded an overall NOAEL (2.6 mg/kg bw, equal to 0.46 mg B/kg bw/day), based on reduced testes weight and histological changes in the testes at a dose of 24 mg/kg bw/day, (4.2 mg B/kg bw/day). This finding is supported by the study with disodium tetraborate decahydrate, in which a (statistically non-significant) reduction in testicular weight and histological changes in the testes were observed at 42 mg/kg bw/day (4.7 mg B/kg bw/day) and severe testicular atrophy at 341 mg/kg bw/day (38 mg B/kg bw/day).

The DS concluded that since DOT is proposed to be classified for effects on reproductive toxicity, the effects on testes will not be considered under STOT-RE. Accordingly, the substance shall not be classified for STOT-RE according to CLP.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Since no information on DOT is available, RAC supported a read across approach using data on boric acid and disodium tetraborate decahydrate. RAC considered that the human data on accidental or intentional poisoning incidents with boric acid and sodium borate reflect symptoms of acute poisoning only were not valid for STOT RE assessment.

With respect to animal investigations, 5 oral studies in mice, rats and dogs on boric acid as well as 5 oral studies in rats and dogs on disodium tetraborate decahydrate are provided, but two of them, oral 2-year studies in dogs, must be discarded due to major methodological deficiencies, as was indicated by the DS. In the remaining studies in mice, rats and dogs, generally, effects on the testes and on blood parameters were found. These included a decrease in testes weight, testicular atrophy, and haematological effects (extramedullary haematopoiesis, decreased Hb and cell volume, presence of haemosiderin in reticular cells of the liver and in proximal tubules of the kidney) indicative of increased red blood cell destruction. According to CLP criteria, range of guideline dosage values for category 2 classification is $10 < C \leq 100$ mg DOT/kg bw/day (corresponding to 20.96 mg/kg bw/day of B as the upper limit concentration). The estimated range of LOAEL values in 3 studies on boric acid (NTP, 1987; Weir, 1962; Weir 1966a) as well as in 3 studies on disodium tetraborate decahydrate (Dixon, 1979; Weir, 1962b, Weir 1966b) was 25-58.5 mg B/kg bw/day. Only in two 90-day oral studies in dogs on boric acid and disodium tetraborate decahydrate (Paynter, 1963a and 1963b), very low LOAEL values (4.2 and 4.7 B/kg bw/day) were detected based on testicular atrophy.

In conclusion, RAC agreed with the DS that the testes effects shall not be considered under STOT–RE and that other effects did not meet the criteria to be classified for STOT-RE according to CLP.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

The DS reported that no information is available for DOT regarding (germ cell) mutagenicity. Since in aqueous solutions at physiological and acidic pHs, low concentrations of simple borates, like DOT, will predominantly exist as undissociated boric acid, the DS considered it justified to make conclusions based on boric acid.

In a comet assay (Single Cell Gel Electrophoresis assay) in B exposed workers, the relation between DNA-strand breaks under neutral and alkaline test conditions) in sperm cells and

previously described sperm quality parameters was investigated. A correlation between blood B levels and mean DNA-strand breaks in sperm was weak, and DNA-strand breaks in sperm were statistically not different between control and exposed groups (Duydu, 2011a).

In vitro studies do not indicate that boric acid induces gene mutations or chromosome aberrations. No original study reports on in vivo genotoxicity effects of borates were available. In a US EPA report an *in vivo* micronucleus test on boric acid in mice is described. It is reported that boric acid did not induce chromosomal and spindle abnormalities in bone marrow erythrocytes. In chronic studies in mice and rats on borates (boric acid and disodium tetrahydrate decahydrate) there are no indications that these compounds have carcinogenic properties. Based on the available data it is concluded by the dossier submitter that boric acid is unlikely to be genotoxic.

The DS concluded that DOT does not meet the criteria for mutagenicity according to CLP.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

As no information on DOT is available, a read across approach to boric acid is considered justified by RAC.

With respect to human data, the comet assay conducted in B exposed workers gave no correlation with blood B levels (Duydu, 2011a).

Three *in vitro* genotoxicity studies gave negative results. The studies consisted in a bacterial reverse mutation test with *S. typhimurium*, up to 2500 µg/plate (Stewart, 1991), an *in vitro* mammalian cell gene mutation test with mouse lymphoma cells up to 5mg/ml (Rudd, 1991) and an *in vitro* mammalian chromosome aberration test in Chinese hamster ovary cells up to 2500 µg/ml with S9 metabolic activation system and up to 2000 µg/ml without S9 metabolic activation system (NTP, 1987).

