Section A7.1.3	Adsorption / Desorption screening test
Annex Point IIA7.7	Section A7.1.3

27.3 Guideline study	Yes OECD 106 Draft, SETAC-Europe and EU Council Directive 95/36/EC
27.4 GLP	Yes
27.5 Deviations	Yes Concentrations changed (increased) because protocol specified concentrations below background level of boron in the soils No analytical certificate was added to report Test performed according to 95/36/EC, not 91/414/EEC as stated in protocol Soil particle analysis, pH, organic carbon, organim matter, total nitrogen and CEC were measured by Levington Agriculture Mass balance determined by single soil extraction, not three times as stated in protocol. These deviations are assumed not to have affected the results of the study.
	MATERIALS AND METHODS
27.6 Test material	As given in section 2, Boric Acid Manufacturing Grade
Lot/Batch number	Not available
Specification	As given in section 2
Purity	+99.9%
Further relevant properties	Water solubility 4.7% at 20°C.
Method of analysis	ICP-AES (Inductively coupled plasma atomic emission spectrometry) (see Annex G of report)
27.7 Degradation products	Degradation products tested: No
Method of analysis for degradation products	
27.8 Reference substance	No
Method of analysis for reference substance	

Section A7.1.3	**Adsorption / Desorption screening test**
Annex Point IIA7.7	**Section A7.1.3**

27.9 Soil types	Four soil types were tested (see table A7_1 _3-1): humic sand soil, sandy loam soil, loam soil, low humic content sand soil
27.10 Testing procedure	Non-entry field
Test system	Tests were conducted in 20 ml polyethylene vials closed with screw caps. 1-gram samples of soil were added with10.0 ml of test solutions. Vials were shaken for approx. 20 hr at 20±2 °C in the dark. Vials were centrifuged and supernatant transferred to new vial for boron analysis. Triplicate samples were tested.
Test solution and Test conditions	Stock solutions were prepared by dissolving 50.4 mg boric acid in 1 liter of 0.01 M $CaCl_2$. For other concentrations, this stock was diluted further. Nominal concentrations were 1.008, 2.016, 10.08 and 50.4 mg/L as boric acid (boron equivalents: 0.176, 0.353, 1.76, and 8.82 mg-B/L). Soil/solution ration: 1 g/10 ml.
27.11 Test performance	Non-entry field
Preliminary test	According to (a)"OECD 106": Yes A stock solution containing 1 g/L of test substance indicated that the background of boron in the soils of the lowest test concentration was more than the test solution and was at the level of the detection limit of ICP.
Screening test: Adsorption	No
Screening test: Desorption	Not performed
HPLC-method	No
Other test	

RESULTS

27.12 Preliminary test	Background levels exceeded test solution concentrations
27.13 Screening test: Adsorption	
27.14 Screening test: Desorption	
27.15 Calculations	Non-entry field

Section A7.1.3 | **Adsorption / Desorption screening test**

Annex Point IIA7.7 | **Section A7.1.3**

Ka , Kd

Soil	Kd (mg/g)	Koc (mg/g)	% organic carbon
Humic sand soil	0.86	61.6	1.4
Sandy loam soil	3.946	438.4	0.9
Loam soil	1.93	214.4	0.9
Low humic content sand soil	0.749	187.2	0.4

Correlation Coefficients (From adsorption isotherms, see Annex C)

Humic sand soil $R^2 = 0.9976$

Sandy loam soil $R^2 = 0.9733$

Loam soil $R^2 = 0.9953$

Low humic content sand soil $R^2 = 0.9427$

Desorption tests were not conducted because the amount in the supernatant was >75% of the total amount added.

See 4.4.1

Ka_{oc} , Kd_{oc}

27.16 Degradation product(s)

APPLICANT'S SUMMARY AND CONCLUSION

27.17 Materials and methods

The test followed OECD 106 (draft) guidelines as specified by EU Council Directive 95/36/EC. A preliminary test indicated that the soil concentrations of boron (background) exceeded the added concentrations, so the test was run using loadings of 1 to 50 mg/L boric acid (0.18 to 8.8 mg-B/L equivalents). The soils were extracted only once (instead of the guideline 3x) because the mass balance calculation showed total recovery of 96% to 107%. No desorption test was conducted because over 75% of material was in the supernatant. X

The soil:solution ratio chosen was 1:10. In the humic sand soil the change in concentration in the liquid phase was so small that the authors reported the adsorption coefficient could not realistically be determined. A review of Annex B (data and calculations) suggests that changes in concentrations for all soils were minimal – about 10% decreases, and that the Ceq values for the low humic content sand soil were as small as for the humic sand soil. This suggests that the soil:solution ratio might have been increased (to obtain greater reductions in test substance in the supernatant) as recommended in ASTM Standard Method E1195-01. X X

In calculation of the isotherms, replicate data appears to have been averaged before calculation of the regression. This reduces the apparent variability of the data (four points are plotted instead of 12) and may have increased the stated fit of the regression line (as shown in the R-square statistic).

Section A7.1.3 | **Adsorption / Desorption screening test**

Annex Point IIA7.7 | **Section A7.1.3**

The raw data (boron concentrations) are presented in Annex G, but no code is provided to link the sample ID number with a treatment group.

Reference: ASTM International, 2001. E1195-01 "Standard Test Method for Determinng a sorption constant (Koc) for an organic chemical in soil and sediments" Volume 11.05 ASTM International, W. Conshohocken, PA.

27.18 Results and discussion

The adsorption values ranged from Kd = 0.749 to 3.95. The authors characterised these as indicating that the test substance is only very slightly adsorbed to humic sand soil and adsorbed to all the other soils.

To compare the adsorption values with measured soil properties, a comparison table is shown below, arranged in order of increasing Kd:

Soil	Low humic content sand soil	Humic sand soil	Loam soil	Sandy loam soil
Kd (ml/g)	0.749	0.860	1.93	3.95
% carbon	0.4	1.4	0.9	0.9
CEC	2	9.8	13.4	10.7
%clay	2	3	26	15
%sand	97	90	40	58
pH	7.4	5.5	7.8	7.7
Koc (ml/g)	187	61	214	438

No clear pattern is evident and consistent. One observation is that the sorption tends to increase with increased clay fraction (or decreased sand fraction): Kd values are <1 for both soils with %clay <5%, or with %sand >90%. This is consistent with other observations that boron tends to bind with clay (Butterwick et al., 1989). Goldberg et al. (2000) also reviewed boron binding, modelling it as the constant capacitance model, a function of binding with surface hydroxyl groups on oxides and clay minerals.

The purpose of this procedure is to help evaluate the mobility of the test substance in soil. The observed Koc values suggest that boron will be mobile in soil. (For comparison, immobile or low mobility substances have Koc values >500 in this type of batch equilibrium test, according to ASTM 2001).

References:

Butterwick, L, N DeOude, K Raymond. 1989. "Safety assessment of boron in aquatic and terrestrial environments." Ecotox Environ Safety 17: 339-371.

Goldberg, S, SM Lesch and DL Suarez 2000. "Predicting boron adsorption by soils using soil chemical parameters in the constant capacitance model." Soil Sci. Soc. Am. J. 64: 1356-1363.

X

Section A7.1.3	Adsorption / Desorption screening test	
Annex Point IIA7.7	Section A7.1.3	
Adsorbed a.s. [%]		
K_a		
K_d		
Ka_{oc}		
Ka/Kd		
Degradation products (% of a.s.)		
27.19 Conclusion	The adsorption values were determined using the standard method in OECD 106. This test was designed to predict mobility in soil, and boron would be classified as medium to very high mobility, per ASTM 2001 scheme.	X
Reliability	A reliability indicator of 2 is suggested: Reliable with limitations. The soil:solution ratio was too low to show reliable changes in the boron concentration of the initial solution/supernatant, so the resulting values may be highly variable. The analytical values were averaged before estimating the isotherms, which may have decreased the apparent variability of the regression equation.	X
Deficiencies	Yes As discussed above, some methodological issues might have been changed to improve the usefulness of the study. No clear pattern with soil properties was evident. However, the intent of the study was to determine the mobility of boron in representative soils and the results are adequate to show that boron should be regarded as mobile in soil.	X

Evaluation by Competent Authorities

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

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

14-01-2005

Materials and Methods

Applicant's summary adequately reflects the report.

Results and discussion

The variation in the calculated adsorbed fraction between replicates is large and authors calculate negative sorption for one replicate of humic sand at 10 µg/mL and low humic sand at 50 µg/mL, regression for humic sand was performed with three concentrations.

Section 5.2, table: K_d in humic sand soil should read 0.862; 1/n values and % silt are not given in applicant's table but were reported by the authors. Revised table is given below

Soil	Low humic content sand soil	Humic sand soil	Loam soil	Sandy loam soil
Kd (ml/g)	0.749	0.862	1.93	3.95
1/n	0.542	0.659	0.802	0.685
% carbon	0.4	1.4	0.9	0.9
CEC (mmol/kg)	20	98	134	107
%clay	2	3	26	15
%sand	97	90	40	58
% silt	1	7	34	27
pH	7.4	5.5	7.8	7.7
Koc (ml/g)	187	62	214	438

The authors suggest that the sorption behaviour might have been influenced by pH, but the pKa of boric acid is between 9.1 and 13.8, which is higher than the pH of the test soils. Thus dissociation is not considered to have biased the results.

According to OECD 106, at least five concentrations should have been included to determine the Freundlich adsorption coefficient, only four concentrations were used in the experiment.
According to OECD 106, the optimal soil solution ratio should have been determined beforehand so that > 20 % adsorption and preferably > 50 % adsorption is achieved. According to OECD 106, sorption coefficients that are based on concentration decrease in the aqueous phase can only be determined accurately when the product of the adsorption coefficient and the soil:solution ratio is > 0.3. This is only the case for sandy loam (Kd x 0.1 = 0.395).
For use in equilibrium partitioning calculations, a Kp is required (see TGD). From the Freundlich equation, $C_{soil} = K_F \times C_{solution}^{(1/n)}$, it follows that the K_F is equal to a partition coefficient Kp (defined as $C_{soil}/C_{solution}$) when 1/n is 1 and sorption is linear. For leaching modelling, a default of 0.9 is used and K_F's with 1/n values between 0.7 and 1.1 are considered to comply with this default. Only loam soil meets this criterion.
As none of the soils meets both criteria (Kd x 0.1 > 0.3 and 0.7 < 1/n < 1.1), no reliable sorption coefficient can be derived from this experiment.

Conclusion	Because of the methodological deficiencies, it is not possible to derive a reliable quantitative estimate of the sorption coefficient from this study. The results of the study indicate that sorption of boric acid to soils is generally low. The study does, however, not allow for a quantitative estimation of the sorption coefficient.
Reliability	3
Acceptability	not acceptable
Remarks	This study should have been submitted under Annex point IIIA XII.1.27.7, Section 7.2.3.1, because it is a full adsorption/desorption study instead of a screening test.

	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 A7_1 _3-1: Classification and physico-chemical properties of soils used as adsorbents

	Soil 1	Soil 2	Soil 3	Soil 4
Sample Identity	*Humic Sand Soil HZ990901*	*Sandy Loam HW990901*	*Loam ZV990901*	*Low humic content sand soil SZ990901*
Soil order				
Soil series				
Classification (USDA Soil texture)	*Sandy loam*	*Sand*	*Loam*	*Sand*
Location	*Heerewaarden, NE*	*Lisse, NE*	*Lelystad, NE*	*Wageningen, NE*
Horizon				
Sand [%]	*58%*	*97%*	*40%*	*90%*
Silt [%]	*27%*	*1%*	*34%*	*7%*
Clay [%]	*15%*	*2%*	*26%*	*3%*
Organic carbon [%]	*0.9*	*0.4.*	*0.9*	*1.4*
Carbonate as $CaCO_3$				
insoluble carbonates [%]				
pH (1:1 H_2O)	*7.7*	*7.4*	*7.8*	*5.5*
Cation exchange capacity (MEQ/100 g)	*10.7*	*2.0*	*13.4*	*9.8*
Total Nitrogen (%)	*0.16*	*0.06*	*0.14*	*0.14*
Extractable cations (MEQ/100 g)				
Ca				
Mg				
Na				
K				
H				
Special chemical/mineralogical features				
Clay fraction mineralogy				

Section 7.2.3.1 **Annex Point IIIA XII.1.27.7**	**Adsorption / Desorption** **Section 7.2.3.1 – Boric Acid**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [x] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Data present in Doc IIIA 7.1.3	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14-02-2005
Evaluation of applicant's justification	An adsorption-desorption study has been submitted which is summarised in Doc. IIIA, Section A.7.1.3
Conclusion	A screening assay is not necessary
Remarks	The adsorption-desorption study should have been submitted under this Annex point, but is summarised under Annex point IIA, 7.7 (screening test).
	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 7.3 Annex Point VII.5	**Phototransformation in air (estimation method) including identity of the products** **Section 7.3.1**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Boric acid behaves like a weak inorganic acid in water due to its reaction with water forming the tetrahydroborate anion ($[B(OH)_4]^-$) and releasing H^+ ions. The equilibrium constant is small enough that the proportion of boric acid in dilute solutions with a neutral pH is >99%. The relative concentration of tetrahydroborate anion becomes dominant at pH values >9 (7.1.1.1.1). The vapour pressure of boric acid is negligible. A direct photochemical decay is not possible as the boric acid has no absorption characteristics. The only known oxidation mechanism of boric acid that could be considered to constitute a decay mechanism in the context of air pollution, is a reaction with hydrogen peroxide in an alkaline aqueous solution resulting in the peroxoborate, commonly referred to as perborate. The only significant perborate in dilute solution is the $[(HO)_3BOOH]^-$ anion. It could be speculated that such a reaction could take place in the air and in particular in rainwater if boric acid should enter the air. However, it is not likely that this reaction to perborate will take place in the air in the presence of hydroxyl radicals and ozone. There are three major reasons for this: 1 The volatility of boric acid is negligible and therefore boric acid will not enter the air. 2 The conditions in the air (*e.g.* usually neutral to acidic pH in rainwater) are not conducive to this reaction. 3 The oxidation product would not be stable in the air as it would react with organic compounds. A calculation of the decay rate of boric acid in air will not be needed. **Reference** [redacted] (2004). Boric Acid (CAS No. 10043-35-3): Statement on Phototransformation in air (estimation method), including identification of breakdown products [redacted]	
Conclusion		
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	17-01-2005
Evaluation of applicant's justification	Applicant's justification is considered valid, with the addition that strictly speaking, the statement that the vapour pressure is negligible, is not true. The vapour pressure has a certain value. However, due to the low value, the *volatility* is negeligible.
Conclusion	Justification for non-submission of data is accepted
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	
Evaluation of applicant's justification	
Conclusion	Discuss if deviating from view of rapporteur member state
Remarks	

Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

Official use only

28 REFERENCE

28.1 Reference

Hamilton, SJ and KJ Buhl 1997. "Hazard evaluation of inorganics, singly and in mistures, to Flannelmouth Sucker Catostomus latipinnis in the San Juan River, New Mexico." Ecotox Environ Safety 38: 296-308

28.2 Data protection

No

Data owner

Authors

Criteria for data protection

No data protection claimed

GUIDELINES AND QUALITY ASSURANCE

Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

28.3 Guideline study	Yes ASTM E729-88a "Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians." In: 1989 Annual Book of ASTM Standards, Vol. 11.04, pp. 378-397.
28.4 GLP	No - GLP was not compulsory at the time the study was performed nor was it necessary for research objectives
28.5 Deviations	No

MATERIALS AND METHODS

28.6 Test material	Study report: boric acid, reagent grade
Lot/Batch number	
Specification	
Purity	
Composition of Product	
Further relevant properties	
Method of analysis	Nominal additions used. Measurements of test substance concentrations in field samples not detailed in this publication.
28.7 Preparation of TS solution for poorly soluble or volatile test substances	
28.8 Reference substance	No
Method of analysis for reference substance	
28.9 Testing procedure	Non-entry field
Dilution water	(see table A7_4_1_1-2)
Test organisms	(see table A7_4_1_1-3)
Test system	(see table A7_4_1_1-4)

Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

Test conditions	(see table A7_4_1_1-5)
Duration of the test	96 hr
Test parameter	Mortality
Sampling	Test substance concentration not measured
Monitoring of TS concentration	No
Statistics	LC50 values calculated by moving average-angle method (Peltier and Weber 1985) Reference: Peltier WH and CI Weber (1985) "Methods for measuring the acute toxicity of effluents to freshwater and marine organisms" 3rd ed. US Environmental Protection Agency, Cincinnati, OH (EPA 600/4-85-013)

RESULTS

If appropriate, include tables. Sample tables are given below

28.10 Limit Test Not performed

Concentration	
Number/ percentage of animals showing adverse effects	
Nature of adverse effects	

28.11 Results test substance Non-entry field

Initial concentrations of test substance	
Actual concentrations of test substance	Not measured
Effect data (Mortality)	96 – hr LC_{50} = 125 mg-B/L (102 – 162 mg-B/L) (see table A7_4_1_1-7)
Concentration / response curve	

Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

Other effects	None reported
28.12 Results of controls	
Number/ percentage of animals showing adverse effects	No mortalities
Nature of adverse effects	
28.13 Test with reference substance	Not performed
Concentrations	
Results	
	APPLICANT'S SUMMARY AND CONCLUSION
28.14 Materials and methods	A static acute bioassay was performed using a site-related dilution water. The dilution water attempted to reconstitute the San Juan River in New Mexico where the test species is found. No trace elements were added to the reconstituted water. Nominal test substance concentrations were used. The procedures followed the ASTM e729-88a standard method.
28.15 Results and discussion	The results indicated a 96 – hr LC_{50} = 125 mg-B/L (102 – 162 mg-B/L). LC_{50} values at 24, 48 and 72 hours were: 1000 mg-B/L, 337 mg-B/L, and 225 mg-B/L, respectively.
LC_0	
LC_{50}	96 – hr LC_{50} = 125 mg-B/L (102 – 162 mg-B/L)
LC_{100}	
28.16 Conclusion	The test meets the appropriate validity criteria, with the exception of measured concentrations (see validity criteria summarized in table table A7_4_1_1-8)
Other Conclusions	
Reliability	A reliability of 2: reliable with limitations, is suggested. The limitations are that test concentrations were not measured, test concentrations were not replicated and procedure is an older standard. However, this is a peer-reviewed publication.

