Section A4.2	**Analytical Methods for Detection and Identification**
Annex Point IIA 4.2	A4.2 (c) Water

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28-04-2005
Materials and methods	Applicant's version is acceptable, but a more extensive method description can be found in the above cited EPA method 200.7. Estimated detection limit in method 200.7 is given as 5.7 µg B/L. The EPA method 6010C "Inductively Coupled Plasma-Atomic Emission Spectrometry" gives an estimated instrumental detection limit (IDL) for boron of 3.8 µg/L.
Conclusion	Boron in aqueous solutions (without particulate material) can be determined by ICP-AES (see e.g. EPA method 200.7), depending on the instrument, detection limits of ca. 4 µg B/L are achievable. LOQ is generally higher than the concentrations found in surface water (mean 95 percentile of monitoring data in 15 EU countries is between 17 and 632 µg B/L).
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	PT 08 - Fungicide, insecticide	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**	*Non-entry field*	
5.2.1	**Organism(s) to be controlled (IIA5.2)**	Dry rot (*Serpula lacrymans*) - preventive Wet rot (*Coniophora puteana, Gloeophyllum trabeum, Poria placenta, Coriolus versicolor*) – preventive Common furniture beetle (*Anobium punctatum*) - preventive House longhorn beetle (*Hylotrupes bajulus*) - preventive Lyctus powderpost beetle (*Lyctus brunneus)* – preventive Subterranean termites *(Reticulitermes santonensis, Reticulitermes flavipes, Reticulitermes lucifugis*) – preventive	X1
5.2.2	**Products, organisms or objects to be protected (IIA5.2)**	<u>Products to be protected:</u> Timbers exposed to risk of attack by wood destroying organisms. For example: Use Class 1 timbers under cover including indoor joinery (preventive) Use Class 2 timbers under cover including indoor roofing timbers – risk of wetting (preventive) Use Class 3 exterior timbers out of ground contact including joinery protected with a surface coating (preventive) Use Class 4a remedial application to timbers in service in ground contact e.g. boron rods inserted into utility poles which are already in service and may have been treated in the past with creosote (curative and preventive).	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)**	*Non-entry field*	
5.3.1	**Effects on target organisms (IIA5.3)**	See summary table section 5.3 and supplied Master Tables of references. In the majority of cases the studies determine toxic thresholds as lying between two of the concentrations of the active substance (boric acid equivalent) used in the study. The highest toxic threshold for the test organisms is 0.4% (for *G. trabeum*). At a BAE retention of 0.4% all organisms listed are prevented from attacking the timber.	X2

Section A5		Effectiveness against target organisms and intended uses	
5.3.2	**Likely concentrations at which the A.S. will be used (IIA5.3)**	PT-08: for preventive pre-treatment of timber commodities in Use Classes 1, 2 and 3 the likely concentration is 2 kg/m^3 BAE (equivalent to 0.4% BAE assuming wood density is 500 kg/m^3) See BS 8417 (2003). Remedial treatment of timber using boron rods often results in effective killing of any decay present and further protection from attack at retentions of 4 kg/m^3 BAE (0.8% BAE).	X3
5.4	**Mode of action (including time delay) (IIA5.4)**		
5.4.1	**Mode of action**	Fungicide, borate ions form chelate complexes with polyols of biological significance (oxidised co-enzymes) leading to a disruption of the organisms metabolic pathways Insecticide, borate ions form chelate complexes with polyols of biological significance (oxidised co-enzymes) leading to a disruption of the organisms metabolic pathways The biochemical effects of boron are largely attributed to the fact that boron (in the form of boric acid or ionized as tetrahydroxy borate anion) forms oxygen compounds that will chelate organic compounds containing adjacent alcohol groups. Vitamins and co-enzymes can react to form complexes with the borate ion within the cell and can produce dramatic changes in metabolism. Borates can also act as an inhibitor in a purely ionic manner, inhibiting the production of enzymes. The toxicity of boron containing compounds to most organisms would appear to be due to the ability to complex with organic compounds. This effect in mirco-organisms has been shown to be more biostatic than biocidal, with organisms appearing to "starve". The fungicidal mechanism of action of borates has been investigated . It was hypothesised that its primary mode of action was in general metabolism by interaction of the borate anion with polyols of biological significance. The oxidised co-enzymes NAD+, NMN+ and NADP+ were suggested as the most likely target of the borate ion. This theory was upheld in tests in vivo and it was concluded that the same mechanism was likely in all organisms, not just decay fungi References – Also listed at the end Lloyd J D (1998) Borates and their biological applications. The International Research Group on Wood Preservation. 21st annual meeting , Maastricht, The Netherlands. IRG Doc no. IRG/WP98-30178. Published by the IRG Secretariat , Stockholm. Lloyd J D & Dickinson D J (1991) Comparison of the inhibitory effects of borate, germanate, tellurate, arsenite and arsenate on 6-phosphogluconate dehydrogenase. The International Research Group on Wood Preservation. 21st annual meeting , Kyoto, Japan. IRG Doc no. IRG/WP1508. Published by the IRG Secretariat , Stockholm. Lloyd J D, Dickinson D J & Murphy RJ (1990) The probable mechanisms of action of boric acid and borates as wood preservatives. The International Research Group on Wood Preservation. 21st annual meeting , Rotorua, NZ. IRG Doc no. IRG/WP1450. Published by the IRG Secretariat , Stockholm. Lloyd J D, Dickinson D J & Murphy RJ (1991) The effect of sorbitol on the decay of boric acid treated Scots pine. The International Research Group on Wood Preservation. 21st annual meeting , Kyoto, Japan. IRG Doc no. IRG/WP1509. Published by the IRG Secretariat , Stockholm	X4
5.4.2	**Time delay**	None	

Section A5		Effectiveness against target organisms and intended uses
5.5	**Field of use envisaged (IIA5.5)**	
	MG02: Preservatives	Product types PT06-13 PT-08
5.6	**User (IIA5.6)**	Industrial Professional and Amateur (General Public)
	Industrial	See Section 2.10
	Professional	
	General public	
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**	No resistance
5.7.1	**Development of resistance**	Not relevant
5.7.2	**Management strategies**	Not relevant
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	To be submitted by EBA – Confidential information

Section 5.3: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
Include respective code(s) for function type(s) given in section 5.1	*Include respective code(s) for product type(s) given in section 5.5*	*Describe specification if deviating from that given in section 2*	*Specify species, strain, sex, weight, growth stage etc. as appropriate*	*Shortly describe test system and application method used in the tests*	*Shortly describe test conditions including concentrations applied and exposure time*	*Describe relevant results; quantify the effects on target organisms; indicate the dependence on the concentrations of the A.S. and the possible existence of a threshold concentration.* *Also describe if results indicate the mode of action and/or the development of resistance.*	*Only author(s) and year of publication/ report; full bibliographic data in footnote*
fungicide	PT-08	Boric acid	*Coniophora puteana* BAM440	DIN wood block over agar	Cultures grown for four weeks at 22°C, test samples and controls introduced and exposed for 12 weeks at 22°C	Toxic threshold concentrations determined as 0.1 – 0.16% BAE (0.5 – 0.8 kg/m^3 BAE) assuming wood density is 500 kg/m^3.	Becker 1966
fungicide	PT-08	Boric acid	*Coriolus (Trametes) versicolor* (L:.Fr.) Pil. 2666	Agar block test pine feeder strips on malt agar nutrient. Vacuum impregnation and diffusion.	Cultures grown for four weeks at 25°C, test samples and controls introduced and exposed for 12 weeks at 25°C. Mean retentions in blocks of 0, 0.1, 1.0 and 10 kg/m^3 BAE	Toxic threshold concentration determined as 0.2% BAE (1.0 kg/m^3 BAE).	Cookson & Pham 1995

fungicide	PT-08	Boric acid	*Gloeophyllum (Lenzites) trabeum* Madison 617	Soil block test following method of Leutritz (1946). Blocks placed on inoculated pine feeder strips in volcanic soil. Vacuum impregnation and diffusion.	Cultures grown for four weeks at 23°C, test samples and controls introduced and exposed for 14 weeks at 23°C. test concentrations of boric acid 0, 0.1, 0.24, 0.48 and 0.9% BAE.	Toxic threshold concentrations determined as 0.2 – 0.4% BAE (1.0 – 2.0 kg/m^3 BAE) assuming wood density is 500 kg/m^3.	Harrow 1950
fungicide	PT-08	Boric acid	*Poria placenta*	Soil block test	12 weeks at 22°C	Toxic threshold concentrations determined as 0.12 – 0.24% BAE (0.6 – 1.2 kg/m^3 BAE) assuming wood density is 500 kg/m^3.	Carr 1957
fungicide	PT-08	Boric acid	*Serpula lacrymans* BAM133	Agar block test	12 weeks at 20°C	Toxic threshold concentrations determined as 0.11 – 0.18% BAE (0.55 – 0.88 kg/m^3 BAE) assuming wood density is 500 kg/m^3.	Bravery & Carey 1983
insecticide	PT-08	Boric acid	*Anobium punctatum* de Geer	Egg laying and larval survival	9 month test. Loadings of boric acid ranged from 0.004 to 3.25 % BAE	Toxic threshold concentrations determined as 0.022 – 0.043% BAE (0.11 – 0.21 kg/m^3 BAE) assuming wood density is 500 kg/m^3.	Spiller 1948
insecticide	PT-08	Sodium metaborate	*Lyctus brunneus*	Egg laying and larval survival	16 weeks	Toxic threshold concentrations determined as 0.06 – 0.14% BAE (0.29 – 0.72 kg/m^3 BAE) assuming wood density is 500 kg/m^3.	Cummins & Wilson 1936
insecticide	PT-08	Borax DOT	*Hylotrupes bajulus*	Borax applied by vacuum impregnation to Corsican pine sapwood. BS 3651 newly hatched larvae introduced into holes.	6 months 0.012% to 1.2% solutions of DOT	For borax the toxic threshold concentrations determined as 0.008 – 0.045% BAE (0.068 – 0.34 kg/m^3 borax). For DOT the toxic threshold concentrations determined as 0.019 – 0.09% BAE (0.077 – 0.39 kg/m^3 DOT).	Taylor 1967
Insecticide/ termiticide	PT-08	Boric acid	*Reticulitermes flavipes*	Subterranean termite attack in a field test in Hawaii	6 months	Toxic threshold concentrations determined as 0.3% BAE (1.5 kg/m^3 BAE) assuming wood density is 500 kg/m^3.	Mauldin 1996

*) References:

List the references cited in alphabethical order with full bibliographic data (Author(s) (year) Title. Source)

Becker G, Hof T, Jacquiot C, Lohwag K, Rennerfelt E & Walchli O (1966) Vergleichsversuche zur Laboratoriumsprufung der pilzwidrigen Wirksamkeit von Holzschutzmitteln. Holz als Roh und Werkstoff 24 (2) 53-58.

Bravery A F & Carey J K (1983) A review of the data on the toxicity of boric acid to fungi and insects attacking wood. Job no. P820648 Building Research Advisory Service 1983.

Carr D R (1957) Toxicities of some waterborne wood preservatives to wood destroying fungi. New Zealand Forest Service, FRI report (unpublished) 8pp.

Cookson L J & Pham K (1996) Relative tolerance of twenty basidiomycetes to boric acid. Mat und Org 29 (3) 187-196.

Cummins J & Wilson H B (1936) The preservation of timber against the attacks of powder post beetle (*Lyctus brunneus*) by impregnation with various chemicals. Journal of the Commonwealth Science and Industry Res., Australia 9(1) 37-56.

Harrow K. M. (1950) Toxicity of water soluble wood preservatives to wood destroying fungi. NZ Journal Sci. & Tech. Vol B 31.

Mauldin J K & Kard B M (1996) DOT treatments to slash pine for protection against Formosan subterranean termite and eastern subterranean termite. J. of Economic Entomology 89 682-688.

Spiller D. (1949) Toxicity of boric acid to the common house borer, *Anobium punctatum*. NZ Journal Sci. & Tech. Vol B 30, 20, 22-30.

Taylor J M (1967) Toxicity of boron compounds to the common furniture beetle and house longhorn beetles. International Pest Control 9(1) 14-17.

Section 5.4.1 References

Lloyd J D (1998) Borates and their biological applications. The International Research Group on Wood Preservation. 21st annual meeting , Maastricht, The Netherlands. IRG Doc no. IRG/WP98-30178. Published by the IRG Secretariat , Stockholm.

Lloyd J D & Dickinson D J (1991) Comparison of the inhibitory effects of borate, germanate, tellurate, arsenite and arsenate on 6-phosphogluconate dehydrogenase. The International Research Group on Wood Preservation. 21st annual meeting , Kyoto, Japan. IRG Doc no. IRG/WP1508. Published by the IRG Secretariat , Stockholm.

Lloyd J D (1993) The mechanisms of action of boron containing preservatives. Thesis submitted for degree of Doctor of Philosophy of the University of London and the Diploma of Membership of Imperial College.

Lloyd J D, Dickinson D J & Murphy RJ (1990) The probable mechanisms of action of boric acid and borates as wood preservatives. The International Research Group on Wood Preservation. 21st annual meeting , Rotorua, NZ. IRG Doc no. IRG/WP1450. Published by the IRG Secretariat , Stockholm.

Lloyd J D, Dickinson D J & Murphy RJ (1991) The effect of sorbitol on the decay of boric acid treated Scots pine. The International Research Group on Wood Preservation. 21st annual meeting , Kyoto, Japan. IRG Doc no. IRG/WP1509. Published by the IRG Secretariat , Stockholm

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28-Feb-05
Materials and methods	Section IIIA5.2 Organisms to be controlled No indication is given in which parts of the Community the organisms to be controlled exist.
Conclusion	Incomplete data.
Reliability	not applicable
Acceptability	not acceptable The notifier is requested to indicate for which part of the Community the organisms to be controlled exist.
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE** 9-Nov-05
Materials and methods	Section IIIA5.3.1 Effects on target organisms a. In section IIIB5.7 some extra information concerning the effect on target organisms is available which is copied here: Preventive efficacy – prevents the use of the treated wood as a food source by the fungus or insect. Criterion in laboratory tests is less than a 3% mass loss in treated blocks when validated by the required mass losses in controls. Products often applied by dip-diffusion or vacuum impregnation. Curative efficacy – kills any decay fungi or insects that are present in the wood. For example fused boron rods slowly diffusing into the heartwood of a utility pole to remedially treat incipient decay. b. Efficacy studies were available on all target organisms mentioned above except for *Reticulitermes santonensis* and *Reticulitermis lucifugis*. The RMS expects that toxic threshold levels for *Reticulitermes santonensis* and *Reticulitermis lucifugis* are similar to toxic threshold levels for *Reticulitermis flavipes* (for which an efficacy study has been carried out), because they belong to the same genus. c. The summary table was incomplete. The RMS added 3 studies submitted for IIIB5.10, one study submitted for IIA and 4 studies submitted for IIB and made a new summary table. This table is appended at the end of the RMS section (see RMS table IIIA5.3). Values from this table are used to assess the efficacy. In addition, study summaries made by the notifier are considered incomplete, because relevant data were not reported. Therefore a new summary table was made by the RMS (see RMS table IIIA5.3). Carr, 1957, is considered as key study for treatment of decay fungi. The study of Cummins and Wilson, 1936, is considered as key study for treatment against larvae of wood-boring insects. The study of Mauldin and Kard, 1996, is considered as key study for treatment against termites. The notifier is requested to summarize these studies. d. Toxic threshold levels depend on the way the test is carried out. Toxic threshold levels are higher when leaching or evaporation occurs during the test period. Toxic threshold levels presented in the current CA-report are derived from experiments where blocks were placed on (damp) feeder strips, nylon nets, glass rests or plastic mesh squares. Experiments where blocks were placed directly on soil or agar were not taken into account, because experimental outcome is influenced by possible leaching during the test period. These experiments are indicated in the table (RMS table IIIA5.3). e. For some studies ranges in toxic threshold levels are given. For these studies several experiments were reported with different results. In most of the cases different toxic threshold levels are the result of different concentration steps used in the experiments. For evaluation of efficacy the highest level is taken as toxic threshold level. f. Distribution of the product through the wood depends on wood type (impregnatability, volumic mass) and on the wood loading procedure. Wood type Density of wood types varies between 360-1000 kg/m^3. Density for pine wood is 500 kg/m^3. Pine wood (pine, fir) is the wood type that is mainly used in the European Union. Therefore toxic threshold levels for fungi, wood-boring insects and termites are based on a default wood density of 500 kg/m^3.

Wood loading procedure

In the table for intended use (table IIB 7.5-2), several wood loading procedures are stated (e.g. vacuum pressure, dipping, spraying, injection), while most efficacy studies have been carried out with vacuum impregnation of solutions of boric acid or borates. In the present CA-report, the way the particular wood load is reached (application method), the time needed to reach this wood load and the homogeneity of the product distribution through the wood are not taken into account, because wood load is considered to be the crucial criterion for assessment of efficacy.

Efficacy studies with different application method or with different product distribution through the wood are therefore not required.

g. Upon dissolution in water (or when boric oxide rods come into contact with water) all boron species are converted into boric acid/borate according to the following formulae:

boric acid: $B(OH)_3$

boric oxide: $B_2O_3 + 3H_2O = 2B(OH)_3$.

disodium tetraborate anhydrous: $Na_2B_4O_7 + 7\ H_2O = 2\ NaB(OH)_4 + 2\ B(OH)_3$.

disodium tetraborate pentahydrate: $Na_2B_4O_7.5H_2O + 2\ H_2O = 2\ NaB(OH)_4 + 2\ B(OH)_3$.

disodium tetraborate decahydrate: $Na_2B_4O_7.10H_2O = 2NaB(OH)_4 + 2B(OH)_3 + 3H_2O$.

disodium octaborate tetrahydrate: $Na_2B_8O_{13}.4H_2O + 9H_2O \leftrightarrow 2NaB(OH)_4 + 6\ B(OH)_3$.

At low boron concentrations ($B \leq 0.025$ M) the following equilibrium is found:

$$B(OH)_3 + 2H_2O \leftrightarrow B(OH)_4^- + H_3O^+$$

In dilute aqueous solutions ($B \leq 0.025$ M) boron species exists as undissociated boric acid $B(OH)_3$ at pH < 7, at pH > 11 the metaborate ion $B(OH)_4^-$ becomes the main species in solution. At inbetween values (pH 7-11) both species are present. At higher boron concentrations (B > 0.025 M) an equilibrium is formed between $B(OH)_3$, polynuclear complexes of $B_3O_3(OH)_4^-$, $B_4O_5(OH)_4^{2-}$, $B_3O_3(OH)_5^{2-}$, $B_5O_6(OH)_4^-$ and $B(OH)_4^-$. In short:

$$B(OH)_3 \leftrightarrow \text{polynuclear anions} \leftrightarrow B(OH)_4^-.$$

In acid solution at pH<5, boron is mainly present as $B(OH)_3$ and in alkaline solution at pH>12.5, boron is mainly present as $B(OH)_4^-$. At inbetween values (pH 5-12) polynuclear anions are found as well as $B(OH)_3$ and $B(OH)_4^-$.

In the presence of metal ions (e.g. Na, Mg, Ca) ion-pair complexes are formed, which further reduce the undissociated boric acid concentration:

$$M^{n+} + B(OH)_4^- \leftrightarrow MB(OH)_4^{(n-1)+}$$

These ion pair complexes are expected to be present in solutions of disodium tetraborate, disodium octaborate and buffered solutions of boric acid and boric oxide.

For all boron species, solutions will contain undissociated boric acid $B(OH)_3$, borate ions $B(OH)_4^-$, alkali metal ion-pair complexes and possibly polynuclear boron complexes. Amounts of each of these compounds depend on pH, boron concentration and concentrations of alkali metals (Na, K).