No original study reports on *in vivo* mutagenicity tests on borates were available, but according to US EPA (2006), boric acid did not induce chromosomal or mitotic spindle abnormalities in bone marrow erythrocytes in the micronucleus assay in Swiss-Webster mice at the doses up to 3500 mg/kg administered orally during two consecutive days (O'Loughlin, 1991).

According to the CLP classification criteria for mutagenicity Category 2, there should be positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, positive evidence obtained from somatic cell mutagenicity tests *in vivo*, in mammals; or other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

In conclusion, RAC agreed with the DS that no classification of DOT as a germ cell mutagen is justified according to CLP.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The DS reported that no information is available for DOT regarding carcinogenicity. Considering the fact that DOT will predominantly exist as undissociated boric acid in physiological conditions, the toxicological properties of boric acid and DOT are expected to be similar. Therefore, a read across from boric acid and other borates to DOT is applied.

No human data are available. In a carcinogenicity oral 2-year study in mice no evidence of a carcinogenic effect of boric acid (at 446 and 1150 mg/kg bw/day) was observed. In two chronic toxicity studies in rats performed with boric acid (at 334 mg/kg bw/day) and on sodium tetraborate decahydrate (at 516 mg/kg bw/day) no indication for a carcinogenic effect of these substances was found. However, it should be noted that in these rat studies only 10 animals/sex were used for macroscopic and histopathological examinations (Weir, 1966a; Weir, 1966b).

The DS concluded that DOT does not meet the criteria for carcinogenicity according to CLP.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

No information is available for DOT. RAC agrees that a read across from boric acid and other borates to DOT is justified.

No human data are available. With respect to animal studies, three oral 2-year key studies in rats and mice are provided. Application of boric acid at 446 and 1150 mg /kg bw/day gave no carcinogenic effects in mice (NTP, 1987). In addition, 334 mg boric acid/kg bw/day as well as 516 mg/kg bw/day of sodium tetraborate decahydrate caused no carcinogenic outcomes in rats (Weir, 1966a and 1966b). Besides, RAC noted that borates are not expected to have mutagenic properties.

With regard to other B compounds, no carcinogenic properties were observed. In conclusion, RAC agreed with the DS that no classification of DOT for carcinogenicity is justified according to CLP.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The DS proposed to classify DOT for reproductive toxicity with Repr. 1B, H360FD ('May damage fertility. May damage the unborn child'), based on read-across from other tested borates (e.g. boric acid) and borate salts (borax or disodium tetraborate decahydrate). Hydrolysis of borates results in the formation of the same chemical entities (boron, B). The resulting classification is comparable to that of the other borates in Annex VI of CLP.

The DS assessed the available epidemiological studies, and noted that the estimated exposure levels in the human studies were lower than the overall NOAELs for testis effects in experimental animals. Thus, the DS argued that human data do not contradict the animal data. Furthermore, an SCL of 4.5% w/w for this classification was proposed using a calculation method which is in line with the other borates already included in Annex VI.

Comments received during public consultation

A total of 47 comments were received during the public consultation on DOT. Most Member states who participated in the public consultation agreed with the proposal to classify DOT as Repr. 1B (H360FD) accordingly to the CLP criteria.

The Polish CA recognised that there are reproductive effects of B compounds in laboratory animals under test conditions, but it questioned if these data meet the criteria for Category 1B classification as argued in the CLH Report submitted to ECHA for boric acid. Based on the total weight of evidence, the Polish CA was of the opinion that the data showed that it is improbable that boric acid will cause reproductive or developmental effects in humans. Therefore, they considered Repr. 2 (H361d: 'Suspected of damaging the unborn child') as the most appropriate classification.

Moreover, the European Borates Association (EBA) and other industry organisations including downstream users (all referred to as EBA below) opposed the proposed classification. EBA stated that there is no evidence of reproductive or developmental effects in humans attributable to B in epidemiology studies with cohorts in China, Turkey and Chile with high exposures to B. According to EBA, workers in B mining and processing industries represent the maximum possible human exposure and a key difference between humans and laboratory animals relative to boric acid toxicity is the large zinc stores in humans compared to laboratory animals. According to EBA, the protective effect of the large zinc stores in the human body may explain the absence of toxicity in humans exposed to high levels of B. Supporting studies conducted with zinc borate were submitted during the boric acid public consultation to support this hypothesis. They were shared with the DS of DOT and commented on in due course (see the RCOM). Additionally, mechanistic data showed that the action of boric acid on histone deacetylase inhibition (HDACi) and Hox genes

occurs at a high dose (1000 mg boric acid/kg bw) and during a very narrow window of gestation (gestation days 8-9) in laboratory animals. According to EBA, these effects were not likely to be relevant to humans, since the dose of 1000 mg/kg bw in humans would be lethal. Based on the total weight of evidence, EBA argued that it is improbable that boric acid will affect fertility in humans. However, they recognised that epidemiological studies of developmental effects being not as robust as the fertility studies, a classification in Category 2 (H361d: 'Suspected of damaging the unborn child') was warranted.