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

Deficiencies	Yes – test concentrations were not measured, test concentrations were not replicated. However, the procedure is basically the same as current revisions and the endpoint is consistent with other acute fish toxicity values (ranging from 233 to >1000 mg-B/L) in the literature.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*08-02-2005*
Materials and Methods	*Applicant's version is adopted:* Test is performed with boric acid, results are expressed on the basis of elemental boron (B). A static acute bioassay was performed using a site-related dilution water. The dilution water attempted to reconstitute the San Juan River in New Mexico where the test species is found. No trace elements were added to the reconstituted water. Nominal test substance concentrations were used. The procedures followed the ASTM e729-88a standard method.
Results and discussion	*Applicant's version is adopted:* The results indicated a 96 – hr LC_{50} = 125 mg-B/L (102 – 162 mg-B/L). LC_{50} values at 24, 48 and 72 hours were: 1000 mg-B/L, 337 mg-B/L, and 225 mg-B/L, respectively.
Conclusion	*Applicant's version is adopted:* The test meets the appropriate validity criteria, with the exception of measured concentrations (see validity criteria summarized in table table A7_4_1_1-8)
Reliability	*Revised version:* A reliability of 2: reliable with limitations, is suggested. The limitations are that test concentrations **(both nominal and measured)** were not **mentioned**, test concentrations were not replicated and procedure is an older standard. **Furthermore, mortality data were also not presented.** However, **the experiment seems to be conducted in a proper manner, based on the material and methods.**
Acceptability	Acceptable, the result 96-hours 125 mg B/L is included in the risk assessment.
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 A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	
Vehicle control performed	No
Other procedures	

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	*Reconstituted water designed to simulate site-specific concentrations of major cations and anions, without trace elements, in the San Juan River near Shiprock New Mexico for November 1985 (a year with average river flows).*
Alkalinity	*103±1 mg/L as CaCO3*
Hardness	*144±1 mg/L as CaCO3*
pH	*7.93±0.32*
Oxygen content	*≥ 72% saturation throughout test*
Conductance	***402±3 µmhos/cm at 25° C***
Holding water different from dilution water	*No – maintained for 2 days in dilution water*

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	*Flannelmouth sucker (Catostomus latipinnis)*
Source	*Obtained in San Juan River near Shiprock, New Mexico, USA*
Wild caught	Yes - *Eggs fertilized from milt from adults caught in San Juan River, New Mexico*
Age/size	*Tested at 12-13 days post-hatch*
Kind of food	*Commerical diet (Biodiet, Bioproducts, Inc., Warrenton Oregon) supplemented with live nauplii of brine shrimp (Artemia sp.)*
Amount of food	
Feeding frequency	
Pretreatment	*Held for 2 days before experiment in dilution water at test temperature and lighting*
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	*Static*
Renewal of test solution	
Volume of test vessels	*3.8 L jars with 3 L test solution*
Volume/animal	*3 L / 10 fish = 300 ml per fish*
Number of animals/vessel	*10 fish per jar*
Number of vessels/ concentration	*One*
Test performed in closed vessels due to significant volatility of TS	*No*

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	*25±1° C*
Dissolved oxygen	*≥ 72% saturation*
pH	*6.7 to 8.9*
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	
Photoperiod	

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal/measured)[1] [mg/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	0	0	0	0				
Others not reported								
Temperature [°C]								
pH								
Oxygen [mg/l]								

[1] specify, if TS concentrations were nominal or measured

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0				
LC_{50}	*337 (n)*	*276-434*	*125 (n)*	*102-162*
LC_{100}				

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
Concentration of test substance ≥80% of initial concentration during test	Not measured	
Criteria for poorly soluble test substances	Not applicable	

Section A7.4.1.2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 *Daphnia magna*

Official use only

29 REFERENCE

29.1 Reference

Maier, KJ and A W Knight, (1991). The toxicity of waterborne boron to Daphnia magna and Chironomus decorus and the effects of water hardness and sulphate on boron toxicity. Arch. Environ. Contam. Toxicol. 20: 282-287

29.2 Data protection

No

Data owner

Criteria for data protection

No data protection claimed

GUIDELINES AND QUALITY ASSURANCE

29.3 Guideline study

Yes – followed US EPA Guideline (USEPA (1975) "Methods for acute toxicity tests with fish, macroinvertebrates and amphibians." EPA 660/3-75-009. Corvallis Oregon)

29.4 GLP

No – GLP not compulsory.

29.5 Deviations

No

MATERIALS AND METHODS

Section A7.4.1.2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 *Daphnia magna*

29.6 Test material	Sodium tetraborate (Na2B4O7 . 10 H2O)
Lot/Batch number	
Specification	Obtained from Sigma Chemical, Inc.
Purity	
Composition of Product	
Further relevant properties	
Method of analysis	Carmine method (American Public Health Association (APHA), (1985) "Standard methods for the examination of water and wastewater." Washington, DC
29.7 Preparation of TS solution for poorly soluble or volatile test substances	
29.8 Reference substance	
Method of analysis for reference substance	
29.9 Testing procedure	*Non-entry field*
Dilution water	*Used reconstituted US EPA moderately hard fresh water(see table A7_4_1_2-2) In related studies, levels of water hardness were varied from 10.6 to 179 mg/L as $CaC3_3$. In another series, sulphate levels were varied from 10.2 to 325.4 mg/L SO^{-2}_4. For these studies, daphnids were exposed to the 48-hr LC50 concentration of boron, but in dilution waters of different hardness or sulphate concentration.*
Test organisms	Daphnia magna from existing laboratory cultures (see table A7_4_1_2-3), neonates used
Test system	(see table A7_4_1_2-4)
Test conditions	(see table A7_4_1_2-5)
Duration of the test	48 hours

Section A7.4.1.2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 *Daphnia magna*

Test parameter	Mortality after 24 and 48 hours
Sampling	No analysis reported
Monitoring of TS concentration	No
Statistics	Probit analysis using the SAS statistical program ((SAS Inc. (1985) User's Guide: Statistics. Version 5. SAS Institute, Inc., Cary, NC) to estimate LC50. Mortality data to compare water hardness and sulphate were analyzed by ANOVA with a Duncan's Multiple Range test for comparison of treatment means.

RESULTS

29.10 Limit Test	Performed
Concentration	Values not reported; used to determine final experimental levels
Number/ percentage of animals showing adverse effects	
Nature of adverse effects	
29.11 Results test substance	
Initial concentrations of test substance	Not reported
Actual concentrations of test substance	Not reported
Effect data (Immobilisation)	Raw data not reported in journal publication. 48-hour LC_{50} = 141 mg-B/L (95 % C.I. = 123 to 159 mg-B/L)
Concentration / response curve	Not reported
Other effects	No significant effects of water hardness or sulphate were observed at any level tested.
29.12 Results of controls	Control mortality below 4%

Section A7.4.1.2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 *Daphnia magna*

29.13 Test with reference substance	Not performed
Concentrations	
Results	
	APPLICANT'S SUMMARY AND CONCLUSION
29.14 Materials and methods	Method reflected 1975 standard guidance. Series of replicates tested and mortality was monitored. To evaluate interactions with water hardness and sulphate levels, the dilution water was modified and daphnids tested at the LC50 as determined in the initial test. Test appears to have followed the guideline. However, no concentration measurements are reported, nor are raw data. This is consistent with publication in scientific journals, but may not meet current OECD data reporting requirements. Numbers of animals tested at each concentration, test duration, etc. appear to meet current guidance.
29.15 Results and discussion	Results of 141 mg-B/L were consistent with subsequent tests at different hardness and sulphate levels: when exposed to the LC50 value, mortalities ranged from 30 to 60% in the subsequent 10 tests. The test results are also consistent with an earlier published study by Gersich (1984) which found a 48-hr LC50 of 144 mg-B/L (95% CI: 115-153 mg-B/L), and slightly below the study of Lewis and Valentine (1981) which found a 48-hr LC50 of 226 mg-B/L (200-246 mg-B/L)
EC_0	
EC_{50}	141 mg-B/L (95% confidence interval : 123-159 mg-B/L)
EC_{100}	
29.16 Conclusion	Validity criteria can probably be considered as fulfilled. There is no discussion of daphnids remaining at surface, but boron does not deplete oxygen nor produce a surface film that might adhere to neonate daphnids. Test concentrations were not reported, beyond a note (Table 3) that it was "as calculated (±8%)" which would permit use of nominal concentrations under current OECD guidance.
Reliability	2 – acceptable with limitations
Deficiencies	Yes - raw data not reported, followed older standard method, not certified GLP, but followed accepted scientific procedures, as acceptable to peer-reviewed technical publication. Overall, a technically valid investigation, suggesting result may be relied upon.

Section A7.4.1.2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 *Daphnia magna*

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*08-02-2005*
Materials and Methods	Test was performed with sodium tetraborate decahydrate ($Na_2B_4O_7$. 10 H_2O) , results are expressed on the basis of elemental boron (B). Applicant's version is adopted. with the following addition: Volume of test vessels in table A7_4_1_2-4 should be stated as "500 mL glass jars containing 100 mL test solution". And this leads to a volume/animal of "10 per jar = 10 mL per individual" (instead of the 50 ml that is mentioned now).
Results and discussion	*Applicant's version is adopted.*
Conclusion	*Revised version:* Validity criteria can probably be considered as fulfilled. There is no discussion of daphnids remaining at surface, but boron does not deplete oxygen nor produce a surface film that might adhere to neonate daphnids. **However, t**est concentrations **(both nominal and measured)** were not reported, beyond a note (Table 3) that it was "as calculated (±8%)" which would permit use of nominal concentrations under current OECD guidance. **Furthermore, immobility data were also not presented**
Reliability	2 – reliable with limitations, raw data not reported, followed older standard method, not certified GLP, but followed accepted scientific procedures, as acceptable to peer-reviewed technical publication. Overall, a technically valid investigation, suggesting result may be relied upon.
Acceptability	Acceptable, the result 48-hours EC_{50} 141 mg B/L is included in the risk assessment.
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 A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	
Vehicle control performed	No
Other procedures	

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	*US EPA reconstituted moderately hard water, modified for hardness and sulphate content in later experiments*
Alkalinity	
Hardness	*See tables below*
pH	
Ca / Mg ratio	*See tables below*
Na / K ratio	*See tables below*
Oxygen content	
Conductance	
Holding water different from dilution water	Not reported

Hardness modification:

Hardness as mg/L CaCO3	(mg/L)			
	NaHCO3	CaSO4.2H2O	MgSO4	KCl
10.6	12	7.5	7.5	0.5
42.5	48	30	30	2
*85	96	60	60	4
127.5	144	90	90	6
170	196	120	120	8

Sulfate modification (values are mg/L):

Sulfate	NaHCO3	CaSO4.2H2O	MgSO4	KCl	CaCl2	MgCL2	NaSO4
10.2	96	7.5	7.5	4	44.8	88.7	0
40.7	96	30	30	4	25.6	50.6	0
*81.4	96	60	60	4	0	0	0
162.7	96	60	60	4	0	0	60.2
325.4	96	60	60	4	0	0	120.3

***US EPA moderately hard water**

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	Lab culture
Source	
Age	Neonate
Breeding method	(Parthenogenic)
Kind of food	Not reported
Amount of food	
Feeding frequency	
Pretreatment	Not reported
Feeding of animals during test	Not reported

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	Not reported
Volume of test vessels	500 ml glass jars
Volume/animal	10 per jar = 50 ml per individual
Number of animals/vessel	10
Number of vessels/ concentration	5
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	*20. ±0.1 °C*
Dissolved oxygen	*8.6 ±0.2 mg O2/L*
pH	Hardness (mg/L as CaCO3) / pH Control: 10.6 / 7.3±0.1 Control: 42.5 / 8.0±0.1 Control: 85 / 8.2±0.1 Control: 127.5 / 8.4±0.1 Control: 170 / 8.6±0.1 Treatment: 10.6 - 170 / 9.1±0.1
Adjustment of pH	No
Aeration of dilution water	Not reported
Quality/Intensity of irradiation	Not reported
Photoperiod	Not reported

Table A7_4_1_2-6: Immobilisation data

Test-Substance Concentration (nominal/effective)[1] [mg/l]	Immobile *Daphnia*						
	Number		Percentage		Oxygen [mg/l]	pH	Tempera-ture [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Not reported							

[1] specify, if TS concentrations were nominal or measured

Table A7_4_1_2-7: Effect data

	EC_{50} [1]	95 % c.l.	EC_0 [1]	EC_{100} [1]
24 h [mg/l]				
48 h [mg/l]	141 mg-b/L (n)	123-159		

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	Not fullfilled
Immobilisation of control animals <10%	X	
Control animals not staying at the surface		?
Concentration of dissolved oxygen in all test vessels >3 mg/l	X	
Concentration of test substance ≥80% of initial concentration during test		?

Criteria for poorly soluble test substances ergänzen		

Section A7.4.1.3 **Growth inhibition test on algae**

Annex Point IIA7.3

Official use only

30 REFERENCE

30.1 Reference

[redacted], (2000) "Determination of the effect of Boric Acid, Manufacturing Grade on the growth of the fresh water green alga, Selenastrum capricornutum. [redacted]

[redacted]

30.2 Data protection

Yes

Data owner

[redacted]

Criteria for data protection

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

GUIDELINES AND QUALITY ASSURANCE

30.3 Guideline study

Yes - OECD Guideline no. 201

30.4 GLP

Yes

30.5 Deviations

No

METHOD

Section A7.4.1.3 **Growth inhibition test on algae**

Annex Point IIA7.3

30.6 Test material	As given in section 2 - Boric Acid Manufacturing Grade
Lot/Batch number	Not available
Specification	As given in section 2
Purity	+99.9%
Composition of Product	
Further relevant properties	Water solubility 4.7% at 20°C.
Method of analysis	ICP-AES (Inductively coupled plasma atomic emissions spectrometry) at wavelength of 249.704 nm and 208.964 nm.
30.7 Preparation of TS solution for poorly soluble or volatile test substances	
30.8 Reference substance	No
Method of analysis for reference substance	
30.9 Testing procedure	Non-entry field
Culture medium	Mineral composition per OECD 201, additional $NaHCO_3$ (150 mg/L) to improve buffer capacity Hardness (Ca + Mg) = 24.2 mg equivalent $CaCo_3$/L Fe-citrate pH 7.3 to 8.4
Test organisms	Selenastrum capricornutum ATCC 22662. Give details on tested organisms in tabular form (see table A7_4_1_3-2)
Test system	Incubation with fluorescent lighting, orbital shaker (see table A7_4_1_3-3)
Test conditions	(See table A7_4_1_3-4)
Duration of the test	76 hours

Test parameter

Cell concentration over time, total cell volume measured as total particle volume (to represent algal biomass). Inhibition determined by comparisons with controls X

Sampling

Sampled at 0, 26, 49.5 and 76 hours

Monitoring of TS concentration

Yes at initiation and termination

Statistics

EC values for growth rate and logistic growth were calculated by a weighted least square fitting of a logistic growth model to the results, per Kooijman, Hanstveit and Oldersma (1983), Water Research 17: 527-538. X

EC values for area under the growth curve (biomass) were calculated by linear interpolation of the plotted percent reduction in growth vs. log concentration of test substance, per OECD Guideline 201.

NOEC values were estimated by comparison of measured and calculated growth values of controls and treatment groups. The NOEC was determined using three factors relating to a 10% change relative to controls: total volume, calculated growth rate (corresponding to the E_rC_{10}) and the area under the growth curve (corresponding to E_bC_{10}). In addition, a No-effect-concentration (NEC) was calculated using the model described in Kooijman, Hanstveit and Nyholm (1996) Water Research 30: 1625-1632, and Kooijman and Bedaux (1996) "The analysis of Aquatic Toxicity Data" VU University Press, Amsterdam.

RESULTS

30.10 Limit Test

Not performed

Concentration

Number/ percentage of animals showing adverse effects

30.11 Results test substance

Non-entry field

Initial concentrations of test substance

Nominal	Measured
Control	1.1 mg/L boric acid
32	30.9
100	95.5
320	301.4

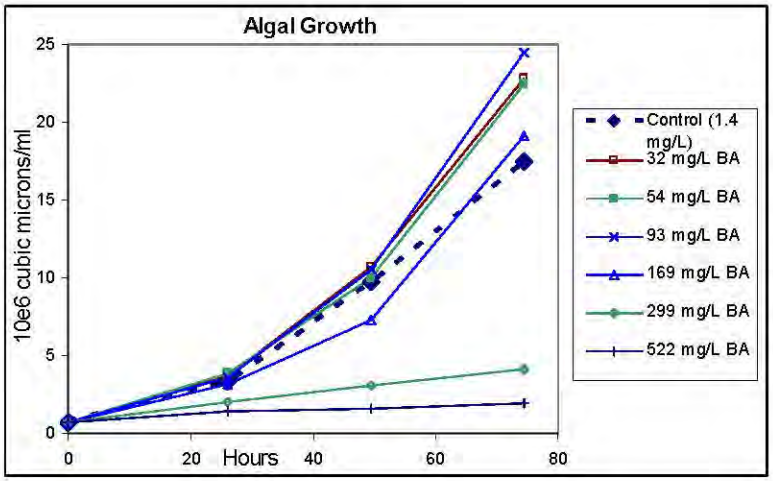
X

Actual concentrations of test substance

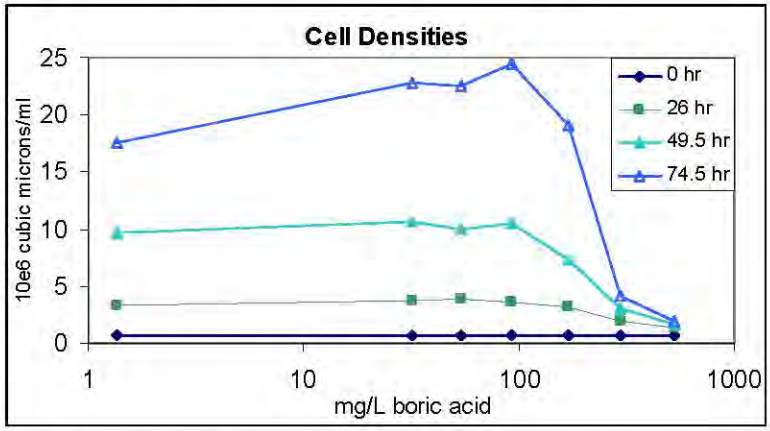
At 74.5 hours:

Nominal	Measured	Average (all measurements)
Control	1.6 mg/L boric acid	1.4 mg/L boric acid
32	32.6	32
100	89.6	93
320	296	299

Growth curves



Concentration / response curve



Cell concentration data

(see table A7_4_1_3-5) X

Effect data (cell multiplication inhibition)

X

Endpoint Values (mg/L boric acid):

Time	Endpoint	Value	(95% Conf Limit)
74.5 hr	E_rC_{50} – growth	277	(240-313)
74.5 hr	E_rC_{10} – growth	185	
74.5 hr	E_bC_{50} – biomass	212	(167-295)
74.5 hr	E_bC_{10} – biomass	130	
74.5 hr	NOEC	93	
74.5 hr	LOEC	169	

The NOEC was determined using three factors relating to a 10% change relative to controls: total volume, calculated growth rate (corresponding to the E_rC_{10}) and the area under the growth curve (corresponding to E_bC_{10}). The group with 169 mg/L had a 13% decrease in cell density. The E_rC_{10} was 185 mg/L. The E_bC_{10} was 130 mg/L The treatment group of 93 mg/L boric acid was therefore identified as the NOEC with the 169 mg/L group becoming the LOEC. The authors calculated the NEC using the model of Kooijman et al. and estimated the NEC to be 157 mg/L, which was taken as support that the NOEC of 93 mg/L was a reasonable approximation.

Note: values are expressed using probable concentrations; authors presented results using nominal concentrations only.