Primary mode of action of boron solutions is the complexation of the borate anion $B(OH)_4^-$ with polyols of biological significance such as oxidised co-enzymes (NAD^+, NMN^+, $NADP^+$) and vitamins.

Efficacy studies with boric acid, disodium octaborate tetrahydrate (DOT), disodium tetraborate decahydrate (Borax) or sodium metaborate showed similar toxic threshold levels based on boric acid equivalents (BAE), when similar target species (fungi, wood-boring insects) were compared in tests with the same wood type (pine, spruce, oak, yellow carrabeen, eucalyptus) as can be seen in the studies summarized in RMS table IIIA.5.3. Compounds used and target organisms tested are indicated in the table (RMS table IIIA5.3).

From this studies it can be concluded that boric acid/borate is indeed the toxic component for all boron species. Therefore efficacy studies with all types of boron species can be used to establish toxic threshold levels for boric acid containing

products. For this purpose, toxic threshold levels for each boron species can either be expressed as equivalent boric acid concentration or as equivalent boron concentration. Because the intended uses were expressed as boric acid equivalents (BAE) all efficacy studies were recalculated as equivalent boric acid concentrations.
h. Of the fungi mentioned in IIIA5.2.1 highest tolerancy for boron species was found for *Gloeophyllum trabeum* syn *Lenzites trabea* with toxic threshold levels of 2.2 kg/m^3 BAE in wood or 0.44 % (w/w) BAE in wood (Carr, 1957)
Of the wood-boring insects mentioned in IIIA5.2.1 highest tolerancy for boron species was found for larvae of *Lyctus brunneus*. Toxic threshold levels for egg larvae were 1.5 kg/m^3 BAE in wood or 0.30% (w/w) BAE in wood. Boron was not effective against larger larvae from *Lyctus brunneus* up to the highest concentration tested of 15 kg/m^3 in wood or 3.0% (w/w) BAE in wood. Next highest tolerancy for boron species was found for larger larvae of *Anobium punctatum* at 9.5 kg/m^3 BAE in wood or 1.9% (w/w) BAE in wood. However, larger larvae are not considered to be target organisms.
For the termites mentioned in IIIA5.2.1, toxic threshold levels for *Reticulitermes flavipes* were 1.5 kg/m^3 BAE in wood or 0.30% (w/w) BAE in wood.
i. None of the studies has been carried out according to present day standards. Evaporative ageing procedures required for class 1-4a have only been carried out in some of the fungi tests (4-6 weeks at air dry conditions or 48 hrs in a ventilated environment). Evaporative ageing (air-drying for 4 weeks) for the most persistent decay fungus *Gloeophyllum trabeum* syn *Lenzites trabea* resulted in the toxic threshold levels of 2.2 kg/m^3 BAE in wood or 0.44 % (w/w) BAE in wood, as mentioned above. No evaporative ageing procedures have been carried out for wood-boring insects or termites. Evaporative ageing procedures according to EN73 are not applicable, because this test is based on evaporation of the active substance. For boric acid, the evaporation of water may cause problems. Once the water is evaporated from the wood, boric acid is converted into the solid state active substance, while efficacy is based on the presence of the borate anion, which is only formed in a humid environment. Therefore, additional studies are required where treated wood is dried (e.g. 4-6 weeks) and is exposed to wood-boring insects and termites. Therefore no final conclusions can be drawn concerning efficacy against all target organisms to be controlled.
Leaching procedures (EN 84 or EN 330) required for class 3-4a have not been carried out. This is acceptable for the Annex I evaluation phase, but for product authorisation phase additional tests are required.
j. Study report Smith, 1969 is incomplete. Because the study describes efficacy data for the most boron tolerant fungi, the complete study report is required.

Conclusion

Efficacy studies were available on all target organisms mentioned above except for *Reticulitermes santonensis* and *Reticulitermis lucifugis*. The RMS expects that toxic threshold levels for *Reticulitermes santonensis* and *Reticulitermis lucifugis* are similar to toxic threshold levels for *Reticulitermis flavipes* (for which an efficacy study has been carried out), because they belong to the same genus.
Efficacy studies with boric acid, disodium octaborate tetrahydrate (DOT), disodium tetraborate decahydrate (Borax) or sodium metaborate showed similar toxic threshold levels based on boric acid equivalents. From this studies it can be concluded that boric acid/borate is indeed the toxic component for all boron species. Therefore efficacy studies with all types of boron species can be used to establish toxic threshold levels for boric acid containing products.
Of the fungi mentioned in IIIA5.2.1 highest tolerancy for boron species was found for *Gloeophyllum trabeum* syn *Lenzites trabea* with toxic threshold levels of 2.2 kg/m^3 BAE in wood or 0.44 % (w/w) BAE in wood.
Of the wood-boring insects mentioned in IIIA5.2.1 highest tolerancy for boron species was found for larvae of *Lyctus brunneus*. Toxic threshold levels for egg larvae were 1.5 kg/m^3 BAE in wood or 0.30% (w/w) BAE in wood. Boron was not effective against larger larvae from *Lyctus brunneus* up to the highest concentration tested of 15 kg/m^3 in wood or 3.0% (w/w) BAE in wood. Next highest tolerancy for boron species was found for larger larvae of *Anobium punctatum* at 9.5 kg/m^3 BAE in wood or 1.9% (w/w) BAE in wood. However,

	larger larvae are not considered to be target organisms. For the termites mentioned in IIIA5.2.1, toxic threshold levels for *Reticulitermes flavipes* were 1.5 kg/m^3 BAE in wood or 0.30% (w/w) BAE in wood.
Reliability	Harrow, 1950, set at 2 Baechler and Roth, 1956, set at 4 (no experimental conditions) Carr, 1957, set at 2, key study for decay fungi Becker, 1966, set at 2 Gallagher, 1968, set at 2 Smith, 1969, set at 4 (incomplete study report) Bechgaard, 1979, set at 2 Doi et al, 1994, set at 4 (leaching not prevented) Cooksen and Pham, 1995, set at 2 Cummins and Wilson, 1936, set at 2, key study for wood boring insects Cummins, 1939, set at 4 (no experimental conditions) Spiller, 1948, set at 4 (no experimental conditions) Taylor, 1967, set at 2 Mauldin and Kard, 1996, set at 2, key study for termites Findley, 1959, set at 4 (no experimental conditions) Becker, 1959, set at 4 (no experimental conditions) Bravery and Carey, 1983, set at 4 (no experimental conditions)
Acceptability	Acceptable for annex I inclusion, however in the product authorisation phase: 1. For use class 1-3, efficacy tests on wood boring insects and termites with the biocidal product are required where treated wood is dried (e.g. 4-6 weeks) and is exposed to wood-boring insects and termites. 2. For use class 3, efficacy tests on decay fungi, wood boring insects and termites with the biocidal product are required where leaching procedures are included, according to present day standards (EN 84). 3. A complete study report from Smith, 1969. 4. Detailed summaries for key studies on decay fungi (Carr, 1957), larvae of wood-boring insects (Cummins and Wilson, 1936) and on termites (Mauldin and Kard, 1996) are required.
Remarks	-

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

9-Nov-05

Materials and methods

Section IIIA5.3.2 Likely concentrations at which the a.s. will be used.
a. The notifier submitted additional information on application rates which should overrule all other concentration rates stated earlier. Full reference: Borax, 2005, Explanatory note on efficacy assessment, application rates, environmental exposure assessment, boric acid. [redacted]
This information is summarized by the RMS as follows:
Boric acid is used in PT8 products either
• as a stand alone product, that is to say there are no other co-formulants or co-biocides.
• as a co-biocide, used to enhance the spectrum of activity of the wood preservative formulation.
• as a co-formulant to assist in the properties of the formulation, e.g. stability.
• or to perform both co-formulant and co-biocide functions.

It is common practice to categorise the application of wood preservatives and their application rates according to the Biological Hazard (Use) Classes, for example UC 1, 2, 3,4 and 5. These increase in the severity of the biological challenge that the timber will be exposed in service.

FORM OF APPLICATION (preventive treatments)	UC1	UC2	UC3	UC4	UC5
Stand alone borate biocide	√	√	√	**X**	**X**
Co-biocide	√	√	√	√	√
Co-formulant	√	√	√	√	√
Co-formulant & biocide	√	√	√	√	√

In UC1, UC 2 and UC3, boric acid is normally used as a biocide. However when incorporated in formulations for use in UC4 and UC5, it is present principally as a formulation aid (e.g. pH control, solubility aid etc) rather than for efficacy purposes.

MAXIMUM APPLICATION RATES (Preventive treatment)

FORM OF APPLICATION (preventive treatments)	UC1 Max application rate* BAE	UC2 Max application rate* BAE	UC3 Max application rate* BAE	UC4 Max application rate* BAE	UC5 Max application rate* BAE
Stand alone borate biocide	2 kg/m3	2 kg/m3	2 kg/m3	N/A	N/A
Co-biocide	2 kg/m3	2 kg/m3	2 kg/m3	2 kg/m3	2 kg/m3
Co-formulant	2 kg/m3	2 kg/m3	2 kg/m3	2 kg/m3	2 kg/m3
Co-formulant & co-biocide	2 kg/m3	2 kg/m3	2 kg/m3	2 kg/m3	2 kg/m3

* expressed in terms of overall loading of boric acid equivalent (BAE) in 1 m3 of timber, that is to assume the heartwood and sapwood ratio of the timber to be treated is 50:50.

TYPICAL APPLICATION RATES (Preventive treatment)

FORM OF APPLICATION (preventive treatments)	UC1 Typical application rate* BAE	UC2 Typical application rate* BAE	UC3 Typical application rate* BAE	UC4 Typical application rate* BAE	UC5 Typical application rate* BAE
Stand alone biocide	1 kg/m3	1 kg/m3	1 kg/m3	N/A	N/A
Co-biocide	0.4kg/m3	0.4 kg/m3	0.4 kg/m3	0.4 kg/m3	0.4 kg/m3
Co-formulant	0.4 kg/m3	0.4 kg/m3	0.4 kg/m3	0.4 kg/m3	0.4 kg/m3
Co-formulant & biocide	0.4 kg/m3	0.4 kg/m3	0.4 kg/m3	0.4 kg/m3	0.4 kg/m3

* expressed in terms of overall loading of boric acid equivalent (BAE) in 1 m^3 of timber, that is to assume the heartwood and sapwood ratio of the timber to be treated is 50:50.

TERMITE TREATMENT APPLICATION RATES (Preventive treatment)

FORM OF APPLICATION (preventive treatments)	UC1 Typical application rate* BAE	UC2 Typical application rate* BAE	UC3 Typical application rate* BAE	UC4 Typical application rate* BAE	UC5 Typical application rate* BAE
Termite treatments (stand alone borate biocide)	4.0kg/m3	4.0kg/m3	4.0kg/m3	N/A	N/A

* expressed in terms of overall loading of boric acid equivalent (BAE) in 1 m3 of timber, that is to assume the heartwood and sapwood ratio of the timber to be treated is 50:50.

Conclusion

The notifier submitted various documents with inconsistent information on dose rates used in the different application methods. The RMS proposes to assess the efficacy against the dose rates as presented in section IIIA5.3.2 and table IIB 7.5-2 and which is also summarized below. The reader is referred to intended uses as proposed by RMS, which is included in Doc IIB, Section 7.5.

Application rates of boric acid containing products for the different types of application as proposed by the RMS.

Treatment process	Intended application method	Normal use (decay fungi and wood-boring insects)	Termite prevention	Intended treatment type
Industrial	vacuum pressure double vacuum	2.0 kg/m^3 BAE in wood or 0.4% (w/w) BAE in wood	8.5 kg/m^3 BAE in wood or 1.7% (w/w) BAE in wood	preventive treatment class 1, 2, 3
Industrial	automated dipping industrial deluge	0.05 kg/m2 BAE on wood	not for termites	preventive treatment class 1, 2, 3
Industrial	automated spraying	0.05 kg/m2 BAE on wood	not for termites	preventive treatment class 1, 2, 3
In-situ	injection	2.0 kg/m^3 BAE in wood or 0.4% (w/w) BAE in wood	not for termites	remedial treatment (curative) class 4a
In-situ	brushing spraying	0.05 kg/m^2 BAE on wood	not for termites	remedial treatment (curative) class 4a

BAE = boric acid equivalents, i.e "expressed as boric acid"

	The notifier proposed to use boric acid containing products: • as a stand alone product, that is to say there are no other co-formulants or co-biocides. • as a co-biocide, used to enhance the spectrum of activity of the wood preservative formulation. • as a co-formulant to assist in the properties of the formulation, e.g. stability. • or to perform both co-formulant and co-biocide functions. In the present CA-report only the proposed use as stand alone product is assessed. No information is available on the other product uses. Therefore only use class 1, 2, 3 and 4a are assessed as is indicated in IIIA5.2.2. At the proposed concentrations of 2.0 kg/m^3 or 0.4% (w/w) BAE for preventive treatment in use class 1, 2, and 3, egg larvae of wood-boring insects and termites will be controlled. Although most fungi (wet rot and dry rot) will be controlled, the most persistent fungi (*Gloeophyllum trabeum* syn *Lenzites trabea)* will not be controlled completely: toxic threshold levels are 2.2 kg/m^3 BAE in wood or 0.44 % (w/w) BAE in wood. Therefore, the notifier is requested to reconsider the proposed dose rates. At the proposed concentrations of 2.0 kg/m^3 or 0.4% (w/w) BAE for remedial treatment in use class 4a, egg larvae of wood-boring insects and termites will be controlled. Although most fungi (wet rot and dry rot) will be controlled, the most persistent fungi (*Gloeophyllum trabeum* syn *Lenzites trabea)* will not be controlled completely: toxic threshold levels are 2.2 kg/m^3 BAE in wood or 0.44 % (w/w) BAE in wood. However, larger larvae of *Anobium punctatum* and *Lyctus brunneus* survive treatment. Therefore boric acid containing products can only be used for preventive treatment and not for remedial (curative) treatment. Use Class 4a remedial application to timbers in service in ground contact is therefore not feasible. At present no final conclusions for use in classes 1, 2, and 3 can be drawn, because evaporative ageing processes (class 1-3) for wood-boring insects and termites and leaching processes (class 3) for all target organisms have not been carried out. -
Reliability	
Acceptability	Acceptable for as part of annex I inclusion. For the product authorisation phase additional information is required: 2. The notifier proposed to use 12% w/v BAE and 35% w/v BAE in treatment solutions. But the solubility for boric acid is about 50 g/L (see Doc IIB 1.3) , which is 5% w/v BAE. Therefore it is impossible to prepare the proposed treatment solutions. The notifier is requested to clarify this issue. 3. For use class 1-3, efficacy tests on decay fungi, wood boring insects and termites, show that at least a concentration of 2.2 kg/m^3 or 0.44% (w/w) BAE in wood is required for efficacy of the boron containing product against decay fungi. The notifier is requested to reconsider the proposed dose rates. 4. At present no final conclusions for use in classes 1, 2, and 3 can be drawn, because evaporative ageing processes (class 1-3) for wood-boring insects and termites and leaching processes (class 3) for all target organisms have not been carried out.
Remarks	In the product authorisation phase clarification on discrepancies in intended use (see question 1) is required for: a. Application concentrations for injection/brushing/spraying in section IIIB2 (max

	12% w/v BAE) and section IIIB5.3 (max 12% w/v BAE) are not consistent with the concentration levels stated in section IIIB5.4 for injection/brushing/spraying (11.5%-17.25% w/v) b. Wood loading of 1.7% w/w BAE in wood (i.e. 8.5 kg/m^3 BAE in wood) for termites in section IIIB2 is not consistent with the wood loading stated in the additional information supplied by the notifier and summarized by the RMS in section IIIA5.3 (4.0 kg/m^3 BAE in wood) c. Wood loading of 2.0 kg/m^3 BAE in wood for fungi and wood boring insects stated in section IIIA5.3.2 is not consistent with the wood loading stated in the additional information supplied by the notifier and summarized by the RMS in section IIIA5.3.2 (0.4-1.0 kg/m^3 BAE in wood for typical application rates). d. Injection at 0.05 kg/m^2 BAE on wood calculated from kg/m^3 BAE in wood dose rates and default conversion factors from OECD guidelines is not consistent with the value of 0.0345 kg/m^2 BAE as listed in the notifier's List of Endpoints.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE** 22-Mar-05
Materials and methods	Section IIIA5.4.1 Mode of action a. Four studies were submitted. Study 1 (Lloyd, 1998) is a review article without methods and is given reliability of 4. Study 2 (Lloyd and Dickinson, 1991) compare activity of other compounds with borates and is considered not relevant here. The study is given reliability of 4. Study 3 and 4 (Lloyd et al, 1990 and 1992) are considered as key studies by the RMS, because the hypothesis for the mode of action is experimentally tested. Studies are given reliability of 2 because of non-GLP and no indications of purity of the active substance. b. The hypothesis for the mode of action assumes that boron compounds are converted as much as possible into the tetrahydroxyborate ion $B(OH)_4^-$. The borate anion acts by complexation with polyols of biological significance such as oxidised co-enzymes (NAD+, NMN+, NADP+), vitamins and others. c. In study 3 (Lloyd et al, 1990) the effect of boric acid (pH=7) was tested in an in-vitro enzyme system (yeast 6-phosphogluconate dehydrogenase) and in-vitro fungal systems (*Trichoderma viride*, *Coriolus versicolor*, and *Coniophora puteana*). The 6-phosphogluconate dehydrogenase was found to be inhibited by the borate anion (41.7 mM borate). Fungal growth was reduced with increasing borate concentrations (0.02-0.12% borate). White rot (*Coriolus versicolor*) was least affected by the borate. The addition of different polyols reduced the inhibitory effect of the borate anion both in the enzyme system and in the fungal system. Highest reduction was found for sorbitol, followed by ribose and then glucose. d. In study 4 (Lloyd et al, 1991) the effect of boric acid (pH=7) was tested in an in-vivo system. Small blocks of scots pine sapwood (Pinus sylvestris, 30x10x5 mm) were treated with a boric acid - sorbitol solution (0-0.14% boric acid, 0-500 mM sorbitol), equivalent to 0-1.2 kg/m^3 boric acid. The blocks were immersed in the solution and treated under vacuum for one hour, followed by 2 hrs of atmospheric pressure. The blocks were left to dry for 2 weeks, while the blocks were turned every 24 hrs. Blocks were sterilized by gamma-irradiation. Blocks were placed on agar plates covered for 95% with cultures of Coniophora puteana (FPRL 11E) or Coriolus versicolor (FPRL 28A). Plates were incubated for 7 weeks at 22 °C. The addition of high sorbitol concentrations (250-500 mM) reduced the inhibitory effect of the borate anion (at 0.45 kg/m3 and higher).