A detailed response to these comments is provided by The Netherlands in the RCOM.

Assessment and comparison with the classification criteria

Studies of reproductive toxicity and repeated dose toxicity studies in mice, rats and dogs clearly indicate that B impairs fertility through an effect on the testes including testicular atrophy and seminiferous tubule degeneration. The effects observed in the different species are similar in nature. Based on the data from the 2 years feeding study on boric acid in rats (Weir, 1996a), the overall NOAEL for fertility is 100 mg/kg bw/day, equal to 17.5 mg B/kg bw/day. This conclusion on the testicular effects and the overall NOAEL is also supported by the study conducted with disodium tetraborate decahydrate (Weir, 1996b). There are no indications that the impaired fertility is secondary to other toxic effects (CAR, 2006).

Developmental toxicity of B was clearly observed in studies in rats and rabbits, the rat being the most sensitive species, with an overall NOAEL of 9.6 mg B/kg bw/day. Malformations consisted primarily of anomalies of the eyes, the central nervous system, the cardiovascular system, and the axial skeleton. The most common malformations were enlargement of lateral ventricles in the brain and agenesis or shortening of rib XIII. There are no indications that the developmental effects are secondary to other toxic effects. In addition, the teratogenicity is possibly caused by an altered hox gene expression, caused by inhibition of histone deacetylases, a mechanism that is likely to be also relevant for humans (see below).

There are a number of cross sectional epidemiological studies available on cohorts of workers studies available from China, Turkey and the US on the potential effects of boron exposure on parameters mainly related to fertility among workers occupationally exposed to B. The average daily boron exposure for the high exposure groups in these studies were estimated to be 1.8 mg B/kg/day (n=16), 0.2 mg B/kg/day (n=39) and 0.4 mg B/kg/day (n=109) (Scialli *et al.,* 2010, Duydu *et al.,* 2011, and Whorton *et al.,* 1994, respectively). Average daily exposure values in these workers were one to two orders of magnitude below the lowest observed adverse effect levels (LOAEL) for fertility in mice (Fail *et al*., 1991, 1998), and for developmental toxicity in rats (Price *et al.*, 1994, 1996).

The Chinese studies (reviewed in Scialli *et al*., 2010) showed the highest B exposure levels, with a small subset (n= 16) of the highly exposed group having an average intake of 1.8 mg B/kg bw/day. The analysis was also conducted on a larger group having an average exposure of 0.45 mg B/kg bw/day (n= 75). Parameters included semen analysis, reproductive outcomes and sperm X:Y ratio: no statistically significant effects were observed in either group compared to controls. It is noted that most study groups contained a rather low number of participants, as illustrated by a local and a regional control group of 15 and 23 persons, respectively, thus decreasing the power of the studies. Some of the parameters showed a large variation (e.g. the total sperm count (±S.D.) in controls was 218±124 million), making it difficult to identify potential effects. Furthermore, the selection of participants in the Chinese study was unclear, as it was not explained how 75 workers were selected out of the 957 interviewed workers. Also, it was not explained why 21 out of 60 workers from a pilot study were selected to participate in the full study, but not the other workers. Overall, it is acknowledged that no effects were found, but it is considered that the power of the studies could have been higher and that there are questions regarding the selection of participants (Scialli *et al*., 2010).

The Turkish studies (Duydu *et al.*, 2011, 2012; Başaran *et al.*, 2012) were initially set up based on the assumption that different occupational categories would give groups with quantitatively different exposure to B. However, high B concentrations in drinking water resulted in high exposure also in the controls (without occupational exposure), and a very poor correlation between occupational air exposure and blood concentrations of B was observed. Therefore, participants were grouped according to blood concentrations of B rather than based on

occupational exposure. It is not clear how well these new groups were matched. Also, the participation rate was very low (about 24%). The estimated average daily B exposure for the high exposure group was 14.45 mg B/day, which can be calculated into an external daily dose of 0.2 mg B/kg bw/day based on an assumed body weight of 70 kg. No adverse effects of B exposure on sperm analysis parameters were found, but the group size (n=39 in the high exposure group) was limited, leading to low statistical power. The B exposure level was still approximately two orders of magnitude lower compared to the rat NOAEL for reproductive and developmental effects; moreover the difference in exposure level between the groups was relatively low.