Other observed effects	Cells in the two highest treatment groups were few in number and microscopic examination showed most of these to have abnormal morphology.
30.12 Results of controls	Mean control cell densities were 1, 5.2, 16.9, and 36 (x10e4 cells/ml) at the four sample times.
30.13 Test with reference substance	Not performed
Concentrations	
Results	

APPLICANT'S SUMMARY AND CONCLUSION

30.14 Materials and methods	Standard test for algal growth inhibition using OECD 201 procedures. Test method involves inoculation of Selenastrum capricornutum into standard medium with test substances and monitoring algal growth for about 3 days. Growth was monitored via automated procedure (Coulter Counter) as the number of particles and the total particle volume. Data were corrected for background particle counts then used to fit growth models. Some of the growth models are not prescribed by the OECD Guideline, but were developed by other work done by the study authors.
30.15 Results and discussion	Boric acid concentrations of 299 and 522 mg/L had clear inhibitory effects. Effects at 169 mg/L boric acid were determined to be significantly different from the controls, but differences were less evident. Cell volume of the 169 mg/L treatment exceeded the control mean at the final sampling period. Cell densities and particle volumes at the three lowest treatments exceeded those of controls.
NOE_rC	93 mg/L boric acid (equivalent to 16.3 mg-B/L)
E_{r50}	277 mg/L boric acid (equivalent to 48 mg-B/L)
E_bC_{50}	212 mg/L boric acid (equivalent to 37 mg-B/L)
30.16 Conclusion	Validity criteria for controls were met. A reasonable dose-response relationship was demonstrated.
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	17-01-2005
Materials and Methods	Applicant's version adequately reflects the report.
Results and discussion	Remarks to applicant's summary of results: Section 4.2.5: Concentrations in table *A7_4_1_3-5* are nominal concentrations in 100 mL before addition of the 1 mL algae suspension. Calculated concentrations of boric acid in the final test solutions with algae are 0, 32, 56, 100, 181, 321 and 562 mg/L. Section 5.2: typo, 522 mg/L should read 562 mg/L. Comments on the study: Blank algal medium contains 185 µg boric acid/L. Expressed as elemental boron, this is equivalent to 32 µg B/L. Actual background concentrations in the control (without algae) were higher: 0.2 and 0.28 mg B/L, equivalent to 1.1 and 1.6 mg boric acid/L. These background concentrations are < 5 % of the lowest test concentration. Only mean cell counts are presented, individual numbers per replicate are not given. Author's derive endpoints based on cell volumes and not on cell numbers. This is done because particle size varied and cell volumes are thus assumed to present a better estimate of the biomass. According to the EU-TGD, the endpoint should be based on growth rate and RMS considers cell numbers more appropriate for the calculation of the E_rC_{50}. Results of the chemical analysis are included as an Annex to the report, but identification of sample codes is not provided. Authors calculate area under the growth curve conform OECD 201 (1984). In discussions on the revision of this guideline, it was made clear that the area should be calculated based on ln-transformed cell counts (see draft July 2002). According to EU-TGD, only growth rate should be used as endpoint. Authors present NOEC and EC_{50} based on nominal concentrations, applicant presents values based on measured concentrations. Applicant's NOEC is based on mean measured concentrations at the corresponding nominal exposure level. It is not clear how applicant's EC_{50}'s are derived, a new model fit with measured concentrations is apparently not performed. Besides, test concentrations 56, 181 and 562 mg boric acid/L were not included in the chemical analysis. Applicant correctly states that some of the statistical procedures as used by the authors are not prescribed by OECD. Author's NOEC is set at the next lower concentration to the estimated No Effect Concentrations (NEC). As the NEC represents an EC_0, this is considered to be over-conservative. Either the NEC or the concentration with no statistical difference with the control should be considered as NOEC. Authors omit time point 74.5 h from the estimation of the NEC because growth was no longer exponential between 49.5 and 74.5 h. It is not clear whether the last sampling point was included in the determination of the area under the growth curve. A comparison of the control growth rate over time intervals 0 – 1, 1 -2 and 2 -3 shows that the coefficient of variation in daily growth rates is > 35 % (OECD validity criterion) when the last time point is included, and 12 - 22 % when the last sampling point is omitted. This indicates that growth was indeed not longer exponential between 49.5 and 74.5 h. Average cell numbers (x 10^4 cells/mL) at respective test concentrations and growth rate per hour, calculated by RMS according to revised draft OECD 201, are given in Table below:

Concentration [mg/L boric acid]	Mean measured concentration [mg/L boric acid]	Time [h] 0	26.0	49.5	74.5	growth rate 0 – 49.5 h [h^{-1}]	% change relative to control
control[1]	1.4	1.0	5.2	16.9	35.9	0.063	-
32	32	1.0	6.0	22.8	48.0	0.062	10.8
56	n.a.	1.0	5.9	21.4	43.3	0.060	8.5
100	93	1.0	5.4	19.4	44.3	0.047	5.0
181	n.a.	1.0	4.1	10.1	30.8	0.018	-18.1
321	300	1.0	1.7	3.8	5.5	0.008	-69.2
562	n.a.	1.0	1.4	1.5	1.6	0.016	-85.6

1: average of two mean values presented in report

n.a.: not analysed

The 49.5 h E_rC_{50} was estimated by non-linear fit assuming a logistic concentration response relationship as 255 mg/L (95 % CI 214 – 303 mg/L), based on nominal concentrations of added boric acid. This is equivalent to 44.6 mg B/L. The NOEC cannot be calculated because cell counts for individual replicates are not given. The 74.5 h E_rC_{50} was not estimated since growth rate was not exponential between 49.5 and 74.5 h.

The 74.5-hours NOE_rC based on cell volumes is 100 mg/L, expressed as nominal concentration of added boric acid (William's test, $p < 0.05$). This is equivalent to 17.5 mg B/L. Concentration in control is 0.24 mg B/l.

Conclusion

Because remarks above mainly consider data treatment and not the performance of the study itself, the recalculated endpoints are considered reliable.

Reliability

1

Acceptability

Acceptable, the 49.5-hours E_rC_{50} 44.6 mg B/ and 74.5-hours NOE_rC 17.5 mg B/L are included in the risk assessment.

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 A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	
Vehicle control performed	No
Other procedures	

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	Selenastrum capricornutum
Strain	ATCC 22662
Source	American Type Culture Collection, Rockville Md, USA
Laboratory culture	Yes
Method of cultivation	Per OECD Guideline 201, using specified algal medium, with additional NaHC03 (150 mg/L) and Fe-citrate
Pretreatment	None
Initial cell concentration	1.0 x. 10^4 cells/ml measured in control cultures

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	100 ml of media in 200 ml conical glass flasks
Culturing apparatus	Incubation at 23 ± 2°C
Light quality	Fluorescent lamps, 60-120 micromol/sec/m^2 as measured with Bottemanne Weather Instruments Photosynthetic Radiometer RA 200Q
Procedure for suspending algae	Orbital shaker at approximately 100 rpm
Number of vessels/ concentration	6 control, 3 vessels/concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	23 ± 2°C (data not reported)
pH	Start: 7.3 to 8.2, End: 7.8 to 8.4
Aeration of dilution water	No
Light intensity	60-120 micromol/sec/m^2 as measured with Bottemanne Weather Instruments Photosynthetic Radiometer RA 200Q
Photoperiod	

Table A7_4_1_3-5: Cell concentration data

Test-Substance Concentration (nominal)[1] [mg/l]	Cell concentrations (mean values) [10e4 cells/ml]							
	measured				Percent of control			
	0 h	26 h	49.5 h	76 h	0 h	26 h	49.5 h	76 h
Control (6 replicates)	1	5.2	16.9	35.85	100%	100%	100%	100%
32 mg/L as boric acid	1	6	22.8	48	100%	115%	135%	134%
57	1	5.9	21.4	43.3	100%	113%	127%	121%
101	1	5.4	19.4	44.3	100%	104%	115%	124%
182	1	4.1	10.1	30.8	100%	79%	60%	86%
324	1	1.7	3.8	5.5	100%	33%	22%	15%
568	1	1.4	1.5	1.6	100%	27%	9%	4%
Temperature [°C]								
pH	7.3 – 8.2			7.8 – 8.4				

[1] TS concentrations were nominal

3. Tables for Applicant's Summary and Conclusion

3.1 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**Yes**	
Concentration of test substance ≥80% of initial concentration during test	**Yes**	

Criteria for poorly soluble test substances		

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA7.4

Official use only

31 REFERENCE

31.1 Reference

[redacted] (2000) "Screening of the effect of Boric Acid, Manufacturing Grade on the respiration rate of activated sludge." [redacted]

[redacted]

31.2 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

31.3 Guideline study

Yes - OECD Guideline no. 209

31.4 GLP

Yes

31.5 Deviations

No

MATERIALS AND METHODS

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA7.4

31.6 Test material	As given in section 2 - Boric Acid Manufacturing Grade	
Lot/Batch number	Not available	
Specification	As given in section 2	
Purity	+99.9%	
Composition of Product		
Further relevant properties	Water solubility 4.7% at 20°C.	
Method of analysis	Not measured.	
31.7 Preparation of TS solution for poorly soluble or volatile test substances		
31.8 Reference substance	Yes – 3,5-dichlorophenol	
Method of analysis for reference substance	Not reported	
31.9 Testing procedure	Non-entry field	
Culture medium	BOD dilution water per NEN 6634 ("Water – Determination of biological oxygen demand after n days (BOD n): Dilution atnd seeding method." Nederlands Normalisatie-institut, Deflt June 1991) Synthetic sewage feed prepared per OECD 209	X
Inoculum / test organism	Activated sludge taken from an oxidation ditch at district of Hazerwoude Dorp, the Netherlands on 11 August 1999. The ditch is used to treat domestic sewage. (see table A7_4_1_4-2)	
Test system	(see table A7_4_1_4-3)	
Test conditions	(see table A7_4_1_4-4)	
Duration of the test	Oxygen consumption measured for 10 minutes after 3 hours incubation	X

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA7.4

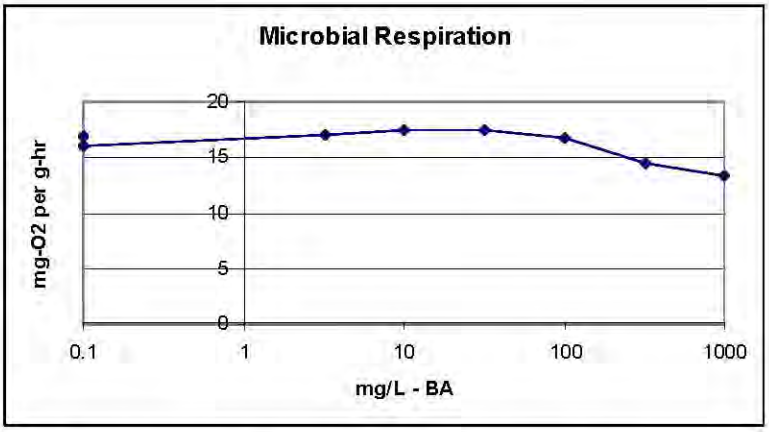
Test parameter	Respiration inhibition
Analytical parameter	Oxygen measurement – consumption of oxygen per gram dry weight active sludge per hour
Sampling	3 hours of incubation
Monitoring of TS concentration	No
Controls	Controls established without test substance or reference substance. Initial control was prepared before test and reference systems, final control was prepared after test and reference systems
Statistics	Systems were not replicated. Responses calculated as percent inhibition of control average, then a line fitted to respiration as function of concentration using a maximum likelihood estimate following method of Kooijman(1981) Water Research 25: 401-408. EC50 estimated from line and EC20, EC80 calculated from slope of line.

RESULTS

31.10 Preliminary test	Not performed
Concentration	
Effect data	
31.11 Results test substance	*Non-entry field*
Initial concentrations of test substance	*0, 3.2, 10, 32, 100, 320 and 101 mg/L – boric acid*
Actual concentrations of test substance	
Growth curves	
Cell concentration data	
Concentration/ response curve	Plot of the Oxygen consumption vs. concentration of test substance

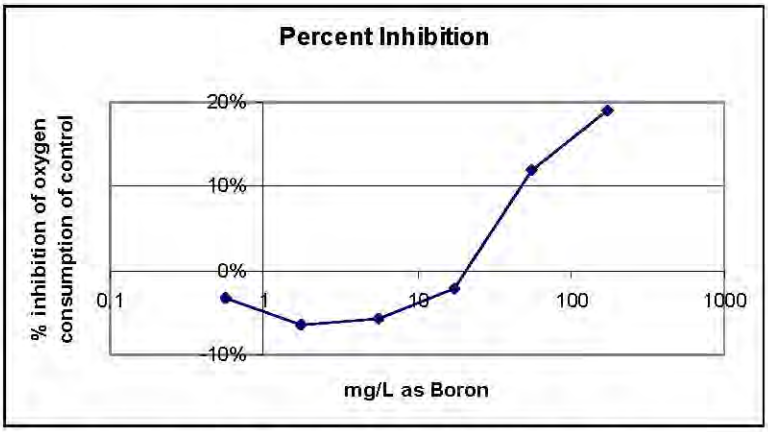
Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4



(Note: Control mis-labelled as 0.1 mg/L to fit on log scale)

Plot of inhibition as percent of control oxygen consumption



Effect data

EC_{50} > 1001 mg/L Boric acid (no C.I. calculatable)
EC_{20} = 638 mg/L Boric acid (95% CI: 495-821 mg/L)
EC_{80} >> 1001 mg/L Boric acid
NOEC stated as 100 mg/L Boric acid

Other observed effects

Initial control: 16.9 mg-O2 /g-hr

31.12 Results of controls

Performed using 3,5-dichlorophenol

31.13 Test with reference substance

5, 12, 30 mg/L DCP

Concentrations

EC_{50} = 11.5 mg/L (95% CI: 6.3 – 21 mg/L)

Results

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA7.4

APPLICANT'S SUMMARY AND CONCLUSION

31.14 Materials and methods

Method measures oxygen consumptions (microbial respiration) of a sewage sludge inoculum in a synthetic medium treated with varying levels of test substance, or with a reference substance (dichlorophenol). Sewage was from a plant that treats domestic sewage. Test substance and microbial inoculum are incubated for 3 hours, then transferred to BOD bottles to measure oxygen consumption in 15 minute period. X

Method is OECD Guideline 209.

31.15 Results and discussion

Inhibition was observed only at extremely high concentrations (320 mg/L or more, boric acid). No EC50 was observed. Maximum inhibition was reported by authors to be 24%; however, the value at 1001 mg/l was reported as 13.3 mg-O2, which is only 19% less than the average control respiration of 16.5. The reference substance demonstrated inhibition within expected OECD range. X

EC_{20}

EC_{20} = 638 mg/L Boric acid (95% CI: 495-821 mg/L)
Restated as boron-equivalents:
EC_{20} = 112 mg-B/L (95% CI: 87-144 mg/L)

EC_{50}

EC_{50} > 1001 mg/L Boric acid
Restated as boron-equivalents:
EC_{50} > 175 mg-B/L

EC_{80}

EC_{80} > 1001 mg/L Boric acid
Restated as boron-equivalents:
EC_{80} > 1001 mg-B/L X

31.16 Conclusion

Test met validity criteria. At higher concentrations, a suitable dose-response pattern was observed.

Reliability

1 – valid without limitation

Deficiencies

No

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA7.4

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	17-01-2005
Materials and Methods	Applicant's summary is acceptable, except for the following points: - 3.4.1 Culture medium: Amount of meat extract in the synthetic sewage feed as mentioned in the study report is significantly higher than recommended by OECD guideline 209 (221 vs. 22 g in 2L), although this might be a typing-error. - 3.4.5 Duration of the test: It is stated that O_2-consumption was measured for 10 minutes, whereas in 5.1 (Materials en methods) a period of 15 minutes is mentioned. This is inconsistent.
Results and discussion	Applicant's summary is acceptable, except for the following points: - The authors state that:"however, the value at 1001 mg/L was reported as 13.3 mg-O_2, which is only 19% less than the average control respiration of 16.5.". This is not correct, because the average control respiration is 17.5 mg-O_2 ((16.9 + 18.0)/2). Therefore, the difference between the value at 1001 mg/L and the average control respiration is 24%. - 5.2.3 EC_{80}: An EC_{80} > 1001 mg/L Boric acid is mentioned, which is restated as >1001 mg/L boron-equivalents. This is incorrect, test result expressed on the basis of elemental boron (B) is > 175 mg B/L.
Conclusion	Test met validity criteria. At higher concentrations, a suitable dose-response pattern was observed.
Reliability	1 – valid without limitation
Acceptability	Acceptable, the result 3-hours EC_{50} > 175 mg B/L is included in the risk assessment.
Remarks	Comments given in the sections above, are most likely to be (related to) typing errors that have to be corrected, but do not affect the validity of the study.
	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*

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	
Vehicle control performed	No
Other procedures	

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not specified
Strain	
Source	Oxidation ditch from sewage treatment plant treating predominantly domestic sewage
Sampling site	District of Hazerswoude Dorp, the Netherlands
Laboratory culture	No
Method of cultivation	
Preparation of inoculum for exposure	Sample centrifuged and supernatant discarded. Sludge washed three times in tap water (twice) and dilution water (once). Suspension was diluted to 4.9 g/L mixed liquor suspended solids with dilution water, aerated vigourously, and kept at 20° C in the dark. Sludge was used the following day.
Pretreatment	None
Initial cell concentration	3.7 mg suspended solids/L

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	Initial mixing and 3 hour incubation was in 1 L glass beakers. Samples were transferred to BOD flasks for measurement of inhibition
Number of culture flasks/concentration	1
Aeration device	Yes
Measuring equipment	WTW OXI 2000 O_2-electrode
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	Not reported
pH	Range from 7.7 to 8.0 in test samples
Aeration of dilution water	Yes – flow not specified other than "vigourously"
Suspended solids concentration	Adjusted to 3.7 mg/L

Section A7.4.2 **Bioconcentration in aquatic organisms**

Annex Point IIA7.5 **Section A7.4.2**

Official use only

32 REFERENCE

Reference

Thompson, J.A.J., J.C. Davis, and R.E. Drew. 1976. "Toxicity, uptake and survey studies of boron in the marine environment." Water Research 10: 869-875.

(Authors' affiliation: Environment Canada)

Data protection

No

Data owner

Published article

Criteria for data protection

No data protection claimed

GUIDELINES AND QUALITY ASSURANCE

Guideline study

No – predates guidelines

GLP

No – Predates GLP and involves field survey

Deviations

MATERIALS AND METHODS

Section A7.4.2 Annex Point IIA7.5	Bioconcentration in aquatic organisms Section A7.4.2	
Test material	Sodium metaborate, analytical grade	
Lot/Batch number		
Specification		
Purity		
Further relevant properties		
Radiolabelling		
Method of analysis	Seawater samples were analysed by modified curcumin colorimetric procedure (Grinstead and Snider, 1967). Uptake experiment analyses used method of Uppstrom (1968). Tissue samples digested in sulphuric acid/hydrogen peroxide, dehydration with acetic anhydride, and measurement of boron-curcumin complex in buffered solution at 545 nm (Drew, 1975) References: Drew RE (1975). "A simplified spectrophotometric curcumin method for the determination of boron in marine shellfish." J. Fish Red Bd Can 32: 813-816. Grinstead, R.R. and S. Snider (1967) "Modification of the curcumin method for low level boron determination." Analyst, Lond 87: 956-969. Uppstrom, L.R. (1968) "A modified method for determination with curcumin and a simplified water elimination procedure." Anlyt Chim Acta 43: 475-486.	X
Reference substance	No	
Method of analysis for reference substance		
Testing/estimation procedure	Non-entry field	

Test system/ performance

Boron uptake was studied in young Pacific oysters (Crassostrea gigas, wet tissue weight 4.0 to 6.29 g). Organisms were exposed in 80-L fibreglass tanks containing 60 L of test solution. A continuous flow of fresh seawater of 500 ml/min were estimated to provide 90% replacement every 6 hours. One tank received no additional boron. Two tanks received 1 mg-B/L above background. Two tanks received 10 mg-B/L above background. Boron was added as a continuous flow of a concentrated borate solution (2.0 ml/min).

30 oysters were placed in each tank with 5 individuals removed on days 8, 16, 36 and 47 after initiating boron addition. Triplicate determinations of boron concentrations were made on the pooled oyster samples. On day 47, boron administration was stopped and oysters were sampled on day 71 to observe depuration.

Field surveys collected 71 samples of seawater at surface and 5 m depth from 4 areas around Vancouver Island in southern British Columbia (Canada). Oyster tissue surveys were collected near groundwood pulp mills, before and after the mills began use of a process that would release borates as a by-product. Estimated boron emissions would be less than 1 mg-B/L. The number of oysters sampled was not reported.

Analyses were conducted as described in section 3.1.6. Standard deviation of the seawater analyses was reported as 0.1 to 2%. Standard deviation of the tissue analysis was about 5%.

Estimation of bioconcentration

Authors did not calculate bioconcentraton factor (BCF) values. Data were presented as comparisons between control and boron-added systems. When significant amounts of boron were added (e.g., 10 mg/L), tissue levels increased. However, when boron additions ceased, tissue concentrations decreased to those of the controls. The authors concluded that "... oysters appeared to take up boron in relation to its availability.... A drop of environmental boron was followed by a drop in internal boron concentration in oysters (Fig. 3), illustrating that the element was not bound in the tissues."

In field surveys, no evident differences were observed between samples taken before or after the change in pulp processing that introduced additional boron emissions. The authors concluded that "the results of the oyster field surveys also suggest that no significant accumulation in tissue was occurring at that time. A slightly higher mean tissue concentration for the November sampling probably reflects the higher salinities existing during a period of lower freshwater runoff."