Conclusion	e. Results substantiate the theory that the polyol complexing ability of the borate anion is responsible for the protection against decay (or insects) of boron treated timber. Primary mode of action is the interaction of the borate anion with polyols of biological significance e.g. oxidised co-enzymes (NAD+, NMN+ and NADP+).
Reliability	study 1 (Lloyd, 1998) set at 4 study 2 (Lloyd and Dickinson, 1991) set at 4 study 3 (Lloyd et al, 1990) set at 2 study 4 (Lloyd et al, 1991) set at 2
Acceptability	acceptable
Remarks	-

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

RMS table IIIA5.3 Summary table of experimental data on the effectiveness of the active substance against target organisms

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
fungicide	PT-08	Boric acid	Three species *Lenzites trabea* syn *Gloeophyllum trabeum* highest boron tolerancy Other relevant species: *Coniophora cerebella (2 strains)* syn *Coniophora puteana, Poria vaporaria* syn *Poria placenta*	Pine (Pinus radiata) blocks treated by vacuum impregnation and diffusion. Blocks were left until air dry (period not stated). No ageing or leaching. Soil block test according to method of Leutritz (1946), mass loss in weight. Treated autoclaved blocks were placed on feeder strips which were placed on the soil surface.	Test concentrations 0, 0.4-3.7 kg/m^3 BAE or 0, 0.10%-0.90% w/w BAE. Blocks exposed for 14 weeks at 23°C.	Highest boron tolerancy for *Lenzites trabea* syn *Gloeophyllum trabeum* on pine (*Pinus radiata*): Toxic threshold concentrations determined as 0.48% w/w BAE (2.0 kg/m^3 BAE). No conversion factors used, actual values from the test.	Harrow 1950
fungicide	PT-08	Sodium borate (assumed to be borax)	Four species *Lenzites trabea* syn *Gloeophyllum trabeum* highest boron tolerancy. Other relevant species: *Poria monticola*.	Pine and oak blocks. Wood treatment conditions not stated. No ageing or leaching. Soil block test, mass loss in weight. Test conditions not stated. Slightly higher toxic threshold levels are found for oak than for pine for *G. trabeum* (factor 1.5) but not for *P. monticola* (factor 0.82)	Not stated.	Highest boron tolerancy for *Lenzites trabea* syn *Gloeophyllum trabeum* on oak: Toxic thresholds 0.22-0.37% w/w BAE (1.11-1.84 kg/m^3 BAE, 0.107-0.177 lb/ft^3 as borax). Conversion factor lb/ft^3 → kg/m3 multiply by 15.99. Conversion factor kg/m^3 → % w/w multiply by 0.2. Conversion factor borax → BAE multiply by 0.65.	Baechler and Roth, 1956
fungicide	PT-08	Boric acid or borax	Three species *Lenzites trabea* syn *Gloeophyllum trabeum* highest boron tolerancy Other relevant species: *Coniophora cerebella* syn *Coniophora puteana, Poria vaporaria* syn *Poria placenta*	Pinus radiata sapwood treated by vacuum impregnation and diffusion. Blocks were air dried for 4 weeks (ageing). No leaching. Soil block test, mass loss in weight. Treated autoclaved blocks were placed on feeder strips which were placed on the soil surface.	Blocks exposed for 12 weeks at 27°C. Test concentrations 0, 0.08-0.37 lb/ft^3 for boric acid or 0, 0.09-0.36 lb/ft^3 for borax.	Highest boron tolerancy for *Lenzites trabea* syn *Gloeophyllum trabeum* on pine *sapwood (Pinus radiata):* Toxic threshold concentrations for **boric acid** determined as 0.26 – 0.44% BAE (1.3 – 2.2 kg/m^3 BAE, 0.08-0.14 lb/ft^3 as boric acid) assuming wood density is 500 kg/m^3. Toxic threshold concentrations for **borax** determined as 0.28%-0.38% BAE (1.4-1.9 kg/m^3 BAE, 0.14-	Carr 1957

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
						0.19 lb/ft^3 as borax). Toxicities of boric acid and borax are in proportion to their boron content. Conversion factor lb/ft^3 → kg/m3 multiply by 15.99. Conversion factor kg/m^3 → % w/w multiply by 0.2. Conversion factor borax → BAE multiply by 0.65.	
fungicide	PT-08	Boric acid	Three species and several strains. *Coniophora cerebella* Pers. EMPA62 syn *Coniophora puteana* highest boron tolerancy Other relevant species: *Merulius lacrymans* (Wulf.) Fr. syn *Serpula lacrymans* and *Polystictus versicolor* (Linn.) Fr. syn *Coriolus versicolor*.	DIN 52176; NF X 41-512; Agar block test, mass loss in weight. Wood type, wood treatment and cultivation conditions not indicated. ASTM D1413 resulted in higher toxic threshold levels. Results considered not reliable because of leaching and evaporation during the test. These results are not taken into account for derivation of the toxic threshold concentration.	Concentration range not indicated. Blocks exposed for 6-18 weeks at 20-25°C.	Highest boron tolerancy for *Coniophora cerebella* Pers. EMPA62 syn *Coniophora puteana* on unknown wood type: Toxic threshold concentrations determined as 0.12 – 0.28% w/w BAE (0.6 – 1.4 kg/m^3 BAE) assuming wood density is 500 kg/m^3. Conversion factor kg/m3 → % w/w multiply by 0.2.	Becker 1966
fungicide	PT-08	Timbor (=DOT)	Two species. *Coniophora cerebella* syn *Coniophora puteana* highest boron tolerancy. Other relevant species: *Poria xantha*.	Scots pine sapwood and heartwood treated by vacuum pressure or vacuum diffusion process. Treated wood dried at 50 °C for 3 hrs and ventilated in a filtered air-flow for 48 hours. Agar block test, mass loss in weight. Blocks placed on glass rests on the agar surface. Toxic threshold levels for Scots pine heartwood were a factor 1.8 lower than for Scots pine sapwood.	Retention in blocks 0, 0.07-0.89 kg/m^3 as borax. Blocks exposed for 12 weeks at 22 °C.	Highest boron tolerancy for *Coniophora cerebella* syn *Coniophora puteana* on pine sapwood: Toxic threshold concentration determined as 0.13% w/w BAE (0.65 kg/m^3 BAE, 0.54 kg/m^3 as DOT). Conversion factor kg/m^3 → % w/w multiply by 0.2. No conversion factor used for DOT to BAE, actual values from the study	Gallagher, 1968

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
fungicide	PT-08	Timbor (=DOT)	*Lenzites trabea* Pers ex Fr FPLV 47B syn *Gloeophyllum trabeum*	Ponderosa pine sapwood treated by vacuum impregnation. No ageing or leaching. Soil block test, mass loss in weight and total carbon dioxide evolution measured by GC-FID (after reduction to methane). ASTM D1413 (1961). Blocks were placed on feeder strips, placed on the soil surface.	Concentration range not stated. Blocks exposed for 12 weeks at 23 °C.	Due to incomplete study report toxic threshold values could not be evaluated (figure 4 and table 2 are missing). Data are required because G. trabeum is considered the most boron tolerable decay fungus.	Smith, 1969
fungicide	PT-08	boric acid	Two species *Lentinus lepideus* BK C-1 highest boron tolerancy, but not relevant for present evaluation. Relevant species for toxic threshold concentration: *Gloeophyllum trabeum* (A570)	Pinus sylvestris L. sapwood treated by vacuum/pressure process. Blocks air dried for 24 hrs. No ageing or leaching. Agar block test, mass loss in weight. Blocks were placed on a nylon net, which was placed on the agar surface.	Retentions in blocks 0.1-1.0 kg/m^3 BAE. Blocks exposed for 6 months at 50°C.	Highest boron tolerancy for *Gloeophyllum trabeum* (A570) on pine (*Pinus sylvestris*) sapwood: Toxic threshold concentration determined as 0.08%-0.18% w/w BAE (0.40-0.92 kg/m^3 BAE). Conversion factor kg/m^3 → % w/w multiply by 0.2.	Bechgaard, 1979
fungicide	PT-08	boric-acid triethanolamine (BTEA); boric acid or Timbor (= DOT)	Four species. *Chaetomium globosum* Kunze IAM 8059 highest boron tolerancy, but not relevant for present evaluation. Relevant species for toxic threshold concentration in sequence of highest boron tolerancy: *Coriolus versicolor* L ex. Fr. Quel FFPR 1030 and *Serpula lacrymans* FFPR 0739.	Yezo spruce (Picea jezoensis) and Japanese beech (Fagus crenata) sapwood treated by vacuum impregnation. JIS A9201 (1991) test without weathering. Soil block test or agar block test (C. globosum only), mass loss in weight. Blocks were placed directly on the soil or agar surface. Toxic threshold levels on Japanese beech (Fagus crenata) were higher than on Yezo spruce (only tested for C. versicolor), but dose rates were not high enough to deduce a toxic threshold level for Japanese beech (> 1.45 or >1.53 kg/m^3 BAE for Timbor and boric acid, respectively).	Retention in blocks 0, 0.39-4.29 kg/m^3 BAE for Tim-bor and 0, 0.40-1.65 kg/m^3 BAE for boric acid. Blocks exposed for 120 days at 26 °C or 20 °C (S. lacrymans only). BTEA is considered not relevant for the present evaluation, because the tri-ethanol amine has synergic effects on boron efficacy. Toxic threshold levels for Timbor and boric acid are similar:0.85 and 0.83 kg/m^3 BAE, respectively. for *S. lacrymans* on Yezo spruce,	Highest boron tolerancy for *Coriolus versicolor* L ex. Fr. Quel FFPR 1030 on Yezo spruce (*Picea jezoensis*) sapwood: For **Tim-bor** toxic threshold concentration determined as 0.77%-0.86% w/w BAE (3.84-4.29 kg/m^3 BAE). **Boric acid** was not tested on the combination C. versicolor and Yezo spruce. Conversion factor kg/m^3 → % w/w multiply by 0.2. Toxic threshold levels in this study were higher compared to other studies, because leaching was not prevented during test. This study is therefore considered as not reliable	Doi et al., 1994

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
						and results are not used in efficacy assessment.	
fungicide	PT-08	Boric acid	Several species *Gloeophylum abietineum* (Fr.) Karst 13851 highest boron tolerancy Other relevant species: *Gloeophyllum trabeum* (Fr.) Murr. 7520, *Serpula lacrymans* S.F. Gray 16508, *Coniophora olivacea* (Fr.) Karst, *Poria sp.* 2422, *Poria subcrassa* Rodway & Cleland 11040, *Trametes versicolor* (L.:Fr.) Pil. syn *Coriolus versicolor.*	Pinus radiata D Don sapwood and Eucalyptus regnans F. Muell heartwood treated by vacuum impregnation and diffusion. Blocks were air dried for 6 weeks (ageing). No leaching. Soil block test, mass loss in weight. Blocks were placed on a plastic mesh square, but not in contact with the feeder strips which were placed on the soil surface. Agar block test resulted in higher toxic threshold levels, results are considered not reliable because of larger concentration intervals. Results from agar block tests are not used for derivation of toxic threshold levels. Toxic threshold levels for pine and eucalyptus were similar for *Poria sp.* 2422, *Poria subcrassa* Rodway & Cleland 11040. The other relevant species were only tested on pine	Mean retentions in blocks of 0 and 0.5-2.0 kg/m^3 BAE for soil block test or 0 and 0.1-10.0 kg/m^3 BAE for agar block test. Blocks exposed for 12 weeks at 25°C.	Highest boron tolerancy for *Gloeophylum abietineum* (Fr.) Karst 13851 on pine (Pinus radiata) sapwood: Toxic threshold concentration determined as 0.4% w/w BAE (2.0 kg/m^3 BAE) in the soil block test. Conversion factor kg/m^3 → % w/w multiply by 0.2.	Cookson & Pham 1995
insecticide	PT-08	Sodium metaborate (assumed $NaBO_2$)	Egg larvae and larger larvae of *Lyctus brunneus* Stephens	Starch-free and starch-containing sapwood of Eucalyptus regnans or Eucalyptus obliqua treated by immersion in boiling solution. Blocks were air dried (period not stated). No ageing or leaching. Larval survival and mass loss in weight of wood. Wood-boring in starch free wood is generally lower than in starch containing wood. Because of the	Test concentrations 0.4-2.3 lb/ft^3 for larger larvae and 0.04-2.8 lb/ft^3 for beetle test (egg larvae). Duration of the test not stated, but at least 9 weeks. Large larvae hardly eat from the wood and pupate almost immediately. Therefore tests were carried out with very small, small and medium sized larvae which	Highest boron tolerancy for Lyctus brunneus on starch-free Eucalyptus obliqua: Toxic threshold concentrations for egg larvae determined as 0.30% w/w BAE (1.5 kg/m^3 BAE or 0.1 lb/ft^3 sodium metaborate) assuming wood density is 500 kg/m^3. Not effective against larger larvae at highest level tested: 6.9% w/w BAE (35 kg/m^3 BAE, 2.3 lb/ft^3	Cummins & Wilson 1936

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
				reduced amount of toxic material passed through the digestive tract, toxic threshold levels for starch free wood is higher. Experiments with larger larvae were only carried out on Eucalyptus obliqua. Results from egg larvae on Eucalyptus obliqua and Eucalyptus regnans were similar.	have sufficient gluttony to ensure proper assessment of efficacy.	sodium metaborate). Conversion factor lb/ft^3 → kg/m3 multiply by 15.99. Conversion factor kg/m^3 → % w/w multiply by 0.2. Conversion factor metaborate (MW 657996) → BAE multiply by 0.94.	
insecticide	PT-08	boric acid or borax or boric acid plus borax	Egg larvae of *Lyctus brunneus* Stephens	Starch containing yellow carrabeen (Sloanea woolsii). Wood treatment not stated. Visual damage to wood. Experimental conditions not stated.	Test concentrations 0.01-0.24 lb/ft^3 BAE for boric acid or 0.04-0.3 lb/ft^3 for borax. Duration of the test not stated.	Boron tolerancy for Lyctus brunneus on yellow carrabeen (Sloanea woolsii) For boric acid, toxic threshold concentration is 0.16% w/w BAE (0.80 kg/m^3 BAE, 0.05 lb/ft^3 BAE). For borax, toxic threshold concentration is 0.08% w/w (0.42 kg/m^3 BAE, 0.04 lb/ft^3 as borax). Toxicity of boric acid, borax or mixtures of borax and boric acid, is considered equal. Because of differences in concentration ranges, final endpoints are slightly different. Conversion factor lb/ft^3 → kg/m3 multiply by 15.99. Conversion factor kg/m^3 → % w/w multiply by 0.2. Conversion factor borax → BAE multiply by 0.65.	Cummins; 1939
insecticide	PT-08	Boric acid	Egg larvae of *Anobium punctatum* de Geer	Pinus radiata D. Don sapwood and Podocarpus dacrydoides sapwood; wood treatment not stated. Larval survival. Efficacy results for Pinus radiata D.	Test concentrations 0.004-3.25 % (w/w) in wood. Duration of the test not stated.	Highest boron tolerancy for *Anobium punctatum* on pine (Pinus radiata) and kabikatea (Podocarpus Dacrydoides) sapwood: Toxic threshold concentrations determined as 0.022 – 0.043%	Spiller 1948

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
				Don sapwood and Podocarpus dacrydoides sapwood are similar.		(w/w) BAE (0.11 – 0.21 kg/m^3 BAE) assuming wood density is 500 kg/m^3. Conversion factor % w/w → kg/m^3 multiply by 5.	
insecticide	PT-08	Borax or DOT	Egg larvae and larger larvae of two species *Anobium punctatum* de Geer highest boron tolerancy Other relevant species: *Hylotrupes bajulus*	Corsican pine sapwood treated by vacuum impregnation. Details on wood treatment not stated. BS 3651 and BS 3652 newly hatched (egg larvae) or larger larvae introduced into holes. Larval survival and mass loss in weight of wood.	Borax test concentrations 0.068-3.4 kg/m3 or 0.013-0.70 % w/w (0.008-0.45 % w/w BAE) for egg larvae and larger larvae (1-3 mg). DOT test concentrations 0.077-7.7 kg/m3 or 0.016-1.6 % w/w (0.019-1.9% w/w BAE). for egg larvae and larger larvae (1.5-5.5 mg). Duration of the test 6-18 months.	Highest boron tolerancy for *Anobium punctatum* on pine sapwood. For borax the toxic threshold concentrations determined as 0.45% w/w BAE (2.2 kg/m^3 BAE, 3.4 kg/m^3 borax) for larger larvae. For DOT the toxic threshold concentrations determined as 1.9% w/w BAE (9.5 kg/m^3 BAE, 7.7 kg/m^3 DOT) for larger larvae. For DOT the toxic threshold concentrations determined as 0.09% w/w BAE (0.45 kg/m^3 BAE, 0.39 kg/m^3 DOT) for egg larvae. Toxicity of boric acid and DOT, is considered equal. Because the test conditions for DOT differ from test conditions for boric acid (length of larvae, test duration), final endpoints are different Conversion factor % w/w → kg/m^3 multiply by 5.	Taylor 1967
termiticide	PT-08	DOT	*Reticulitermes flavipes*	Slash pine (Pinus elliottii Engelm. variety elliottii) treated by vacuum/pressure impregnation. Air dried for 24 hrs. No ageing or leaching. Laboratory test with no choice (only treated wood) or choice (both	DOT loadings equivalent to 0.37-2.9 kg/m^3 BAE or 0.10-0.54% (w/w) BAE (by analytical determination). Duration of the laboratory test 4 weeks at 25-28 °C. Duration of the field test 18 months.	Boron tolerancy for *Reticulitermes flavipes* on pine (Pinus elliottii). For DOT, toxic threshold concentrations determined as 0.30% BAE (1.5 kg/m^3 BAE) in the choice laboratory test.	Mauldin and Kard, 1996

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
				treated and untreated wood available). Subterranean termite attack in a field test in Gulfport, MS, USA (non-leaching conditions and protected from rain). Termite mortality and mass loss of weight in wood.		Field tests in USA are considered not relevant for EU. No conversion factors used, actual values from study report.	
fungicide; insecticide	PT-08	Boric acid or borax or sodium borate (assumed to be borax)	Review article on decay fungi (e.g. *Coniophora cerebella* syn *Coniophora puteana, Lenzites trabea* syn *Gloeophyllum trabeum, Poria vaporaria* syn *Poria placenta, Polystictus versicolor* syn *Coriolus versicolor, Merulius lacrymans* syn *Serpula lacrymans*) and wood boring insects (egg larvae and larger larvae of *Anobium punctatum, Hylotrupes bajules, Lyctus brunneus*).	Not stated	Not stated	For boric acid highest toxic threshold levels for decay fungi were determined as 0.12%-0.40% w/w BAE (0.6-2.0 kg/m^3 BAE). For egg larvae, highest toxic threshold levels were 0.04%-0.12% w/w BAE (0.2-0.6 kg/m^3 BAE). For borax (or sodium borate) highest toxic threshold levels for decay fungi were determined as 0.065%-0.38% w/w BAE (0.32-1.9 kg/m^3 BAE, 0.5-2.9 kg/m^3 borax) Toxicity of boric acid and borax, is considered equal. Because the test conditions for borax differ from test conditions for boric acid, final endpoints are slightly different Conversion factor kg/m^3 → % w/w multiply by 0.2. Conversion factor % w/w → kg/m^3 multiply by 5. Conversion factor borax → BAE multiply by 0.65.	Findlay, 1959
fungicide; insecticide	PT-08	Boric acid or borax	Review article on decay fungi (e.g. *Coniophora cerebella* syn *Coniophora puteana, Lenzites trabea* syn *Gloeophyllum trabeum, Poria vaporaria* syn *Poria placenta, ,Merulius lacrymans* syn	Not stated	Not stated	For boric acid highest toxic threshold levels for decay fungi were determined as 0.072%-0.28 % w/w BAE (0.36-1.4 kg/m^3 BAE) if American test methods are omitted. Highest toxic threshold levels for	Becker, 1959

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
			Serpula lacrymans) and wood boring insects (egg larvae and larger larvae of *Anobium punctatum, Hylotrupes bajules, Lyctus brunneus*).			egg larvae were 0.03%-0.12% w/w BAE (0.15-0.6 kg/m^3 BAE) after 12 weeks. Highest toxic threshold levels for larger larvae were 0.072%-1.5% w/w BAE (0.36-7.4 kg/m^3 BAE) after 16-24 weeks. For borax toxic highest threshold levels for decay fungi were determined as 0.065%-0.21% w/w BAE (0.32-1.0 kg/m^3 BAE, 0.5-1.6 kg/m^3 borax) if American test methods are omitted. Highest toxic threshold levels for egg larvae were 0.023%-0.084% w/w BAE (0.12-0.42 kg/m^3 BAE, 0.18-0.65 kg/m^3 borax) after 12 weeks. Highest toxic threshold levels for larger larvae were 0.091%-0.34% w/w BAE (0.46->1.7 kg/m^3 BAE, 0.7->2.6 kg/m^3 borax) after 24 weeks. Toxicity of boric acid and borax, is considered equal. Because the test conditions for borax differ from test conditions for boric acid, final endpoints are slightly different Conversion factor kg/m^3 → % w/w multiply by 0.2. Conversion factor % w/w → kg/m^3 multiply by 5. Conversion factor borax → BAE multiply by 0.65.	
fungicide; insecticide	PT-08	Boric acid or borax or DOT (=TIMBOR = Polybor) or sodium metaborate	Review article on decay fungi (*Coniophora puteana, Gloeophyllum trabeum, Poria placenta, Coriolus versicolor, Serpula lacrymans*) and wood boring insects (egg larvae and larger larvae of *Anobium punctatum, Hylotrupes bajules, Lyctus brunneus*).	Not stated.	Not stated	Highest toxic threshold concentrations determined as 0.016%-0.42% w/w BAE (0.08-2.1 kg/m^3 BAE) for decay fungi (if ASTM values are deleted) and 0.008%-0.2% w/w BAE (0.04-1.0 kg/m^3 BAE) for egg larvae and 0.008%-1.8% w/w BAE (0.04-9.2 kg/m^3 BAE) for larger larvae	Bravery & Carey 1983

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
						assuming wood density is 500 kg/m^3. Conversion factor kg/m^3 → % w/w multiply by 0.2.	