No epidemiological studies on possible adverse pregnancy outcomes in female workers are available.

In addition to the non-occupational exposure data presented in the Boric Acid CLH Report (Page 110), the highest non-occupational exposures were found in communities from Northern Chile in which the estimated intake of B was 21 to 27 mg B/day, which correlated to naturally high B concentrations in local rivers (Barr *et al*., 1993). In a recent study of populations in Chile, the exposure levels of B in drinking water and urine was measured from volunteers in Arica, an area in the North of Chile with high levels of naturally occurring B (Cortes *et al*. 2011). The concentration of B in urine varied between 0.45 and 17.4 mg/l, with a median of 4.28 mg/l and it was found to correlate with tap water sampled from the homes of the volunteers (r=0.64). Espinoza-Navaro *et al*., 2010 analysed sperm for total sperm count, sperm concentration, volume, vitality, pH, morphology, overall motility and grade A for motility in a sample of 102 healthy young males aged 18 to 30 years residing in Arica, Chile. The volunteers also completed a questionnaire about their fertility, habits and andrologic diseases. Males sampled in Arica had normal sperm values in comparison with international reports (Espinoza-Navarro *et al*. 2010). No analysis was apparently performed on potential developmental effects of high environmental B exposure.

The overall negative epidemiological studies on male fertility effects of B should be considered as additional information, due to several limitations in the study design. As pointed out by Scialli *et al*. (2010) the available human studies show no clear evidence of adverse effects on male fertility at these exposure levels, which is quite different than showing no evidence for such effects. In contrast, experimental studies in animals showed clear and significant reproductive toxicity in four different species. For effects on fertility, the lowest effect level (LOAEL) was 27 mg B/kg/day in mice (Fail *et al.*, 1991, 1998), and for developmental toxicity 13.3 mg B/kg/day in rats (Price *et al.,* 1994, 1996). The highest occupational exposure levels in the two occupational cohorts and in the environmental exposed cohort were, thus, 15-135 times lower than the animal LOAEL for fertility effects and 7-66 times lower than the animal LOAEL for developmental toxicity. Assuming a similar sensitivity of humans as in the four laboratory species studied, it would have been unlikely to observe any adverse effects on human male fertility at those exposure levels. Also, effects on female fertility and developmental effects in humans were not studied in the studies, which anyway had human exposure levels below the animal LOAELs for these effects. In line with CLP, Annex 1, Section 1.1.1.4, it is overall concluded that human data showing no clear evidence do not contradict the animal data.

Several studies on zinc borate were announced and/or submitted by EBA during or after public consultation of boric acid. They were shared with the DS of DOT and commented (see the RCOM). The study reports (final or drafts) were made available through CIRCA BC to the RAC. Non-confidential executive summaries for Hofman-Huther, 2013; Durand, 2013; Kirkpatrick, 2013a; Kirkpatrick, 2013b; Edwards, 2013 and Edwards, 2014 were provided by EBA. It was stated by EBA (see RCOM) that zinc interacts with boric acid in the body, reducing the toxicity of boric acid. A reason for this assumption is that zinc borate is less toxic than other borates in experimental studies. EBA further proposed that higher zinc stores in humans than in the experimental animals will provide some protection in humans against the toxic effects of boron, and that this species difference raises doubt about the human relevance of the reproductive toxicity seen in animals.

The RAC acknowledged that zinc borate *in vivo* in rats appears to have a higher LOAEL than other borates, but did not find the argumentation for the protective nature of zinc convincing. Firstly, there is no proposed mechanism for this zinc/borate interaction. Secondly, the unpublished *in vitro* study by Durand (2013), referred to in the RCOM and submitted after public consultation as evidence for a protective effect of zinc, suffers from not showing any negative effects of boric acid that zinc can protect against. Thirdly, if tissue levels of zinc affect the toxicity of borates, it is