The authors presented a summary of boron levels found in various marine shellfish in British Columbia (Canada) which ranged from 0.9 to 5.5 mg/kg B (wet weight). They concluded that "in all species tested, levels of boron approximating that in the water are attained in the tissues. There is no evidence to suggest that bioaccumulation of boron does occur."

RESULTS

Non-entry field

Experimental data

Mortality/behaviour

No mortality reported. Authors reported that all oysters were observed open and actively pumping a good portion of the test time.

Lipid content

Not reported

Concentrations of test material during test

Boron concentrations in 71 seawater samples averaged 3.53 mg-B/L (standard deviation 26.1%0 at surface and 3.86 mg/L (standard deviation 28.8%) at 5 m depth.

Background boron levels in oyster tissue were reported as 3.84 (3.67 to 4.01) mg/kg wet weight. Data were presented as a graph of tissue concentration vs. time. At day 8, tissue levels were only slightly changed from day 0. Maximum tissue concentrations were observed on day 36. Values (in mg/kg) estimated from Fig. 3 are tabled below

Added Boron	Day 8	Day 36	Day 47	Day 71
0	3.6	5.9	4.7	3.4
1	3.8	6.9	5.6	3.8
1	3.8	7.3	5.2	3.8
10	4.2	12.5	11.3	3.4
10	4.2	13.8	10.3	4.2

Depuration was started on day 47. By day 71, all tissue levels were at background.

Mean boron levels in field surveys were:

Crofton site: 3.2 mg/kg(sd = 0.5, range 2.6 to 3.8) in May 1973 (before process change), and 4.0 mg/kg (sd= 0.7, range 2.8 to 4.5) in November 1973 (after process change)

Powell River site: 3.6 mg/g (sd = 0.3, range 3.1 to 3.9) in May 1973 (before process change), and 3.8 mg/kg(sd = 1.2, range 2.6 to 5.1) in June 1974 (after process change).

The authors did not calculate BCF values or uptake/depuration rates. See section 5.2 for a re-creation of BCF values

Bioconcentration factor (BCF)

No BCF values were calculated by authors. See section 5.2

Uptake and depuration rate constants

No rates were calculated by the authors.

Depuration time

Based on limited data, virtually all additional boron was eliminated (return to background levels) within the 24 days after boron addition was stopped. The authors state that the rate of clearance is not known.

Metabolites

Other Observations

Estimation of bioconcentration

The authors conclude that there is no evidence to suggest that bioaccumulation of boron does occur.

In addition to the oyster studies reviewed here, the authors exposed salt-water acclimated sockeye salmon (Oncorhynkus nerka) at 0, 10 and 10 mg-B/L. Tissue levels were elevated in sockeye after 3 weeks exposure, but the authors concluded that the tissue levels "were not vastly different from water boron levels, suggesting no evidence for active bioaccumulation of boron in sockeye tissues."

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

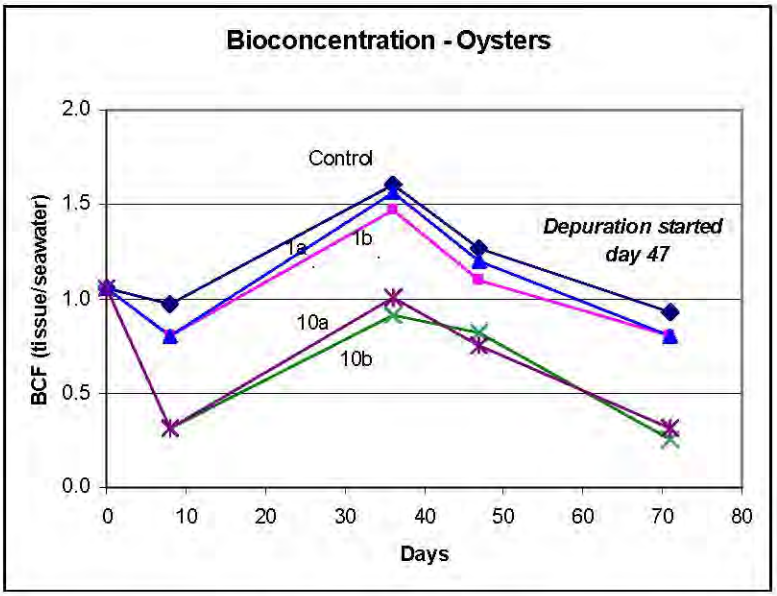
The study followed two distinct approaches: a laboratory study and field surveys. The laboratory study preceded the publication of OECD standard 305 and even preceded the earlier ASTM E-1022-84 standard practice. However, the test design is very similar: aquatic organisms are exposed to elevated concentrations of test substance in the water and tissue concentrations are measured over time. Test substance additions are stopped and decreases in tissue concentrations (depuration) is measured. A flow-through exposure system is used.

Concentrations of boron in the exposure systems was not measured. Because boron occurs naturally in seawater, the test is therefore an indication of changes associated with added boron.

The field survey reflects a desirable before/after design with samples taken before and after process changes occurred that would increase the amount of boron in the system. The analytical methods may not have been as sensitive as current techniques, but it appears that non-detects (and therefore sensitivity of method) were not a problem.

Results and discussion

To re-evaluate the data, values on Figure 3 were translated to estimates as shown in section 4.1.3. To estimate water concentrations of boron, the background concentration was assumed to be that measured in the 71 seawater samples (mean concentration of 3.7 mg-B/L). The ratio of tissue concentration/water concentration could then be calculated. As shown in the graph of calculated BCF values below, the values at day 37 and day 46 were about the maximum observed.



However, these values are in the range of 1 to 1.5, which clearly indicate that BCF values are not very high (BCF values of concern typically are set at 300 to 1000).

Many aspects of the current OECD 305 procedure are important for lipid-soluble organic test substances, but are not critical for inorganics. Consequently, the absence of lipid concentrations, or the choice of test concentrations are not critical limitations.

The field survey results could also be expressed as the ratio of tissue concentrations to ambient water concentrations. Using the mean seawater concentration as before (3.7 mg-B/L) and the field survey oyster concentrations as given in Section 4.1.3, BCF estimates range from 0.87 to 1.07. This also supports the authors' conclusion that bioconcentration is not a significant process for boron. The precision of this approach is limited by the lack of simultaneously measured concentrations, but the results also suggest that bioconcentration is not a significant issue.

Conclusion

ASTM E1022-94 contains a number of acceptability criteria. No evaluation can be made of criteria regarding temperature excursions, dissolved oxygen concentrations, or treatment of disease. No mortality of the organisms was reported, suggesting that potential bioconcentration was not affected by toxicity, and meeting one of the ASTM criteria. Test concentrations were not measured. (The OECD 305 guideline specifies testing of fish but does not discuss bivalves.)

While acknowledging these limitations, the general finding of the studies is that boron is not actively bioconcentrated in these aquatic organisms. The study reaches this conclusion based on both laboratory experimentation and on field survey.

Reference:

ASTM International, 2003. E1022-94 (Reapproved 2002). Standard

Guide for conducting bioconcentration tests with fishes and saltwater bivalve molluscs. Volume 11.05 Standards on Disc.

Reliability

2, Acceptable with limitations: test conditions (temperature, oxygen) not reported; concentrations of test substance not measured in test.

Deficiencies

Yes

Yes – the laboratory procedures lack analytical measurement of test substance concentrations during the test, as well as other data in current protocols to ensure the test is done within standard conditions.

The combination of field survey and laboratory testing makes the case much stronger than either single approach would. In addition, the results show no significant bioconcentration: estimated BCF values are about 1.0, not the BCF values of 300 or greater that characterize POP or PBTs. Therefore, the study is adequate to support the basic absence of bioconcentration, and its conclusion, that boron occurs in organisms in direct proportion to its concentration in the environment, is valid.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	18-01-2005
Materials and Methods	Applicant's summary is acceptable:
Results and discussion	Applicant's version is adopted.
Conclusion	Applicant's version is adopted.
Reliability	3 - limitations: test conditions (temperature, oxygen) not reported; concentrations of test substance not measured in test
Acceptability	Acceptable as supporting evidence, the result maximum BCF in the range 1 – 1.5 L/kg for molluscs is included in the risk assessment.
Remarks	It has to be noted that: This study can not be seen as bioaccumulation study according to OECD 305 but it only provides additional data. There are no measured test concentration in water phase, no (from the author) calculated BCF values, no uptake or depuration rate constants, no clearance time (CT50), no lipid content in oysters, test conditions (e.g. temperature, oxygen content) not reported, little information on condition of exposure. Therefore the calculated accumulation values are indicative showing a low accumulation potential for molluscs and that salmoids show lack of bioaccumulation
	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
Reliability	Discuss if deviating from view of rapporteur member state
Findings	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Remarks	

Section 7.4.3.2 **Annex Point IIIA XIII 2.2**	**Effects on reproduction and growth rate of fish**	
	33 REFERENCE	**Official use only**
Reference	[redacted] (2000) "Early Life Stage test under semi-static conditions with Boric Acid, Manufacturing Grade and the zebra fish Brachydanio rerio [redacted]	
Data protection	Yes	
Data owner	[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 Guideline no. 210*	
GLP	Yes	
Deviations	No	
	METHOD	

Section 7.4.3.2 **Annex Point IIIA XIII 2.2**	**Effects on reproduction and growth rate of fish**	
Test material	*As given in section 2 - Boric Acid Manufacturing Grade*	
Lot/Batch number	*Not available*	
Specification	As given in section 2	
Purity	*+99.9%*	
Composition of Product		
Further relevant properties	*Water solubility 4.7% at 20°C.*	
Method of analysis	*ICP-AES (Inductively coupled plasma atomic emissions spectrometry) at wavelength of 249.704 nm and 208.964 nm.*	
Preparation of TS solution for poorly soluble or volatile test substances		
Reference substance	*No*	
Method of analysis for reference substance		
Testing procedure	*Non-entry field*	
Dilution water	*Dilution water was DSWL-E, prepared from ground water with added salts, See Table A7_4_3_2-2*	
Test organisms	*Brachydanio rerio, zebrafish (see table A7_4_3_2-3)*	
Handling of embryos and larvae (OECD 210/212)	*Initially, 45 fertilized eggs added to each container, then after 24 hours, 20 eggs were retained and others removed.*	
Test system	*Semistatic with 3 renewals per week. Twenty fish per container with 800 ml per beaker, four replicates of each concentration (see table A7_4_3_2-4)*	
Test conditions	*25±1°C, pH 7.2 to 8.0, dissolved oxygen 7.1 to 9.0 mg/L, 16/8 photoperiod (see table A7_4_3_2-5)*	
Duration of the test	*34 days*	

Section 7.4.3.2 **Annex Point IIIA XIII 2.2**	**Effects on reproduction and growth rate of fish**	
Test parameter(s)	*Survival (mortality) during test, length and average weight at conclusion of test. Fish condition was evaluated but not used to establish the NOEC and LOEC.*	X
Examination / Sampling	*Survival monitoring when test solutions replenished (Monday, Wednesday, and Friday). Length and weights measured at conclusion of test.*	X
Monitoring of TS concentration	*Yes* *Boron concentrations measured in clean samples from selected concentrations when test solutions replenished on days 4, 11, 18, 25, and 32. Concentrations measured in the spent samples from those same concentrations two days later, i.e., on days 6, 13, 20, 27 and 34.*	
Statistics	*Mortality was evaluated using binomial model at 95% significance level. Growth was evaluated using two-tailed Dunnett-test at 95% or 99% significance level. LC50 estimated using log-logistic model as implemented in Koojiman, Water Res. 15: 107-119 (1981).*	
	RESULTS	
Range finding test	*Performed*	
Concentrations	*Results not reported*	
Number/ percentage of animals showing adverse effects	*Results not reported*	
Nature of adverse effects	*Results not reported*	
Results test substance	*Non-entry field*	
Initial concentrations of test substance	*Nominal: 0, 0.18, 0.56, 1.8, 5.6, 18, 56 mg-B/L* *Not measured on day 0*	
Actual concentrations of test substance	***Concentrations expressed as mg-B/L*** *Average concentrations from measurements on 10 days are shown for the four groups that were monitored. All 43 measurements were used in simple linear regression. "Probable" concentrations represent the best estimate of actual boron concentrations present. Probable concentrations are the average measured value, or an estimate from the regression if actual measured value not available.*	X

Group	Nominal	Measured	Regression*	Probable**
Control	0	0.56	0.53	0.56

Section 7.4.3.2 Annex Point IIIA XIII 2.2	**Effects on reproduction and growth rate of fish**	

1	0.18	-	0.72	0.72
2	0.56	1.1	1.1	1.1
3	1.8	-	2.4	2.4
4	5.6	6.3	6.4	6.3
5	18	19	19	19
6	56	-	59	59

**Regression estimate obtained from linear regression of all measured values vs. nominal (r-squared = 0.998). Regression equation: Y = 1.434*X + 0.5288 where Y = predicted boron concentration and X = nominal concentration.*

***Authors reported endpoints using nominal concentrations based on their determination that measured values were within 20% of nominal. However, they subtracted control concentrations from all values. This underestimates boron, especially at lower test concentrations.*

For example, nominal concentration (0.56) of group 2 is 50% of measured concentration. The Probable concentration uses average measured values when available or the regression estimate.

Effect data

Hatching rates and Egg Mortality (cumulative) by Treatment Group

Values are presented as N-M, where N =cumulative number of eggs hatched and M=cumulative mortality of eggs

Da	C	1	2	3	4	5	6
1	0-0	0-0	0-0	0-0	0-0	0-0	0-0
2	0-0	0-0	0-0	0-0	0-0	0-0	0-0
3	15-0	6-0	17-0	6-0	9-0	26-0	16-0
4	80-0	80-0	79-0	73-0	51-0	80-0	45-0
5	-	-	80-0	80-0	80-0	-	62-0
6	-	-	-	-	-	-	66-3
7	-	-	-	-	-	-	71-3
8	-	-	-	-	-	-	71-9

Note: On day 0, 180 eggs were distributed to each group. On day 1, 80 viable eggs were retained for each group (20 per container).

Larval Fish Mortality (cumulative) by Treatment Group

Values show cumulative mortality of larvae. Initial number of surviving larvae was 80 for all groups except highest dose where only 71 larvae hatched.

X

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	

y							
8	0	0	0	0	0	0	8
11	0	0	0	0	0	0	46
13	0	0	0	0	0	1	71*
15	0	0	0	1	0	4	-
18	0	0	0	1	0	6	-
20	0	0	0	2	0	6	-
22	0	0	0	2	0	7	-
25	0	0	0	2	0	8	-
27	0	0	0	2	0	10	-
29	0	1	0	2	0	11	-
32	0	1	0	2	0	11	-
34	0	1	0	2	0	11*	-

**Significantly different from control (binomial test, p=0.05)*

Larval Fish Growth Measured by Length

Values show average length (cm) of surviving fish by replicate within treatment group. Standard deviation is shown on line below.

Rep	C	1	2	3	4	5	6
A	1.34	1.29	1.38	1.35	1.36	1.11	-
	0.15	0.26	0.15	0.14	0.19	0.14	
	7	1	7	7	3	7	-
B	1.36	1.37	1.36	1.38	1.31	1.08	-
	0.17	0.21	0.17	0.18	0.19	0.11	
	6	6	3	5	5	3	-
C	1.34	1.32	1.36	1.47	1.33	1.17	-
		0.20	0.24	0.13	0.24	0.11	
	.204	5	4	2	8	8	-
D	1.34	1.32	1.35	1.36	1.36	1.16	-
		0.21	0.18	0.24	0.18	0.12	
	.204	7	2	1	5	0	-
Grou						1.13	
p	1.34	1.33	1.36	1.36	1.34	‡	-
SD	0.17	0.22	0.19	0.18	0.20	0.13	-

‡Significantly less than control (two-tailed Dunnett-test, p=0.01)

Larval Fish Growth Measured by Dry Weight

Values show average dry weight (mg) of all fish per test vessel, divided by number of fish. Group average and standard deviation is also shown.

Re p	C	1	2	3	4	5	6

Section 7.4.3.2 Annex Point IIIA XIII 2.2	**Effects on reproduction and growth rate of fish**	

A	3.4	2.89	3.5	2.95	2.68	1.92	-
B	3.54	2.96	2.56	3.16	2.3	1.59	-
C	3.28	2.61	2.85	3.69	2.78	0.87	-
D	3.38	2.62	2.74	2.98	2.74	1.56	-
		2.77				1.49	
Grp	3.40	*	2.91	3.20	2.63‡	‡	-
SD	0.11	0.18	0.41	0.34	0.22	0.44	-

**Significantly less than control (two-tailed Dunnett-test, p=0.05)*

‡Significantly less than control (two-tailed Dunnett-test, p=0.01)

The authors consider the lesser growth (measured as weight) in Group 1 to be an outlier that does not fit in the dose response relationship and therefore is not used to establish the NOEC or LOEC values.

No malformations were reported.

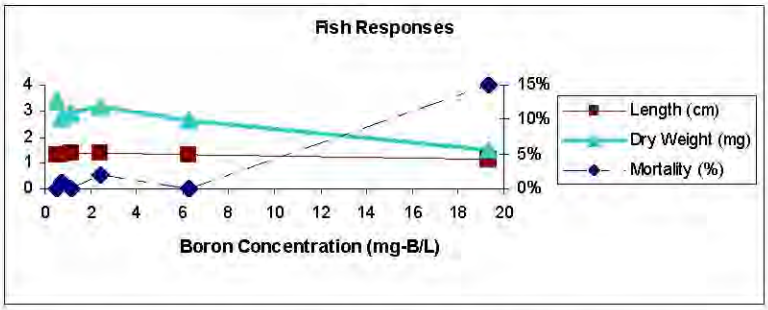
At the two highest concentrations, some slow swimming fish and fish with disturbed swimming behaviour were observed. These visual observations were not quantified.

The NOEC and LOEC values for condition were reported to be 5.6 and 18 mg-B/L (nominal concentrations), which correspond to 6.3 and 19 mg-B/L (measured concentrations).

Endpoint Values:

Time	Endpoint	Value (mg-B/L)
6 day	LC_{50}	>100
34 day	LC_{50}	26
34 day	Mortality - NOEC	6.3
34 day	Mortality – LOEC	19
34 day	Growth (length) – NOEC	6.3
34 day	Growth (length) – LOEC	19
34 day	Growth (dry weight) – NOEC	2.4
34 day	Growth (dry weight) = LOEC	6.3

(Note: values are expressed using probable concentrations; authors presented results using nominal concentrations only. Authors summary states that NOEC for growth (dry weight) was same as NOEC for growth (length), but statistics summary indicates NOEC for growth (dry weight) was lower, as shown in table above.)