Section A6.1.1 **Acute Toxicity**

Annex Point IIA6.1 **Section A6.1.1; Oral Route; Rat; LD_{50}**

Official use only

REFERENCE

Reference

[redacted] Boric acid. Acute Oral Administration -Rats Final [redacted] 1962 (TX-62-5) (Unpublished)

[redacted]

(Electronic File)

Data protection

Yes

Data owner

[redacted]

Companies with letter of access

Curent Access

[redacted]

Criteria for data protection

Data on new a.s. for first entry to Annex I/IA

GUIDELINES AND QUALITY ASSURANCE

Guideline study

No

No specific guidelines were available at the time of this study. Although only old data is available for boric acid, there are a number of studies in rats (and mice and dogs), which confirm the low acute oral toxicity of boric acid. In addition, data (including GLP/OECD protocols) on other borates (boric oxide, disodium tetraborate anhydrous, disodium tetraborate decahydrate, disodium tetraborate pentahydrate and disodium octaborate tetrahydrate) confirm that borates are of low acute oral toxicity. Further testing of boric acid is therefore not justified in the interests of protecting laboratory animals.

GLP

No

GLP was not compulsory at the time the study was performed

Deviations

Not relevant

MATERIALS AND METHODS

As given in section 2

Test material

Not known

Lot/Batch number

As given in section 2

Specification

Fine soft white powder

1.1.1.1 Description

>99%

1.1.1.2 Purity

Stable

1.1.1.3 Stability

Non-entry field

Test Animals

Species	Rat
Strain	Sprague Dawley
Source	
Sex	
Age/weight at study initiation	Males: 267-302 g: Females: 214 – 248 g
Number of animals per group	5
Control animals	No
Administration/ Exposure	Oral *Fill in respective route in the following, delete other routes*
Post exposure period	14 days
	Oral
Type	Gavage
Concentration	Gavage 2.0; 2.52; 3.16; 3.98;5.01 and 6.31 g/kg bw
Vehicle	0.5 % aqueous methyl cellulose
Concentration in vehicle	50% w/v
Total volume applied	
Controls	
Examinations	Clinical observations and Pathology
Method of determination of LD_{50}	Litchfield and Wilcoxon, , Weil, Thompson,
Further remarks	

2 RESULTS AND DISCUSSION

Clinical signs	Clinical signs included depression; laboured breathing or rapid respiration; bloody crust around nose; ataxia; marked diarrhoea; ptosis; and other CNS effects
Pathology	Autopsies indicated congestion of lungs kidneys and adrenals; inflammation of pylosirc portion of stomach and small intestine.
Other	*Describe any other significant effects*
LD_{50}	LD_{50} male = 3450 mg/kg bw LD_{50} females = 4080 mg/kg bw LD_{50} males + females = 3765 mg/kg bw

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods	LD_{50} carried out to pre guideline and GLP rules.
Results and discussion	LD_{50} 3.45 – 4.08 g/kg bw
Conclusion	Non-entry field
Reliability	*2*
Deficiencies	Although only old data is available for boric acid, there are a number of studies in rats (and mice and dogs), which confirm the low acute oral toxicity of boric acid. In addition, data (including GLP/OECD protocols) on other borates (boric oxide, disodium tetraborate anhydrous, disodium tetraborate decahydrate, disodium tetraborate pentahydrate and disodium octaborate tetrahydrate) confirm that borates are of low acute oral toxicity. Further testing of boric acid is therefore not justified in the interests of protecting laboratory animals.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 Feb 2005
Materials and Methods	The applicant states that the purity is >99%. In the study report no purity data are provided. The study author merely states that "for the purpose of the study the material was considered to be free of impurities.
Results and discussion	The version of the applicant is adopted. The number of animals that died were 0/5, 0/5, 2/5, 4/5, 5/5 and 5/5 in the 2.0; 2.51; 3.16; 3.98;5.01 and 6.31 g/kg bw groups respectively.
Conclusion	The version of the applicant is adopted.
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.2 Annex Point IIA6.1	Acute Toxicity Section A6.1.2; Dermal Route; Rat; LD_{50} Limit Test	Official use only

REFERENCE

Reference

[redacted] Acute Dermal Toxicity Screen in Rabbits; Primary skin irritation study in rabbits of boric acid. [redacted] 1982, [redacted]

Electronic File

Data protection

Yes

Data owner

[redacted]

Companies with letter of access

Current Access:
[redacted]

Criteria for data protection

Data on new a.s. for first entry to Annex I/IA

GUIDELINES AND QUALITY ASSURANCE

Guideline study

Yes

FIFRA (40 CFR 163) Acceptable protocol at the time

GLP

No data

Although not carried out to modern protocols and GLP, the data is acceptable particularly as data is available to indicate the absorption through humans skin is negligible > 0.5%. In addition, acceptable data on other borates indicates that dermal acute toxicity is not an issue. Therefore further testing is not warranted.

Deviations

See above

MATERIALS AND METHODS

As given in section 2

Test material

OA 107-3

Lot/Batch number

As given in section 2

Specification

2.1.1.1 Description

White powder

2.1.1.2 Purity

>99%

2.1.1.3 Stability

Stable

	Non-entry field
Test Animals	
Species	Rabbit
Strain	New Zealand White
Source	Harlan F Plummer
Sex	Male and Female
Age/weight at study initiation	1623 –2922 grams
Number of animals per group	5 male; 5 female
Control animals	No
Administration/ Exposure	Dermal
Post exposure period	14 days
	Dermal
Area covered	Not specified but implies > 10 % of body surface The skin of all of the animals was abraded longitudinally every 2-3 cm , deep enough to penetrate the stratum corneum, but not cause bleeding.
Occlusion	Semi occlusive
Vehicle	Physiological saline
Concentration in vehicle	Substance moistened with 1.5 ml saline
Total volume applied	Dosage to 2 g/kg bw
Duration of exposure	24 h
Removal of test substance	Moist towel
Controls	None
Examinations	Clinical observations, necropsy, histopathology or other

Method of determination of LD_{50}	Not relevant – Limit test
Further remarks	On removal of binders the binders and exposed areas were moist or dry with sample indicating incomplete absorption of sample.

3 RESULTS AND DISCUSSION

Clinical signs	Clinical changes were limited t transient diarrhoea in 2 rabbits and some incidences of erythema (9), oedema (3), atonia (2), desquamation (4) at 24 hours and later times after treatment.
Pathology	No gross necropsy findings were observed. Observations included one animal with gas filled intestine, one animal with pale yellow coloured kidneys; 5 animals with enlarged or swollen or pale fallopian tubes.
Other	
LD_{50}	LD_{50} > 2000 mg/kg bw No lethal effect at limit dose

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods	Protocol to FIFRA (40 CFR 163), which was an acceptable protocol at the time. Limit test in which rabbits were treated with 2g/kg bw boric acid. The skin of all of the animals was abraded longitudinally every 2-3 cm , deep enough to penetrate the stratum corneum, but not cause bleeding. Although not carried out to modern protocols and GLP, the data is acceptable particularly as data is available to indicate the absorption through humans skin is negligible > 0.5%. In addition, acceptable data on other borates indicates that dermal acute toxicity is not an issue. Therefore further testing is not warranted.
Results and discussion	LD_{50} > 2000 mg/kg bw indicating no acute dermal toxicity. Clinical changes were limited to transient diarrhoea in 2 rabbits and some incidences skin irritation 24 hours and later times after treatment. No gross necropsy findings were observed.
Conclusion	Non-entry field
Reliability	2
Deficiencies	See above

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 February 2005
Materials and Methods	The applicant states that the purity is >99%. In the study report no purity data are provided, only a lot number is given.
Results and discussion	The version of the applicant is adopted.
Conclusion	The version of the applicant is adopted.
Reliability	2
Acceptability	acceptable
Remarks	In the heading it says that the species is the rat, whereas in this study rabbits were used.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.3 **Acute Toxicity**

Annex Point IIA6.1 **Section A6.1.3; Inhalation Route; Rat; LC_{50} Limit Test**

Official use only

REFERENCE

Reference

[redacted]. Acute inhalation toxicity limit on boric acid [redacted] 1997

[redacted]

Electronic File

Data protection

Yes

Data owner

[redacted]

Companies with letter of access

Curent Access

[redacted]

Criteria for data protection

Data on new a.s. for first entry to Annex I/IA

GUIDELINES AND QUALITY ASSURANCE

Guideline study

Yes

OECD Guide-line 403 "Acute Inhalation Toxicity" (USEPA.FIFRA 40 CFR Part 160.

GLP

Yes

Deviations

Yes

The stability; characterisation, identity and verification of the test substance was the responsibility of the study sponsor. Also highest dose was limited. However, this was a repeat study carried out at the request of the US EPA to confirm that the highest dose obtainable was 2 mg/l. It was deemed by the US EPA to be an acceptable study

MATERIALS AND METHODS

Test material

As given in section 2

[redacted]

Lot/Batch number

Lot #7B10

Specification

As given in section 2

3.1.1.1 Description

White powder

3.1.1.2 Purity

>99%

3.1.1.3 Stability

Stable

Test Animals	Non-entry fieLC
Species	Rat
Strain	Sprague-Dawley
Source	Ace animals Inc; Boyertown, PA
Sex	
Age/weight at study initiation	Young adults: Males 205-255 grams; Females 179-208 grams
Number of animals per group	5 male; 5 female
Control animals	No
Administration/ Exposure	Inhalation
Postexposure period	14 days
Concentrations	**Inhalation** Nominal concentration 2000 mg/m³ Analytical concentration ..... 2120 ±140 mg/m³
Particle size	Not an aerosol study
Type or preparation of particles	Sample was ground in a ball mill for 24 hours MMAD 3.5 µm ± GSD 1.81µm Top dose ~ 2 mg/l was the highest that was obtainable under the conditions of the test
Type of exposure	Whole body
Vehicle	Not relevant
Concentration in vehicle	Not relevant
Duration of exposure	4 h
Examinations	Clinical observations, Pathology
Method of determination of LC_{50}	Not relevant – Limit Test

Further remarks

This study was a repeat study carried out at the request of the US EPA to confirm that the highest dose obtainable was 2 mg/l.
None

Controls

4 RESULTS AND DISCUSSION

Clinical signs

Animal observations were limited due to the accumulation of test material on the walls of the exposure chamber. During the first 1.5 hours of exposure, ocular and nasal discharge, hypoactivity and haunched posture were noted. Ocular discharge and or nasal discharge persisted in most animals after removal from the chamber. All animals recovered by day two after removal from chamber.

Pathology

No specific findings observed except red lung discolouration consistent with CO2 inhalation (caused by euthanasia technique). All tissue sand organs were normal.

Other

LC_{50}

LC_{50} > 2.12.mg/L (2g/m^3)
No lethal effect at limit dose

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

Acute inhalation toxicity limit on boric acid. The Sample was ground in a ball mill for 24 hours to give a MMAD 3.5 µm ± GSD 1.81µm
Top dose ~ 2 mg/l was the highest that was obtainable under the conditions of the test

Results and discussion

LC_{50} > 2.12.mg/L (2g/m^3). Animal observations were limited due to the accumulation of test material on the walls of the exposure chamber. This was a repeat study carried out at the request of the US EPA to confirm that the highest dose obtainable was 2 mg/l. It was deemed by the US EPA to be an acceptable study

Conclusion

LC_{50} > 2.12.mg/L (2g/m^3).

Reliability

1

Deficiencies

No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 Feb 2005
Materials and Methods	In the study report it is stated that the sponsor characterized the composition of test substance to be 100 % boric acid. The nominal concentration of boric acid is reported to be 26.06 mg/L (26.06 g/m^3). Otherwise the version of the applicant is accepted.
Results and discussion	The version of the applicant is adopted.
Conclusion	The version of the applicant is adopted.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.1.4 **Acute Eye Irritation**

Annex Point IIA6.1.4 Section A6.1.4Rabbit Eye Irritation Study

Official use only

1 REFERENCE

Reference

[redacted] Primary eye irritation of boric acid [redacted] 1989

[redacted]

Electronic File

Data protection

Yes

Data owner

[redacted]

Companies with letter of access

Curent Access

[redacted]

Criteria for data protection

Data on new a.s. for first entry to Annex I/IA

GUIDELINES AND QUALITY ASSURANCE

Guideline study

Yes

FIFRA (40 CFR 158, 162); TSCA (40 CFR 798). Although not carried out to an OECD protocol, the study has been carried out to an US EPA acceptable protocol and meets the requirements of OECD 405, although the report lacks detail

GLP

Yes

Deviations

Although the studies on boric acid have not been carried out to OECD protocols, two studies have been carried out to US Government Guidelines and evaluated under EU 67/548/EEC rules and indicate that boric acid is not an eye irritant. Further testing of boric acid is therefore not justified in the interests of protecting laboratory animals.

MATERIALS AND METHODS

Test material	As given in section 2
Lot/Batch number	**8M8E**
Specification	As given in section 2
1.1.1.1 Description	White powder
1.1.1.2 Purity	>99%
1.1.1.3 Stability	Stable

Test Animals	Non-entry field
Species	Rabbit
Strain	New Zealand White
Source	Approved USDA supplier
Sex	Male and Female
Age/weight at study initiation	Not reported
Number of animals per group	3 male; 3 female
Control animals	No
Administration/ Exposure	
Preparation of test substance	*Test substance was used as delivered*
Amount of active substance instilled	100 mg
Exposure period	24h followed by rinsing with physiological saline
Post exposure period	21 days
Examinations	
Ophthalmoscopic examination	No
1.1.1.4 Scoring system	Scoring in report according to Draize, but scoring reported here according to EU 67/548/EEC
1.1.1.5 Examination time points	60min, 24h, 48h, 72h, 4d, 7d, 21d
Other investigations	
Further remarks	

2 RESULTS AND DISCUSSION

Clinical signs	Table A6_1_4E-1.

Average score	Non-entry field
Cornea	0.00
Iris	0.11
Conjunctiva	Non-entry field
2.1.1.1 Redness	0.94
2.1.1.2 Chemosis	0.56
Reversibility	Yes Chemosis and Redness reversed by seven days
Other	
Overall result	Not classifiable in the EU under Directive 67/548/EEC. Classified in US Category III (40 CFR 156) "Corneal involvement or irritation clearing in 7 days or less. Non Irritant under US CPS (16 CFR 15000.42)

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods	Eye irritation study in New Zealand white rabbits to FIFRA (40 CFR 158, 162); TSCA (40 CFR 798). . 100 mg boric acid was instilled in the eyes for 24 hours followed by rinsing with physiological saline
Results and discussion	Minor effects on the iris and effects on conjunctivae redness and Chemosis were reversed by day 7. Changes included colouration and texture of the eye and blistered appearance to conjunctiva. Boric acid is used up to 5% in eye washes
Conclusion	Not classifiable in the EU under directive 67/548/EEC.
Reliability	2
Deficiencies	Although the studies on boric acid have not been carried out to OECD protocols, two studies have been carried out to US Government Guidelines and evaluated under EU 67/548/EEC rules and indicate that boric acid is not an eye irritant. Further testing of boric acid is therefore not justified in the interests of protecting laboratory animals.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 Feb 2005
Materials and Methods	The applicant states that the purity is >99%. In the study report no purity data are provided, only a lot number is given.
Results and discussion	The version of the applicant is adopted.
Conclusion	The version of the applicant is adopted.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.1.4 Acute Eye Irritation

Annex Point IIA6.1.4

Section A6.1.4Rabbit Eye Irritation Study

Appendix

Table A6_1_4E-1. Results of eye irritation study

Use this table, if relevant effects occur.

	Cornea	Iris	Conjunctiva	
			redness	chemosis
score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to4
60 min	0.17	0.83	1.00	1.67
24 h	0.00	0.33	1.00	0.83
48 h	0.00	0.00	1.00	0.50
72 h	0.00	0.00	0.83	0.33
Average 24h, 48h, 72h	0.00	0.11	0.94	0.56
Area effected				
Maximum average score (including area affected, max 110)				
Reversibility*		c	c	c
average time for reversion		By 48 h	By 7 days	By 7 days

Section A6.1.4 **Acute Dermal Irritation**

Annex Point IIA6.4 Section A6.1.4 : Rabbit Skin Irritation Study

Official use only

3 REFERENCE

Reference

[redacted] 1982). Acute Dermal Toxicity Screen in Rabbits; Primary skin irritation study in rabbits of boric acid. [redacted]

Electronic File

Yes

Data protection

Data owner [redacted]

Companies with letter of access

Curent Access

[redacted]

Criteria for data protection

Data on new a.s. for first entry to Annex I/IA

GUIDELINES AND QUALITY ASSURANCE

Section A6.1.4 **Annex Point IIA6.4**	**Acute Dermal Irritation** Section A6.1.4 : Rabbit Skin Irritation Study
Guideline study	Yes FIFRA (40 CFR 163) Acceptable protocol at the time
GLP	Yes.
Deviations	Although not carried out to modern protocols, data from other irritation studies on boric acid confirm the results Therefore further testing is not warranted in the interest of animal welfare.
	MATERIALS AND METHODS
Test material	As given in section 2
Lot/Batch number	OA 107-3
Specification	As given in section 2
3.1.1.1 Description	White powder
3.1.1.2 Purity	>99%
3.1.1.3 Stability	Stable
Test Animals	Non-entry field
Species	Rabbit
Strain	New Zealand White
Source	Harlan F Plummer
Sex	Male and Female
Age/weight at study initiation	1623 –2922 grams
Number of animals per group	3 male; 3 female
Control animals	No
Administration/ Exposure	Dermal

Section A6.1.4 **Annex Point IIA6.4**	**Acute Dermal Irritation** Section A6.1.4 : Rabbit Skin Irritation Study
Application	Non entry field
3.1.1.4 Preparation of test substance	0.5 grams of test substance was moistened with 0.5 ml physiological saline
3.1.1.5 Test site and Preparation of Test Site	Hair was clipped from the saddle area of rabbit and two areas on each rabbit were abraded by making epidermal incisions with a hypodermic needle sufficiently deep to penetrate the epidermis, but not to induce bleeding Each rabbit was treated on two intact and two abraded areas
Occlusion	Occlusive
Vehicle	Physiological saline
Concentration in vehicle	
Total volume applied	0.5 gram test substance
Removal of test substance	Moistened towel
Duration of exposure	24 h
Post exposure period	72 h
Controls	None
Examinations	
Clinical signs	Ye
Dermal examination	Yes
3.1.1.6 Scoring system	Draize, 1959
3.1.1.7 Examination time points	24h, 72h
3.1.2 Other examinations	None

Section A6.1.4 **Acute Dermal Irritation**

Annex Point IIA6.4 Section A6.1.4 : Rabbit Skin Irritation Study

Further remarks

4 RESULTS AND DISCUSSION

Average score See Table A6_1-4S-1

Erythema 0.105

Edema 0

Reversibility Not relevant

Other examinations

Overall result Non Irritant

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods FIFRA (40 CFR 163) Hair was clipped form the saddle area of rabbit and two areas on each rabbit were abraded by making epidermal incisions with a hypodermic needle sufficiently deep to penetrate the epidermis, but not to induce bleeding, therefore each rabbit was treated on two intact and two abraded areas with 0.5 grams boric acid under and occlusive dressing

Results and discussion No irritancy was observed and although not carried out to modern protocols, data from other irritation studies on boric acid confirm the results Therefore further testing is not warranted in the interest of animal welfare.