difficult to explain rather similar LOAELs in the experimental animals (in the range of 13-79 mg B/kg/day in mice, rats, rabbits and dogs) despite e.g. perhaps 40-fold higher zinc concentrations in dog liver than in mouse liver (see RCOM). It is also noted that the lethal dose of boric acid is much lower in humans than in rats, so apparently humans are more sensitive than rats to acute exposure despite the alleged protection from zinc in humans. A specific protective action of zinc against reproductive/developmental effects might not be ruled out, but the evidence is still limited. It is possible that zinc quantitatively affects the toxicity of borates at some conditions, as well as boron might impair the physiological functions of zinc, an essential trace element involved in fertility and development in both animals and humans. These statements bring about a certain scientific interest but there is at present not sufficient evidence to generally support them; most importantly, there is no reason to challenge the relevance for humans of the toxicity of borates observed in experimental animals.

The EBA stated that the mechanism of action (MoA) for developmental toxicity of borates involves histone deacetylase inhibition (HDACi) and affected expression of the Hox genes, and that these effects are high dose phenomena in animals making the likelihood of similar effects in humans low. The evidence comes from studies with single exposure of pregnant mice to 1000 mg/kg boric acid on gestation day 8, causing a high incidence of malformations and showing evidence of inhibition of histone deacetylase and a shifted expression of Hoxc6 and Hoxa6. RAC noted that this MoA might be plausible, but there is no proof that the altered histone deacetylase is only a high dose effect. On the other hand, if these effects only occur at high exposure levels, they may not represent the most sensitive and relevant MoA for the developmental toxicity of borates. Lower exposure levels were not tested so it is unclear to what extent these effects are relevant MoAs for the borates. Even if these effects are indeed the relevant MoA, it is not clear why they would not be relevant for humans. Finally, it is noted that this MoA is proposed for developmental toxicity, but not for adverse effects on fertility.

The EBA also highlighted that B is likely to be an essential mineral in mammals, and that homeostatic control of B concentrations in the cells will decrease the risk of toxic effects. RAC noted that in its opinion on the upper tolerable intake level of B, the European Food Safety Authority concluded that, although it may have a beneficial effect on bone calcification and maintenance, B has not been established to be an essential nutrient for humans and no specific biochemical function has been identified in higher animals or man (EFSA, 2004). Therefore, the statement on the essentiality of B appears unsupported. In the unlikely situation that essentiality at very low intake levels will be demonstrated, the RAC further notes that B is still toxic to reproduction and development in experimental animals above certain exposure levels, and cannot see how the essentiality will affect the inherent toxicological properties of B.

It is stated in the EBA comments that the studied workers (in B mining and processing industries) represent the maximum possible human exposure, and that the data show that it is improbable that borates will cause effects on fertility or development in humans. RAC had no possibility to assess the exposure potential for the different B substances in different uses, but noted that the classification criteria do not consider exposure assessments. Rather, it is the inherent toxicological properties of the substances that lead to classification. Finally, the available epidemiological investigations dealt with male fertility only, with several methodological limitations; they did not cover developmental effects at all.

Based on the total weight of evidence, toxicity data from four different species (mice, rats, rabbits and dogs) provide clear evidence of an adverse effect of B (and consequently of DOT) on sexual function, fertility, and development in the absence of other toxic effects. No evidence of reproductive toxicity was observed in the epidemiological studies but they were designed to cover only male fertility effects and had methodological limitations. Therefore, the epidemiological studies do not lead to doubt as to the relevance of the animal toxicity data to humans at similar dose levels as causing toxicity in experimental animals. In line with CLP, Annex 1, Section 1.1.1.4, it was concluded overall that the negative human data do not contradict the animal data. Therefore, there is no evidence that the effects observed in animals are not relevant to humans.

Regarding SCLs, the DS proposed an SCL of 4.5% in line with the method used to determine SCLs for several other borates (the so-called 'German' method; BAuA, 1998) in Annex VI of CLP. The SCLs for boric acid and other borates were derived from the overall NOAEL for embryotoxic/teratogenic effects of 9.6 mg B/kg bw/day, based on a reduction in mean fetal body

weight/litter and an increased incidence in short rib XIII at 76 mg/kg bw/day (13.3 mg B/kg bw/day) (Price *at al*., 1996).