Section 7.4.3.2 **Annex Point IIIA XIII 2.2**	**Effects on reproduction and growth rate of fish**	
Concentration / response curve	 *At 59 mg-B/L (not shown), mortality was 100% and occurred by day 13.*	
Other effects	*No malformations were observed. Visual observations noted that swimming and feeding behavor were normal for the control and Groups 1 through 4. Group 5 and 6 fish showed some slow swimming or disturbed behaviour.*	
Results of controls		
Number/ percentage of animals showing adverse effects	*All controls survived.*	
Nature of adverse effects	*None observed*	
Test with reference substance	Not performed	
Concentrations		
Results		
	APPLICANT'S SUMMARY AND CONCLUSION	
Materials and methods	*Standard test for early life stage in fish under static renewal procedures. Dilution water used a ground water that was high in the test substance, so that exposure in control was significantly greater than zero and actual exposures at low concentrations exceed 20% deviation from nominal. Therefore, use of nominal concentrations does not seem justified. Measured concentrations throughout the test allowed construction of a linear regression with very good fit (r-squared = 0.998). Endpoints are reported using probable concentrations based on the measured values or linear regression. Measured concentrations were consistent throughout the study (as shown by fit of regression line), so concentration estimates not time weighted.* *No deviations from the GLP protocol were reported.*	X

Section 7.4.3.2 Annex Point IIIA XIII 2.2	**Effects on reproduction and growth rate of fish**	
Results and discussion	*Mortality was evident at higher concentrations (19 and 59 mg-B/L). Complete mortality was observed in the highest concentration within two weeks of test initiation. No significant mortalities were observed at 6.3 mg-B/L or lower.* *Effects on growth (measured as length) were also significant in the 19 mg-B/L group, with average length about 84% that of control group. Average fish length in other groups typically equalled or slightly exceeded the control group.* *Effects on growth (measured as dry weight of all fish in a test vessel) were significantly reduced in the 6.3 and 19 mg-B/L groups. Average dry weight in those groups was 77% and 44% of control group, respectively.*	
NOEC	*NOEC – survival for 34 day egg/larval exposure : 6.3 mg-B/L* *NOEC – growth measured as length: 6.3 mg-B/L* *NOEC – growth measured as dry weight: 2.4 mg-B/L* *Boric acid contains 17.5% boron by weight, so the above results can be restated based on boric acid:* *NOEC – survival for 34 day egg/larval exposure : 36 mg/L as boric acid* *NOEC – growth measured as length: 36 mg/L as boric acid* *NOEC – growth measured as dry weight: 14 mg/L as boric acid*	X
LOEC	*LOEC – survival for 34 day egg/larval exposure : 19 mg-B/L* *LOEC – growth measured as length: 19 mg-B/L* *LOEC – growth measured as dry weight: 6.3 mg-B/L* *Boric acid contains 17.5% boron by weight, so the above results can be restated based on boric acid:* *LOEC – survival for 34 day egg/larval exposure : 109 mg/L as boric acid* *LOEC – growth measured as length: 109 mg/L as boric acid* *LOEC – growth measured as dry weight: 36 mg/L as boric acid*	X
Conclusion	*Validity criteria were met. Dissolved oxygen remained high throughout the test. Temperature remained within the targeted range of 25±1°C. No mortalities or other adverse effects were observed in the control group.* *Results were demonstrated for all endpoints. Complete mortality was shown in highest treatment group (59 mg-B/L). Effects were evident in mortality, growth as length and growth as dry weight in the 19 mg-B/L group. At the next lower treatment (6.3 mg-B/L), mortality and growth as length were not significantly different from control, but growth as*	

Section 7.4.3.2 **Annex Point IIIA XIII 2.2**	**Effects on reproduction and growth rate of fish**	
	weight was reduced to about 77% of the control group average.	
Other Conclusions		
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	16-09-2005	
Materials and Methods	Test was performed with boric acid, results are expressed on the basis of elemental boron (B). Applicant's version is adopted with minor remarks: 3.4.7. Test parameters: The applicant states that fish condition was evaluated but not used for the determination of a NOEC/LOEC. This is not true. 3.4.8. Examination/sampling: The applicant does not mention the evaluation of fish condition which is performed at each test solution replacement time.	
Results and discussion	4.2.2. Actual concentrations and 5.1 Materials and methods: The applicant states that "*Authors reported endpoints using nominal concentrations based on their determination that measured values were within 20% of nominal. However, they subtracted control concentrations from all values. This underestimates boron, especially at lower test concentrations.*" However, background concentrations also occur in the environment. In the risk assessment, Predicted Environmental Concentrations (PEC's) are therefore based on added concentrations. Subsequently, toxicity values also have to be based on added concentrations. The applicant included the background concentrations of the test solution in this study (approx. 0.56 mg/L) in his endpoint calculations. This is considered inappropriate. 4.2.3 Effect data: Some minor differences are observed between the data reported by the applicant in this summary and the data reported in the study report. However, this did not affect the outcome of the study. 4.2.3: Effect data and 5.2.: Results and discussion: The applicant reported endpoint values based on concentrations including background concentrations. This is not considered correct (see above) and the correct endpoint values should be: Time / Endpoint / Value (mg-B/L) 34 day / LC_{50} / 24 34 day / Mortality - NOEC / 5.6 34 day / Mortality – LOEC / 18 34 day / Growth (length) – NOEC / 5.6	

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	

34 day	Growth (length) – LOEC	18
34 day	Condition – NOEC	5.6
34 day	Condition - LOEC	18
34 day	Growth (dry weight) – NOEC	1.8
34 day	Growth (dry weight) – NOEC	5.6

It should be noticed that nominal as well as extrapolated measured concentrations lead to a $NOEC_{added}$ of 1.8 mg B/l. The measured boron concentration in the control was 0.56 mg B/l.

7 Januari 2008 the applicant submitted a statistical analysis for the derivation of the NOEC (see second section of remarks field). This analysis consisted of Tukey's multiple comparison of means among the different concentrations excluding the control group.
This statistical method considers the null hypothesis that two means are different within a test with several groups (Zar, 1984). The notifier considered that on basis of this analysis the dry weights of groups exposed to nominal concentrations 0.18, 0.56, 1.8 and 5.6 mg B/L were statistically indistinguishable. By principle, however, the approach as applied by the notifier is considered to be incomplete and not in line with the guidance in OECD guideline 210. The OECD guideline proposes the Dunnett's test as already included in the dossier of the applicant in the first place. The guidelines states that the NOEC/LOEC values should be derived by comparison of the individual concentrations with the control and NOT by comparison of means of groups, excluding the control. The approach proposed by the applicant is therefore not accepted.

3 July 2008 the applicant submitted an additional argumentation. This argumentation is discussed by the RMS in the first section of the remarks field. On basis of the available information, however, the RMS sees no reason to change the conclusions of the study. Boric acid is included on Annex I, this gives the notifier the opportunity to extent doc II and III for the product authorisation phase.

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	
Conclusion	Applicant's version is acceptable, except for the comments expressed above. Therefore, a NOEC of 1.8 mg B/L can be used for risk assessment.	
Reliability	1	
Acceptability	Acceptable, the result 34-days NOEC 1.8 mg B/L is included in the risk assessment.	
Remarks	Subscript of letter notifier to RMS including RMS comments to notifier's argumentation (also included are copies of sections of the original report): The EBA believes that the change to the aquatic ecotoxicity endpoint (PNECaquatic, added) is not scientifically justified and that the critical NOEC from the zebrafish early-life-stage study should be 5.6 mg B/L. This value was reported by the study authors and in the initial evaluation by the RMS. **Reaction RMS:** The RMS, in line with the conclusions of the author's report, considers that the NOEC is 1.8 mg/L. The authors quote: "At 5.6 and 18 mg B/L growth of the surviving fish was significantly retarded (p=0.05 and p=0.01 respectively). The significantly lesser growth (p=0.05) at the lowest test substance concentration (0.18 mg B/L), measured as dry weight, is considered to be an outlayer that does not fit in the dose response relationship and therefore is not taken into account to establish the NOEC and LOEC values for growth. **Therefore the NOEC and LOEC with respect to growth is 1.8 and 5.6 mg B/l respectively**". In fact the NOEC of 1.8 mg B/L was already included in doc IIIA as prepared by the notifier.	

TNO report

V99.168
3 March 2000

16 of 35

3.4.3 Growth

The total length per fish and the total dry weight, as determined for the four replicates of each test solution and the control medium are recorded in Annex D. A summary of the results is given in Table 3. The highest concentration tested without a significant effect on growth (measured as weight) was 1.8 mg $B.l^{-1}$.

At 5.6 and 18 mg $B.l^{-1}$ growth of the surviving fish was significantly retarded ($p = 0.05$ and $p = 0.01$ respectively). The significant lesser growth ($p = 0.05$) at the lowest test substance concentration (0.18 mg $B.l^{-1}$), measured as dry weight, is considered to be an outlayer that does not fit in the dose response relationship and is therefore not taken into account to establish the NOEC and LOEC values for growth. Therefore the NOEC and LOEC with respect to growth were 1.8 and 5.6 mg $B.l^{-1}$ respectively.

In the revised draft CAR, the RMS states that it now considers the NOEC to be 1.8 mg B/L, rather than 5.6 mg B/L. In the revised (April 2008) Document IIIA, section 7.4.3.2, the RMS asserts that the OECD 210 guideline requires use of the NOEC derived by use of Dunnett's procedure and that the statistical analysis provided by Rio Tinto Minerals (RTM) is invalid1.
The OECD 210 guideline states that "Dunnett's method may be found useful" but also states that "care must be taken where applying such a method to ensure that chamber to chamber variability is estimated and is acceptably low." The OECD

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	

guideline also states that other examples are available.
RTM provided an alternative statistical analysis for the dry weight data because the existence of an outlier group provides basic evidence of a problem in variability. The dry weight value for the lowest treatment group (nominally 0.18 mg B/L) was considered by the study authors to be an outlier. Both RMS' original evaluation (May 2006) and latest evaluation (April 2008) accepted this interpretation.
The existence of an outlier group suggests caution in applying standard statistical analyses to the larger data set. In fact, the mean dry weight of the outlier group was about the same as for the 4th treatment group (nominally 5.6 mg B/L), so the clear question is whether the 4th treatment group should be considered equally anomalous.

To answer that question, RTM calculated a multiple comparison commonly described as Turkey's multiple comparison procedure. For the dry weight data, the Turkey procedure was used to compare the 4th treatment group with all other groups. The conclusion is that the dry weight of 4th treatment group was not significantly different from treatment groups 1 through 3. In other words, the dry weights of groups exposed to 0.18, 0.56, 1.8 and 5.6 mg B/L cannot be statistically distinguished from each other.

Reaction RMS: The data set indicates a reasonably consistent concentration - effect relationship. The dunnett's test was applied and considered valid. We see no reason to assume that also the 4th treatment group (nominally 5.6 mg B/L) should be considered as invalid. Overlooking the raw data it is clear that for length the NOEC is 5.6 mg B/L, but for dry weight the NOEC is 1.8 mg B/L, both with regard to individual data and mean values.
The guideline recommends the NOEC/LOEC values to be derived by comparison of the individual concentrations with those of the control and NOT by comparison of means of groups. The Tukey assessment provided by the notifier is a comparison of the means of the different exposure concentrations and no comparison with the control. The Tukey procedure as prepared by the notifier does not fulfil the requirements of the guideline and therefore is not applicable.

Table 3 *Summary of results on hatching, mortality and growth of eggs/larvae of* Brachydanio rerio *exposed to several concentrations of Boric Acid, Manufacturing Grade.*

Boron concentration ($mg.l^{-1}$)	% of eggs hatched after 6d	% mortality after 34d	Growth		
			No. of fish	Length[1] (cm)	Dry weight[1] (mg)
0	100	0	80	1.34±0.17	3.40±0.11
0.18	100	1	79	1.33±0.22	2.77±0.18 [3]
0.56	100	0	80	1.36±0.19	2.91±0.41
1.8	100	2	78	1.39±0.18	3.19±0.34
5.6	100	0	80	1.34±0.20	2.62±0.22 [4]
18	100	15	68	1.13±0.13 [4]	1.48±0.44 [4]
56	82	100	0	-	-

[1] Mean and standard deviation (dry weight calculated as explained in the text).
[2] Mortality significantly higher than that of the control animals (binomial test; p = 0.05).
[3] Significantly less than control (two-tailed Dunnett-test, p = 0.05)
[4] Significantly less than control (two-tailed Dunnett-test, p = 0.01)

Visual observations by the authors reported "smaller fish" in treatment groups 1 through 4 (nominal concentrations 0.18, 0.56, 1.8, and 5.6 mg B/L). In reporting on fish "condition" (a judgment based on visual appearance, length and weigh),

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	
	the study authors reported the NOEC based on condition to be 5.6 mg B/L. The authors, while stating the statistical results, did not rely on the dry weight measurements as the representative value for the test. The report summary and Table 2 reported the NOEC for growth to be 5.6 mg B/L and indicated that it reflected a combination of length and weight. This statistical conclusion is consistent with the observations about smaller fish in these 4 treatment group, and supports the authors' interpretation that the NOEC should be considered as 5.6 mg B/L. **Reaction RMS:** In all groups a few smaller fish were observed. These account for not more than 3 fish per treatment group and is only a minor difference in length. This difference will not have influenced the calculations. It may have confused the notifier that at other places in the report for the same growth parameter a higher NOEC was indicated. The Dunnett's test and conclusions, however, remain unchanged. Authors themselves carried out the Dunnett's test and concluded that the NOEC is 1.8 mg B/l. The use of the lowered NOEC results in a PNEC of 0.18 mg B/L. However, RMS previously noted that the NOEC from mesocosm studies were 0.7 mg/L, which is suggested as confirming a PNEC of 0.64 mg/L (Comment 138). The newly revised PNEC contradicts this earlier comment by RMS. Further, Rowe et al. (1998)2 reported a NOEC of 13 mg B/L (9.2 mmol) for zebrafish, in sharp contrast with the use of the RMS-recommended NOEC. The RMS estimated a PNEC derived from an SSD, but has put that approach aside in lieu of the use of a single species value in concluding the ecological hazard assessment in the Biocides dossier review. The EBA suggests that the SSD approach may need to be revisited before finalization of the CAR for boric acid. **Reaction RMS:** It should be emphasised that these mesocosms studies all are carried out without fish and therefore cannot replace ecotoxicity tests with fish. The value of the mesocosm might be used for assessment of the other taxonomic groups, however, these mesocosms have not been evaluated in line with the BPD and are not included doc IIIA and therefore cannot be used for the assessment. **Reaction RMS:** We agree with the notifier that the SSD method would be preferable. Precondition is, however, that all tests used in the SSD have been evaluated and included in doc IIIA, as was decided at TM level. Because most of the studies used in the SSD were not included in doc IIIA and have not been evaluated in line with the BPD requirements, these could not be used for the PNEC derivation. Therefore the only option remained was to use the in doc IIIA evaluated studies. In doc IIA an extensive discussion is included on the data to be used for PNEC derivation and still is considered valid as it is. On basis of the available information we see no reason to change the conclusions. As boric acid is included on Annex I, this gives the notifier the opportunity to extent doc II and III for the product authorisation phase. *7 Januari 2008* The notifier gave the following comments:	

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	

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THE NETHERLANDS

7 January 2008

Dear Dr Okkerman

RE: Boric acid, disodium tetraborates, disodium octaborate tetrahydrate and boric oxide.

At the Biocides Technical Meeting, TMV-07, in December 2007, the Notifier objected to the selection of a proposed NOEC value for fish because it was neither consistent with the study report, nor with the summary prepared by the Notifier, nor with the evaluation and conclusion originally prepared and included by the RMS in its draft Assessment Report. At this meeting, the RMS agreed to review its derivation of the proposed NOEC value.

This letter presents the Notifier's technical objections to using the newly proposed NOEC value of 1.8 mg B/L and justification for why the value of 5.6 mg B/L should be used.

Use of a single NOEC to derive a $PNEC_{aquatic, added}$.

The RMS proposed in November 2007 that the ecological hazard assessment rely on a single aquatic chronic NOEC. In the previous technical meeting (TMIV-07), the RMS proposed reliance on a species sensitivity distribution, but was requested to elaborate the data used in the proposed SSD. Rather than provide information about the data, the RMS proposed use of the single NOEC value to derive a $PNEC_{aquatic, added}$. The selected NOEC is from a study of the early life stage toxicity to the fish *Brachydanio rerio* (Hooftman et al., 2000) that was submitted by the Notifier in the original dossier in 2004.

From a pragmatic perspective, the Notifier agrees that use of a single NOEC value may be a useful way to make progress in the Biocides assessment process. Both the single NOEC and the SSD approaches are consistent with the TGD. As noted by the RMS, only one exposure scenario is likely to be of concern for aquatic toxicity, and use of either the SSD or the single NOEC approach is not expected to alter the risk characterization of this scenario. We indicated our agreement at the meeting in December.

Derivation of NOEC from the Fish Study

The proposed NOEC value is not consistent with the study, nor with the evaluations made by the study authors (Hooftman et al., 2000), the Notifier, or the RMS in its initial evaluation (Boric acid: Document IIA, May 2006). All these previous evaluations reported the critical endpoint as the NOEC for growth as 5.6 mg B/L.

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	

The RMS in November 2007 suggested that the fish study results in question were mis-interpreted by the study authors, by the Notifier, and by RMS in its initial review and presentation in Document IIA. The new proposal was that a NOEC of 1.8 mg B/L be used, based on growth as dry weight.

We do not agree that a NOEC of 1.8 mg B/L represents an accurate interpretation of the fish study as conducted by TNO Nutrition and Food Research Institute and the study report (Hoofman et al., 2000) submitted for RMS review with the original dossier in 2004. The study followed the OECD 210 protocol for early life stage growth and survival of the zebrafish (*Brachydanio rerio*). The NOEC for the endpoints based on mortality, growth, and condition were stated to be 5.6 mg B/L.

The study authors reported a NOEC for growth (as length and weight) of 5.6 mg B/L. We believe that this is an accurate summary and correct interpretation of the results. We therefore suggest that a NOEC of 5.6 mg B/L be used from this study in any derivation of PNEC values.

Details of the Fish Study by Hoofman et al., 2000.

The data on fish dry weight and results for other endpoints are attached as Annex 1 to this letter. The NOEC for every endpoint (mortality, growth, condition) was stated by the study authors to be 5.6 mg B/L in both the initial summary (page 2 of report) and table of results (Table 2, page 15).

As noted by the study authors (and in the summary submitted by the Notifier in 2004), a statistical comparison of mean dry weight with the control group showed significant differences between controls and the nominal boron concentrations of 0.18, 5.6, and 18 mg B/l (Table 3 of the report). The study authors determined that the result at 0.18 mg B/L was considered to be an outlier and was not taken into account. The report stated that the mean dry weight of the 5.6 mg B/L group was less than the control using the two-tailed Dunnett test. However, the study authors did not select this treatment as the LOEC in either their overall summary (page 2), nor their presentation of study results (Table 2 page 15).

We note that the mean dry weights of the control through 5.6 mg B/L treatment groups were similar with overlapping data points. As shown in Annex 1, the mean dry weight values were not a consistent variable with increasing borate exposure, the largest mean value was in the controls, but the next largest mean value was in the 1.8 mg B/L treatment group (Dose 3). This suggests that dry weight was too variable an endpoint to use by itself and that the study authors may reasonably have considered dry weight in combination with fish length as a more reliable indicator of chemical effects.

The similarity of mean dry weights is confirmed by a simple multiple comparison procedure. Using the Tukey multiple comparison test with equal sample sizes (also known as the "honestly significant difference" test, see Annex 2), one concludes that the mean dry weight of Dose 5 (18 mg B/L) is different from the other means, but that the mean dry weights of the Doses 1 through 4 (0.18 through 5.6 mg B/L) are not statistically different from each other.

Hence, the 18 mg B/L treatment is concluded to be the LOEC, making the next lower treatment (5.6 mg B/L) the NOEC for growth.

Development of Aquatic SSD is not necessary for risk characterization under the BPD.

In its November 2007 statement, the RMS also states its intention to derive a SSD as a separate document. However, the RMS noted difficulty in obtaining and reviewing some

2

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	

of the studies it proposed to use for the SSD so it would not use the SSD in the BPD assessment.

As noted by the RMS, the derived PNEC would likely not differ greatly from that obtained from a single chronic NOEC. Thus as a practical matter, further work on a SSD would possibly lengthen the BPD review with little benefit to be gained in terms of the risk assessment and its practical impact on the regulation of borates under the BPD. We also note that the Risk Assessment under the Existing Substances Regulation (EEC/793/93) process is underway and development of a SSD may be appropriate within that framework. We therefore suggest that there is no need for the RMS to develop a SSD for the purposes of the BPD assessment of borates.

Recommendations

We believe that the LOEC should be 18 mg B/L, and the NOEC for growth be 5.6 mg B/L. We also suggest that for BPD purposes there is no need for the RMS to develop a SSD for borates.

We look forward to hearing your response to these recommendations.