Conclusion Non-irritant

Reliability *2*

Deficiencies

Section A6.1.4 **Acute Dermal Irritation**

Annex Point IIA6.4 Section A6.1.4 : Rabbit Skin Irritation Study

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 Feb 2005
Materials and Methods	The version of the applicant is adopted. For clarity reasons it should be mentioned that 0.5 grams of test substance was applied to each of the 4 areas, so the total amount of test substance per animal was 2 grams.
Results and discussion	The version of the applicant is adopted.
Conclusion	The version of the applicant is adopted.
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.4 **Acute Dermal Irritation**

Annex Point IIA6.4 Section A6.1.4 : Rabbit Skin Irritation Study

Table A6_1-4S-1. Table for skin irritation study

Site	time	Erythema	Edema
Intact	24 h	0	0
	72 h	0.25	0
Abraded	24 h	0	0
	72 h	0.17	0
average score	24h, 72h	0.105	0

Section A6.1.5 **Skin sensitisation**
Buehler Test

Annex Point IIA6.1.5

Official use only

5 REFERENCE

Reference

[redacted] (1994), Dermal sensitization test-Buehler method on boric acid. [redacted]

[redacted]

Electronic File

Data protection

Yes

Data owner

[redacted]

Companies with letter of access

Curent Access

[redacted]

Criteria for data protection

Data on new a.s. for first entry to Annex I/IA

GUIDELINES AND QUALITY ASSURANCE

Guideline study

Yes

OECD Guide-line 406 "Skin Sensitization"

GLP

Yes

Deviations

No

MATERIALS AND METHODS

In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.

Section A6.1.5 **Annex Point IIA6.1.5**	**Skin sensitisation** Buehler Test
Test material	As given in section 2
Lot/Batch number	Lot #4H25-3611
Specification	As given in section 2
5.1.1.1 Description	White powder
5.1.1.2 Purity	>99%
5.1.1.3 Stability	Stable
5.1.1.4 Preparation of test substance for application	*a) for induction: used as delivered moistened with distilled water (95%w/v)* *b) for challenge: used as delivered moistened with distilled water (95%w/v)*
5.1.1.5 Pre-test performed on irritant effects	Yes

Section A6.1.5 **Annex Point IIA6.1.5**	**Skin sensitisation** Buehler Test
Test Animals	Non-entry field
Species	Guinea pigs
Strain	Hartley albino
Source	Davidson's Mill Farms, South Brunswick, NJ
Sex	
Age/weight at study initiation	Young adult males: 314 -411 grams; Young adult females: 282-376 grams
Number of animals per group	Test Group: 20 animals Naive Control: 10 animals Positive Control: 20 animals Positive Naive Control: 10 animals
Control animals	Yes
Administration/ Exposure	State study type: Non-Adjuvant
Induction schedule	day 0 – day –7 – day 21 *Table A6_1_5-1.*
Way of Induction	Topical Occlusive
Concentrations used for induction	0.4 g 95% w/w/boric acid moistened with distilled water to enhance skin contact
Challenge schedule	Day 28; Table A6_1_5-1.
Concentrations used for challenge	95% w/w/boric acid moistened with distilled water to enhance skin contact
Rechallenge	No
Scoring schedule	24h, 48h after challenge
Removal of the test substance	After 6 hours test substance wiped off with water

Section A6.1.5 Annex Point IIA6.1.5	Skin sensitisation Buehler Test
Positive control substance	Dinitrochlorobenzene
Examinations	Non-entry field
Pilot study	No
Further remarks	

6 RESULTS AND DISCUSSION

Results of pilot studies	No pilot study
Results of test	See Table A6_1_5-2
24h after challenge	0/20
48h after challenge	0/20
Other findings	
Overall result	Non -sensitiser

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods	OECD Guide-line 406 "Skin Sensitisation" method (Buehler test) using 95% w/w boric acid moistened with distilled water to enhance skin contact
Results and discussion	Very faint erythema seen in one animal at induction stage and 2 animals at challenge stage and also in one naïve control. No other adverse effect observed
Conclusion	Non-sensitiser
Reliability	1
Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2 Feb 2005
Materials and Methods	In the study report it is stated that the sponsor characterized the composition of test substance to be 100 % boric acid. The induction schedule was day 0 - day 7-day 14. On day 28 a challenge dose was applied.
Results and discussion	The version of the applicant is adopted.
Conclusion	The version of the applicant is adopted.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_1_5-1. Detailed information including induction/challenge/scoring schedule for skin sensitisation test

Treatments	Buehler test	Observations/Remarks *give information on irritation effects*
	day of treatment	
Induction 1	day 0	Very faint erythema (0.5) observed at one test site at 24 hours after first induction dose. No other irritation observed
Induction 2	7	No irritation observed
Induction 3	14	No irritation observed
challenge	28	No irritation observed
(rechallenge)		
scoring 1	29	Very faint erythema (0.5) observed at two test sites at 24 hours after challenge dose. Irritation persisted at one site for 48 hours. Very faint erythema (0.5) observed at one test site at 24 hours in one naive control.
scoring 2	30	

Table A6_1_5-2. Result of skin sensitisation test

	Number of animals with signs of allergic reactions / number of animals in group		
	Negative control	**Test group**	**Positive control**
scored after 24h	***0 / 10***	***0 / 20***	***10/20***
scored after 48h	***0 / 10***	***0 / 20***	***7/20***

Section A6.2 **Percutaneous absorption (in vivo test)**

Annex Point IIA6.2 Section A6.2 Human In vivo

Official use only

7 REFERENCE

Reference

[redacted] (1996). In Vivo Percutaneous Absorption of Boric Acid, Borax and Octaborate Tetrahydrate (DOT) in Man. [redacted] (Unpublished)

Electronic File

Also Published

Wester RC, Hui X, Hartway T, Maibach HI, Bell K, Schell MJ, Northington DJ, Strong P and Culver, BD. In vivo percutaneous absorption of boric acid, Borax and disodium octaborate tetrahydrate in humans compared to in vitro absorption in human skin from infinite to finite doses. Toxicol Sciences 45 42-51 (1998)

Data protection

Yes

Data owner

[redacted]

Companies with letter of access

Current Access

[redacted]

Criteria for data protection

Data on new a.s. for first entry to Annex I/IA

GUIDELINES AND QUALITY ASSURANCE

Guideline study

No

Human Study specifically designed and therefore no specific guidelines available, but designed to comply with US 40 CFR, 160

GLP

Yes

Deviations

Not relevant

MATERIALS AND METHODS

As given in section 2

Test material	
Lot/Batch number	As given in section 2
Specification	
7.1.1.1 Description	White powder
7.1.1.2 Purity	>99%
7.1.1.3 Stability	Stable
7.1.1.4 Radiolabelling	^{10}B
Test Animals	Non-entry field
Species	Humans
Strain	
Source	
Sex	Male & female
Age/weight at study initiation	Age 22 -50
Number of animals per group	8/groups
Control animals	Internal controls (i.e. baseline boron measured)
Administration/ Exposure	Dermal both intact and abraded skin
Preparation of test site	Skin was washed and a 30 cm x 30 cm area marked on back
Concentration of test substance	5% Boric acid ; 5% Borax or 10% DOT in distilled water
Specific activity of test substance	
Volume applied	3 ml/900 cm^2

Size of test site

900 cm²

Exposure period

After 5 days during which urine samples were collected the test substance was applied topically; air-dried and a commercial white T-shirt worn for 24 hours during which time urine was collected. At 24 hours the T-shirt was removed and analysed. The exposed areas were analysed for transepidermal water loss (TEWL) and then washed carefully with soap and distilled deionised water and all washing analysed. On day 11 the TEWL was measured and the treatment site dosed with 1.8 ml of 2% SDS (sodium lauryl sulphate) to cause irritation. On day 12 the TEWL was measured and the test substance was applied again topically; air-dried and a commercial white T-shirt worn for 24 hours during which time urine was collected. At 24 hours the T-shirt was removed and analysed. The exposed areas were analysed for transepidermal water loss (TEWL) and then washed carefully with soap and distilled deionised water and all washing analysed.

Sampling time

See above – Sample time 24 hours

Samples

Urine sampled as well as T-shirts worn and skin washings samples – see above

RESULTS AND DISCUSSION

Toxic effects, clinical signs

No adverse effects

Dermal irritation

No skin Irriation observed

Recovery of labelled compound

BA -76.5%; Borax 72%; DOT 78.5% Since the skin was washed 10 times and less 1 % was found I the last wash, it is assumed that most of the substance unaccounted for was in lost to outside clothing (over the T-shirt) an bedding during the 24 hour dosing period

Percutaneous absorption

Substance	% Dose Absorbed (95% CI)	Flux µg/cm²/hr	Permeability Kp cm/hr.
5 % Boric Acid	0.226 ± 0.125	0.009	1.8×10^{-7}
5 % Borax[1]	0.210 ± 0.194	0.009	1.8×10^{-7}
10% DOT[2]	0.122 ± 0.10	0.010	1.0×10^{-7}

[1] Disodium tetraborate decahydrate

[2] Disodium octaborate tetrahydrate

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

This study was designed to address absorption of typical solutions used in wood preservation and other biocidal uses.

Human Volunteers (8 per group) Group I, group II, and group III received two separate topical application of B^{10}-enriched 5% Boric Acid, 5% Borax, and 10% DOT solutions on their back skin, respectively and the in vivo percutaneous absorption was determined for a 24-hour dosing period. One dose was applied on day 5 under normal skin conditions and the other on day 12 under irritated skin conditions created by applying 2% SLS solution. Twenty- four hours after each topical dose, residual chemical on the dosed skin site was removed by skin wash. Urine samples were collected every 24 hours for 17 days. Urine samples from day 1 to day 4 were used to establish base boron levels and isotope ratios in the urine. The samples from day 5 to day 11 and day 12 to the end were used to compare absorbed level under normal skin and irritated skin conditions. To evaluate the dosing site skin condition, TEWL measurement and skin visual scoring were taken each time before dosing (including SLS treatment) and washing. To control any boron intake some food/beverage restrictions were instituted and daily detailed records were required. Boron analysis was done using inductively coupled mass spectrometry

Results and discussion

Approximately one-half of the administered topical dose was recovered after 24 hours in the T-shirt covering the dosed skin area and the skin washes. Pre-treatment with the potential skin irritant 2~ sodium lauryl sulphate had no effect on boron skin absorption for all three different dosage forms. No skin irritation was noted for any of the dosage forms.

Substance	% Dose Absorbed (95% CI)	Flux $\mu g/cm^2/hr$	Permeability Kp cm/hr.
5 % Boric Acid	0.226 ± 0.125	0.009	1.8×10^{-7}
5 % Borax[1]	0.210 ± 0.194	0.009	1.8×10^{-7}
10% DOT[2]	0.122 ± 0.10	0.010	1.0×10^{-7}

[1] Disodium tetraborate decahydrate

[2] Disodium octaborate tetrahydrate

Conclusion

Low skin absorption. For risk assessment where an absorbed dose is used the mean plus the standard deviation is used as a conservative absorption figure Boric acid = 0.351% absorption; Borax = 0.404 % absorption; DOT = 0.132 % absorption.

Reliability

1

Deficiencies

No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2 Feb 2005
Materials and Methods	The version of the applicant is accepted.
Results and discussion	In the studies total recovery of the applied dose ranged from 48.8-63.6%. Accordingly 36.4-51.2% of the applied dose is not accounted for. This may be due to loss to outside clothing and bedding, as suggested by the study authors. However, part of the lost dose may be located in the body or in the skin at the application site, which in that case should be considered as being absorbed. As such, the absorption estimates from this study are unreliable. On the other hand, toxicokinetic studies also indicate that borates have a low dermal absorption and low potential for accumulation in the body. In this respect the present data are in line with dermal absorption data from other studies. Therefore, based on this study and other data a dermal absorption borates of 0.5% can be assumed as a reasonable worst case estimate.
Conclusion	Reasonable worst case estimate for dermal absorption of borates is 0.5%.
Reliability	3
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2 Toxicokinetics, metabolism and distribution

Annex Point IIA6.2	Section A6.2

The toxicokinetics of boric acid; boric oxide; boric oxide disodium octaborate tetrahydrate and the sodium tetraborate (anhydrous; pentahydrate and decahydrate) are similar in rats and humans with respect to absorption, distribution, and metabolism (Dourson et al., 1998; Murray, 1998). A

ABSORPTION

Oral Absorption

As decreed in Section 3 the simple inorganic borates existe as undissociated boric acid at physiological pH and also at acidic pH (similar to gastric pH). Boric acid and the simple sodium borates given orally are readily and completely absorbed in humans and animals. Animals investigated include rats (Ku et al., 1991), rabbits (Draize & Kelly, 1959), sheep (Brown et al., 1989) and cattle (Owen, 1944; Weeth et al., 1981) as shown by the levels of boron in urine, blood or tissues. In adult human volunteers given a single oral dose of 131 mg B (as boric acid dissolved in water), 94% of the administered dose was excreted in the urine over a 96 hour period (Schou et al, 1984). Similar absorption was observed based on urinary excretion of boron in 6 volunteers drinking curative spa water with a high boron content (daily dose of 102 mg B) for two weeks (Job, 1973). In another study greater than 90% was absorbed in human volunteers taking in 3% boric acid in an aqueous solution or as a waterless emulsifying ointment spread onto biscuits Jansen, 1984a). In a series of human volunteer studies conducted in the early 1900s, in which large doses of boric acid were repeatedly administered orally, approximately 80% of an administered dose was recovered in the urine, while 1% was recovered in the faeces (Wiley, 1904). Reports involving accidental human ingestion, particularly in infants, where new-born infants died after accidentally ingesting boric acid, provide further evidence of oral absorption (Wong, 1964)

Inhalation Absorption

Inhaled sodium borate dust is readily absorbed as demonstrated by the blood and urine levels among groups of workers occupationally exposed to various levels of boron (Culver et al., 1993; 1994b).In rats, inhaled boron oxide aerosol was readily absorbed, based on the increased levels of boron excreted in the urine following inhalation exposure (Wilding et al., 1959).

Dermal Absorption

Dermal absorption of borates across intact skin is insignificant in all species evaluated, including human new-born infants (Friis-Hansen et al., 1982), adult humans (Beyer et al., 1983; Hui et al, 1996; Wester et al, 1998), rabbits (Draize and Kelley, 1959), and rats (Nielsen, 1970). Borates have been demonstrated to penetrate damaged or abraded skin (Draize and Kelley, 1959; Nielsen, 1970, Stüttgen et al., 1982). However, the use of an ointment-based vehicle may prevent or reduce the absorption though diseased skin compared to an aqueous jelly based vehicle (Nielsen, 1970 and Stüttgen et al, 1982), although the results by Stüttgen et al. (1982) have a number of flaws and are therefore not conclusive. Skin absorption data was obtained in human volunteers. Volunteers were dosed on a 900 cm^2 area (30cm x 30 cm) area of the back with ^{10}B enriched boric acid or borax (5% in aqueous solution), or disodium octaborate tetrahydrate (10% in aqueous solution). Twenty-four hours later the residual dose was removed by washing. Boron was measured in the urine (Hui et al, 1996; Wester et al, 1998). The absorption rates are given below.

Dermal Absorption in Humans of boric acid, disodium tetraborate decahydrate and disodium octaborate tetrahydrate

	% Dose Absorbed ± SD	Rate of Absorption Flux µg/cm²/hr	Permeability C (Kp) (cm/
Boric Acid (5 %)	0.226 ± 0.125	0.009	1.9 x 10
Disodium tetraborate decahydrate (5 %)	0.210 ± 0.194	0.00875	1.8 x 10
Disodium octaborate tetrahydrate (10 %)	0.122 ± 0.10	0.00975	1.0 x 10

The percutaneous absorption of disodium tetraborate decahydrate can be read across to disodium tetraborate pentahydrate and disodium tetraborate anhydrous

Disodium tetraborate pentahydrate only slightly less hydrated than the decahydrate. Anhydrous disodium tetraborate is the anhydrous salt of disodium tetraborate decahydrate and disodium tetraborate pentahydrate. For practical purposes one part of anhydrous disodium tetraborate is equivalent to 1.45 parts of disodium tetraborate pentahydrate; 1.9 parts of disodium tetraborate decahydrate; 1.02 parts disodium octaborate tetrahydrate and in aqueous solution 1.23 parts of boric acid. Anhydrous disodium tetraborate is hygroscopic and takes up water to form a hydrated salt and like the other borates, in solution it will exist as undissociated boric

Acute dermal limit studies carried out on both hydrated forms and disodium octaborate tetrahydrate indicated the LD_{50} to be > 2000 mg/kg bw. In these studies, limited symptoms were seen with the tetraborates and no symptoms with disodium octaborate tetrahydrate suggesting minimal dermal absorption. In an acute dermal limit study on boric acid, the rabbit skin was abraded to increase the absorption. Even in this study there was limited symptoms observed and the acute dermal LD_{50} was > 2000 mg/kg bw. This data supports minimal absorption, which is supported by the results of the human percutaneous absorption study

Since anhydrous disodium tetraborate and disodium tetraborate pentahydrate will form the various similar borates in the moistened form that it is applied to the skin, they are unlikely to be absorbed at any greater rate than the other borates tested.

Boric oxide is the anhydride of boric acid and it is hygroscopic and takes up water to form boric acid. The data for boric acid is therefore relevant for boric oxide

DISTRIBUTION

There is no substantiated evidence of boron accumulation in humans or other animals although bone contains higher levels than other tissues. (Alexander et al, 1951; Forbes et al., 1954; Forbes and Mitchell, 1957; Jansen et al, 1984b; Ward, 1987; Treinen and Chapin, 1991; Ku et al., 19911993; Culver et al., 1994b).

Absorbed boron rapidly distributes throughout the body water in humans and animals. In a study of workers occupationally exposed to 10 mg/m3 of airborne borax (0.22 mg B/kg/day), there was no progressive accumulation of boron in soft tissues during the working week as measured by blood and urine levels (Culver et al., 1993; 1994b). Similarly, Jansen et al. (1984a, b) concluded from pharmacokinetic studies of human volunteers that there was no tendency for boron to accumulate following a single i.v. dose of 600 mg of boric acid (approximately 105 mg B). Tissue levels of boron generally reached steady-state within three to four days among rats fed boric acid in the diet or drinking water for 28 days (Treinen and Chapin, 1991) or 3 – 4 days (Ku et al., 1991). Thus, boron does not accumulate in soft tissues with time in either humans or animals.

In both humans and animals, boron levels in soft tissue are comparable to plasma levels, while a greater concentration of boron in bone is observed relative to other tissues.

