Table A7_5_1_1-3: Test system

Criteria	Details
Artificial soil test substrate	Composition of the test substrate: sphagnum peat: kaolin clay: fine industrial sand in ratio 1:2:7 based on dry weight Calcium carbonate added to give pH of 6.0± 0.5 before adding test substance. Soil components were mixed with distilled water and calcium carbonate in polyethylene bag. Water was added to yield a moisture content of about 55% after addition of test solution. Initial moisture was 35%, then was 55% after addition of test substance solution.
Test mixture	Test substance concentrations were 0, 1, 3.2, 10, 32, 100, 320 and 1000 mg/L boric acid.
Size, volume and material of test container	1-litre glass jars with a glass lid (domestic preserving jars)
Amount of artificial soil (kg)/ container	775 g
Nominal levels of test concentrations	0,1, 3.2, 10, 32, 100, 320, 1000 mg/kg artificial soil
Number of replicates/concentration	4 with animals + 1 for physical measurements
Number of earthworms/test concentration	4 * 40 = 160
Number of earthworms/container	40
Light source	Not specified
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7_5_1_2-4: Test conditions

Criteria	Details
Test temperature	Day 0: 19.4° C in Control vessel D. Day 7: 19.5° C in Control vessel D. Day 14: 19.7° C in Control vessel D.
Moisture content	Day 0: 54.96% in Control vessel E. Day 14: 54.66% in Control vessel A.
pH	Day 0: 6.0 in Control Day 0: 6.1 in 1000 group Day 14: 6.2 in Control Day 14: 6.2 in 1000 group
Adjustment of pH	Not adjusted after test initiation
Light intensity / photoperiod	Lux 556 to 582 in test pot array
Relevant degradation products	

Table A7_5_1_2-5: Mortality data

Test Substance Concentration (nominal)[1] [mg/kg artificial soil]	Mortality			
	Number		Percentage	
	7 d	14 d	7 d	14 d
0	0	0	0	0
1	0	0	0	0
3.2	0	1*	0	2.5%
10	0	0	0	0
32	0	0	0	0
100	0	0	0	0
320	0	1*	0	2.5%
1000	0	0	0	0
Temperature [°C]	19.5	19.7		
pH		6.2		
Moisture content		54.88%		

[1] TS concentrations were nominal

* Worm not recovered, assumed dead.

Table A7_5_1_2-6: Effect data

	14 d [mg/kg soil][1]	95 % c.l.
LC_0	>1000	
LC_{50}	>1000	
LC_{100}	>1000	

[1] based on nominal concentrations of boric acid

Table A7_5_1_2-7: Validity criteria for acute earthworm test according to OECD 207

	fulfilled	Not fulfilled
Mortality of control animals < 10%	X	

Section 7.5.1.3 Annex Point IIIA XIII 3.4	Terrestrial plant toxicity	

Official use only

INTRODUCTORY NOTE:

Boron is an essential micronutrient for normal growth of plants and yet is phytotoxic at higher concentrations. At low boron concentrations, plants will show adverse effects and much of the research into boron and terrestrial plants has been to determine when boron deficiency is of concern. Consequently, the dose-response pattern should be imagined as a U-shaped curve, with adverse effects observed at both low and high boron concentrations.

The concentrations where deficiency and toxicity begin vary significantly among plants, and the ranges overlap such that concentrations which are toxic to some plants result in deficiencies to other species.

Investigations into plant/boron interactions date back to the 1940's so much of the available data do not resemble current ecotoxicity study designs. The studies typically extend longer than current terrestrial plant toxicity tests, such as the OECD 208 protocol, because the symptoms of boron deficiency and toxicity were seen in harvested yield or fruit/seed production. Data have been summarized and reviewed by a number of independent scientists and the following information relies on the published summaries, rather than detailed evaluation of a single laboratory study.

Three studies are described in detail as these studies involve replicated test units at several treatment levels. While such studies resemble toxicity-study designs, the other data must also be considered.

Section 7.5.1.3 **Terrestrial plant toxicity**
Annex Point IIIA XIII 3.4

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Data protection

No

<u>Data owner</u>

Authors

Section 7.5.1.3 **Terrestrial plant toxicity**
Annex Point IIIA XIII 3.4

Criteria for data protection

No data protection claimed

GUIDELINES AND QUALITY ASSURANCE

Guideline study

No. The TNsG identifies the relevant guideline for Acute Toxicity to Plants is OECD 208, which is intended to determine possible toxic effects of soil-incorporated solid or liquid chemical substances on the emergence of seedlings and the early stages of growth of a variety of terrestrial plants after a single application.
Because of the essentiality of boron to plants, available studies go beyond short term (acute) evaluations and typically involve continuous, not single, application. Also, available studies typically focus on yield, total biomass, or other measures of phytotoxicity in mature plants, rather than seedling emergence and early growth. Finally, available studies seldom attempt to determine an EC50 but rather report threshold values, i.e., exposures where decreases in yield or biomass or phytotoxic symptoms are initially observed. (This is comparable to the NOEC/LOEC endpoints used in chronic studies.)

GLP

No – GLP not available when most studies performed or study not designed as standard toxicity test.

Deviations

No – Not guideline studies

METHOD

Section 7.5.1.3 Annex Point IIIA XIII 3.4	Terrestrial plant toxicity	
Test material	Most used boric acid	X
Lot/Batch number	Information not available (Review articles)	
Specification	Not specified	
Purity	Information not available	
Composition of Product	Information not available	
Further relevant properties	Information not available	
Method of analysis	Methods vary, including colorimetric and spectrometric (e.g., azimethine-H and ICP methods).	
Preparation of TS solution for poorly soluble or volatile test substances	No preparing – borates dissolved in water	
Reference substance	Information not available	
Method of analysis for reference substance		
Testing procedure		
Dilution water	For laboratory tests such as hydroponic studies or sand culture studies, dilution water was a prepared nutrient solution. For field studies, test material (usually as a commercial product) was applied directly or mixed with other nutrients and applied as standard fertilizer.	X
Test plants	See Figures and Tables at A7_5_1_3-X, below. A large variety of plants have been tested. Gupta and Cutcliffe (1984) tested Phaseolus and Brassica. Aitken and McCallum (1988) tested Helianthus Riley et al. (1984) tested Hordeum	
Test system	See Tables A7_5_1_3-4 a, b, c.	
Test conditions	See table A7_5_1_3-5 a, b, c.	
Test duration	See table A7_5_1_3-5 a, b, c.	
Test parameter	Phaseolus – dry weight of bean Brassica – fresh weight of cabbage head Helianthus – dry weight of above-ground biomass	

Section 7.5.1.3 **Terrestrial plant toxicity**
Annex Point IIIA XIII 3.4

Hordeum – harvest index, leaf necrosis, plant growth form

Sampling

Samples taken at harvest (beans, cabbage), after 14 d (sunflower) and at maturity (barley)

Method of analysis of the plant material

Samples typically dried, ashed and analyzed for boron by ICP-AES or azimethine-H method.

Quality control

Not reported

Statistics

Varied with test design; typically analysis of variance with LSD comparisons.

RESULTS

Results test substance

Non-entry field

Applied initial concentration

Gupta and Cutcliffe: See Table A7_5_1_3-6a. Application rates were 0, 2.2, 4.4 and 8.8 kg-B/ha. Soil concentrations were measured after harvest.

Aitken and McCallum: See Table A7_5_1_3-6b. Application rates were 0, 0.25, 0.5, 1, 2, 4, 6, 8 and 12 kg-B/ha. Soil in containers was measured after harvest

Riley et al.: See Table A7_5_1_3-6c. Exposure rates in containers were 0, 0.5,,1, 2, 4 and 8 mg-B/kg soil. Measured values were <0.5, <0.5, <0.5, 1, 2.1 and 4.9 mg-B/kg using mannitol/CaCl2 extraction procedure.