[redacted]

Copied to:
Erik van der Plassche, Head of Sector, ECB
Wim de Coen, ECB

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	

Annex 1: Fish Mean Dry Weight Data

Hoeftmar et al. (2000) reported the results of a 34 day early life stage fish test conducted under the OECD 210 Guidelines using zebrafish. The test substance was boric acid expressed as mg B/L added. Seven exposure groups were tested with four replicate systems in each treatment group. At the conclusion of the test, total dry weight of all fish from each test vessel was determined using a Mettler drying apparatus and the average dry weight per fish was reported.

Nominal exposure concentrations were:

Control	0 mg B/L
Dose 1	0.18 mg B/L
Dose 2	0.56 mg B/L
Dose 3	1.8 mg B/L
Dose 4	5.6 mg B/L
Dose 5	18 mg B/L
Dose 6	56 mg B/L

Test Results:

	Control	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
% eggs hatched after 6 days	100%	100%	100%	100%	100%	100%	82%
% mortality after 34 days	0%	1.1%	0%	2.5%	0%	15%	100%
Length (cm) ± std deviation	1.34 ±0.17	1.33 ±0.22	1.36 ±0.19	1.39 ±0.18	1.34 ±0.20	1.13* ±0.13	-
Dry Weight (mg) ± std deviation	3.40 ±0.11	2.77** ±0.18	2.91 ±0.41	3.19 ±0.34	2.62* ±0.22	1.48* 0.41	-

*Significant difference (two-tailed Dunnett's test vs. control), p<0.01
** Significant difference (two-tailed Dunnett's test vs. control), p<0.05

Mean Fish Dry Weight (Total mg per replicate, divided by number of surviving fish):

	Replicate 1	Replicate 2	Replicate 3	Replicate 4
Control	3.40	3.54	3.26	3.38
Dose 1 (0.18 mg B/L)	2.69	2.96	2.51	2.62
Dose 2 (0.56 mg B/L)	3.50	2.56	2.85	2.74
Dose 3 (1.8 mg B/L)	2.95	3.16	3.68	2.98
Dose 4 (5.6 mg B/L)	2.88	2.30	2.78	2.74
Dose 5 (18 mg B/L)	1.92	1.59	0.97	1.56
Dose 6 (56 mg B/L)	no data			

4

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	

Annex 2: Multiple Comparisons of Means

Tukey's multiple comparison of means considers the null hypothesis that two means are different within a test with several groups (Zar, 1984)[1]. The mean values are ranked and the difference between means is divided by the Standard Error (SE) calculated as:

$SE = \sqrt{(s^2/n)}$ where

s^2 – Error-Mean Square as derived from the ANOVA table and

n = number of replicates in treatment group

The test statistic, q, is calculated as the difference in group means divided by SE. The critical value is known as a "Studentized range" and is dependant on α (the significance level), ν (the error degrees of freedom for the ANOVA) and k (the total number of means being tested). The significance level is an experiment-wise error rate, not a comparison-wise error rate – it represents the probability of falsely rejecting at least one null hypothesis during the course of comparing all the pairs of means.

The mean values of fish dry weight are ranked, and the test statistic q is calculated as the difference between means divided by SE. The critical value of the test statistic, q, was determined to be 4.495, for α = 0.05, ν = 18 and k = 6 (Table B.5, Zar 1984). The largest mean is compared with the smallest, then with the next smallest, etc. The second largest mean is then compared with the smallest in a similar sequence, and so on. If no significant difference is found between two means, it is concluded that no significant difference exists between the means enclosed by these two and no test is calculated.

For the fish dry weight data, six groups were compared (no fry survived in the highest treatment group). ANOVA calculations led to a Error Mean Square value of 0.09548, with 4 replicates per group, or SE = 0.154. The results of the comparisons are shown in the Table below.

The comparisons show two groups of overlapping means:

Control Dose 3 Dose 2 Dose 1 Dose 4 Dose 5

This suggests that only Dose 5 (18 mg B/L) represents a statistically distinguishable difference from the other sets of mean dry weight values. Mean values from Doses 1 through 4 (2.18 through 5.6 mg B/L) were not statistically different from each other, and mean values from control through Dose 3 were not statistically different.

No mortalities were observed in the control, 0.56 and 5.6 mg B/L groups, with minimal mortalities in the 0.18 mg B/L (1 fish) and the 1.8 mg B/L (2 fish) groups. Average length in the 5.6 mg B/L group was the same (1.34 cm) as in the control group. The study authors reported no difference in visual condition of the fish in Doses 1 through 4 vs. the controls. In contrast, there were 15% mortalities and reduced average fish length (1.13 cm) in the Dose 5 (18 mg B/L) group.

Thus, the only clear effect was seen at Dose 5 (18 mg B/L) which should be concluded to be the LOEL. By convention, the next lowest concentration would be taken as the NOEL. This is Dose 4, the 5.6 mg B/L exposure group.

[1] Zar, J.H., 1984. Biostatistical Analysis, 2nd Edition, Prentice-Hall, Inc., Chapter 12 "Multiple Comparisons" pp. 185-190.

Section 7.4.3.2 Annex Point IIIA XIII 2.2	Effects on reproduction and growth rate of fish	

Calculation of Tukey's Honestly Significant Difference (per Zar, 1984)

Comparison	Mean 1	Mean 2	Difference	q	q-critical	Conclusion
C vs 18	3.4	1.5	1.9	12.40	4.495	*Means not equal*
C vs 5.6	3.4	2.6	0.8	5.02	4.495	*Means not equal*
C vs 0.18	3.4	2.8	0.6	4.08	4.495	*No significant difference*
C vs 1.8	3.4	3.2	*Not tested because within range accepted as equal*			
C vs 0.56	3.4	2.9	*Not tested because within range accepted as equal*			
1.8 vs 18	3.2	1.5	1.7	11.07	4.495	*Means not equal*
1.8 vs 5.6	3.2	2.6	0.6	3.69	4.495	*No significant difference*
1.8 vs 0.56	3.2	2.9	*Not tested because within range accepted as equal*			
1.8 vs 0.18	3.2	2.8	*Not tested because within range accepted as equal*			
0.56 vs 18	2.9	1.5	1.4	9.24	4.495	*Means not equal*
0.56 vs 5.6	2.9	2.6	0.3	1.86	4.495	*No significant difference*
0.56 vs 0.18	2.9	2.8	*Not tested because within range accepted as equal*			
0.18 vs 18	2.8	1.5	1.3	8.32	4.495	*Means not equal*
0.18 vs 5.6	2.8	2.6	0.1	0.94	4.495	*No significant difference*

	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 A7_4_3_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	*Give the concentration (% v/v)*
Vehicle control performed	No
Other procedures	

Table A7_4_3_2-2: Dilution water

Criteria	Details
Source	*Synthetic freshwater DSWL-E prepared from ground water and salts. Ground water is from a locality near Linschoten (the Netherlands)*
Salinity	
Hardness	*212 mg/L as $CaCO_3$*
pH	*8.0 to 8.2 after aeration*
Oxygen content	*8.6 to 9.0 mg/L*
Conductance	
Holding water different from dilution water	No

Table A7_4_3_2-3: Test organisms

Criteria	Details
Species/strain	*Brachydanio rerio (zebrafish)*
Source	*Atlanta hatchery, Hellevoertsluis, (Netherlands)*
Wild caught	*No*
Age/size	*Fertilized eggs*
Kind of food	Rotifers, *Artemia* nauplii
Amount of food	*(not specified)*
Feeding frequency	*Rotifers upon larvae hatching, Artemia after 10 days*
Post-hatch transfer time	
Time to first feeding	*Hatching began at day 3*
Feeding of animals during test	*Yes – rotifers and* Artemia
Treatment for disease within 2 weeks preceeding test	*None*

Table A7_4_3_2-4: Test system

Criteria	Details
Test type	*Semistatic*
Renewal of test solution	*Solutions renewed every Monday, Wednesday and Friday from stock solutions freshly made*
Volume of test vessels	*1-liter glass beakers containing 800 ml TS*
Volume/animal	*800 ml per 20 animals = 40 ml*
Number of animals/vessel	*20 (after 24 hours)*
Number of vessels/ concentration	*4*
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-5: Test conditions

Criteria	Details
Test temperature	*Maximum: 25.7 °C, minimum 24.1°C*
Dissolved oxygen	*Maximum: 8.8 mg/L, minimum 7.1 mg/L*
pH	*Maximum: 8.0, minimum 7.2*
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	*Not reported*
Photoperiod	*16/8 h photoperiod with 30 minute transition*

Table A7_4_3_2-6: Validity criteria for fish tests according to OECD Guidelines 210/212

	fulfilled	Not fullfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	**Yes**	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	**Yes**	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	**Yes**	
Test substance concentrations maintained within ± 20% of mean measured values	**Yes**	
No effect on survival nor any other adverse effect found in solvent control	**Not applicable**	
Further criteria for poorly soluble test substances	**Not applicable**	

Table A7_4_3_2-7: Validity criteria for fish test according to OECD Guideline 215

	Fulfilled	Not fullfilled
Concentration of dissolved oxygen in all test vessels > 60% saturation		
Difference of water temperature < 1° C between test chambers at any time during test; temperature within a range of 2° C of the temperature for specific test species		
Mortality of control animals <10%		
Increase of fish weight sufficient for detection of the minum variation of growth rate considered as significant		
Criteria for poorly soluble test substances		

Section 7.4.3.4
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Official use only

34 REFERENCE

Reference

[redacted] (2000) "Semi-static reproduction test with Boric Acid, Manufacturing Grade and Daphnia magna. [redacted]

[redacted]

Data protection

Yes

Data owner

[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 Guideline no. 211

GLP

Yes

Deviations

No

METHOD

See Dossier Guidance for level of detail required in summarizing test and study reports

Section 7.4.3.4 Annex Point IIIA XIII 2.4	**Effects on reproduction and growth rate with an invertebrate species**	
Test material	As given in section 2 - Boric Acid Manufacturing Grade	
Lot/Batch number	Not available	
Specification	As given in section 2	
Purity	+99.9%	
Composition of Product		
Further relevant properties	Water solubility 4.7% at 20°C.	
Method of analysis	ICP-AES (Inductively coupled plasma atomic emissions spectrometry) at wavelength of 249.704 nm and 208.964 nm.	
Preparation of TS solution for poorly soluble or volatile test substances		
Reference substance	No	
Method of analysis for reference substance		
Testing procedure	Non-entry field	
Dilution water	Dilution water was DSWL-E, prepared from ground water with added salts, See Table A7_4_3_4-2	
Test organisms	Daphnia magna Straus (see table A7_4_3_4-3)	X
Handling of offspring	Offspring were counted and removed when test solutions were replaced.	
Test system	Semistatic with 3 renewals per week. One daphnid per container with 50 ml per beaker, ten replicates of each concentration (see table A7_4_3_4-4)	X
Test conditions	20±1°C, pH 7.2 to 8.0, dissolved oxygen 7.4 to 9.1 mg/L, 16/8 photoperiod (see table A7_4_3_4-5)	X
Duration of the test	21 days	
Test parameter	Survival (mortality) during test, number of live young at each test solution replacement time and "condition" (swimming behaviour, colour, size of parent daphnids)	

Section 7.4.3.4 Annex Point IIIA XIII 2.4	Effects on reproduction and growth rate with an invertebrate species
Examination / Sampling	Survival and number of young daphnids monitored when test solutions replenished (Monday, Wednesday, and Friday). Condition was qualitatively monitored by comparing control and test solution daphnids at the same time.
Monitoring of TS concentration	Yes Boron concentrations measured in clean samples from selected concentrations when test solutions replenished on days 5, 12, and 19. Concentrations measured in the spent samples from those same concentrations two days later, i.e., on days 7, 14, and 21. Measurements were taken for the control, group 2, 4 and 6 concentrations.
Statistics	Mortality was evaluated using binomial model at 95% significance level. Reproduction was evaluated using two-tailed Dunnett-test at 95% or 99% significance level comparing control vs. test groups. The LC50 for mortality was estimated using log-logistic model as implemented in Koojiman, Water Res. 15: 107-119 (1981). The EC50 for growth was estimated using a maximum likelihood fitting procedure on a logistic model.
	RESULTS
	If appropriate, include tables
Range finding test	Performed
Concentrations	Results not reported
Number/ percentage of animals showing adverse effects	Results not reported
Nature of adverse effects	Results not reported
Results test substance	Non-entry field
Initial concentrations of test substance	Nominal: 0, 1.8, 3.2, 5.6, 10, 18, 32, 56 mg-B/L Not measured on day 0

Actual concentrations of test substance

Average concentrations from measurements on 7 days are shown for the four groups that were monitored. All 29 measurements were used in simple linear regression. "Probable" concentrations represent the best estimate of actual boron concentrations present. Probable concentrations are the average measured value, or an estimate from the regression if actual measured value not available.

Concentrations expressed as mg-B/L

Group	Nominal	Measured	Regression*	Probable**
Control	0	0.57	0.71	0.57
1	1.8	-	2.6	2.6
2	3.2	3.92	4.1	3.9
3	5.6	-	6.6	6.6
4	10	11	11	11
5	18	-	19	19
6	32	34	34	34
7	56	-	59	59

Regression estimate obtained from linear regression of all measured values vs. nominal (r-squared = 0.997). Regression equation: Y = 1.0435 X + 0.7078 where Y = predicted boron concentration and X = nominal concentration.

**Authors reported endpoints using nominal concentrations based on their determination that measured values were within 20% of nominal. However, they subtracted control concentrations from all values. This underestimates boron, especially at lower test concentrations. For example, nominal concentration (1.8) of group 1 is 69% of measured concentration. The Probable concentration uses average measured values when available or the regression estimate.

Effect data

The total numbers of living offspring per surviving parent animal are shown in the table below. Average, standard deviations, and coefficients of variation for each group are also presented. For the highest test concentration, no parents survived. Day of parent animal death is noted.

X

Total Live Offpsring per parent animal at test termination

Replicate	Control	Group 1	Group 2	Group 3
1	138	147	142	98
2	117	137	107	129
3	128	137	129	178
4	123	166	126	147
5	d-21*	161	118	136
6	163	141	116	d-20*
7	124	100	d-19*	d-16*
8	106	d-19*	116	141
9	198	130	135	156
10	135	117	107	129
Ave	136.8	137.3	121.9	129.3
Std Dev	27.9	20.5	12.2	23.2
Co Var	20.4%	14.9%	10.0%	16.7%
Parental deaths	1 of 10	1 of 10	1 of 10	2 of 10

* Parental daphnid died on this day

Total Live Offpsring per parent animal at test termination

(Continued)

Replicate	Group 4	Group 5	Group 6	Group 7
1	163	144	d-19*	d-7*
2	d-19*	111	0	d-9*
3	142	91	0	d-12*
4	150	120	13	d-12*
5	91	74	0	d-12*
6	d-21*	124	2	d-13*
7	80	124	9	d-14*
8	139	97	0	d-14*
9	131	37	0	d-15*
10	163	144	24	d-17*
Ave	132.4	106.6	5.8	0
Std Dev	31.1	33.1	8.5	-
Co Var	23.5%	31.0%	156.8%	-
Parental deaths	2 of 10	0 of 10	1 of 10	10 of 10

* Parental daphnid died on this day

Endpoint Values:

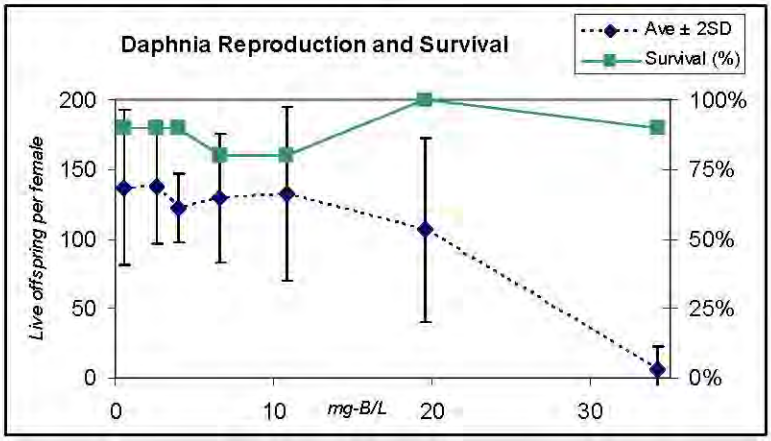
Time	Endpoint	Value (95% confidence interval)
21 day	EC50 (reproduction)	24 mg-B/L (21 to 27)

21 day	NOEC (reproduction)	11 mg-B/L
21 day	LOEC (reproduction)	19 mg-B/L
7 day	LC50 (mortality)	63 mg-B/L
21 day	LC50 (mortality)	36 mg-B/L
21 day	NOEC (mortality)	34 mg-B/L
21 day	LOEC (mortality)	59 mg-B/L
21 day	NOEC (condition)	19 mg-B/L
21 day	LOEC (condition)	34 mg-B/L

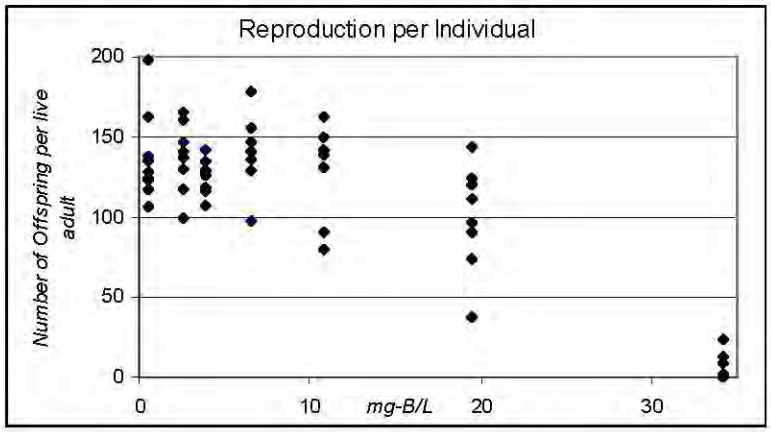
Reproduction and Survival Response Curve

Concentration / response curve

Average number of living offspring per surviving adult are shown. Error bars represent ±2 standard deviations. Parental survival is also shown. Mortality in the highest concentration was 100% and is not shown.



Number of live offspring per surviving adult is shown for each replicate (individual parental daphnid) below.



Other effects

Effects on size and condition (swimming behaviour, colour or any other observable morphological or behavioural criterion) were visually estimated. For Groups 1-5, no effects were observed. Some individuals in Groups 6 and 7 were smaller, paler or greener, or moved slower than the control animals.

Results of controls

Control parental survival was 90%. Average number of living offspring was 136.8±27.9.