The most complete study of boron distribution conducted to date examined tissue disposition of boron in reproductive organs and other selected tissues in adult male rats fed boric acid, providing approximately 100 mg B/kg bw/day for up to seven days (Ku et al., 1991; 1993). All tissues examined, except bone and adipose tissue, appeared to reach steady state boron

levels by three to four days. Bone achieved the highest concentration of boron (2 to 3 times plasma levels), and bone boron levels continued to increase throughout seven days of dietary administration (Ku et al., 1991). In contrast, adipose tissue concentration was approximately 20 % of the plasma level. No other tissues showed any appreciable accumulation of boron over plasma levels. In dogs, an accumulation in the brain, liver and fat was reported after a high single does of 2000 mg/kg bw boric acid (Pfeiffer et al., 1945). However, the accuracy of the analytical procedures in that study is questionable.
Previous studies also show a greater concentration of boron in bone relative to other tissues in humans (Alexander et al., 1951; Forbes et al., 1954;) and rats (Forbes and Mitchell, 1957). Boron levels in a number of tissues have been measured (Abou-Shakra, 1989; Ciba and Chrusciel, 1992; Ward 1987; Sabbioni et al., 1990; Shuler et al., 1990; Minoia et al., 1990; 1994). In mice, boron distribution appeared to be homogenous in the tissues examined, except for higher levels in the kidney (bone was not analysed) (Locksley and Sweet, 1954; Laurent-Pettersson et al., 1992), but higher levels were found in bone in another study (Massie et al., 1990). In vivo and in vitro studies indicate that boric acid has a strong affinity for cis -hydroxyl groups. This may explain the higher concentrations of boric acid in bone, owing to the binding of to the cis -hydroxyl groups of hydroxyapetite.

METABOLISM

Boric acid is not metabolised in either animals or humans, owing to the high energy level required (523kJ/mol) to break the B - O bond (Emsley, 1989). Other inorganic borates convert to boric acid at physiological pH in the aqueous layer overlying the mucosal surfaces prior to absorption. Additional support for this derives from studies in which more than 90% of administered doses of inorganic borates are excreted in the urine as boric acid. Boric acid is a very weak and exclusively monobasic acid that is believed to act, not as a proton donor, but as a Lewis acid, i.e., it accepts OH-. Because of the high pKa, regardless of the form of inorganic borate ingested (e.g., boric acid, borax or boron associated with animal or plant tissues), uptake is almost exclusively (>98%) as undissociated boric acid.

EXCRETION

In both humans and animals, boron is excreted in the urine regardless of the route of administration. It is excreted with a half-life of < 24 hours in humans and animals. Boric acid is slowly eliminated from bone.
In humans, 99 % of a single i.v. dose of boric acid was excreted in the urine; the plasma half-life was calculated to be 21 hours using a three compartment toxicokinetic model (Jansen et al., 1984b). Following oral intake of an aqueous solution of boric acid, the urinary recovery was 94 % (Jansen et al., 1984a); more than 50 % of the oral dose was eliminated in the first 24 hours, consistent with the 21 hour half-life in the i.v. study. Sutherland et al. (1999) showed in a boron balance study that only 8% of dietary boron is excreted in faeces. Half-lives ranging 13-28.7 hours have also been reported from various poisoning cases (Astier et al., 1988; Litovitz et al., 1988).
Elimination half-lives for animals have not been stated explicitly in the scientific literature, but they can be calculated or estimated from the data in the literature. In mice, assuming first order kinetics for elimination, the half-life was estimated to be approximately one hour, and in rat < 12 hours (Farr and Konikowski, 1963; Ku et al. 1991; 1993). In rabbits, 50 to 66% of an orally administered dose of boric acid was excreted in the urine in the first 24 hours after dosing (Draize and Kelley, 1959). A recent study indicated that the half-life may be only 3 hours (Vaziri et al., 2001) in both pregnant and non-pregnant rats.
The major determinant of boric acid excretion is expected to be renal clearance since boric acid is excreted unchanged in the urine. Rats and mice generally have faster rates of renal clearance than humans since the glomerular filtration rates as a function of body mass are generally higher in rats and mice than in humans.
Clearances of 40.4 ± 3.2 ml/min/1.73m^2 for sodium tetraborate in male rats and 40 ml /min/1.73m2 for boron in mice (Usuda et al., 1998; Farr and Konikowski, 1963) have been reported, although there are methodological and/or analytical limitations in both studies. In more recent studies boric acid clearance rates in non-pregnant rats and pregnant rats ranged from 29.0 ± 5.7 to 31.0 ± 4.5 and from 32.2 ± 5.1 to 35.6 ± 5.7 ml/min/1.73m^2, respectively (Vaziri et al., 2001).
In humans, Jansen et al (1984b) determined a clearance rate of 55 ml/min/1.73m^2 following an i.v. dose of 600 mg of boric acid (105 mg B). Farr and Konikowski (1963) also reported

a similar value of 39 ml/min/1.73m^2 in humans given 35 mg B/kg intravenously as sodium pentaborate, although there are methodological and analytical limitations to this 40 year old study. In a more recent study, renal clearance rates in humans were 68.30 ± 35.0ml/min/1.73m^2 for pregnant subjects and 54.31 ± 19.35 ml/min/1.73m^2 for non-pregnant subjects (Pahl et al., 2001). This indicates about 20 –25% greater clearance in pregnant humans.

A comparison of the renal clearance between rats and humans in terms of body surface area indicated that humans clear boric acid slightly faster than rats (~1.7 -1.9 times as fast), while a comparison by bodyweight indicates that humans may clear boric acid more slowly than rats (~ 3 - 4 times slower). (Pahl et al., 2001; Vaziri et al., 2001).

Summary of Toxicokinetics of Inorganic Borates in rats and humans

Absorption	• Readily absorbed orally and by inhalation (of respirable particles) • No dermal absorption except through severely damaged skin
Distribution	• Rapidly distributed through body water • No accumulation in tissues
Metabolism	• Not metabolised • Exists mainly as boric acid in whole blood
	•
Excretion	• Excreted almost exclusively in the urine • Half-life < 24 hours • Renal clearance is approximately 3 time faster in rats than humans based on a body weight comparison

Conclusion

There is little difference between animals and humans in absorption, distribution, and metabolism. Differences in renal clearance is the major determinant in the differences between animals and humans, there being an approximate 3 fold difference between rats and humans.

Absorption via the oral route is nearly 100%. Similarly, 100% of inhaled (respired) borates are absorbed across the lung. Dermal absorption though intact skin is extremely low. A figure of 0.4% for boric acid and borax has been used for this risk assessment as a conservative worse case approach.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 Feb 2005
Materials and Methods	Not applicable
Results and discussion	Not applicable
Conclusion	The applicant provides an extensive overview of the toxicokinetic data of borates. The conclusions in the summary table of the applicant are adopted. The evaluation of the toxicokinetics of borates in DOC IIA will be based on the present overview of the applicant and studies from published literature.
Reliability	0. This is an overview of toxicokinetic data obtained from a larger number of studies from published literature, with varying quality and reliability.
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

References

1	Dourson, M, A. Meier, B. Meek, A. Renwick, E. Ohanian, and K. Poirier, Boron tolerable intake: re-evaluation of toxicokinetics for data-derived uncertainty factors. Biol. Trace Elem. Res. 66(1-3), 453-463 (1998).
2	Murray, F.J. A comparative review of the pharmacokinetics of boric acid in rodents and humans. Biol. Trace Elem. Res. 66:331-341 (1998).
3	Ku, W.W., R. E. Chapin, R. F. Moseman, R. E. Brink, K. D. Pierce, and K. Y. Adams, Tissue disposition of boron in male Fischer rats, Toxicol. Appl. Pharmacol. 111,145-151 (1991)
4	Draize J H and Kelley E A (1959). The urinary excretion of boric acid preparations following oral administration and topical applications to intact and damaged skin of rabbits. Toxicol. Appl. Pharmacol 1, 267-276
5	Brown, P.H., N. Bellaloui, R.N. Sah, E.Bassil, H. Hu. 2002. "Uptake and Transport of Boron" pp. 87 – 101 in: H.E. Goldback, P.H. Brown, B. Rerkasem, M. Thellier, M.A. Wimmer and R.A. Bell (eds.) Boron in Plant and Animal Nutrition. Kluwer Academic/Plenum Publishers. New York.
6	Owen E C (1944). The excretion of borate by the dairy cow. J Dairy Res. 13, 243-248.
7	Weeth H J, Speth C F and Hanks D R (1981). Boron content of plasma and urine as indicators of boron intake in cattle. Am. J. Vet. Res. 42, 474-477
8	Schou, J. S., J. A. Jansen, and B. Aggerbeck, Human pharmacokinetics and safety of boric acid, Arch. Toxicol. 7, 232-235 (1984).
9	Job, C,. Absorption and excretion of orally administered boron, Z. Angew. Bader-und Klimaheilkunde 20, 137-142 (1973).
10	Jansen, J.A., J. S. Schou, and B. Aggerbeck, Gastrointestinal absorption and in vitro release of boric acid from water-emulsifying ointments, Fd. Chem. Toxicol. 22, 49-53 (1984a).
11	Wiley H W (1904). Influence of food preservatives and artificial colours on digestion and health, I-Boric acid and borax. US Department of Agriculture, Bureau of Chemistry, Bulletin 84, Washington DC, 1-477. Summarised in Jansen WF, The squad that ate poison, FDA Consumer, Dec. 1981 - Jan 1982, 6-11.
12	Wong L C, Heimbach M D, Truscott D R and Buncan B D .Boric acid poisoning: report of 11 cases. Canad. Med. Assoc. J. 90, 1018-1023 (1964).
13	[redacted] 1993
14	Culver, B.D., P. T. Shen, T. H. Taylor, A. Lee-Feldstein, H. Anton-Culver, and P. L. Strong, (1994b) The relationship of blood-and urine-boron to boron exposure in borax-workers and the usefulness of urine-boron as an exposure marker, Environ. Health Perspect. 102(7), 133-137.
15	Wilding J L, Smith W J, Yevitch P, Sicks M E, Ryan S G and Punte CL (1959). The toxicity of boron oxide. Am. Ind. Hyg. J 20, 284-289.
16	Friis-Hansen B, Aggerbeck B and Jansen JA: Unaffected blood boron levels in new-born infants treated with a boric acid ointment, Fd Chem Toxicol 20:451, 1982.
17	Beyer KH, W.F. Bergfeld, W.O. Berndt, R.K. Boutwell, W.W. Carlton, D.K. Hoffmann and A.L. Schroeter, FDA Cosmetic Ingredient Review Expert Panel, Final report on the safety assessment of sodium borate and boric acid, J. Am. Coll. Toxicology 2, 87-125 (1983).
18	[redacted] (1996) [redacted]
19	Wester RC, Hui X, Hartway T, Maibach HI, Bell K, Schell MJ, Northington DJ, Strong P and Culver, BD. In vivo percutaneous absorption of boric acid, Borax and disodium octaborate tetrahydrate in humans compared to in vitro absorption in human skin from infinite to finite doses. Toxicol Sciences 45 42-51 (1998)
20	Nielsen G H (1970). Percutaneous absorption of boric acid form boron-containing preparations in rats. Acta. Pharmacol. Toxicol. 28, 413-424.
21	Stüttgen, G, Siebel, Th., and Aggerbeck, B. Absorption of Boric Acid through Human Skin Depending on the Type of Vehicle. Arch Dermatol Res. 272: 21-29, (1982).
22	Alexander, G.V, R. E. Nusbaum, and N. S. MacDonald, The boron and lithium content of human bones, J. Biol. Chem. 192, 489-496 (1951).
23	Forbes, R.M., A. R. Cooper, and H. H. Mitchell, On the occurrence of beryllium, boron, cobalt, and mercury in human tissues, J. Biol. Chem. 209, 857-864 (1954).
24	Forbes, R.M. and H. H. Mitchell, Accumulation of dietary boron and strontium in young and adult albino rats, Arch. Ind. Health 16, 489-492 (1957).
25	Jansen, J.A., J. Andersen, and J. S. Schou, Boric acid single dose pharmacokinetics after intravenous administration to man, Arch. Toxicol. 55,64-67 (1984b).
26	Ward, N.L., The determination of boron in biological materials by neutron irradiation and prompt gamma-ray spectrometry, J. Radioanal. Nucl. Chem. 110(2), 633-639 (1987).
27	Treinen K.A. and R. E. Chapin, Development of testicular lesions in F344 rats after treatment with boric acid, Toxicol. Appl. Pharmacol. 107, 325-335 (1991).

28	Ku, W.W., R. E. Chapin, R. N. Wine, and B. C. Gladen, Testicular toxicity of boric acid (BA): Relationship of dose to lesion development and recovery in the F344 rat. Reprod. Toxicol. 7, 305-319 (1993).
29	Pfeiffer CC, L.F. Hallman and I. Gersh, Boric acid ointment. A study of possible intoxication in the treatment of burns, J. Amer. Med. Assoc. 128, 266-274 (1945)
30	Abou-Shakra, F R, Havercroft, J. K. and Ward, N I, (1989), Lithium and Boron in Biological Tissues and Fluids. Trace Elements in Medicine, 6, 142 -146.
31	Ciba J and Chrusciel A (1992) Spectrophotometric determination of boron in human hair with Azomethine H. Fresenius J Anal Chem. 342, 147-149
32	[redacted] (1990)
33	Shuler TR, Pootrakul P, Yamsukon P and Nielsen FH, (1990). Effect of thalassemia/haemoglobin E disease on macro, trace and ultratrace element concentrations in human tissue. J. Trace Elem. Exp. Med. 3, 31-43
34	Minoia, C., Sabbioni, E., Apostoli, P., Pietra, R., Pozzoli, L., Gallorini, M., Nicolaou, G., Alessio, L. and Capodaglio, E., Trace element reference values in tissues from inhabitants of the European Community 1. A study of 46 elements in urine, blood and serum of Italian subjects. The Science of the Total Environment, 95, 89-105 (1990)
35	Locksley HB and Sweet. Tissue distribution of boron compounds in relation to Neutron-capture Therapy of cancer. Proc. Soc. Exp. Biol. Med. 86, 56 –63 (1954)
36	Laurent-Pettersson M, Delpech B and Thellier M . The mapping of natural boron in histological sections of mouse tissues by the use of neutron capture radiography. Histochem. J. 24, 939-950. (1992)
37	Massie HR, Aiello VR, Shumway AE and Armstrong T, (1990). Calcium, iron, boron, collagen and density changes in bone with aging in C57BL/65 mice. Exp. Gerontol. 469-481
38	Emsley, J. (1989). The Elements. p. 32, Clarendon, Oxford
39	Sutherland, B., Strong, P.L. and King, J.C. (1998). Determining Human Dietary Requirements for Boron. Biological Trace Element Research. 66, 193-204.
40	Astier A, Baud F and Fourneir A (1988). Toxicokinetics of boron after an acute accidental intoxication by boric acid. J Pharm. Clin. 7, 57-62.
41	Litovitz T L, Klein-Schwartz W, Oderda G M and Schmitz B F. Clinical manifestations of toxicity in a series of 784 boric acid ingestions. Am. J. Emerg. Med. 6, 209-213. (1988)
42	Farr L E and Konikowski T (1963). The renal clearance of sodium pentaborate in mice and men. Clin. Chem. 9, 717-726.
43	Vaziri, N.D., F. Oveisi, B.D. Culver, M.V. Pahl, M.E. Anderson, P.L. Strong and F.J. Murray. 2001. The effect of pregnancy on renal clearance of boron in rats given boric acid orally. Toxicological Science 60, 257-263.
44	Usuda, K., Kono, K., Orita, Y., Dote, T., Iguchi, K., Nishiura, H., Tominga, M., Tagawa, T., Goto, E., and Shirai, Y. Serum and urinary boron levels in rats of sodium tetraborate. Arch. Toxicol. 72, 468-474 (1998)
45	Pahl, M.V., B.D. Culver, P.L. Strong, F.J. Murray and N. Vaziri. 2001. The effect of pregnancy on renal clearance of boron in humans: A study based on the normal dietary intake of boron. Toxicological Science 60, 252-256.

Section 6.3 Annex Point IIA 6.3	Short-Term Repeated Dose Toxicity (28day)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [x] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	▪ **Oral 6.3.1** An adequate oral 90 day subchronic study has been carried out in mice therefore no 28 day study is necessary and the interests of animal welfare and protecting Laboratory animals no further testing is deemed necessary according to TNGs Chap 2 P 38 Guidance on Data Requirements ▪ **Dermal 6.3.2** A dermal repeated dose study is not necessary. TNsG P39 states that Repeated dose toxicity (dermal) is required 'where potential dermal exposure is significant and route-to-route extrapolation is not possible'. From dermal absorption studies (see Doc IIIA A_6.2 Percutaneous Boric acid.doc; Doc IIIA A_6.2 Percutaneous BO read across.doc; DOC IIIA A_6.2 Percutaneous DOT.doc; Doc IIIA A_6.2 Percutaneous Tetraborates.doc) no significant absorption occurs for any borate. In addition, due to the simple toxicokinetics of borates extrapolation from route to route is possible. Therefore no further animal testing is warranted. ▪ **Inhalation 6.3.3** An inhalation repeated dose study is not necessary Repeated dose toxicity (inhalation) instead of a oral route is required is TNsG P39 ' For volatile substances (vapour pressure >1x 10- Pa) or in cases where the potential inhalation exposure is significant, an inhalation study is required instead of the oral'. However the vapour pressures of all the borates is low and 100% absorption by inhalation can be assumed therefore extrapolations can be made. Therefore no further animal testing is warranted.	
Undertaking of intended data submission [ ]	In the interests of animal welfare and protecting Laboratory animals no further testing is deemed necessary	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	8 March 2005
Evaluation of applicant's justification	The justification of the applicant is adequate
Conclusion	The justification of the applicant is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (2 year) – Oral Dogs Boric Acid

Official use only

8 REFERENCE

Reference

[redacted] (1966) Two-Year Dietary Feeding -- Dogs. Boric Acid. Final Report. Hazleton Laboratories. [redacted]

This study was published in summary form in Weir RJ and Fisher RS. (1972) Toxicological studies on borax and boric acid. Toxicol Appl Pharmacol. 23(3):351-64. Unfortunately, the published version does not always accurately reflect the original study reports. Thus, it is necessary to evaluate the original study reports to appreciate the limitations of these studies.

Data protection

Yes

Data owner

[redacted]

Companies with letter of access

[redacted]

Criteria for data protection

Data on new a.s. for first entry to Annex I

GUIDELINES AND QUALITY ASSURANCE

Guideline study

No. No guidelines available at the time the study was conducted.

GLP

No. GLP was not compulsory at the time the study was performed.

Deviations

No Guidelines available at the time

MATERIALS AND METHODS

Test material

Boric acid [redacted].

Lot/Batch number

Not stated..

Specification

Not stated.

8.1.1.1 Description

"Soft, fine, white powder without noticeable odour"

8.1.1.2 Purity

Boron content of the test substance varied between 98.7 and 100.0% of the theoretical value. [redacted].

8.1.1.3 Stability

Test material expected to be stable.

Test Animals

Species

Dog

Strain

Purebred Beagle

Source

Unknown

Sex

Male and female

Age/weight at study initiation

Body weights of the dogs ranged from 4.2-10.3 kg during the first week of the study. The dogs were described as "young," but no specific age was provided.

Number of animals per group

4 males and 4 females per group at the start of the study.
However, these were sacrificed at different time intervals.
Consequently, the number of dogs per sex per group varied from 1-2 at any given time of sacrifice.

Control animals

Yes, the control group was employed as a common control with studies of borax. Separate control groups were used for the 2-year and 38-week studies, since they were started at different times.

Administration/ Exposure

Oral (diet)

2 years, except the highest dose (38 weeks).

Duration of treatment

Initially, groups of 4 male and 4 female dogs were fed diets containing 0, 0.033, 0.067, or 0.20% boric acid for up to 2 years. Because no effects were observed in this initial portion of the study, additional groups of 4 male and 4 female dogs were fed diets containing 0 or 0.67% boric acid for up to 38 weeks.

Dogs were sacrificed at various time intervals, so the duration of exposure varied depending on the time of sacrifice of each dog. In the initial portion of the study, one male and one female from each group were sacrificed at one year. The remaining dogs were sacrificed at 2 years, except for one male and one female in the control and 0.20% groups, which were sacrificed 3 months post-exposure (2 years). In the second portion of the study, two males and two females were sacrificed at 26 weeks in the high dose (0.67%) and its concurrent control group. At 38 weeks, the one male and one female were sacrificed in the high dose and two male and two female concurrent control group dogs were sacrificed. One male and one female dog at the high dose were sacrificed 25 days post-exposure (38 weeks) to study recovery.