Phytotoxicity rating

Gupta and Cutcliffe: Not reported

Aitken and McCallum: Not reported

Riley et al: Not reported

Plant height

Gupta and Cutcliffe: Not reported

Aitken and McCallum: Not reported

Riley et al: Not reported

Plant dry weights

Gupta and Cutcliffe: See Table A7_5_1_3-6a. For cabbage, no decrease at any application rate up to 8.8 kg-B/ha, approximately equal to 6.3 mg-B/kg soil. For beans, no decrease at 2.2 kg-B/ha application were observed, approximately equal to 1.6 mg-B/kg-soil, and application rates of 4.4 kg/ha reduced bean yield at two of four sites. No decreases were observed during a second year at any site

Aitken and McCallum: See Table A7_5_1_3-6b. For sunflower, toxicity was observed in some, but not all, soil types at application rates up to 12 kg-B/ha. In most cases, decreased yield was observed at 4 kg-B/ha application. By measuring soil porewater concentrations, a bi-linear (or broken-stick) pattern of soil solution B and relative yield was identified. Fitting that model to the data, a threshold of 2.4 mg-B/L was found; above that value, yield reduction due to toxicity occurred. Fitting an alternative model gave a threshold of 1.9 mg-B/L.

Riley et al et al: See Table A7_5_1_3-6c. For barley, toxicity was observed in harvest index (relative grain yield) only at the highest boron addition (8 mg/kg).

Section 7.5.1.3 **Terrestrial plant toxicity**
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Root dry weights

Gupta and Cutcliffe: Not reported

Aitken and McCallum: Not reported

Riley et al: Not reported

X

Root length

Gupta and Cutcliffe: Not reported

Riley et al: Not reported

Aitken and McCallum: Not reported

Number of dead plants

Gupta and Cutcliffe: Not reported

Aitken and McCallum: Not reported

Riley et al: Not reported

Effect data

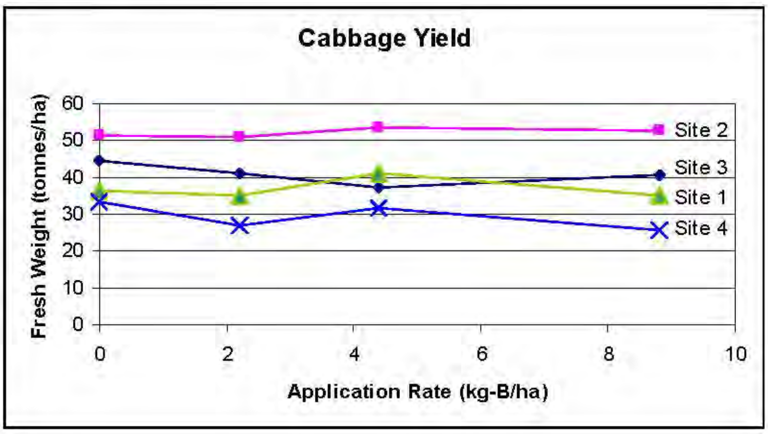
Gupta and Cutcliffe: See Table A7_5_1_3-6a

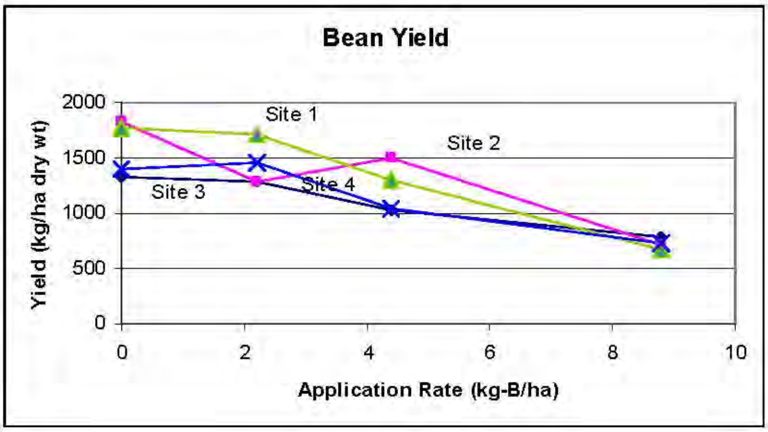
Aitken and Mc Callum: See Table A7_5_1_3-6b

Riley et al: See Table A7_5_1_3-6c. As noted in 4.1.4, effects on harvest index were observed only at the highest treatment of 8 mg/kg. No marked effects on tillers were observed at any treatment. However the average height of the primary tiller and the percentage of tillers that lodged were significantly increase at 2 mg/kg or greater treatment. Leaf necrosis followed a similar pattern, with significant areas affected at 2 mg/kg or greater treatment.

Concentration / response curve

Gupta and Cutcliffe 1984:





Section 7.5.1.3 **Terrestrial plant toxicity**
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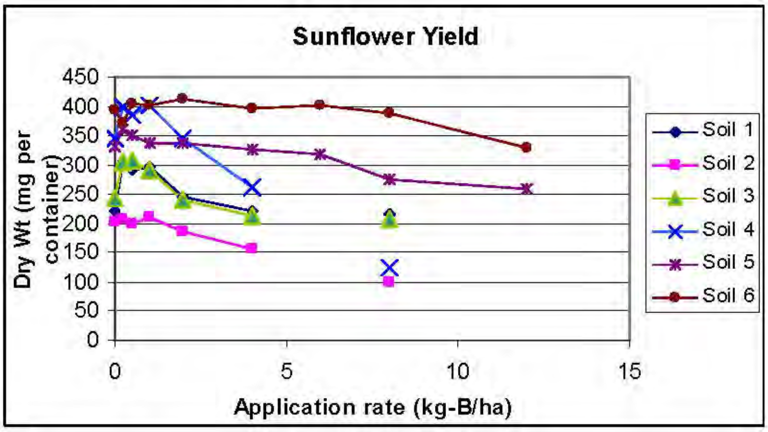
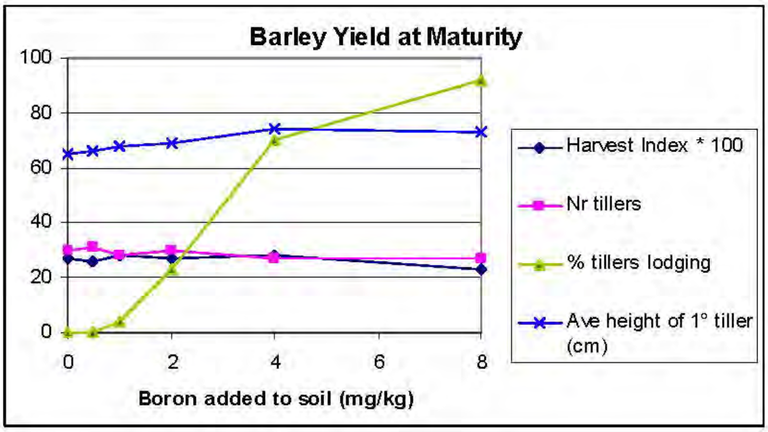
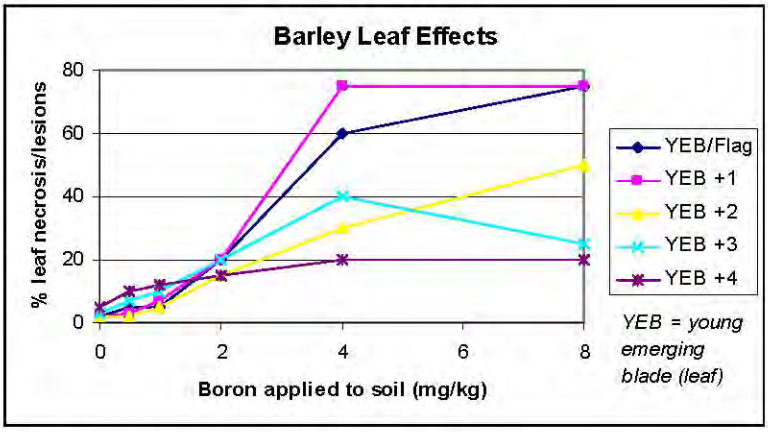
Aitken and McCallum (1988)



Riley et al (1984):





Other effects

Gupta and Cutcliffe: Not reported

Aitken and McCallum: Not reported

Riley et al: Not reported

Results of controls

Number/ percentage of plants showing adverse effects	Gupta and Cutcliffe: See Table A7_5_1_3-6a Aitken and McCallum: Not reported Riley et al.: Not reported
Nature of adverse effects	Gupta and Cutcliffe: Not reported Aitken and McCallum: Not reported Riley et al.: Not reported
Test with reference substance	Not performed
Concentrations	
Results	

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

Bioavailable boron is frequently estimated in the field using the fraction that is hot-water-soluble, which varies from 0.4% to 4.7% of the total boron (Eisler, 2000). However, there appear no simple translator relationships to predict soil water concentrations from soil content or from supplied nutrient concentrations (Goldberg, et al., 2000). As noted in Section 4.1, binding to soil chemicals may reduce available borates.