However, RAC concluded that the SCL for DOT should be determined according to the new guidance for the setting of specific concentration limits of the EU expert group (version 4.0. November 2013). The fetal incidence of short rib XIII malformation was 1.2 and 1.5% at the LOAEL (13.3 mg B/kg bw/day) and the highest dose tested (25 mg B/kg bw/day) respectively (Price *et al*., 1996). As the incidences are low, it is not possible to derive an ED_{10}. In this instance the LOAEL should be used for setting the SCL, according to the guidance. Correcting for the percentage of B (w/w), the LOAEL of 13.3 mg B/kg bw/day corresponds to a LOAEL of 63.5 mg/kg bw/day for DOT as it contains 20.96% B. DOT thus belongs to the medium potency groups (4 mg/kg bw/day < ED_{10} (LOAEL) < 400 mg/kg bw/day). None of the modifying factors apply. For medium potency substances, the general concentration limit (GCL) applies. As borates are classified in category 1B, the GCL is 0.3% (see Table 3.7.2 of CLP).

Conclusion

In conclusion, based on the adverse developmental and fertility effects of borates in rats and rabbits, RAC concluded that DOT should be classified with Repr. 1B, H360FD ('May damage fertility. May damage the unborn child.') according to CLP with no specific concentration limits.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The DS proposed no classification for environmental hazards, based on an evaluation concluding that DOT is not rapidly/readily degradable, has no potential for bioaccumulation, and none of the numerous toxicity studies give effect levels below the classification thresholds for CLP or DSD.

Comments received during public consultation

Two MSCAs and one industry organisation (EBA) supported the proposal for no classification. However, EBA disagreed on the approach chosen for the assessment of data, stating e.g., that the specific metal guidance of the CLP should have been used for the assessment of the data, and that the data from fresh water organisms should be given preference over data from marine organisms. In addition, one MS pointed out that one additional toxicity study was available that was not presented in the dossier, but also noted that the data did not deviate from the rest of the data base and thus, did not affect the proposal for no classification.

Assessment and comparison with the classification criteria

Boron is a metalloid that has properties in between those of metals and non-metals. The DS chose not to use the specific metal guidance on the CLP when assessing the need to classify for environmental effects. RAC agreed with this approach.

RAC noted that all borate compounds dissociate to boric acid, an inorganic substance for which the degradation criteria do not apply, and supported the conclusion that DOT should be considered not readily/rapidly degradable. Measured log K_{ow} for boric acid is about -1, and measured BCFs (although of uncertain quality) around 1 L/kg, indicating negligible potential for bioaccumulation. There are no toxicity studies on DOT as such. However, as all borate compounds dissociate in water, with boric acid as the main product, toxicity data from other borate compounds (boric acid, anhydrous sodium tetraborate and hydrated sodium tetraborates) have been used for the assessment. Concentrations of the above substances have been converted to concentration of elemental boron, and then back to concentration of DOT. RAC supported this procedure. It is noted that boric acid is not classified for environmental hazards.

Acute and chronic data are available for all three trophic levels (fish, invertebrates, and algae).

The lowest acute toxicity LC_{50} values reported in the dossier are 353, 119, and 213 mg/l, for fish, invertebrates and algae, respectively. The RAC notes that the lowest invertebrate EC_{50} (for the

marine shrimp *Litopenaeus vannamei*) is obtained from a study using a salinity of 3 ‰, whereas a more than threefold higher EC_{50} (315 mg/l) was obtained in the same study at a salinity of 20‰. An alternative LC_{50} value of 305 mg/l could be chosen from a study on *Hyalella Azteca* as the lowest acute effect value for invertebrates. In either case, all acute effect levels are above the thresholds for classification under CLP (1 mg/l).

The lowest chronic toxicity values are 3.3 (LC_{10}), 29 (NOEC) and 24 mg/l (NOEC), for fish, invertebrates and algae, respectively. All chronic effect levels are above the thresholds for classification under CLP (1 mg/l).

RAC noted that some of these studies use marine species. RAC supported the use of reliable marine toxicity data, and the CLP also endorse this (CLP 4.1.1.2.2). Overall, RAC concluded that the data do not fulfil the criteria for classification under CLP, and that DOT should not be classified for environmental hazards.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier submitter's proposal

The DS proposal contained no information as to the potential hazards to the ozone layer, and no classification is proposed.

Comments received during public consultation

The dossier did not contain any information on this topic, and no comments were received.

Assessment and comparison with the classification criteria

The DS proposal did not include this endpoint, making it difficult for RAC to assess hazards to the ozone layer. The RAC noted that the vapour pressure of DOT is very low, less than 5-10 Pa at ambient temperature, and that no other borate (e.g. boric acid) is classified for hazards to the ozone layer. In conclusion, RAC concluded that no classification for this endpoint seems plausible.

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ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and rapporteurs' comments (excl. confidential information).