Frequency of exposure

Seven days per week

Postexposure period

One male and one female from the control and 0.20% groups were placed on the control diet for 3 months postexposure to evaluate recovery and disappearance of stored boron.

Oral

8.1.1.4 Type

In food

8.1.1.5 Concentration

0, 0.033, 0.067, 0.20, 0.67% boric acid in the diet
These concentrations provided doses of 0, 1.7, 3.8, 10.9, and 41 mg B/kg/day, based on the actual body weight and food consumption data in the study.
Others in the scientific literature have described these doses as 0, 1.5, 2.9, 8.8, and 29 mg B/kg/day, but these calculations are based on standard assumptions regarding body weight and food consumption, not on the actual data from this study.
Food consumption was ad libitum.

8.1.1.6 Vehicle

8.1.1.7 Concentration in vehicle

8.1.1.8 Total volume applied

8.1.1.9 Controls

Plain diet (Wayne Dog Meal). The diet was not analyzed for the background level of boron. Boron is an essential plant element, and plant-derived foods (such as fruits and vegetables) are significant dietary sources of boron. The background level of boron in the control diet was not considered in the calculations of the dose levels.

Examinations

Observations	
8.1.1.10 Clinical signs	Yes. The dogs were observed daily for appearance, behaviour, and gross signs of systemic toxicity or pharmacological effects.
8.1.1.11 Mortality	Yes. Daily throughout the study.
Body weight	Yes. Weekly.
Food consumption	Yes. Weekly.
Water consumption	No.
Ophthalmoscopic examination	No.
Haematology	Yes. Except for the high dose and its concurrent control group, all surviving dogs initially and at 1, 3, 6, 12, 18, 24, and in some cases, 27 months. At the high dose (0.67%) and its concurrent control group, all dogs initially and at 4, 12, 26, and 38 weeks. Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, sedimentation rate.
Clinical Chemisty	Yes. Except for the high dose and its concurrent control group, all surviving dogs initially and at 1, 3, 6, 12, 18, 24, and in some cases, 27 months. At the high dose (0.67%) and its concurrent control group, all dogs initially and at 4, 12, 26, and 38 weeks. Parameters: glucose, blood urea nitrogen. In addition, serum glutamic-pyruvic transaminase and serum glutamic oxaloacetic transaminase were done on all dogs sacrificed at the one year interval and on the control and 0.20% groups sacrificed after two years of exposure.

Urinalysis	Yes. Except for the high dose and its concurrent control group, all surviving dogs initially and at 1, 3, 6, 12, 18, 24, and in some cases, 27 months. At the high dose (0.67%) and its concurrent control group, all dogs initially and at 4, 12, 26, and 38 weeks. Parameters: appearance, specific gravity, pH, protein, glucose, acetone, blood, and microscopic findings.
Sacrifice and pathology	Except for the high dose and its concurrent control group, one male and one female from each group were sacrificed at one year. The remaining dogs were sacrificed at 2 years, except for one male and one female in the control and 0.20% groups, which were sacrificed 3 months post-exposure (2 years). Two males and two females were sacrificed at 26 weeks in the high dose (0.67%) and its concurrent control group. At 38 weeks, the one male and one female were sacrificed in the high dose and two male and two female concurrent control group dogs were sacrificed. One male and one female dog at the high dose were sacrificed 25 days post-exposure (38 weeks) to study recovery.
Organ Weights	Yes. All dose groups. Organs: liver, kidneys, adrenals, testes, thymus, spleen, brain, heart, thyroid
Gross and histopathology	Yes. All dose groups. Organs: brain, thyroid, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, lungs, testes
Other examinations	Ejaculate specimens were collected from two male dogs each from the control group and the 0.20% group prior to the 2-year sacrifice for determination of sperm counts and motility. Blood and urine samples were taken from each dog initially and at 1, 3, 6, 12, 18, 24, and 27 months for subsequent analysis for boron content. Samples of various organs were frozen for subsequent analysis for boron content. One male and one female per group were selected for this determination at 12, 24, and 27 months. These organs included: brain, liver, kidney, body fat, and muscle. Pharmacokinetic Study. A single male dog from the control and 0.20% groups were placed in a metabolism cage for one month at the beginning of the study, two weeks at the one-year interval, and three months at the two-year interval to study the pharmacokinetics of boron. One female dog from both of these groups was added for the three-month terminal pharmacokinetic study. With the exception of the terminal balance study, urine and faeces were collected at 24-hour intervals; blood was collected weekly. In the terminal study, urine and faeces were collected 24-hours prior to compound removal and daily thereafter for a period of five weeks; blood was also collected 24 hours prior to compound removal, three times per week for the next 5 weeks, and weekly thereafter until termination (13 weeks). During the 28th week, one male from the control and the test groups (except the high dose) was selected for a 6-day study to determine the pH and various electrolytes in the blood and urine for the purpose of evaluating the acid-base balance.

Statistics	No.
Further remarks	This study was conducted in a parallel with a 2-year and 38-week study of borax in dogs. The studies were identical in design, and they employed a common control group.

RESULTS AND DISCUSSION

Observations	
Clinical signs	No effects attributable to the test material with the possible exception of diarrhoea at the high dose. (3/8 diarrhoea on 2-4 occasions. Soft stools in all dogs on 2 –5 occasions)
Mortality	No mortalities at any dose.
Body weight gain	No effects attributable to the test material. One dog at the high dose began to show a slight decline in weight.
Food consumption and compound intake	No effects.
Ophtalmoscopic examination	
Blood analysis	
Haematology	No effects.
Clinical chemistry	No effects.
Urinalysis	No effects.
Sacrifice and pathology	
Organ weights	Testis weight and testis/body weight ratios were lower than controls at the high dose (0.67%) at both the 26- and 38-week sacrifices. The testicular weight and testis/body weight ratio of the one male on the 3-week recovery phase was greater than the controls, which were sacrificed at the earlier time intervals. (There was no concurrent control male for the one male on the 3-week recovery phase.) No other treatment-related effects.
Gross and histopathology	At 0.67%, uniform spermatogenic arrest which progressed to complete atrophy of the seminiferous epithelium in various numbers of tubules in 3 dogs. However, according to the authors, similar effects observed in a control dogs were severe enough to introduce some doubt regarding whether the effect was treatment-related. No histological effects at concentrations below 0.67% were considered compound-related.
Other	At 0.67%, two dogs were azospermic, and the investigators were unable to obtain a sample from the other two dogs. Based on only one dog, the testicular degeneration appeared to be readily reversible. Two of four concurrent control dogs had normal sperm counts, one had a low sperm count, and the investigators were unable to obtain a sample from one

control dog. At 0.67%, 2 of 2 dogs with semen sample exhibited zero motility. Among controls, 2 of 3 dogs with semen samples exhibited zero motility.

Boron levels in blood, urine and feces were within background levels within four days after the dogs were removed from the test diet; this time may have been shorter but smaller intervals were not explored.

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

This study was conducted in the mid-1960s before OECD guidelines were established.

Results and discussion

Groups of 4 male and 4 female beagle dogs were fed diets containing 0, 0.033, 0.067, or 0.20% boric acid to provided doses of 0, 1.7, 3.8 or10.9 mg B/kg/day. No toxicity attributable to boric acid was discerned at these doses. Since no toxicity was observed at doses up to 8.8 mg B/kg/day, additional groups of 4 male and 4 female dogs were fed diets containing 0 or 0.67% boric acid to provide doses of 0 or 41 mg B/kg/day for 38 weeks. Testicular effects (i.e., decreased testes weight, decreased sperm count, and histological evidence of reduced spermatogenesis) were observed at 26 weeks.

According to the authors, "These findings, although based on a small number of animals and complicated by degenerative changes in the testis of one of the control dogs, indicate that the ingestion of boric acid for 26 and 38 weeks caused spermatogenic arrest and testicle atrophy. However, based on only one animal, the testicle degeneration appears to be of a reversible nature, and spermatogenesis takes place within a rather short time after withdrawal of the material."

Conclusion

The testes is a key target organ for boric acid in the dog, as it was in rodent species. Adverse effects on the testes were observed in dogs at a dose level of 41 mg B/kg/day (0.67% boric acid in the diet), a dose level that did not produce other toxic effects.

LO(A)EL

The study authors considered 0.67% boric acid in the diet (233 mg boric acid/kg/day or 41 mg B/kg/day) to be the LOAEL based on the presence of testicular effects.

NO(A)EL

The study authors considered 0.20% boric acid in the diet (62.4 mg boric acid/kg/day or 10.9 mg B/kg/day) to be the NOAEL.

Other

4

Reliability

This study was reliable for its intended purpose, which was to identify target organs and toxicological endpoints in the dog (a non-rodent species). This study is not considered suitable for purposes of quantitative risk assessment. The International Programme on Chemical Safety (IPCS, 1998) evaluated this study, as well as the 2-year study of borax in dogs, and concluded: "Confidence in this study is low, and it was considered not suitable for inclusion into the [quantitative] risk assessment because of (1) small and variable numbers of dogs, (2) variable background lesions in controls leading to uncertainty regarding the strength of the response to treatment, (3) lack of GLP, and (4) other, more recent studies of greater scientific quality with findings at a similar or lower intake level of boron (Ku et al., 1993; Price et al., 1994)."—IPCS (1998) Environmental Health Criteria 204. Boron. P. 86.

Deficiencies

Yes. In a study of this age, it is not surprising that there are deficiencies. These include:

- The number of dogs used was low, with only four dogs per dose level; these dogs were sacrificed at three different time intervals, resulting in variable group sizes of 1-2 males/group/sacrifice interval.
- In the 38-week study, there were testicular lesions, including testicular degeneration in 3 out of 4 control dogs, and the fourth control dog had a low sperm count. Although a compound-related effect is apparent at the highest dose, firm conclusions about the NOAEL cannot be drawn.
- There is a large gap between the NOAEL and LOAEL, and the NOAEL and LOAEL were determined from portions of the study performed at different times. In addition, there is evidence that the effect at the LOAEL may be reversible which indicates that the LOAEL may be close to the NOAEL. This is consistent with findings in rats.
- Lack of statistical analysis
- Dogs were from unknown source
- Age unknown
- Disease and dietary history of dogs is unknown
- Previous exposure to drugs, pesticides, chemicals unknown
- Dogs sacrificed at different time intervals (12, 24, 27 months; 26, 38, 41 weeks), sometimes with no concurrent control
- The dog is not the most appropriate species for type of study for reasons such as seasonal breeding performance, inbreeding factors, and insufficient historical background data.
- Limited analysis of test material
- No testing to determine homogeneity of diet
- Some dogs were housed in metabolism cages for part of the study

- Some dogs were catheterized; others were not.
- One dog was in a fight in the exercise pen during cage cleaning; after the fight, the dog went into convulsions for 15 minutes; treated with drugs and put back onto the study.
- Background level of boron in the dog chow was never measured.

Despite these deficiencies, this study is valid for its intended purpose, which was to identify target organs and toxicological endpoints in the dog. This study did not reveal any target organs or toxicological endpoints that were not identified in rodent species. The results indicate that the testes is a key target organ for boric acid toxicity in the dog, as it was in rodent studies. The results do not indicate that the dog is a more sensitive species than rodents. A series of sophisticated studies of the reproductive toxicity of boric acid was conducted by the U.S. National Toxicology Program in the 1980s and 1990s.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	4 feb 2005
Materials and Methods	The applicants version is acceptable.
Results and discussion	The applicant's version is adopted.
Conclusion	In view of the deficiencies in the study it is considered not appropriate to establish a NOAEL and LOAEL from this study. The study identifies the testis as the target organ for boron.
Reliability	4
Acceptability	not acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.4 **Sub Chronic Oral toxicity**

Annex Point IIA6.4 *6.4.1. Repeated Dose (90) day Study Mouse.*

Official use only

9 REFERENCE

Section A6.4 **Sub Chronic Oral toxicity**

Annex Point IIA6.4 *6.4.1. Repeated Dose (90) day Study Mouse.*

Reference	National Toxicology Program (NTP) Technical Report Series No. 324. 1987, Toxicology and Carcinogenesis Studies of Boric Acid in B6C3F1 Mice (feed studies), October 1987, US Department of Health and Human Services.
	Electronic File
Data protection	No

GUIDELINES AND QUALITY ASSURANCE

Guideline study	In compliance with OECD Guideline 408, although mouse is not generally a recommended species. NTP only publishes the summary report, therefore no individual animal data and full details of pathology, blood and clinical data are not available. However, the results obtained are confirmed in other studies therefore in the interests of animal welfare no further testing is necessary.
GLP	Yes
Deviations	Yes

MATERIALS AND METHODS

Test material	As given in section 2
Lot/Batch number	
9.1.1.1 Description	Technical grade boric acid
9.1.1.2 Purity	99.7%
9.1.1.3 Stability	Stable
Test Animals	Non-entry field
Species	Mouse
Strain	B6C3F1
Source	Charles River Breeding Laboratories, MI, USA

Section A6.4	**Sub Chronic Oral toxicity**
Annex Point IIA6.4	*6.4.1. Repeated Dose (90) day Study Mouse.*

Sex	male and female
Age/weight at study initiation	7-8 weeks old
Number of animals per group	10 males/10 females per group
Control animals	yes
Administration/ Exposure	Oral/
Duration of treatment	13 weeks for control and top dose group, 16 weeks for other dose groups
Frequency of exposure	5 days per week in diet
Postexposure period	none
Oral	
9.1.1.4 Type	in food given ad libitum
9.1.1.5 Concentration	0,1200, 2500, 5000, 10000, 20000 ppm of boric acid. Equivalent to 0, 194 (34), 405 (71), 811 (142), 1622 (284), 3246 (568) mg boric acid (mg B)/kg bw per day males; and 0, 169 (47), 560 (98), 1120 (196), 2240 (392), 4480 (784) mg boric acid (mg B)/kg bw per day females.
9.1.1.6 Vehicle	dry mix with diet
9.1.1.7 Concentration in vehicle	*as above*
9.1.1.8 Total volume applied	
9.1.1.9 Controls	plain diet
Examinations	
Observations	
9.1.1.10 Clinical signs	yes twice per day
9.1.1.11 Mortality	yes
Body weight	yes weekly.

Section A6.4 **Annex Point IIA6.4**	**Sub Chronic Oral toxicity** *6.4.1. Repeated Dose (90) day Study Mouse.*
Food consumption	yes
Water consumption	no
Ophthalmoscopic examination	no
Haematology	no
Clinical Chemisty	no
Urinalysis	no
Sacrifice and pathology	
Organ Weights	not reported
Gross and histopathology	yes all dose groups organs: brain, spinal cord (if neurologic signs), pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, bronchi, gonads, uterus, mammary gland, prostate, urinary bladder, gall bladder, mandibular lymph nodes, skin, eyes (if abnormal), gross lesions and tissue masses.
Other examinations	none
Statistics	as needed
Further remarks	**RESULTS AND DISCUSSION**
Observations	See Table A6 4-1.
Clinical signs	Symptoms included nervousness, and at high dose levels had haunched appearance, dehydration, foot lesions scaly tails.
Mortality	Eight out of the ten males and six out of the ten females from the 20000 ppm group died and one of the ten males from the 10000 ppm group died before end of study.

Section A6.4	**Sub Chronic Oral toxicity**
Annex Point IIA6.4	*6.4.1. Repeated Dose (90) day Study Mouse.*

Body weight gain	The mean bodyweights in the 5000, 10000 and 20000 ppm groups were 10%, 17% and 23% lower than controls in males, and 8%, 10% and 18% lower in females.
Food consumption and compound intake	food consumption increased by about 40% by the 12th week in the 3 lower dose groups. Because of excessive scattering at the two highest dose levels intakes were unreliable.
Ophtalmoscopic examination	not done
Blood analysis	not done
Haematology	not done
Clinical chemistry	not done
Urinalysis	not done
Sacrifice and pathology	
Organ weights	not reported
Gross and histopathology	Incidences of extra medullary heamatopoiesis of spleen observed of varying severity in all dose groups for both males and females and hyperkeratosis and/or acanthosis of the stomach observed at the highest dose only in both males and females. At doses of 5,000 ppm (142 mg B/kg bw for the male) and above, degeneration or atrophy of the seminiferous tubules was observed.
Other	none

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods	In compliance with OECD Guideline 408, although mouse is not generally a recommended species. NTP only publishes the summary report, therefore no individual animal data and full details of pathology, blood and clinical data are not available. However, the results obtained are confirmed in other studies therefore in the interests of animal welfare no further testing is necessary.
Results and discussion	At doses $\geq$ 5,000 ppm (811 mg boric acid/kg bw) (142 mg B/kg bw) for the male), degeneration or atrophy of the seminiferous tubules was observed, along with dose related incidence of extra medullary heamatopoiesis of spleen in all dose groups

Section A6.4	**Sub Chronic Oral toxicity**
Annex Point IIA6.4	*6.4.1. Repeated Dose (90) day Study Mouse.*

Conclusion	Seminiferous tubular atrophy was observed at doses≥142mg B/Kg bw per day.
LO(A)EL	Critical effect in males was testis atrophy at doses ≥811 mg boric acid/kg bw(≥ 142mg B/kg bw) per day. In females deaths were observed at 4480 mg boric acid/kg bw (784mg B/kg bw)per day.
NO(A)EL	1200 ppm in diet equivalent to 194(34) mg boric acid(B)/kg bw per day.
Other	
Reliability	2
Deficiencies	NTP only publishes the summary report, therefore no individual animal data and full details of pathology, blood and clinical data are not available. However, the results obtained are confirmed in other studies therefore in the interests of animal welfare no further testing is necessary.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14 February 2005
Materials and Methods	The version of the applicant is accepted
Results and discussion	At all dose levels extra medullary haematopoiesis of the spleen was observed. This may be indicative of an increased breakdown of red blood cells induced by boric acid. In a 2 year carcinogenicity study also extramedullary haematopoiesis was observed in both treatment groups (2500 and 5000 ppm).
Conclusion	Based on the extra medullary haematopoiesis of the spleen at all dose levels, a NOAEL cannot be determined. The LOAEL in this study is 1200 ppm, equivalent to 194 and 169 mg/kg bw/day in males and females respectively.
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_4-1. Results Mouse 90 day of repeated dose toxicity study

Parameter	Control		1200ppm		2500ppm		5000ppm		10000ppm		20000ppm		dose-response +/-
	m[a]	f	m	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f	
number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	
Mortality	0	0	0	0	0	0	0	0	1	0	8	6	
clinical signs	0	0	+/-	+/-	+/-	+/-	+	+	+	+	++	++	
body weight gain (g)	11.7	9.4	10.2	8.4	10.5	8.3	7.3	7.2	5.2	6.6	2.7	4.1	
food consumptn g/kg bw (wk 12)	140	190	165	229	164	219	199	271	456*	431*	753*	1138 *	
Organ x testes degeneration	0/10		0/10		0/10		2/10		8/10		8/10		
spleen extramed. hematopoiesis	1	0	3	2	5	4	5	6	10	10	1	2	

*unreliable because of food scatter

Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (90 Day) – Oral Dogs Boric Acid

Official use only

1 REFERENCE

Reference

[redacted] (1963) 90 Day Dietary Feeding -- Dogs. Boric Acid.

This study was published in summary form in Weir RJ and Fisher RS. (1972) Toxicological studies on borax and boric acid. Toxicol Appl Pharmacol. 23(3):351-64. Unfortunately, the published version does not always accurately reflect the original study reports. Thus, it is necessary to evaluate the original study reports to appreciate the limitations of these studies.