Studies have often used hydroponic or sand cultures with boron applied as part of a nutrient solution. This simplifies attempting to measure soil boron concentrations by assuming that the nutrient solution concentration is the actual exposure.

Field or container studies have applied boron as part of a fertilizer mixture with a single application. This mimics typical agricultural practice. Soil concentrations, when measured, are usually taken after harvest. Gupta and Cutcliffe (1984) illustrate the latter approach. In addition, they measured yields a second year after a single boron application, finding that boron levels had subsided and also that no adverse effects on beans were observed after any application. Aitken and McCallum (1988) used containers, but obtained soil pore water by centrifugation.

All the terrestrial plant studies are more elaborate than the acute plant study profiled in OECD 208. The endpoints are not mortality and most frequently are growth or yield at maturity. Consequently, these studies are appropriately considered as chronic or possible sub-chronic results.

Results and discussion

For the Gupta and Cutcliffe data, toxicity thresholds were observed at 4.4 kg-B/ha application rate for the bean Phaseolus vulgaris but not at 2.2 kg-B/ha. Application rates of up to 8.8 kg-B/ha had no adverse effect on yield of cabbage (Brassica oleracea var capitata). Estimated initial soil loadings of 2.2 and 4.4 kg-B/ha would be 1.6 and 3.1 mg-B/kg-soil, respectively.

Aitken and McCallum (1988) found a toxicity threshold for sunflower Helianthus annuus of 1.9 to 2.4 mg-B/L in soil porewater, depending on which model is fitted to the data.

Riley et al (1984) found no effects to barley at 1 mg/kg soil addition.

At additions of 2 to 8 mg/kg increases in plant growth form and leaf damage were observed. Only at 8 mg/kg was grain production affected.

The narrow band between toxicity threshold and deficiency is illustrated by comparing Riley et al.'s results with another study using the same cultivar of barley. Wongmo et al (2004) reported deficiency in field studies of Hordeum vulgaris cv Stirling where measured boron levels were 0.15 mg/kg. Addition of boron at 1.1 kg/ha increased yield significantly.

The observed differences in toxicity thresholds among these four species are suggestive of the larger range of deficiency and toxicity thresholds. Several reviews of plant requirements and tolerance values are available: Eisler, 2000; Eisler 1990; ECETOC 1997; Butterwick et al., 1989; and Gupta et al., 1985, Keren and Bingham, 1985, and Sprague 1972.

A table showing soil solutions producing the best growth and those producing visible signs of injury is shown below, taken from Sprague, 1972 (Table: Terrestrial Plants: Relationship of Boron to Growth and Injury). The plants are classified as "sensitive", "semi-tolerant" or "tolerant" based on the ratio of growth in trace or 1 mg-B/L culture solutions to the growth in 5, 10 and 15 mg-B/L culture solutions. Sensitive species growth in high-B solutions was 50% or less than growth in low-B solutions. Tolerant species growth in high-B solutions was more than 100% of growth in low-B solutions. The figure below shows relative growth of representative species, with growth of tolerant species (e.g., turnip and beet) enhanced at higher boron soil solution concentrations. Sprague noted that over 70% of the plantings did best with more than a trace of boron and 46% did best with more than 1 mg-B/L.

Similar tables are presented by Gupta et al, (1985), Keren and Bingham (1985), Eisler (1990) and others. A representative table from Eisler (1990) is shown below (Table: Boron Toxicity to some Terrestrial Plants). These illustrate that boron levels associated with adverse effects vary among species. Further, test results are seldom reported as boron concentrations in the soil. Rather, values are the boron concentrations in the nutrient solutions that were applied in the experiments. In addition, most tests used sand or sandy soils to minimize potential adsorption of boron to the solid phase. This reflects the understanding that the concentration of boron in the solution bathing the root system is the bioavailable amount (Keren and Bingham 1985).

Crommentuijn et al. (1995) proposed an ecotoxicological "serious soil concentration" (SSC) value of 7.0 mg/kg. This value was based (in part) on only 3 terrestrial plant species. The SSC value derived from these was 2.2 mg/kg (the geometric mean of the EC50/10 and NOEC values). However, this seems a significant under-representation of available data: the review by Gupta et al. (1985) presented tolerance values for 53 species of crops and ornamentals. Tolerance values represent the threshold for visible symptoms to occur, often without measurable decrease in crop yield (Butterwick et al. 1989), so can be considered equivalent to NOEC values. Further, the lowest value cited was for barley (Hordeum vulgare L. cv. Stirling); the author of that value later published a fuller account that pointed out the variability of critical tissue levels in barley. Riley et al. (1994) reported that critical toxic concentrations of boron in shoots varied between 40 and 150 µg in their work and that only at the highest test concentrations (application 8 µg-B/g-soil) was the harvest index reduced.

$\underline{EC_{20}}$

$\underline{EC_{50}}$

$\underline{EC_{80}}$

Conclusion

The essentiality of boron to plants means that extremely low concentrations of boron can be problematic to the environment. Boron has been added as a critical micronutrient for decades, at rates that reflect the needs of the species being cultivated. Recommended application rates have been published (Borax, 2002) that suggest what range of rate might be suited to address boron deficiency in particular species, as shown in the accompanying table (Table: Estimated Boron Concentration in Soil with Fertilization). However, practitioners rely on measures of boron concentration in plant tissue, as well as soil measurements, to determine the need for boron application.

Application of boron could increase the amount of boron in soil. To estimate potential soil boron calculations, recommended application rates for various products were used with an assumed soil density (1400 kg/cubic meter) and a mixing depth of 20 cm. These factors were used by van de Plassche et al. (1999) to estimate soil additions of boron from agricultural applications.

As shown in the accompanying table (Table A7_5_1_3-X d), boron soil concentrations could range from 0.2 to 2.7 mg-B/kg-soil following a single broadcast application of a typical product at recommended rates. These values slightly exceed those calculated (0.16 to 2.0 mg-B/kg) by Mortvedt et al. (1992) for several crops with application rates of 0.45 to 5.7 kg/ha.

These values exceed, without exception, the Maximum Permissible Addition (MPA) of 0.1 mg-B/kg presented by van de Plassche et al. (1999). The MPA is intended to be a concentration that can be added without concern for ecotoxicological effects. Unfortunately, the scheme fails to accommodate essential elements by attempting to set very conservative standards. Restriction of micronutrient concentrations to the MPA would be expected to impart adverse effects on the relevant biota – not from toxicological effects, but from deficiency effects.

The data for terrestrial plants represent chronic or subchronic studies, extending over all or most of the species' life-cycle. In many cases, the studies involve field plots rather than laboratory studies. Using standard approaches, the data could generate a "PNEC" value. However, given the tolerance and even requirement of some species, that value would not be without its own adverse effects from deficiencies. Consequently, the conclusions reached by numerous reviewers remains valid: boron is essential to plants, but is phytotoxic at higher concentrations. The thresholds for deficiency and toxicity vary between species and even within species and may be quite narrow (e.g., less than 10x), making generalizations dangerous.