Test with reference substance	Not performed	
Concentrations		
Results		
	APPLICANT'S SUMMARY AND CONCLUSION	
Materials and methods	The method followed that described by OECD Guideline 211. No deviations were reported. Dilution water used a ground water that was high in the test substance, so that exposure in control was significantly greater than zero and actual exposures at low concentrations exceed 20% deviation from nominal. Therefore, use of nominal concentrations does not seem justified. Measured concentrations throughout the test allowed construction of a linear regression with very good fit (r-squared = 0.997). Endpoints are reported using probable concentrations based on the measured values or linear regression. Measured concentrations were consistent throughout the study (as shown by fit of regression line), so concentration estimates not time weighted.	X
Results and discussion	Mortality was evident at the highest concentration (59 mg-B/L). All parental animals died within 17 days of test initiation. No significant mortalities were observed at lesser test concentrations Effects on reproduction (measured as average offspring per surviving adult) were significant in the 19 and 34 mg-B/L groups, with average numbers about 78% and 4% that of control group, respectively. Average offspring in other groups typically equalled or slightly exceeded the control group.	
NOEC	NOEC – survival for 21 days: 34 mg-B/L NOEC – reproduction: 11 mg-B/L NOEC – condition: 19 mg-B/L Boric acid contains 17.5% boron by weight, so the above results can be restated based on boric acid: NOEC – survival for 21 days: 195 mg/L as boric acid NOEC – reproduction: 62 mg/L as boric acid NOEC – condition: 111 mg/L as boric acid	
LOEC	LOEC – survival for 21 days: 59 mg-B/L LOEC – reproduction: 19 mg-B/L LOEC – condition: 34 mg-B/L Boric acid contains 17.5% boron by weight, so the above results can be restated based on boric acid: LOEC – survival for 21 days: 338 mg/L as boric acid	

	LOEC – reproduction: 111 mg/L as boric acid LOEC – condition: 195 mg/L as boric acid
EC_{50} (EC_X)	Validity criteria were met. Dissolved oxygen remained high throughout the test. Temperature remained within the targeted range of 20±1°C. Mortalities in the control group met acceptability criterion.
Conclusion	Results were demonstrated for all endpoints. Complete mortality was shown in highest treatment group (59 mg-B/L). Effects were evident in mortality, growth as length and growth as dry weight in the 19 mg-B/L group. At the next lower treatment (6.3 mg-B/L), mortality and growth as length were not significantly different from control, but growth as weight was reduced to about 77% of the control group average. Mortality was evident at highest concentration (59 mg-B/L). No significant mortalities were observed in other groups. Effects on reproduction (measured as number of living offspring per surviving adult) were significant in the 19 mg-B/L group, with average number about 77% that of control group.
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	07-02-2005
Materials and Methods	Test performed with boric acid, results are expressed on the basis of elemental boron (B). The applicant states that:" Dilution water used a ground water that was high in the test substance, so that exposure in control was significantly greater than zero and actual exposures at low concentrations exceed 20% deviation from nominal. Therefore, use of nominal concentrations does not seem justified. Measured concentrations throughout the test allowed construction of a linear regression with very good fit (r-squared = 0.997). Endpoints are reported using probable concentrations based on the measured values or linear regression. Measured concentrations were consistent throughout the study (as shown by fit of regression line), so concentration estimates not time weighted." However, risk assessment should be based on added concentrations, and since the authors of the study report corrected all measured concentrations for the concentration of boron in the dilution water, which resulted in measured concentrations which were 99-108% of nominal, the use of nominal concentrations is considered acceptable.

Results and discussion

4.2.3 Effect data:

A couple of values in the offspring tables, as reported by the applicant in this summary, were different from the values as reported in the actual study report. However, the differences are minor and are not likely to have influenced the outcome of the study.

5.2.1. NOEC (based on nominal added concentrations):

NOEC – survival for 21 days: 32 mg-B/L

NOEC – reproduction: 10 mg-B/L

NOEC – condition: 18 mg-B/L

Boric acid contains 17.5% boron by weight, so the above results can be restated based on boric acid:

NOEC – survival for 21 days: 183 mg/L as boric acid

NOEC – reproduction: 57 mg/L as boric acid

NOEC – condition: 103 mg/L as boric acid

5.2.2 LOEC (based on nominal added concentrations):

LOEC – survival for 21 days: 56 mg-B/L

LOEC – reproduction: 18 mg-B/L

LOEC – condition: 32 mg-B/L

Boric acid contains 17.5% boron by weight, so the above results can be restated based on boric acid:

LOEC – survival for 21 days: 320 mg/L as boric acid

LOEC – reproduction: 103 mg/L as boric acid

LOEC – condition: 183 mg/L as boric acid

Measured concentration in control is 0.57 mg/l.

5.2.3 EC_{50} (EC_x) (adopted from applicant):

Validity criteria were met. Dissolved oxygen remained high throughout the test. Temperature remained within the targeted range of 20±1°C. Mortalities in the control group met acceptability criterion.

Results were demonstrated for all endpoints. Complete mortality was shown in highest treatment group (59 mg-B/L). Effects were evident in mortality, growth as length and growth as dry weight in the 19 mg-B/L group. At the next lower treatment (6.3 mg-B/L), mortality and growth as length were not significantly different from control, but growth as weight was reduced to about 77% of the control group average.

Conclusion	*5.3 Conclusion (adopted from applicant):* Mortality was evident at highest concentration (59 mg-B/L). No significant mortalities were observed other groups. Effects on reproduction (measured as number of living offspring per surviving adult) were significant in the 19 mg-B/L group, with average number about 77% that of control group.
Reliability	1
Acceptability	Acceptable, the result 21-days NOEC 10 mg B/L are included in the risk assessment.
Remarks	Note that the NOECs and LOECs that are used by RMS are different from the values stated by the applicant.

	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 A7_4_3_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	
Vehicle control performed	No
Other procedures	

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	Synthetic freshwater DSWL-E prepared from ground water and salts. Ground water is from a locality near Linschoten (the Netherlands)
Salinity	
Hardness	212 mg/L as $CaCO_3$
pH	8.0 to 8.2 after aeration
Ca / Mg ratio	Ca/Mg = 1.41 mmol/L / 0.71 mmol/L = 2.0
Na / K ratio	Na/K = 1.56 mmol/L / 0.21 mmol/L = 7.4
Oxygen content	8.6 to 9.0 mg/L
Conductance	
TOC	1.6 mg/L
Holding water different from dilution water	No

fSection A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

Official use only

35 REFERENCE

Reference

Ref 1: Bowen, J.E. and H.G. Gauch, 1966. Nonessentiality of boron in fungi and the nature of its toxicity. Plant Physiol 41 (2): 319-324

Ref 2: Crommentuijn, G.H., R. Posthumus and D.F. Kalf, 1995. Derivation of the ecotoxicological serious soil contamination concentration: Substances evaluated in 1993 and 1994. Report nr 715810 008, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

Data protection

No

Data owner

Authors

Criteria for data protection

No data protection claimed

GUIDELINES AND QUALITY ASSURANCE

Guideline study

No – studies predate guidelines or were conducted for independent research

GLP

No – GLP not compulsory at time studies were performed

Deviations

Not guideline study

MATERIALS AND METHODS

fSection A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

Test material	Ref 1: Boric acid Ref 2: not specified
Lot/Batch number	Not specified
Specification	Not specified
Purity	Not specified
Composition of Product	Not specified
Further relevant properties	Not specified
Method of analysis	Ref 1: Curcumin-oxalic acid method with concentration step Ref 2: Not specified
Reference substance	Not specified
Method of analysis for reference substance	
Testing procedure	
Soil sample / inoculum / test organism	Ref. 1 used test organisms obtained from ATCC. Ref. 2 appeared to use collected soil inocula. See Table A7_5_1_1_1-1 and Table A7_5_1_1_1-2.
Test system	See Table A7_5_1_1_1-3
Application of TS	See Table A7_5_1_1_1-4
Test conditions	See Table A7_5_1_1_1-5 and below.

Table: Ref 2 Test conditions for microbial and enzyme inhibition

	Process	Soil Type	pH	% o.m	% clay	Temp °C
A	Nitrification	Loam	5.8	4.39	23	30
B	Nitrification	Clay loam	7.8	6.36	30	30
C	Nitrification	Silty clay loam	7.4	9.7	34	30
D	Nitrification	Unspecified	6.6	5.02	45	30
E	Dehydrogenase	Composite	-	2.27	-	27
F	Dehydrogenase	Composite	-	2.27	-	27
G	Arylsulfatase	Unspecified	6.2	4.64	29	-
H	Arylsulfatase	Unspecified	7.6	5.51	30	-
I	Arylsulfatase	Unspecified	6.5	4.95	26	-

fSection A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

J	Arylsulfatase	Unspecified	7.0	9.04	34	-
K	Urease	Unspecified	5.1	2.57	17	37
L	Urease	Unspecified	6.1	5.64	30	37
M	Urease	Loam	5.8	4.39	23	37
N	Urease	Clay loam	7.8	6.36	30	37
O	Urease	Clay loam	6.8	7.4	42	37
P	Urease	Silty clay loam	7.4	9.27	34	37

Test parameter

Ref. 1:

S. cerevisae – reduced cell replication (doublings/day)

Others – reduced biomass (dry weight per flask)

Ref 2: Enzyme inhibition

Analytical parameter

Not specified

Duration of the test

Ref 1: Not specified; growth rate of fungi suggests several days would be required to observe formation of conidia. X

Ref 2: Varied; see table in section 4.2.6 below

Sampling

Ref 1: S. cerevisae sampled at 4 hour intervals; other fungi at conclusion of test (dry weight) X

Monitoring of TS concentration

No

Controls

Ref 1: Controls grown in zero-B media. Boron concentration of zero-B media measured as less than 0.005 mg-B/L

Ref. 2: No information provided

Statistics

Not specified. Results in Ref. 2 appear to simply be the % enzyme inhibition at specified exposure for nitrification, arylsulfatase and urease results, rather than an interpolation to standard endpoint (e.g., EC50).

RESULTS

fSection A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

35.1 Range finding test	Not performed	
Concentration		
Effect data		
35.2 Results test substance	Non-entry field	
Initial concentrations of test substance	Not reported	X
Actual concentrations of test substance	Not reported	X
Growth curves	Not reported	X
Cell concentration data	Raw data not reported	X
Concentration/ response curve	Not reported	X

Effect data

Ref 1 Results:

Species	Boron	Cell doublings/day
Saccharomyces cerevisae	0 mg/L	8.91
	1.0 mg/L	8.59
	5 mg/L	8.56
	50 mg/L	7.49*
	Boron	**Dry weight (mg/flask)**
Aspergillus niger	0 mg/L	60.6
	500 mg/L	55.8
	1000 mg/L	56.2**
	1300 mg/L	49.1*
Neurospora crassa	0 mg/L	49.9
	100 mg/L	46.6
	250 mg/L	17.8*
Penicillium chrysogenum	0 mg/L	96.8
	500 mg/L	112.9
	4000 mg/L	84.4*

fSection A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

* Signifiant toxic inhibition of growth observed
**Conidia foramtion inhibited

Ref 2 Results of Enzyme Inhibition

	Process	Soil Type	End-point	Exposure time	Result (mg/kg-dry wt)
A	Nitrification	Loam	EC14	20 d	54.05
B	Nitrification	Clay loam	EC70	20 d	54.05
C	Nitrification	Silty clay loam	EC14	20 d	54.05
D	Nitrification	Unspecified	EC70	20 d	54.05
E	Dehydrogenase	Composite	EC50	24 h	363
F	Dehydrogenase	Composite	EC50	24 h	176
G	Arylsulfatase	Unspecified	EC70	0.5 h	270.25
H	Arylsulfatase	Unspecified	EC65	0.5 h	270.25
I	Arylsulfatase	Unspecified	EC72	0.5 h	270.25
J	Arylsulfatase	Unspecified	EC60	0.5 h	270.25
K	Urease	Unspecified	EC98	2 h	54.05
L	Urease	Unspecified	EC13	2 h	54.05
M	Urease	Loam	EC18	2 h	54.05
N	Urease	Clay loam	EC11	2 h	5.4
N	"	"	EC14	2 h	54.05
O	Urease	Clay loam	EC27	2 h	54.05
P	Urease	Silty clay loam	EC13	2 h	5.4
			EC1		

fSection A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

5

Crommentuijn et al. reported the following values:

Nitrification:	NOEC	38 mg/kg
Urease:	NOEC	11.7 mg/kg
Dehydrogenase	EC50	253 mg/kg

Using these values, they derived an "HC50" described as the hazardous concentration for 50% of microbial processes of 21 mg-B/kg.

fSection A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

Other observed effects

Ref 1. reported inhibiation of conidia of A. niger at 1000 mg-B/L, although growth as dry weight was not inhibited.

Results for Ref. 1 shown in Table above

35.3 Results of controls

Not performed

35.4 Test with reference substance

Concentrations

Results

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

Bowen and Gauch (1966) used zero-B culture media for several fungi and found that boron was not essential for growth. (This was the initial intent of the study.) Higher boron concentrations were evaluated using cell doublings per day for the yeast Saccharomyces cervisae and dry weight per flask for other fungi (Aspergillus niger, Neurospora crassa, and Penicillium chrysogenum).

Crommentuijn et al. (1995) cite work done by others (see references cited in Table A7_5_1_1-1). The cited work predates current standardized methods and minimal information about the studies is provided. Crommentuijn et al. used the studies in work on behalf of the Directorate General for Environmental Protection, Directorate for soil in order to derive "Serious Soil Contamination Concentrations" i.e., as information to set soil concentrations requiring intervention.

Results and discussion

Bowen and Gauch found that S. cerevisae growth was affected by 50 mg-B/L, and other fungi were affected only at higher concentrations ranging from 250 mg-B/L for N. crassa to 4000 mg-B/L for P. chrysogenum.

X

Crommentuijn et al. used the cited data to determine NOEC for nitrification and urease inhibition as 38 and 11.7 mg/kg, respectively, and an EC50 for dehydrogenase of 253 mg/kg.

NOEC

Fungi – no effects at 5 to500 mg/L for the four species tested.

Microbial processes – 11.7 to 38 mg/kg

EC_{10}

EC_{50}

Dehydrogenase inhibition - 176 to 363 mg/kg

Conclusion	Bowen and Gauch (1966) evaluated several species of fungi and found toxic effects at 50 to 4000 mg-B/L with severe inhibition above 1000 mg-B/L. Crommentuijn et al. (1995) reported a range of microbial processes including nitrification and enzyme activity (dehydrogenase, arylsulfatase and urease). The lowest effect concentration was 5.4 mg-B/kg (dry weight) withan 11% reduction in urease enzyme activity, with other threshold values reported at 54 mg-B/kg.
Reliability	2 – reliable with limitations. Results taken from published literature that predates more recent standardized protocols.
Deficiencies	Yes – details of procedures and raw data are not part of publication. However, procedures are similar to recent protocols. All tests used concurrent controls (zero-B or low B conditions) so represent effects of added boron.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	18-01-2005
Reference 1	**Bowen and Gauch, 1965**
Materials and Methods	Test was performed with boric acid, results are expressed on the basis of elemental boron (B). Applicant's summary adequately reflects test methods
Results and discussion	The experiments were performed to determine 1) whether boron was essential for the growth of yeast (*Saccharomyces cerevisiae*) and other fungi (*Aspergillus niger*, *Neurospora crassa*, and *Penicillium chrysogenum* and 2) the toxic levels of boron (applied as boric acid) for these species. The main findings are as follows: 1) From a comparison of fungi growth in boron-free culture medium and medium with boron, it was concluded that boron is not essential for the growth of the selected fungi 2) Significantly reduced fungi growth was observed at 50 mg B/L for *S. cerevisiae*, at 1300 mg B/L for *A. niger*, at 250 mg B/L for *N. crassa* and at 4000 mg B/L for *P. chrysogenum*. NOEC, defined as next lower test concentration without significant effects is 5, 1000, 100 and 500 mg B/L for the respective species (see Section 4.2.6). Comments on the study: Experiments were performed in early 1960's and do not meet the current quality criteria for performing and reporting toxicity studies. A lot of basic information is missing (See missing information in Sections 3.1, 3.3 and 4.2). There is, however, no agreed test protocol for a soil test with fungi and the experiment appears to be performed well. Temperature (23 - 25 °C) is above the average in temperate climate zones, but is within the higher range that may be encountered during summer. For *P. chrysogenum*, the spacing in test concentrations was large (0, 500 and 4000 mg B/L), and the NOEC of 500 mg B/L may thus be a lower estimate. All tested fungi are common to soil and thus considered relevant species. Tests were, however, performed in nutrient solution and a direct conversion of the NOEC's to soil concentrations is not possible. The results may be used for an indicative risk assessment based on pore water concentrations.
Conclusion	Boron is not essential for the growth of the fungi *Saccharomyces cerevisiae*, *Aspergillus niger*, *Neurospora crass* and *Penicillium chrysogenum*. At 23 – 25 °C, the NOEC for growth was determined as 5, 1000, 100 and 500 mg B/L for the respective species. All tested fungi are common to soil and thus considered relevant species. Tests were, however, performed in nutrient solution and a direct conversion of the NOEC's to soil concentrations is not possible.
Reliability	2
Acceptability	Acceptable, the NOEC's of 5, 1000, 100 and 500 mg B/L for the growth of the fungi *Saccharomyces cerevisiae*, *Aspergillus niger*, *Neurospora crass* and *Penicillium chrysogenum*, respectively, will be included in the risk assessment.
Remarks	

Reference 2

Crommentuijn et al., 1995

Applicant refers to the NOEC's of 38 and 11.7 mg B/kg for nitrification and urease activity, and the EC_{50} of 253 mg B/kg for dehydrogenase activity, that were derived by Crommentuijn et al. (1995) on the basis of literature data. These values asre geometric means of different endpoints, that in turn were established by applying factors to the test concentrations, depending on the observed effect percentage (e.g. the NOEC of 38 mg/kg is the geometric mean of 54.05/10 and 54.05/2, because in the tests 50 – 80 and 10 – 20 % effect was observed at 54.05 mg/kg and in this case, the NOEC is considered to be equivalent to 0.1 and 0.5 times the effect concentration; the EC_{50} of 253 mg/kg is the geometric mean of two underlying EC_{50}'s of 363 and 176 mg/kg). This way of data treatment is applied as part of the Dutch procedure for setting general environmental quality criteria. The above mentioned NOEC's and EC_{50} should therefore not be used as such for the present goal and underlying references are summarised and evaluated separately below.

1. Tabatabai, M.A. 1977. Effects of trace elements on urease activity in soils. Soil. Biol. Biochem. 9, 9-13

Materials and Methods

The effect of disodium tetraborate decahydrate (borax; $Na_2B_4O_7.10H_2O$) on urease activity was tested in six soils. Soil samples (5 g, air-dried, 2 mm sieved) were put into 50 mL flasks and equilibrated with 1.5 mL of a solution containing 2.5 or 25 µmol boron (final concentration 0.5 or 5 µmol B/g soil). After 30 min., the soil was treated with 0.2 mL toluene as a bacteriostat, 7.5 mL 0.05 M tri(hydroxymethyl)aminomethane buffer, pH 9.0, and 1 mL 0.2 M urea. The flask was stoppered and incubated at 37 °C for 2 hours, after which the volume was made up with 2.5 M KCl containing 100 ppm Ag_2SO_4. Released NH_4-N was determined in a 20 mL aliquot of the soil suspension. A control was included, 1 mL 0.2 M urea was added to soil treated with 1.5 mL water after addition of about 30 ml KCl- Ag_2SO_4 reagent and before making up the volume of the flask.

Results and discussion

Results are summarised in the table below, concentrations are expressed on the basis of elemental boron (B):

Soil type	OM [%]	N [%]	pH	Urease activity [µg NH_4-N/ g soil 2h]	Conc. [mg B/kg]	Incubation time of B [h]	Temp. [°C]	Inhibition[1] [%]
loam 1	1.5	0.13	5.1	18	54	0.5	37	98
loam 2	2.6	0.21	5.8	43	54	0.5	37	18
loam 3	3.3	0.25	6.1	33	54	0.5	37	13
clay loam 1	3.7	0.31	7.8	150	5.4	0.5	37	11
clay loam 1	3.7	0.31	7.8	150	54	0.5	37	14
clay loam 2	4.4	0.39	6.8	210	54	0.5	37	27
silty clay loam	5.5	0.48	7.4	263	5.4	0.5	37	13
silty clay loam	5.5	0.48	7.4	263	54	0.5	37	15

1: as compared to water control

Temperature during exposure is not given. Temperature 37 °C during urease assay is not relevant for field conditions, contact time of 0.5 h is considered rather short. The results indicate that the EC_{50} is > 54 mg B/kg except for loam 1, for which soil almost 100 % effect is found at that concentration. A reliable estimate of the EC_{50} cannot be made on the basis of the available data.

Conclusion

Although the experiment itself can be considered as reliable, the results are is considered not relevant for the purpose of this evaluation because of the experimental set-up and the fact that an estimate of the EC_{50} cannot be made.

Reliability

4

Acceptability

Not acceptable, the results will not be included in the risk assessment.