Data protection

Yes

Data owner

[redacted]

Companies with letter of access

[redacted]

Criteria for data protection

Data on new a.s. for first entry to Annex I

GUIDELINES AND QUALITY ASSURANCE

Guideline study

No. No guidelines available at the time the study was conducted.

GLP

No. GLP was not compulsory at the time the study was performed.

Deviations

No guidelines available at the time the study was conducted.

MATERIALS AND METHODS

In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values depending on the true methodological parameters.

Test material

Boric acid [redacted]

Lot/Batch number

Not stated..

Specification

Not stated.

1.1.1.1 Description

"Fine, white powder without noticeable odor"

1.1.1.2 Purity

Not stated.

1.1.1.3 Stability

Test material expected to be stable.

Test Animals

Species

Dog

Strain

Purebred Beagle

Source

Unknown

Sex

Male and female

Age/weight at study initiation

Body weights of the dogs ranged from 4.2-11.5 kg during the first week of the study. The dogs were described as "young," but no specific age was provided.

Number of animals per group

5 males and 5 females per group

Control animals

Yes. The control group was employed as a common control with the 90-day study of borax

Administration/ Exposure

Oral (diet)

Duration of treatment

90 days.

Frequency of exposure

Seven days per week

Postexposure period

No.

Oral

1.1.1.4 Type

In food

1.1.1.5 Concentration	0, 0.01, 0.1, 1.0% boric acid in the diet These concentrations provided doses of 0, 0.4, 4.4, and 33 mg B/kg/day, based on the actual body weight and food consumption data in the study. Others in the scientific literature have described these doses as 0, 0.4, 4.4, and 44 mg B/kg/day, but these calculations are based on standard assumptions regarding body weight and food consumption, not on the actual data from this study. Food consumption was ad libitum.
1.1.1.6 Vehicle	
1.1.1.7 Concentration in vehicle	
1.1.1.8 Total volume applied	
1.1.1.9 Controls	Plain dry diet (Wayne Dog Feed) for seven days per week. The dry diet for each dog was supplemented with a 100-gram ration of canned meat (Hill Packing Company) five days per week. Neither diet was analyzed for the background level of boron. Boron is an essential plant element, and plant-derived foods (such as fruits and vegetables) are significant dietary sources of boron. The background level of boron in the control diet was not considered in the calculations of the dose levels.

Examinations

Observations	
1.1.1.10 Clinical signs	Yes. The dogs were observed daily for appearance, behavior, and gross signs of systemic toxicity or pharmacological effects.
1.1.1.11 Mortality	Yes. Daily throughout the study.
Body weight	Yes. Weekly.
Food consumption	Yes. Weekly.
Water consumption	No.
Ophthalmoscopic examination	No.
Haematology	Yes. All dogs initially and at 2, 4, and 13 weeks. Parameters: Haematocrit, haemoglobin concentration, total and differential leukocyte count, sedimentation rate.
Clinical Chemisty	Yes. All dogs initially and at 2, 4, and 13 weeks. Parameters: glucose, blood urea nitrogen.
Urinalysis	Yes. All dogs initially and at 2, 4, and 13 weeks. Parameters: appearance, specific gravity, pH, protein, glucose, acetone, bilirubin, blood, and microscopic findings.

Sacrifice and pathology	All dogs were sacrificed after 13 weeks.
Organ Weights	Yes. All dose groups. Organs: liver, kidneys, adrenals, testes, spleen, brain, thyroid
Gross and histopathology	Yes. All dose groups. Organs: brain, thyroid, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, lungs, testes, pituitary, urinary bladder, bone, bone marrow
Other examinations	The liver and spleen of two control dogs and one dog that received boric acid were stained for ferric iron by Perl's method.
Statistics	Yes, but statistical methods were not described. Level of significance (p value) not stated.
Further remarks	**This study was conducted in a parallel with a 90 day study of borax in dogs. The studies were identical in design, and they employed a common control group.**

RESULTS AND DISCUSSION

Observations	
Clinical signs	No effects attributable to the test material.
Mortality	No mortalities at any dose.

Body weight gain	No effects attributable to the test material. The authors stated: "Body weights showed some fluctuation during the study but were within ±1.0 kg of the starting weights. The most pronounced changes were noted in Female Dog No. 4949 (0.1%) and Female Dog No. 4952 (1.0%). Both dogs throughout the study showed a persistent partial acceptance of the basal laboratory diet containing the test material."
Food consumption and compound intake	According to the authors, there was variable acceptance of the diet containing the test material.
Ophtalmoscopic examination	
Blood analysis	
Haematology	No effect attributable to the test material. One female dog (No. 4952; 1.0%) showed a slight but steady decrease in the haematocrit and haemoglobin values during the study; this was one of the dogs which showed a persistent partial acceptance of the basal laboratory diet containing the test material.
Clinical chemistry	No effects.
Urinalysis	No effects.
Sacrifice and pathology	
Organ weights	No effect on organ weights was observed at any dose in female dogs. At 1.0%, the authors reported a statistically significant decrease in testes/body weight, testes/brain weight, and thyroid/body weight ratios in the male dogs. Similar, significant effects on testes/body weight and testes brain weight ratios were noted in a 90-day study in dogs given the same high dose of boron in the form of borax. At 1.0%, significant increases in liver/body weight and liver/brain weight ratios were reported in females, but not males. The only statistically significant effect on organ weight at the middle dose (0.1%) was a decrease in testes/body weight; absolute testes weight and testes/brain weight ratio were not significantly affected. No significant effect on any of these parameters was observed in a in a 90-day study in dogs given the same middle dose of boron in the form of borax.
Gross and histopathology	Treatment-related histological changes were clearly evident at the high dose (1.0%), but not at lower doses. Severe testicular atrophy was reported in 5 out of 5 high dose dogs. In high dose female dogs, there was a tendency for the zona reticularis in the adrenal glands to be increased in width. The thyroid glands in two high dose female dogs were infiltrated by lymphoid tissue and one of them was atrophied; the authors considered these changes "probably coincidental since a similar lesion has been seen previously in control beagles."
Other	In Dog No. 5049 (a control female), ferric iron was demonstrated in one dog by Perl's method in some of the Kupfer cells and in the splenic pulp.

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

This study was conducted in the early 1960s before OECD guidelines were established.

Results and discussion

Groups of 5 male and 5 female beagle dogs were fed diets containing 0, 0.01, 0.1, or 1.0% boric acid for 90 days to provided doses of 0, 0.4, 4.4 or 33 mg B/kg/day, respectively. The authors reported no clinical signs of toxicity attributable to boric acid at any dose. There was variable acceptance of the diet containing the test material. Yet, body weights showed some fluctuation during the study but were within ± 1.0 kg of the starting weights.

The most significant indication of toxicity was a decrease in testicular weight and histological evidence of testicular atrophy in 5 out of 5 male dogs at the high dose (1.0%). Unfortunately, the report did not include the raw data on the histological evaluation. The reason that this is important is that other studies in beagle dogs in the same laboratory showed an unusually high incidence of testicular lesions, as high as 3 out of 4 controls in one study. However, it is highly likely that the high dose in this study produced a treatment-related adverse effect on the testes. Similar effects were seen in a subsequent study of dogs given 0.67% boric acid in the diet for 26-38 weeks.

At the mid-dose (0.1%), the investigators reported a statistically significant (undefined statistical test) decrease in testes ratio/body weight, but not testes/brain weight ratio. No significant effect on these parameters was observed in a in a 90-day study in dogs given the same mid-dose of boron in the form of borax or in a subsequent study in which dogs were given twice this dose of boric acid for up to 2 years. Histogical examination of the testes at the mid-dose showed little evidence of testicular effects, and the spermatogenic epithelium was described as "intact and active." Therefore, the mid-dose (0.1%) is considered a NOAEL in this study.

Conclusion

The testes is a key target organ for boric acid in the dog, as it was in rodent species. Adverse effects on the testes were observed in dogs given 1.0% boric acid in the diet (33 mg B/kg/day) for 13 weeks. These findings are consistent with a subsequent study by the same team of investigators that reported similar testicular effects in dogs administered a diet containing 0.67% boric acid (41 mg B/kg/day) for 26 or 38 weeks.

LO(A)EL

1.0% boric acid in the diet (189 mg boric acid/kg/day or 33 mg B/kg/day) based on the presence of testicular effects.

NO(A)EL

0.1% boric acid in the diet (25.1 mg boric acid/kg/day or 4.4 mg B/kg/day).

Other

Reliability

4

This study is considered reliable for its intended purpose, which was (1) to identify target organs and toxicological endpoints in the dog (a non-rodent species) and (2) to identify appropriate dose levels for a subsequent 2-year study in dogs. This study is not considered appropriate for purposes of quantitative risk assessment.

Deficiencies

Yes. In a study of this age, it is not surprising that there are deficiencies. These include:

-The number of dogs used was small (5 males and 5 females per group)

-In other dog studies conducted around the same time in the same laboratory, an unusually high incidence of testicular lesions were reported among control animals. In fact, in a 38-week study of boric acid, testicular lesions were observed in 3 out of 4 control dogs, and the fourth control dog had a low sperm count. As a result, firm conclusions about the NOAEL cannot be drawn.

-The statistical methods were not described.

-Dogs were from unknown source

-Age of the dogs is unknown

-Disease and dietary history of dogs is unknown

-Previous exposure to drugs, pesticides, chemicals unknown

-Limited analysis of test material

-No testing to determine homogeneity of diet

-Background level of boron in the dog chow was never measured.

Despite these deficiencies, this study is valid for its intended purpose, which was (1) to identify target organs and toxicological endpoints in the dog (a non-rodent species) and (2) to identify appropriate dose levels for a subsequent 2-year study in dogs. This study did not reveal any target organs or toxicological endpoints that were not identified in rodent species. The results indicate that the testes is a key target organ for boric acid toxicity in the dog, as it was in rodent studies. The results do not indicate that the dog is a more sensitive species than rodents. A series of sophisticated studies of the reproductive toxicity of boric acid was conducted by the U.S. National Toxicology Program in the 1980s and 1990s.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11 February 2005
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	Recalculation of the dose levels on the basis of the original boric acid consumption data yielded daily intake levels of 2.4 (0.4), 24 (4.2) and 201 (35.2)mg/kg bw/day for the 0.01, 0.1 and 1.0% groups respectively. Data between brackets indicate intake of boron/kg bw/day. Treatment-related effects (weight and histopathology) on testes were already observed at the dose of 0.1% boric acid. Testes weight

treatment	testes weight (g)	ratio to body weight (%)
control	17.2	0.20
0.01 %	18.8	0.21
0.1 %	14.3	0.15
1.0 %	10.4	0.12

Compared to the control animals absolute testes weights of the 0.1 and 1.0 % groups are reduced by 17 and 40 %, respectively. The reduction at 0.1% was not statistically significant. Relative testes weight at 0.1 and 1.0% were significantly reduced by 25 and 40% respectively. Histopathological examination of the testes of the 0.1% group revealed that the spermatogenic epithelium was intact and active. However, at this dose of boric acid, in the testes of the males histological changes, described as 'artifactual distortion of the tubules in the outer one-third of the glands' were observed. Although these changes are described as artifactual, it is striking that they were found in all males at this dose, but not in males of the control or the low dose groups. A similar effect was found in the 90-day dog study with disodium tetraborate decahydrate at equimolar boron levels. Therefore these histological changes observed at the mid-dose are considered to be a consequences of a boron-related alteration of the structure of the testes. Since the testes appear to be the primary target organ for boron the effects on testes weight and histological changes at the mid dose are considered to be toxicologically relevant. This conclusion is supported by data from dose-response modeling (see end of this document.

At 1.0% severe atrophy of the testes was found.

It was noted that in this 90-day dog study and in the 90-day dog study with disodium tetraborate decahydrate it was not mentioned which statistical tests were used to determine whether changes in testicular weight were significant, which could be a reason for criticism on the study.

We therefore subjected the individual animal data (boron consumption, testes weight and testes/body weight ratio) of the present study and the disodium tetraborate decahydrate to a dose-response modeling, in order to provide a Bench Mark type approach using all the individual data in this study

It is reported that slight extramedullary haematopoiesis is observed in most

	instances in the 0.1 and 1% groups. No detailed histopathological information is provided. In comparison to the initiation of the study, at the end of the treatment period the 1 % group a reduction of 11 and 14 % in cell volume and a reduction of 16 and 17 % in Hb was observed in males and females respectively. In animals of the control group these parameters were slightly increased at the end of the study. The extramedullary haematopoiesis and haematological findings in the high dose animals are indicative of an increased red blood cell destruction at this dose.
Conclusion	LOAEL: 0.1% boric acid, equal to 24 mg boric acid/kg bw/day or 4.2 mg B/kg bw/day, based on the reduction in testes weight and the distortion of the tubules in the outer one-third of the testes. NOAEL: 0.01 % boric acid , equal to 2.3 mg boric acid/kg/day or 0.4 mg B/kg bw/day. (see also dose-response modeling at the end of this document and the justification of the choice of critical endpint and overall NOAEL)
Reliability	3
Acceptability	acceptable
Remarks	Although there are a number of deficiencies in the study, in repeated dose studies with borates in different species, the testes are consistently identified as a major target organ for toxicity. The present adverse effects on testes with boric acid are confirmed by a 90 days study in which dogs were treated with borax. Therefore the present study is acceptable for risk assessment purposes.

COMMENTS FROM TMIIIO7

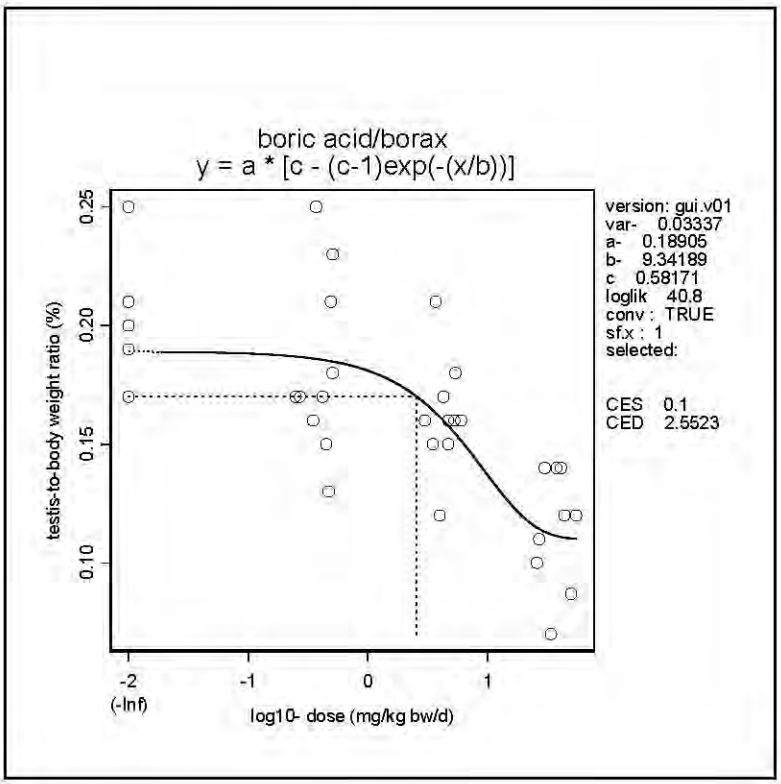
Date	TM 12th of July 2007
Materials and Methods	Although the RMS presented a complete toxicological profile of the substance, including the 90 day dog studies with dose modelling and a justification of the choice of critical endpoint and overall NOAEL, to demonstrate the scientific bases of the NOAEL based on the 90 day dog studies, the TM replied that there are deficiencies in the 90 day dog study compared to the teratogenicity study and that the NOAEL is not reliable and as in other regulatory programms not be used for risk assessment purposes. It was observed that by choosing the NOAEL from the 90 day dog study the AOEL derived was lower than the general background exposure
Results and discussion	No reliable NOAEL is set.
Conclusion	The study is not acceptable for risk assessment purposes. The justification is not valid anymore.
Reliability	4
Acceptability	Not acceptable
Remarks	

Dose-response modeling performed by CA

It was noted that in this 90-day dog study and in the 90-day dog study with disodium tetraborate decahydrate it was not mentioned which statistical tests were used to determine whether changes in testicular weight were significant, which could be a reason for criticism on the study. We therefore subjected the individual animal data (boron consumption, testes weight and testes/body weight ratio) of the present study and the disodium tetraborate dechaydrate to a dose-response modeling, in order to provide a Bench Mark type approach using all the individual data in this study. For this a program developed by RIVM (PROAST) was used. PROAST is a widely accepted dose-response modeling program used also in international evaluations (e.g. within the WHO/FAO JECFA committee and EMEA/CVMP). Our analysis indicates that no statistical difference could be found between the studies with boric acid and disodium tetraborate decahydrate. Therefore, the data are allowed to be pooled providing a control group of n=5 and a treatment group with n=9-10/dose. This analysis indicated that the critical effect dose for a 5 or 10 % change in testicular weight (critical effect size) was 1.2 (90% confidence interval (CI) = 0.59-3.01) mg B/kg bw/day or 2.6 (90% CI =1.27-6.39) mg B/kg bw/day respectively. These values of the critical effect doses are in between the NOAEL and LOAEL determined from the boric acid study, with the lower confidence limits close to the NOAEL of 0.46 mg/kg bw/day. This dose response analysis strongly supports the choice of the NOAEL being 0.46 mg/kg bw/day.

Dose-response modeling of the data on relative testes weights of the dogs form the 90-day studies with boric acid and disodium tetraborate decahydrate:

Critical Effect Doses and 90% confidence intervals (calculated by profile likelihood method) for a change in relative testis weight of 5% and 10%.



Critical Effect	Critical Effect Dose

Size	(90% C.I.) in mg/kg bw/d
-5%	1.189 (0.593 – 3.013)
-10%	2.552 (1.269 – 6.388)

Justification of the choice of critical endpoint and overall NOAEL

Introduction

For the notification of the use of simple borates as biocidal products, the toxicology of boric acid, boric oxide, disodium tetraborate decahydrate (borax), disodium tetraborate pentahydrate, anhydrous borax and disodium octaborate tetrahydrate was evaluated on the basis of original study reports and data from published literature.
The purpose of the evaluation is to set the acceptable operator exposure level (AOEL) and establish whether occupational exposure may lead to health effects. This process involves 3 separate/independent steps :
hazard identification (determination of the most adequate Point of Departure)
determination of appropriate assessment factors,
Risk characterization

Based on outcome of the risk assessment it may be necessary to take regulatory actions, i.e. risk management, which is outside the scope of the evaluation.

We noticed that the Point of Departure (or critical effect) we used for the present evaluation differs from the Point of Departure used in evaluations performed within other frameworks. In the present document a justification of our choice of the Point of Departure is given and a possible explanation for the apparent discrepancy between the present evaluation and the evaluations within other frameworks is given.
On the basis of the available data we considered that the most sensitive effect induced by borates was testes weight reduction and atrophy. The most sensitive species for testicular effects appears to be the dog; the LOAEL for testicular effects in two 90-day feeding studies in dogs with boric acid and borax was 4.2 mg Boron/kg bw/day. The overall NOAEL in these studies was 0.46 mg Boron/kg bw/day. We noticed that the NOAEL and LOAEL are respectively 15 - 20 and 140 - 183 times higher than the human average daily intake of 0.023 - 0.03 mg/kg bw/day.

Justification of the choice of critical endpoint and overall NOAEL

In all the studies available in the mouse, the rat and the dog, the testes were identified as the major target for boron. In these species borates induce a reduced testis weight and testes atrophy (see table). However, the dog appears to be most sensitive species.

Testicular effects in mouse, rat and dog

Compound	Route	duration of study	Species Strain	Results	NOAEL (mg B/kg bw/d)	LOAEL (mg B/kg bw/d)	Reference
Boric acid	Oral in diet	13 weeks	Mouse, B6C3F1	Degeneration and atrophy of the seminiferous tubules.	71	142	National Toxicology Program (NTP) Technical