Reliability	1 - Reliable
Deficiencies	Yes/No (If yes, discuss the impact of of deficiencies and implications on results. If relevant, justify accepability of study

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE** 28-01-2005
Reference	**1. Gupta and Cutcliffe (1984)**
Materials and Methods	Applicant's summary adequately reflects description in paper, except for test substance which was Borate 65. Borate 65 contains 20 % boron as anhydrous disodium tetraborate ($Na_2B_4O_7$), authors report application rates on the basis of elemental boron as kg B/ha.
Results and discussion	Cabbage: applicants summary is correct, addition of $Na_2B_4O_7$ at rates up to 8.8 kg B/ha does not affect cabbage yield

Beans: Applicant's table on dry bean seed yield is copied below with addition of asterisks for statistically significant differences as compared to control treatment

Year	Nominal	Site 1	Site 2	Site 3	Site 4
1	0	1332	1830	1771	1406
1	2.2	1292	1279	1719	1458
1	4.4	1035	1507	1302*	1042*
1	8.8	779*	713*	677*	729*
2	0 aged	1373	2099	1329	1198
2	2.2 aged	1593	1901	1458	1121
2	4.4 aged	1561	2486	1406	965
2	8.8 aged	1658	1965	1277	1121

From this table, NOEC's of 2.2 (site 3 and 4) and 4.4 kg B/ha (site 1 and 2) can be derived for beans. With incorporation to a depth of 10 cm, and assuming a dry soil bulk density of 1500 kg/m^3, 2.2 kg B/ha is equivalent to 1.5 mg B/kg, 4.4 kg B/ha to 2.9 mg B/kg dwt soil and 8.8 kg/ha to 5.9 mg/kg dwt soil

Conclusion	The marketable yield of beans and cabbage was determined after addition of Borate 65 (20 % boron as anhydrous disodium tetraborate) in a field experiment The lowest NOEC for marketable yields of beans is 2.2 kg/ha, equivalent to 1.5 mg B/kg dwt soil. The NOEC for cabbage is ≥ 8.8 kg B/ha, equivalent to ≥ 5.9 mg B/kg.
Reliability	2
Acceptability	Acceptable, the NOEC of 1.5 mg B/kg dwt soil for marketable yield of beans will be included in the risk assessment.
Remarks	

Reference

2. Aitken and McCallum (1988)

Materials and Methods

Applicant's summary is acceptable, addition to test methods:
Test compound was boric acid, application rates are reported on the basis of boron as kg B/ha.
Soil was incubated for 7 days after addition of the test substance

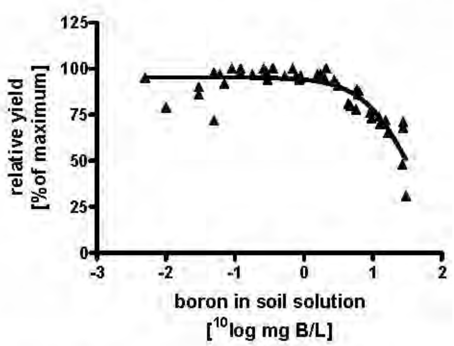
Results and discussion

In view of the duration of the study (14 days), the experiment can be considered as a short-term test and the endpoint should be an EC_{50}. EC_{50} values for dry matter yield were estimated by non-linear regression of a sigmoid concentration-respons curve based on added boron. For sandy loam, a model was used that accounts for positive effects at lower concentrations (Van Ewijk and Hoekstra, 1993[1]). Results are expressed as kg B/ha, based on nominal application rates. The EC_{50} in kg B/ha was recalculated by RMS into mg B/kg soil using the dimensions of the test containers (∅ 6 cm, 11 cm height, assumed soil depth 10 cm) and assuming a dry soil bulk density of 1500 kg/m^3. The latter was used because the application was made on dry soil and moisture content was adjusted afterwards. Results are given in the table below.

Soil	Effect concentration [kg B/ha]				r^2	EC_{50}
	EC_{10}	95 % CI	EC_{50}	95 % CI		[mg B/kg dwt soil]
loamy sand 1	-	-	-	-	-	-
sand 1	2.18	1.44 - 3.32	7.67	6.53 - 9.02	0.9870	5.11
sandy loam			6.59	-63.4 - 76.6	0.9679	4.39
sand 2	2.17	1.08 - 4.35	5.72	4.35 - 7.56	0.9591	3.81
loamy sand 2	5.48	3.28 - 9.13	23.2	12.8 - 42.0	0.9127	15.5
silty clay	10.8	8.59 - 13.5	15.7	9.49 - 26.1	0.8080	10.5

The EC_{50} value for sandy loam is considered less reliable because of the large confidence limits.

The toxic threshold concentrations of 1.9 – 2.4 mg B/L as reported in the applicant's summary were obtained by plotting the dry weight yield as percentage of the maximum yield for all soils against ^{10}log-transformed concentrations in soil solution. Latter were obtained by centrifugation of the test soil. The threshold concentration was estimated by fitting two straight intersecting lines through the points, r^2 = 0.85. The values of 1.9 – 2.4 mg B/L represent a No Effect Concentration.
EC_{50}-values were estimated by RMS by fitting a sigmoid concentration-response model through the data, resulting EC_{50} was 33.7 mg B/L (95 % CI 25.3 - 44.7), EC_{10} was 5.57 mg B/L (95 % CI 3.28 – 9.45), r^2 was 0.7489).



From the data it appears that sub-optimal growth may occur at soil solution of < 0.1 mg B/L.

[1] Van Ewijk, P.A., Hoekstra, J.A. (1993) Calculation of the EC_{50} and its confidence interval when subtoxic stimulus is present. *Ecotoxicol. Environ. Saf.* **25**, 25-32.

Conclusion	From the results of the study it is concluded that the EC_{50} of boric acid for growth of sunflowers is 5.72 – 23.2 kg B/ha, based on nominal added application rates. This is equivalent to 3.81 – 15.5 mg B/kg dwt soil. The geometric mean is 6.74 mg B/kg. Based on measured concentrations of boron in soil solution, obtained by centrifugation of the test soil, the EC_{10} and EC_{50} were 5.57 and 33.7 mg B/L. Sub-optimal growth may occur at boron levels in soil solution of < 0.1 and > 2.5 mg B/L.
Reliability	2
Acceptability	Acceptable, the geometric mean 14-days EC_{50} of 6.74 mg B/kg will be included in the risk assessment.
Remarks	
Reference	**3. Riley et al. (1994)**
Materials and Methods	Applicant's summary is acceptable, addition to test methods: Test compound was boric acid, results are expressed on the basis of boron as μg B/g soil (= mg B/kg). Plants were examined at three stages, early tillering after 35 days, booting after 85 days and at maturity. Latter is presented as 130 days by applicant, this cannot be found in the paper which only states that last fertiliser application was on day 120.

Results and discussion

Applicant's summary of results is acceptable, some additions can be made. Authors present ratio of root dry weight to shoot dry weight, as a measure of effect on roots as compared to that on shoots, and give total affected leaf area. A summary of effect parameters is presented below. Significant differences from control are indicated by asterisks. EC_{10} values for the respective endpoints were calculated by non-linear regression, assuming a sigmoid concentration response relationship.

Concentration added [mg B/kg]	Root/shoot ratio[1]	Harvest index[2,3]	# Tillers[3]	Tillers lodged[3] [%]	Total leaf area affected[3] [%]
0	0.34	0.27	30	0	3
0.5	0.34	0.26	31	0	6
1	0.33	0.28	28	4	9
2	0.29	0.27	30	23*	15
4	0.24*	0.28	27*	70*	40
8	0.20*	0.23*	27*	92*	45
EC_{10}	1.24	7.87	-	1.38	1.04
95 % CI	0.42 – 3.64	-	-	1.20 – 1.60	0.16 – 6.89
r^2	0.9769	0.84	-	0.9993	0.9388

1: at booting, 85 days

2: harvest index = grain yield as proportion of whole shoot dry weight

3: at maturity (> 120 days)

At the lowest level of added boron, leaf area of older leaves was decreased as compared to the control. At higher levels, the affected leaf area was increased. In combination with an increased total leaf area, the net area of unaffected leaves remained constant.

Information on the relationship between soil boron concentrations and uptake by plants, necessary for the assessment of risks for the food chain, can additionally be derived form this article. The concentration of boron in the whole plant, straw (=whole plants minus grains) and grains and corresponding BCF's are given in the following tables

Concentration in soil	Residues [mg/kg dwt] in		
[mg B/kg dwt]	Whole plant	Straw	Grain
0	8	11	2
0.5	19	54	2
1	39	52	3
2	88	120	3
4	190	260	4
8	390	510	6

Concentration in soil	BCF in		
[mg B/kg dwt]	Whole plant [kg_{soil}/kg_{plant}]	Straw [kg_{soil}/kg_{straw}]	Grain [kg_{soil}/kg_{grain}]
0	-	-	-
0.5	38	108	4.0
1	39	52	3.0
2	44	60	1.5
4	48	65	1.0
8	49	64	0.8

Conclusion

From the results of this study it appears that the lowest NOEC for phytotoxic (% Tillerslodged) effects of boric acid on barley is 1 mg B/kg dwt soil, based on nominal added concentrations. The calculated EC_{10} in agreement with this value, the lowest EC_{10} being 1.04 mg B/kg. The NOEC for root/shoot ratio is 2 mg B/kg dwt and the NOEC for relative grain yield at maturity is 4 mg B/kg dwt soil.