Remarks

2. Liang, C.N. and Tabatabai, M.A. 1977. Effects of trace elements on nitrogen mineralisation in soils. Environ. Pollut. 12, 141-147

Materials and Methods

The effect of anhydrous disodium tetraborate ($Na_2B_4O_7$) on nitrogen mineralisation was tested in four soils. Duplicate soil samples (10 g, air-dried, 2 mm sieved) were put into 250 mL flasks and treated with 3 mL of a solution containing 5 or 50 µmol boron (final concentration 0.5 or 5 µmol B/g soil) or with 3 mL water. Final moisture content was ca. 60 % of WHC. Soils were incubated at 30 °C for 20 days after which NH_4-N, NO_3-N and NO_2-N were extracted with 50 mL 2 M KCl. NH_4-N and the sum of NO_3- and NO_2-N were determined by a steam distillation method, NO_2-N by a diazotisation and coupling reaction method. Inhibition of mineralisation was calculated as the sum of NH_4- and NO_3-N produced in the boron treated soils relative to the control

Results and discussion

NO_2-N was not detected in any of the soils. Total N produced in the control soils was between 46 and 71 µg N/g soil. NH_4-N and NO_3-N were significantly lower in all soils, except for NH_4-N in loam. Results of the boron treated soils are summarised in the table below, concentrations are expressed on the basis of elemental boron (B):

Soil type	OM	pH	Clay	N	Initial inorganic N	Conc.	Incubation time of B	Temp.	Inhibition[1]
	[%]		[%]	[%]	[µg/g soil]	[mg B/kg]	[d]	[°C]	[%]
loam	4.4	5.8	23	0.21	8	54	20	30	14
silty clay	5.0	6.6	45	0.25	4	54	20	30	7
clay loam	6.4	7.8	30	0.31	3	54	20	30	7
silty clay loam	9.3	7.4	34	0.46	6	54	20	30	14

1: as compared to water control; average of two replicates

The incubation temperature of 30 °C is considered relatively high in comparison to field conditions. In view of the test duration, the test can be considered as chronic and a NOEC should be derived. This is not possible because only one concentration is tested. However, the average effect level is ca. 10 %, and the exposure concentration of 54 mg B/kg is considered as the 20-days EC_{10}.

Conclusion

$Na_2B_4O_7$, added at a concentration of 54 mg B/kg soil, inhibited nitrogen mineralisation by 7 – 14 % as compared to the control after 20 days of incubation at 30 °C. The average effect level is 10 %, and the 20-days EC_{10} is considered as 54 mg B/kg.

Reliability

3

Acceptability

Not Acceptable, because the too high test temperature of 30 °C and because this is not a dose response study

Remarks

3. Liang, C.N. and Tabatabai, M.A. 1978. Effects of trace elements on nitrification in soils. J. Environ. Qual. 7, 291 - 293

Materials and Methods

The effect of anhydrous disodium tetraborate ($Na_2B_4O_7$) on oxidation of added NH_4-N was tested in three soils. Duplicate soil samples (10 g, air-dried, 2 mm sieved) were put into 250 mL flasks and treated with 3 mL of a solution containing 50 µmol boron (final concentration 5 µmol B/g soil) and 2 mg NH_4-N (as $(NH_4)_2SO_4$) or with NH_4-N only. Final moisture content was ca. 60 % of WHC. Soils were incubated at 30 °C for 10 days after which NH_4-N, NO_3-N and NO_2-N were extracted with 50 mL 2 M KCl. NH_4-N and the sum of NO_3- and NO_2-N were determined by a steam distillation method, NO_2-N by a diazotisation and coupling reaction method. Inhibition of nitrification was calculated as the sum of NO_3- and NO_2-N produced in the boron treated soils relative to the control.

Results and discussion

NO_2-N was initially not present in the soils. Total N produced in the control soils was between 46 and 71 µg N/g soil. NH_4-N and NO_3-N were significantly lower in all soils, except for NH_4-N in loam. Results of the boron treated soils are summarised in the table below, concentrations are expressed on the basis of elemental boron (B):

Soil type	OM	pH	N	Initial inorganic N	Conc.	Incubation time of B	Temp.	Inhibition[1]
	[%]		[%]	[µg/g soil]	[mg B/kg]	[d]	[°C]	[%]
loam	4.4	5.8	0.21	8	54	10	30	74
clay loam	6.4	7.8	0.31	3	54	10	30	92
silty clay loam	9.3	7.4	0.46	6	54	10	30	74[2]

1: as compared to water control

2: value recalculated from authors, give 42 % but total sum of NO_3- and NO_2-N is 43.2 µg/g as compared to 166.1 µg/g in control

Incubation temperature of 30 °C is relatively high in comparison to field conditions. From the results it appears that the 10-days EC_{50} is < 54 mg B/kg. In view of the test duration, a NOEC (or EC_{10}) should be derived but this is not possible because only one concentration is tested that has pronounced effects. The results can therefore not be used.

Conclusion

The 10-days EC_{50} of $Na_2B_4O_7$ for nitrification is < 54 mg B/kg. Although the experiment itself can be considered as reliable, the experimental set-up is considered not relevant for the purpose of this evaluation. In view of the test duration, a NOEC (or EC_{10}) should be derived, but this is not possible because only one concentration is tested that has pronounced effects.

Reliability

4

Acceptability

Not acceptable, the results will not be included in the risk assessment.

Remarks

4. Al-Khafaji, A.A. and Tabatabai, M.A. 1979. Effects of trace elements on arylsulfatase activity in soils. Soil Sci. 127, 129-133

Materials and Methods

The effect of anhydrous disodium tetraborate ($Na_2B_4O_7$) on arylsulfatase activity was tested in four soils. Duplicate soil samples (1 g, air-dried, 2 mm sieved) were put into 50 mL flasks and treated with 1 mL of a solution containing 2.5 or 25 µmol boron (final concentration 2.5 or 25 µmol B/g soil) or with 1 mL water. After 30 min. of equilibration, arylsulfatase activity was assayed by colorimetric determination of *p*-nitrophenol after incubation for 1 hour at 37 °C with 0.25 mL toluene, 3 mL 0.5 M acetate buffer (pH 5.8) and 1 mL of a 5mM buffered potassium *p*-nitrophenol sulfate solution. Inhibition of arylsulfatase activity in the boron treated soil was calculated relative to the control.

Results and discussion

Results of the boron treated soils are summarised in the table below, concentrations are expressed as elemental boron (B):

Soil type	OM [%]	pH	N [%]	Initial AS activity[1]	Conc. [mg B/kg]	Incubation time of B [h]	Temp. [°C]	Inhibition[2] [%]
clay loam	4.6	6.2	0.23	77	270	0.5	37	70
clay loam	5.5	7.6	0.27	142	27	0.5	37	8
clay loam	5.5	7.6	0.27	142	270	0.5	37	65
loam	4.9	6.5	0.25	150	27	0.5	37	31
loam	4.9	6.5	0.25	150	270	0.5	37	72
clay loam	9.0	7.0	0.42	190	270	0.5	37	60

1: µg released *p*-nitrophenol/g soil.h

2: as compared to water control

Exposure was only 30 min., which is considered too short, and temperature during exposure is not given. Temperature 37 °C during arylsulfatase assay is not relevant for field conditions. The results indicate that the 30 min.-EC_{50} is between 27 mg and 270 mg B/kg. A reliable estimate of the EC_{50} can, however, not be made on the basis of the available data.

Conclusion

The 30 min.-EC_{50} of $Na_2B_4O_7$ on arylsulfatase activity is between 27 mg and 270 mg B/kg. A reliable estimate of the EC_{50} can, however, not be made on the basis of the available data. Although the experiment itself can be considered as reliable, the experimental set-up is considered not relevant for the purpose of this evaluation.

Reliability

4

Acceptability

Not acceptable, the results will not be included in the risk assessment.

Remarks

5. Rogers, J.E. and Li, S.W. 1985. Effect of metals and other inorganic ions on soil microbial activity: soil dehydrogenase assay as a simple toxicity test. Bull. Environ. Contam. Toxicol. 34, 858-865

Materials and Methods

The effect of disodium tetraborate decahydrate (borax; $Na_2B_4O_7.10H_2O$) on dehydrogenase activity was tested in a composite soil (2.3 % OM, WHC 22 %) either enriched or unenriched with 1% alfalfa. Duplicate soil samples (1 g, air-dried) were put into 25 mL centrifuged tubes and 0.2 mL of a 3% (w/v) solution of 2,3,4-triphenyl tetrazoliumchloride solution was added. Samples were treated with 0.5 mL of a 0.5 % (w/v) glucose solution containing the test compound. Anion concentration tested were 0, 30, 150, 300, 500, 1000 and 5000 mg BO_3/kg soil. Tubes were incubated at 27 °C in the dark for 24 hours. Methanol was used to extract TTC-formazan. Soil dehydrogenase activity, expressed as µg TTC-formazan produced per gram soil per 24 hours, was quantified by comparison to standard curve. Inhibition of dehydrogenase activity in the boron treated soil was calculated relative to the control.

Results and discussion	A slight increase in activity of 9 % and 4 % as compared to the control was observed in unenriched soil at concentrations 30 and 150 mg BO_3^-/kg, respectively. No increase was observed in the enriched soil. The EC_{50} was estimated by non-linear regression of a sigmoid concentration-effect relationship, using the data of the authors. Resulting EC_{50} was 826.8 and 2260 mg BO_3^-/kg for unenriched and enriched soil, respectively. This is equivalent to 152 and 363 mg B/kg. Incubation temperature of 27 °C is considered relatively high as compared to field conditions, but is not considered exceptional for short periods of time as used in this study
Conclusion	$Na_2B_4O_7.10H_2O$ had a slight stimulating effect in unenriched soil at concentrations of 30 and 150 mg BO_3^-/kg soil. Stimulation was not present in enriched soil. The 24-h-EC_{50} for inhibition of dehydrogenase activity was determined as 152 and 363 mg B/kg for unenriched and enriched soil, respectively.
Reliability	2
Acceptability	Acceptable, the 24-hours EC_{50}'s of 152 and 363 mg B/kg for unenriched and enriched soil, respectively, are included in the risk assessment.
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 A7_5_1_1-1: Microbial sample / Inoculum (*if applicable; include separate table for different samples*)

Ref 1 (Bowen & Gauch):

Criteria	Details
Nature	*Microbial samples from ATCC collection*
Sampling site:	
Geographical reference on the sampling site	
Data on the history of the site	
Use pattern	
Depth of sampling [cm]	
Sand / Silt / Clay content [% dry weight]	
pH	*S. cerevisae: pH 6.5; others: initial pH 6.7*
Organic carbon content [% dry weight]	
Nitrogen content [% dry weight]	
Cation exchange capacity [mmol/kg]	
Initial microbial biomass	*See Table A5_5_1_1-2*
Reference of methods	
Collection / storage of samples	*Samples dried, ashed for boron analysis*
Preparation of inoculum for exposure	*See Table A5_5_1_1-2*
Pretreatment	*See Table A5_5_1_1-2*

Ref 2 (Crommentuijn et al.) provided no information about testing procedures. Soil types were stated, so one infers that inocula were derived from soil samples. Crommentuijn et al cite the following publications:
Nitrification: Liang, CN and MA Tabatabai, 1977. Effects of trace elements on nitrogen mineralization in soils. Environ. Pollut. 12: 141-147.
Dehydrogenase: Rogers, JE and SW Li, 1985. Effect of metals and other inorganic ions on soil microbial activity: soil dehydrogenase assay as a simple toxicity test. Bull. Environ. Contam. Toxicol. 34: 858-865.
Arylsulfatase: Al-Khafaji, AA and MA Tabatabai, 1979. Effects of trace elements on arylsulfatase activity in soils. Soil Science 127(3): 129-133.
Urease: Tabatabai, MA, 1977. Effects of trace elements on urease activity in soils. Soil Biol. Biochem. 9: 9-13.

Table A7_5_1_1-2: Test organism (*if applicable*)

Ref 1 (Bowen and Gauch)

Criteria	Details
Species	*Saccharomyces cerevisiae*
Strain	*Mayer-Gebruder strain ATCC No. 7752*
Source	*ATCC*
Sampling site	
Laboratory culture	Yes
Method of cultivation	*Liquid culture in basal culture solution at 25°C at pH 6.5 under continuous illumination and bubbling of 1% CO_2-in-air mixture*
Preparation of inoculum for exposure	*Sub-cultured 3x in B-free media*
Pretreatment	*None*
Initial cell concentration	*Initial Optical Density (OD) at 450 mμ of 1.00 obtained by centrifugation*

Ref 1. (Bowen and Gauch)

Criteria	Details
Species	*Aspergillus niger* *Neurospora crassa* *Penicillium chryogenum*
Strain	*A. niger van Tieghem (ATCC No. 6275)* *N. crassa Beadle (ATCC No. 10336)* *P. chryogenum Thom (ATCC No. 10238)*
Source	*ATCC*
Sampling site	
Laboratory culture	Yes
Method of cultivation	*Surface cultures in basal media at 23 to 25°C at pH 6.5 under continuous illumination*
Preparation of inoculum for exposure	*Culture in B-free medium*
Pretreatment	*None*
Initial cell concentration	*Not reported*

Ref 2 (Crommentuijn et al.) provided no information about testing procedures.

Table A7_5_1_1-3: Test system

Ref 1 (Bowen and Gauch)

Criteria	Details
Culturing apparatus	*B-free test tubes (S. cerevisae) or B-free extraction flasks*
Number of vessels / concentration	*Not specified*
Aeration device	*1% CO_2-in-air bubbling (S. cerevisae)*
Measuring equipment	*Curcumin-oxalic acid measurement of B. Zero-B solutions were concentrated 1000x and contained less than 0.0005 mg/L of boron*
Test performed in closed vessels	*No*

Ref 2 (Crommentuijn et al.) provided no information about testing procedures.

Table A7_5_1_1-4: Application of test substance

Ref 1 (Bowen & Gauch)

Criteria	Details
Application procedure	*Boric acid added to liquid culture media*
Carrier	*None*
Concentration of liquid carrier [% v/v]	
Liquid carrier control	
Other procedures	

Ref 2 (Crommentuijn et al.) provided no information about testing procedures.

Table A7_5_1_1-5: Test conditions

Ref 1. (Bowen & Gauch)

Criteria	Details
Organic substrate	*Chemically-defined basal medium used*
Incubation temperature	*S. cerevisae - 25°C. Others 23-25°C*
Soil moisture	*Soil not used*
Method of soil incubation	
Aeration	*1% CO_2-in-air bubbled to S. cerevisae to keep the cells suspended*

Ref 2 (Crommentuijn et al.) provided no information about testing procedures.

Section A7.5.1.2 **Earthworm, acute toxicity test**
Annex Point IIIA XIII 3.2

Official use only

36 REFERENCE

Reference

[redacted] (2000) "The acute toxicity of Boric Acid, Manufacturing Grade to the worm species, Eisenia fetida in a 14d test. [redacted]

[redacted]

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 Guideline no. 207

GLP

Yes

Deviations

Yes – worms added randomly to each of the test containers. This is not assumed to have affected the results of the study. X

METHOD

See Dossier Guidance for level of detail required in summarizing test and study reports

Section A7.5.1.2
Annex Point IIIA XIII 3.2

Earthworm, acute toxicity test

Test material	As given in section 2 - Boric Acid Manufacturing Grade	
Lot/Batch number	Not available	
Specification	As given in section 2	
Purity	+99.9%	
Composition of Product		
Further relevant properties	Water solubility 4.7% at 20°C.	
Method of analysis	Not analysed	
Reference substance	No	
Method of analysis for reference substance		
Testing procedure	Non-entry field	
Preparation of the test substance	The test substance was diluted in water (see table A7_5_1_2-1)	
Application of the test substance	Test material was weighed out and dissolved in Ultrapure water. Aliquots were added to dry soil and mechanically mixed.	
Test organisms	Eisenia fetida maintained in laboratory culture (see table A7_5_1_2-2)	X
Test system	(see table A7_5_1_2-3)	
Test conditions	(see table A7_5_1_2-4)	
Test duration	14 days	
Test parameter	Survival, condition and weight	
Examination	Survival and condition monitored on days 0, 7, 14. Weight monitored on days 0 and 14.	
Monitoring of test substance concentration	No	
Statistics	Mortality was evaluated using binomial model at 95% significance level. Growth was evaluated using two-tailed Dunnett-test at 95% or 99% significance level.	

Section A7.5.1.2 **Earthworm, acute toxicity test**
Annex Point IIIA XIII 3.2

RESULTS

Filter paper test	Not performed
Concentration	
Number/ percentage of animals showing adverse effects	
Nature of adverse effects	
Soil test	Non-entry field
Initial concentrations of test substance	1.0, 3.2, 10, 32, 100, 320 and 1000 mg – boric acid/kg dry weight artificial soil
Effect data (Mortality)	There were no mortalities in the controls There were no mortalities in the highest concentration tested. Single worms were not recovered in the 3.2 and 320 mg/L treatments.
Concentration / effect curve	LC_0 > 1000 mg/l boric acid LC_{50} > 1000 mg/L boric acid LC_{100} >>1000 mg/L Boric acid No effects on mortality were observed.
Other effects	No adverse effects on behaviour or condition were observed in any concentration tested. There was no significant weight difference at any concentration tested.
Results of controls	
Mortality	No mortalities were observed
Number/ percentage of earthworms showing adverse effects	0%
Nature of adverse effects	No adverse effects observed
Test with reference substance	Not performed

X

Section A7.5.1.2 **Earthworm, acute toxicity test**
Annex Point IIIA XIII 3.2

Concentrations		
Results		
	APPLICANT'S SUMMARY AND CONCLUSION	
Materials and methods	Test material was applied by mixing water solution with dry synthetic soil. Test was conducted according to OECD 207. The only reported deviation from protocol was to assign worms at random to test chambers, which is not expected to have affected the study results.	
Results and discussion	No adverse effects were observed at any concentration. Although the test material was not measured, it is not volatile and is stable.	
LC_0	> 1000 mg/L – boric acid (>175 mg-B/L)	X
LC_{50}	> 1000 mg/L – boric acid (>175 mg-B/L)	X
LC_{100}	> 1000 mg/L – boric acid (>175 mg-B/L)	X
Conclusion	Validity criteria were fulfilled	
Other Conclusions		
Reliability	1	
Deficiencies	Yes – the absence of measured concentrations is a deficiency. However, the clarity of the results (no adverse effects at any concentration) permits confidence that the test material is of low toxicity to this representative soil organism.	X

Section A7.5.1.2 **Earthworm, acute toxicity test**
Annex Point IIIA XIII 3.2

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	18-01-2005
Materials and Methods	Comments to applicants summary: Section 2: Random allocation of worms to test containers is conform the guideline and is not a deviation from the guideline. Unit mg/L throughout applicant's summary should read mg/kg dwt soil
Results and discussion	No control mortality, one dead worm at 3.3 and 320 mg/kg dwt soil. Weight loss of worms over the 14-days test period was 4.5 ± 3.0 % in the control, weight loss in the boric acid treatments ranged from 3.8 to 10.9 % and was not significantly different from the control.
Conclusion	Comments to applicant's summary: Section 5.3.3: Analytical determination of concentrations is not needed for compounds that are stable in soil, the absence of measurements is thus not considered a deficiency. LC_{50} > 1000 mg/kg dwt soil as boric acid. Expressed as elemental boron, this is equivalent to > 175 mg B/kg dwt soil
Reliability	1
Acceptability	Acceptable, the result 14-days LC_{50} > 175 mg B/kg dwt soil is included in the risk assessment.
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 A7_5_1_2-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	Distilled water ("Ultrapure water")
Alkalinity / Salinity	
Hardness	
pH	
Oxygen content	
Conductance	
Holding water different from dilution water	No
In case of the use of an organic solvent	
Dispersion	No
Vehicle	No
Concentration of vehicle	
Vehicle control performed	
Other procedures	

Table A7_5_1_1-2: Test organisms

Criteria	Details
Species/strain	Eisenia foetida
Source of the initial stock	Blades Biological, Crowden-Edenbridge, Kent, England
Culturing techniques	Cultured in horse-manure, garden soil mixture (1:1) and 24± 2 °C.
Age/weight	Average weight = 467 g, range 355 to 599 g per individual
Pre-treatment	