The BCF is 38 – 49 kg_{soil}/kg_{plant}, 52 -108 kg_{soil}/kg_{straw} and 0.8 – 4.0 kg_{soil}/kg_{grains}, all on a dry weight basis.

Reliability

2

Acceptability

Acceptable, 2 mg B/kg dwt soil for root/shoot ratio and 4 mg/kg dwt soil for relative grain yield and the BCF for whole plants of 38 – 49 kg_{soil}/kg_{plant}, 52 -108 kg_{soil}/kg_{straw} and 0.8 – 4.0 kg_{soil}/kg_{grains} (all dwt/dwt) will be included in the risk assessment.

The NOEC of 1 mg B/kg dwt soil for phytotoxicity (% tillers lodged) is not used in the risk assessment, as this parameter can be considered as ecologically less relevant.

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_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	None
Vehicle control performed	No
Other procedures	Not applicable – TS is water soluble, not volatile

a. Gupta and Cutcliffe (1984): Beans and Cabbage

Table A7_5_1_3-2a: Dilution water

Criteria	Details
Source	None – field experiments
Alkalinity / Salinity	Not reported – natural precipitation only
Hardness	Not reported
pH	Not reported
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	No

Table A7_5_1_3-3 a: Test plants

	Family	Species	Common name	Source (seed/plant)
Dicotyledonae	Mustards	Brassica oleracea var. capitata	Cabbage	Transplanted
	Legumes	*Phaseolus vulgaris*	Field beans	Seeded
Monocotyledonae				

Table A7_5_1_3-4 a: Test system

Criteria	Details
Test type	Field plots
Container type	Field plot
Seed germination potential	Not reported
Identification of the plant species	Not reported
Number of replicates	Four replicate plots at four levels of boron
Numbers of plants per replicate per dose	Not reported
Date of planting	Bean seeds planted early June, Cabbage planted late June/early July
Plant density	Beans 5 cm apart, cabbage 60 cm
Date of test substance application	Before planting
High of plants at application	Not reported
Date of phytotoxicity rating or harvest	Beans harvested mid- to late September. Cabbage harvested 2nd or 3rd week of October (1979 – 1982)
Dates of analysis	Not reported

Table A7_5_1_3-5 a: Test conditions

Criteria	Details
Test type	Field test in randomized block design, at four locations on Prince Edward Island, Canada
Method of application	Single application, broadcast by hand, incorporated to 10 cm depth.
Application levels	0, 2.2, 4.4 and 8.8 kg-B/ha
Dose rates	Assuming soil density of 1400 kg/m^3 and incorporation into 10 cm, initial soil concentrations would be: 0, 1.57, 3.14 and 6.29 mg-B/kg-soil
Substrate characteristics	Soils classed as orthic humoferric podzols, ranged from loamy sand to fine sandy loam. Slopes of 2-5%, well-drained with no impeding horizon (3 sites) or impeding layer within 50 cm (1 site)
Watering of the plants	Not watered or irrigated. Rainfall ranged from 334 to 430 mm during June-September, with average being 330 mm.
Temperature	Not reported
Thermoperiod	Not reported
Light regime	Not reported
Relative humidity	Not reported
Wind volatility	Not reported
Observation periods and duration of test	Beans: planted early June, harvested mid- to late September. Cabbage: transplanted late June, early July, harvested 2nd or 3rd week of October
Pest control	Not reported
Any other treatments and procedures	Followed current vegetable Production Guides for the Atlantic Provinces of Canada

Table A7_5_1_3-6 a: Effective phytotoxicity after test termination

A. Boron application rates and measured soil concentratons

	Nominal (kg-B/ha)	Measured (HWsB) mg-B/kg-soil Site 1	Site 2	Site 3	Site 4
Year 1	0	0.5	0.5	0.3	0.3
Year 1	2.2	0.7	0.7	0.6	0.7
Year 1	4.4	1.2	1.1	0.9	1
Year 1	8.8	1.5	1.4	1.9	1.6
Year 2	0 aged	0.6	0.6	0.4	0.5
Year 2	2.2 aged	0.7	0.7	0.5	0.6
Year 2	4.4 aged	0.7	0.9	0.8	0.7
Year 2	8.8 aged	1	1.2	1.1	0.8

B. Cabbage, tonnes/ha fresh yield

	Nominal (kg-B/ha)	Site 1	Site 2	Site 3	Site 4
Year 1	0	44.4	51.3	36.5	33.3
Year 1	2.2	41.3	51.2	35	26.9
Year 1	4.4	37.2	53.5	41	31.6
Year 1	8.8	40.6	52.5	35	25.6
Year 2	0 aged	31.3	41.9	35.5	26.4
Year 2	2.2 aged	29.1	45.7	35	23.5
Year 2	4.4 aged	27.2	42.8	36.2	29.7
Year 2	8.8 aged	31.9	45.6	35.4	29.4

C. Cabbage, % of Zero Application Sites

	Nominal (kg-B/ha)	Site 1	Site 2	Site 3	Site 4	Mean
Year 1	0	-	-	-	-	
Year 1	2.2	93%	100%	96%	81%	**92%**
Year 1	4.4	84%	104%	112%	95%	**99%**
Year 1	8.8	91%	102%	96%	77%	**92%**
Year 2	0 aged*	70%	82%	97%	79%	**82%**
Year 2	2.2 aged	66%	89%	96%	71%	**80%**
Year 2	4.4 aged	61%	83%	99%	89%	**83%**
Year 2	8.8 aged	72%	89%	97%	88%	**87%**

*Comparison of Year 2 with Year 1 at zero application sites

D. Beans, kg/ha dry bean seed yield

	Nominal (kg-B/ha)	Site 1	Site 2	Site 3	Site 4
Year 1	0	1332	1830	1771	1406
Year 1	2.2	1292	1279	1719	1458

Year 1	4.4	1035	1507	1302	1042
Year 1	8.8	779	713	677	729
Year 2	0 aged	1373	2099	1329	1198
Year 2	2.2 aged	1593	1901	1458	1121
Year 2	4.4 aged	1561	2486	1406	965
Year 2	8.8 aged	1658	1965	1277	1121

E. Beans, % of Zero Application Sites

	Nominal (kg-B/ha)	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Mean**
Year 1	0	-	-	-	-	
Year 1	2.2	97%	70%	97%	104%	**92%**
Year 1	4.4	78%	82%	74%	74%	**77%**
Year 1	8.8	58%	39%	38%	52%	**47%**
Year 2	0 aged*	103%	115%	75%	85%	**95%**
Year 2	2.2 aged	116%	91%	110%	94%	**102%**
Year 2	4.4 aged	114%	118%	106%	81%	**105%**
Year 2	8.8 aged	121%	94%	96%	94%	**101%**

*Comparison of Year 2 with Year 1 at zero application sites

b. Aitken and McCallum (1988): Sunflower

Table A7_5_1_3-2 b: Dilution water

Criteria	**Details**
Source	Deionized water mixed with boric acid for initial boron treatment
Alkalinity / Salinity	Not reported
Hardness	Not reported
pH	Not reported
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	No

Table A7_5_1_3-3 b: Test plants

	Family	Species	Common name	Source (seed/plant)
Dicotyledonae	Compositae	Helianthus annus cv Hysun 31	Sunflower	Transplant seedlings
Monocotyledonae				

Table A7_5_1_3-4 b: Test system

Criteria	Details
Test type	Growth chamber, 16/8 hr illumination
Container type	Polypropylene, 6 cm diameter, 11 cm high
Seed germination potential	Not reported
Identification of the plant species	Cv Hysun 31
Number of replicates	3 per treatment
Numbers of plants per replicate per dose	2 plants per container, 6 per treatment
Date of planting	Not reported
Plant density	Not reported
Date of test substance application	Not reported; before transplantation
High of plants at application	Not reported
Date of phytotoxicity rating or harvest	Not reported; 14 days after transplanting
Dates of analysis	Not reported

Table A7_5_1_3-5 b: Test conditions

Criteria	Details
Test type	Containers in growth chamber
Method of application	Applied as boric acid in solution,mixed with soil and allowed to incubate for 7 days
Application levels	Soils 1-4: 0, 025, 0.5, 1, 2, 4 and 8 kg-B/ha Soils 5-6: 0, 025, 0.5, 1, 2, 4, 6, 8, and 12 kg-B/ha
Dose rates	kg per ha
Substrate characteristics	Soil Type pH %oc %(w/w)<0.002mm 1 loamy sand 5.9 0.7 8% 2 sand 5.7 0.5 2% 3 sandy loam 5.7 1.1 12% 4 sand 5.1 0.1 1% 5 loamy sane 4.8 0.7 10% 6 silty clay 4.8 1.7 49%
Watering of the plants	Watered daily with DI water to constant weight
Temperature	30°C during day, 25°C at night
Thermoperiod	See above
Light regime	16 hr day, 8 hr night
Relative humidity	70%
Wind volatility	Not relevant
Observation periods and duration of test	Harvested after 14 days
Pest control	Not reported
Any other treatments and procedures	Basal nutrients (N K S Ca Mg Cu Zn Mn Mo P) supplied as solution at initiation. Plant tops cut at soil level, washed in DI water, dried and weighed. Boron concentrations in soils extracted with centrifugation and measured with ICP-AES. HWsB also measured in soils 4-6.

Table A7_5_1_3-6 b: Effective phytotoxicity after test termination

Yield of Sunflower top (dry weight, mg/container, mean of 3 replicates)

Application Rate	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6
0	220	203	243	345	332	393
0.25	304	208	304	400	360	373
0.5	292	199	306	384	351	405
1	296	209	290	401	338	402
2	246	186	239	345	338	413
4	221	155	214	262	326	396
6					318	402
8	215	100	207	125	275	388
12					258	329

Relative change in Sunflower Yield (% of Zero application controls)

Application Rate	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6
0.25	138%	102%	125%	116%	108%	95%
0.5	133%	98%	126%	111%	106%	103%
1	135%	103%	119%	116%	102%	102%
2	112%	92%	98%	100%	102%	105%
4	100%	76%	88%	76%	98%	101%
6					96%	102%
8	98%	49%	85%	36%	83%	99%
12					78%	84%
Decrease at:	none	4	4	4	8	12

C. Riley et al. (1984): Barley

Table A7_5_1_3-2 c: Dilution water

Criteria	Details
Source	Not reported
Alkalinity / Salinity	Not reported
Hardness	Not reported
pH	Not reported
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	No

Table A7_5_1_3-3: Test plants

	Family	Species	Common name	Source (seed/plant)
Monocotyledonae				Cultivar:
	Gramineae	Hordeum vulgaris	Barley – 2 row	Stirling

Table A7_5_1_3-4c: Test system

Criteria	Details
Test type	Container
Container type	Plastic with polythene liners,16.5 cm diameter, 3.5 kg soil per container
Seed germination potential	Not reported.
Identification of the plant species	See above
Number of replicates	3 replicate containers per treatment
Numbers of plants per replicate per dose	Planted 20 germinating seeds, culled to 10 per container
Date of planting	Not reported
Plant density	10 per container
Date of test substance application	Before planting
High of plants at application	Before planting
Date of phytotoxicity rating or harvest	Harvest 1: day 35, harvest 2: day 85, final harvest: day 130
Dates of analysis	Not reported

Table A7_5_1_3-5c: Test conditions

Criteria	Details
Test type	Container in glasshouse
Method of application	Boric acid mixed with soils before potting
Application levels	0, 0.5, 1, 2, 4 and 8 mg-B/kg-soil
Dose rates	See above
Substrate characteristics	Soil: virgin sandy soil from Lancelin, air-dried, sieved, pH 5.6, clay content 20%, organic carbon 0.69%.
Watering of the plants	Moistened to 75% of field capacity by weight, used deionized water
Temperature	Not reported
Thermoperiod	Not reported
Light regime	Not reported
Relative humidity	Not reported
Wind volatility	Not reported
Observation periods and duration of test	Not reported
Pest control	Not reported
Any other treatments and procedures	Not reported

Table A7_5_1_3-6c: Effective phytotoxicity after test termination

Barley leaf area affected by B addition to soil (at maturity)

Boron added (µg/g)	YEB/Flag	YEB +1	YEB +2	YEB +3	YEB +4
0	2	2	2	3	5
0.5	5	3	2	7	10
1	5	7	5	10	12
2	20	20	15	20	15
4	60	75	30	40	20
8	75	75	50	25	20

YEB = youngest emerging leaf blade

Barley yield measures (at maturity)

Boron added (µg/g)	Harvest Index	# tillers	% tillers lodging	Ave height of 1° tiller (cm)
0	0.27	30	0	65
0.5	0.26	31	0	66
1	0.28	28	4	68
2	0.27	30	23*	69*
4	0.28	27	70*	74*
8	0.23	27	92*	73*

* = Difference from control exceeded LSD for p=0.05

Table A7_5_1_3-7: Validity criteria for terrestrial plant toxicity according to EPA OPPTS 850.4150 (vegetative vigor test)

	Fulfilled	Not fulfilled
Adverse effect > 25 % on one or more plant species (EPA)	X	

A7_5_1_3-X Survey and Review Articles

Studies of boron effects on terrestrial plants have shown that the ranges of deficiencies and toxicities overlap, as illustrated in the Figure A7_5_1_3-X.a from Sprague (1972) below. Reviews have characterized plants as sensitive, semi-sensitive, or tolerant, as shown in the tables A7_5_1_3-Xb below from Sprague (1972) and A7_5_1_3-Xc from Eisler (1990).

Agricultural practices apply boron in many areas. Shorrocks (1997) reports that boron deficiencies have been reported in over 80 countries and on 132 crops. Table A7_5_1_3-X d shows recommended boron application rates, and the probable resulting soil concentrations, assuming standard soil density and depth of tillage.

Figure A7_5_1_3-X .a. ***Boron Effects on Plant Growth (Fig. 3 from Sprague, 1972. Plant weight relative to growth in trace boron concentrations is shown. Data from Eaton, 1944.)***

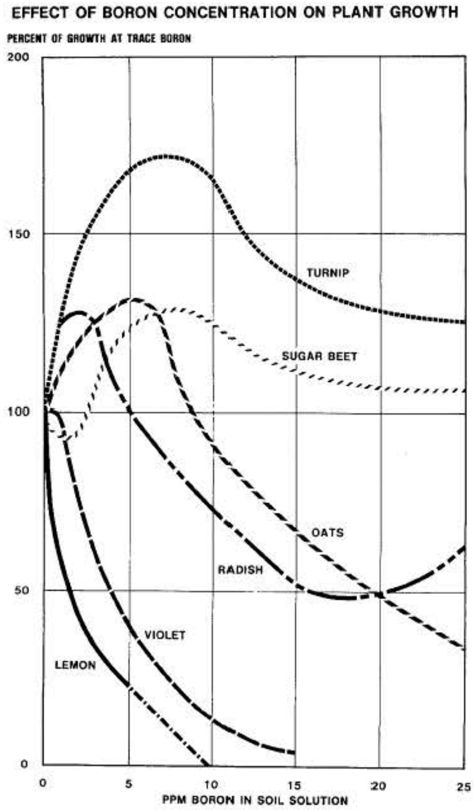


Table A7_5_1_3-Xb

Terrestrial Plants: Relationship of Boron to Growth and Injury.

(From Sprague, 1972, using data from plants in sand cultures using nutrient solutions containing trace boron (0.03 to 0.04 mg-B/L), 1, 5, 10, 15, and 25 mg-B/L. Data from Eaton 1944.)

TABLE V

Plant Growth as Affected by Boron

PLANT	BORON CONC. FOR BEST GROWTH ppm	LOWEST CONC. FOR INJURY ppm	RELATIVE TOLERANCE PCT.	BORON DEFICIENCY SYMPTOMS WITH TRACE BORON*
	SENSITIVE PLANTS			
Blackberry *(Rubus sp.)*	Trace	1	–	S
Lemon *(Citrus limonia osbeck)*	Trace	1	–	None
Elm *(Cimusamericana L.)*	1	1	–	S
Cherry *(Prunus avium L.)*	1	5	–	S
Peach *(Prunus persica (L) Batsch)*	1	5	–	2
Persimmon *(Diospyros kaki L.f.)*	1	1	–	S
Fig *(Ficus carica L.)*	1	5	–	S
Strawberry *(Fragaria sp.)*	1	5	–	None
Lupine *(Lupinus hartwegi Lindl.)*	1	5	–	M
Grape *(Vitisvinifera L.)*	1	5	9	S
Violet *(Viola odorata L.)*	Trace	5	20	None
Pansy *(Viola tricolor L.)*	Trace	5	24	None
Kidney bean *(Phaseolus vulgaris L.)*	1	1	–	None
Cowpea *(Vigna sinensis (Torner) Savi)*	Trace	5	30	D
Jerusalem artichoke *(Helianthus tuberosus L)*	1	1	34	None
Larkspur *(Delphinium sp.)*	1	5	43	S
Zinnia *(Zinnia elegans Jacq.)*	Trace	1	45	None
	SEMI-TOLERANT PLANTS			
Barley *(Hordeum vulgare L.)*	Trace	5	51	D
Pea *(Pisum sativum L.)*	1	5	55	None
Lima bean *(phaseolus lunatus L.)*	Trace	1	57	S
Sweet potato *(Ipomoea batatas (L) Lam.)*	Trace	5	63	S
Onion *(Allium cepa L.)*	Trace	1	68	None
Carrot *(Daucus carota L.)*	Trace	10	70	None
Red pepper *(Capsicum frutescens L.)*	Trace	5	71	None
Kentucky bluegrass *(poa pratensis L.)*	5	1	86	None
Corn *(Zea mays L.)*	1	5	53	None
Potato *(Solanum tuberosum L.)*	1	1	78	S
Cabbage *(Brassica oleracea var. capitata L.)*	1	10	78	None

TABLE V (continued)

Plant Growth as Affected by Boron

PLANT	Boron Conc. For Best Growth ppm	Lowest Conc. For Injury ppm	Relative Tolerance Pct.	Boron Deficiency Symptoms With Trace Boron*
		SEMI-TOLERANT PLANTS		
Milo *(Sorghum vulgare Pers.)*	Trace	5	(8)	M
Calendula *(Calendula officinalis L.)*	Trace	5	80	None
Radish *(Raphanus sativus L.)*	1	10	60	S
Oats *(Avena sativa L.)*	5	5	86	None
Celery *(Apium graveolens L.)*	15	25	89	S
Mustard *(Brassica sp.)*	1	10	94	None
Parsley *(Petroselinum crispum (Mill.) Nym.)*	5	15	95	None
Alfalfa *(Medicago sativa L.)*	10	15	106	None
Lettuce *(Lactucca sativa L.)*	5	1	96	None
Tobacco *(Nicotiana tomentosa Ruiz and Pav.)*	15	10	96	None
Vetch *(Vicia atropurpurea Desf.)*	5	5	98	None
Tomato *(Lycopersicon esculentum Mill.)*	10	5	99	None
California Poppy *(Eschscholtzia californica Cham.)*	5	5	99	None
		TOLERANT PLANTS		
Turnip *(Brassica rapa L.)*	5	25	115	None
Common beet *(Beta vulgaris L.)*	5	15	112	S(?)
Leaf beet *(Beta vulgaris var. cicla L.)*	5	25	110	W
Muskmelon *(Cucumis melo L.)*	5	5	107	D
Sweet clover *(melilotus indica (L.) All.)*	5	10	110	None
Sweet pea *(Lathyrus odoratus L.)*	10	5	113	S
Sugar beet *(Beta vulgaris var. crassa Alef.)*	5	15	121	None
Oxalis *(Oxalis bowiei Herb.)*	10	None	121	S
Cotton *(Gossypium hirsutum L.)*	10	10	130	S
Artichoke *(Cynara scolymus L.)*	5	5	123	S
Asparagus *(Asparagus officinalis L.)*	51	25	217	S

* S=*presence of leaf or other morphological abnormalities.*
M=*time of flowering or ripening substantially affected.*
D=*trace—boron plants more severely attacked by mildew.*
W=*severe wilting.*

Table A7_5_1_3-X c.
Boron Toxicity to some Terrestrial Plants (Eisler, 1990, Table 4)

Species, Dose and Other Variables	*Effects*
Bigleaf maple, *Acer macrophyllum*	
0.9–5.4 mg B/L in saturated soil extracts	Reduced growth; >25% foliar damage; leaf residues of 76–324 mg B/kg ash weight (AW)
Madrone, *Arbutus menziesii*	
2.2–5.4 mg B/L in saturated soil extracts	Growth inhibition; >25% foliar damage; leaf residues of 216–540 mg B/kg AW
Beet, *Beta vulgaris*	
Soil B solutions	
5 mg/L	Optimal growth
15 mg/L	Injury evident
Broccoli, *Brassica oleracea italica*	
Grown in nutrient solutions containing	
0.08 mgB/L	Chlorophyll levels and net photosynthetic rates were significantly lower than those for plants grown in 0.41–0.81 mg B/L solutions
4.1 and 8.1 mg B/L	Leaf damage evident; lower chlorophyll levels and lower net photosynthetic rate than 0.4 and 0.8 mg B/L groups
Rhodes grass, *Chloris gayana*	
Grown in fly ash containing 3 mg hot-water-soluble B/L	Toxic. Residues >149 mg/kg dry weight (DW)
Lemon, *Citrus limonia osbeck*	
Soil B concentrations	
0.03–0.04 mg/L	Optimal growth
1 mg/L	Injury evident
Soybean, *Glycine max*	
Grown in soils amended with scrubber sludge residues (4.1 g B/kg) from coal-fired power plant for 2–3 years	Higher sludge B levels of 2 mg B/kg soil surface at year 1, and 1.2 mg B/kg at year 2 produced signs of B toxicity, including decreased growth and elevated residues in leaf (>83 mg/kg DW) and in seeds (>47 mg/kg DW)
Sunflower, *Helianthus annuus*	
50 mg B/L growth medium	Adversely affects phospholipid composition and synthesis in roots and microsomes from seedlings by inhibition of choline phosphotransferasse
10 mg B/L growth medium	Tolerated level

Barley, *Hordeum vulgare*	
Residues, in mg B/kg DW	
0.5–1.0 in soil	Residues of 46–100 mg/kg DW in leaves
30 in shoots	Damage to older leaves
50–70 in shoots	Reduction of 10% in DW of shoots [7]
60–80 in leaf	Toxicity evident
80–120 in shoots	Toxic signs, but no yield reductions
120–130 in shoots	Grain yield reduced 10%
Alfalfa, *Medicago sativa*	
850–975 mg B/kg DW plant	Reduced yield
Rice, *Oryza sativa*	
Whole plant B residues	
38 mg/kg DW	No signs of toxicity
43–55 mg/kg DW	Signs of toxicity evident
Soil waters	
2.5–5 mg B/L	Toxic
French bean, *Phaseolus vulgaris*	
Grown in fly ash containing	
3 mg hot-water-soluble B/L	Toxic. Residues >209 mg/kg DW
Residues in whole plant	
9–12 mg B/kg DW	Slow flowering and pod formation; general yellowing of tips
>125	Reduced growth; burned older leaves dark brown
Digger pine, *Pinus sabiniana*, seedlings	
13–17 mg B/L in saturated soil extracts	Growth reduction; foliar damage >25%; needle residues 1,242–1,512 mg B/kg AW
Pear, *Pyrus communis*	
82–164 kg B/ha applied to soil around pear trees in a nonirrigated orchard over a 6-year period	Toxicity observed during application and during 4 years postapplication. Toxicity was associated with residues, in mg B/kg DW, of 90–115 in blossom clusters and 45–55 in fruit. Within 5 years postapplication, soil B levels were <2 mg/kg, and all visible signs of toxicity had disappeared
Vegetation, various species	
2,244 kg borates/ha (2,000 lbs/acre)	Total kill of most species
Soil B concentrations	
1 mg/L	Optimal growth
5 mg/L	Injury evident
Plant residues	
>98 mg B/kg DW	Marginal burning and dark brown tips of older leaves

Table A7_5_1_3-X d.

Estimated Boron Concentration in Soil with Fertilization

Crop	Product (lbs/acre)	Application Rate (lbs-B/acre)	(kg-B/ha)	B in Soil (mg-B/kg-soil)
Alfalfa	6	0.90	1.0	0.4
	24	3.6	4.0	1.4
Cocoa	18	2.7	3.0	1.1
	36	5.4	6.1	2.2
Corn	4	0.60	0.67	0.2
	9	1.4	1.5	0.5
Cotton	3	0.45	0.50	0.2
	13	2.0	2.2	0.8
Flowers	3	0.45	0.50	0.2
	13	2.0	2.2	0.8
Grape	12	1.8	2.0	0.7
	45	6.8	7.6	2.7
Grasses	3	0.45	0.50	0.2
	6	0.9	1.0	0.4
Hops	9	1.4	1.5	0.5
	18	2.7	3.0	1.1
Plantain	13	2.0	2.2	0.8
	36	5.4	6.1	2.2
Rapeseed oil	6	0.90	1.0	0.4
	15	2.3	2.5	0.9
Root crops	6	0.90	1.0	0.4
	18	2.7	3.0	1.1
Soy bean	3	0.45	0.5	0.2
	6	0.9	1.0	0.4
Strawberry	3	0.45	0.50	0.2
	6	0.9	1.0	0.4
Tea	5	0.75	0.84	0.3
	9	1.4	1.5	0.5
Vegetables	6	0.90	1.0	0.4
	13	2.0	2.2	0.8

Assumes use of product with 15% boron content, soil mixing depth of 20 cm, soil density 1400 kg/cubic meter

Rates show range recommended for broadcast applications.

d Reference: "Boron in soils and plant nutrition," US Borax Inc., 2002

Section 7.5.3.1.2 **Annex Point IIIA XIII 1.1**	**Short-term toxicity on birds**

Official use only

37 REFERENCE

Reference	[redacted]. 1984. "A Dietary LC50 Study in the Bobwhite with Boric Acid." [redacted]
Data protection	No
Data owner	
Criteria for data protection	No data protection claimed

GUIDELINES AND QUALITY ASSURANCE

Guideline study	Yes – ASTM E857-81 "Standard Practice for conducting subacute dietary toxicity tests with avian species." American Society for Testing and Materials, 1982 US Environmental Protection Agency Office of Pesticide Programs. "Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Subsection 71-2. (October 1982)
GLP	No – GLP not compulsory at time of study (1984)
Deviations	No

METHOD

Section 7.5.3.1.2 **Annex Point IIIA XIII 1.1**	**Short-term toxicity on birds**
Test material	Kerr McGee Chemical Corporation "Boric Acid Purity 99.9% 3/13/84"
Lot/Batch number	
Specification	
Purity	Reported as 99.9% active substance
Composition of Product	
Further relevant properties	White crystalline material
Method of analysis in the diet	Not measured
Administration of the test substance	See table A7_5_3_1_2-1
Reference substance	No
Method of analysis for reference substance	Not measured
Testing procedure	Non-entry field
Test organisms	Bobwhite quail 11-day old chicks (see table A7_5_3_1_2-2)
Test system	Chicks maintained in battery brood chambers, 10 per chamber. 5 groups of controls and 5 treatment groups were tested. Test substance added to diet, which was available ad libitum during 5-day exposure period. Basal diet without test material was presented both before the test and during the 3-day post-exposure period (see table A7_5_3_1_2-3)
Diet	Standard basal diet was used, supplemented with 2% corn oil during the 5-day exposure period, and then basal diet only (no corn oil) was used during 3-day post-exposure period. For treatment groups, test substance was mixed into small portion of basal diet, then mixed with 65 ml corn oil plus additional basal diet (mass of basal diet + test substance = 2940 g).
Test conditions	Temperature at 37±1°C, photoperiod 14/10. (see table A7_5_3_1_2-4)
Duration of the test	5 days exposure, 3-days post-exposure monitoring
Test parameter	Mortality. Average weight and behavioural observations included.

Section 7.5.3.1.2 Annex Point IIIA XIII 1.1	Short-term toxicity on birds
Examination / Observation	Mortalities observed daily. Average weights measured on days 0, 5 and 8. Behavioural observations daily.
Statistics	None applied. No mortalities were observed in highest treatments, so endpoints reflect lack of observed effect.
	RESULTS
37.1 Limit Test / Range finding test	Not performed
Concentration	
Number/ percentage of animals showing adverse effects	
Nature of adverse effects	
37.2 Results test substance	Non-entry field
Applied concentrations	Test substance presented in feed at 0, 562, 1000, 1780, 3160 and 5620 ppm additions (as boric acid).
Effect data (Mortality)	No mortalities were observed at higher concentrations (see table A7_5_3_1_2-5). Mortalities in controls (3/50 or 6%) and in low exposure groups were not considered to be related to the test substance. LD_{50} > 5620 ppm (boric acid)

Body weight

Group	Change in average weight (g)			Feed consumed (g/bird per day)	
	Day 0-5	Day 5-8	Day 0-8	Day 0-5	Day 5-8
Control 1	9	5	14	8	7
Control 2	7	4	11	8	9
Control 3	7	5	12	9	10
Control 4	7	6	13	7	12
Control 5	7	5	12	6	10
Ave-Ctrl	7.4	5	12.4	7.6	9.6
562 ppm	7	5	12	10	13
1000 ppm	8	6	14	11	10
1780 ppm	6	6	12	8	9
3160 ppm	3	4	7	6	8
5620 ppm	3	6	9	9	11

Feed consumption

See table above. Value considered an estimate due to wastage by the birds.

Concentration / response curve

No mortalities were observed at higher exposures.

Other effects

Some individuals in the 5620 ppm group displayed lethargy and reduced reaction to external sound and noise on days 4 and 5 only. At all other times and in all other groups, the test birds were normal in appearance and behaviour.

In the control group, no external lesions were observed on the bird found dead on day 1. One bird had caught its leg in the pen wire (day 6) and one bird showed lesions of nose picking, a cannibalistic form of aggression. The mortality in the 1000 ppm group was attributed to cannibalism.

37.3 Results of controls

Number/ percentage of animals showing adverse effects

3 of 50 (see Table A7.5.3.1.2-5)

Nature of adverse effects

37.4 Test with reference substance

Not performed

Concentrations

Results

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

This test of dietary exposure followed the US EPA Pesticide Assessment Guidelines and ASTM E857-81. Eleven-day-old bobwhite chicks were exposed to diets containing the test substance for 5 days, and then observed for 3 additional days. Diet and water were available ad libitum. Birds were maintained in battery brooder boxes at about 37°C on a 14/10 hr photoperiod. Ten chicks were housed in a box. 5 control groups were tested, treatment groups were not replicated (single box per exposure group)

Boron concentrations in diet or water were not measured.

Results and discussion

No mortalities related to test substance were observed. The maximum exposure was to 5620 ppm boric acid, or 984 ppm as boron.

Body weights of the 3160 and 5620 ppm groups were 78% and 86% of the control average (26.8 ± 1.5 g), respectively. Although no statistic calculation was reported, the average weights of the 3160 and 5620 ppm groups were more than 2 standard deviations less than the mean control group weight, suggesting that weight was significantly reduced. Consequently, the NOEC was reported as 1780 ppm boric acid, or 312 ppm as boron. X

Although it is likely that the basal diet contributed a significant amount of dietary boron, no measurements were reported. Thus the actual dietary intakes were probably more than suggested by the nominal concentrations of boric acid added to the diet.

<u>LD_{50}</u>

None obtained. Estimate as greater than 5620 ppm boric acid (984 ppm as boron).

Conclusion

Validity criteria were met: less than 10% mortality was observed in controls. No mortalities related to the test substance were observed at the highest exposures, up to 5620 ppm added boric acid (or 984 ppm added boron). Weight gain in the two highest exposures appeared less than the control average, suggesting a LOEC of 3160 ppm boric acid (553 ppm added boron equivalent) and a NOEC of 1780 ppm boric acid (312 ppm added boron equivalent).

<u>Reliability</u>

2 – reliable with restrictions (test substance not measured in diet)

<u>Deficiencies</u>

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	18-01-2005
Materials and Methods	Applicant's summary adequately reflects report
Results and discussion	Section 5.2: Applicant suggests that weight was significantly reduced. Data indicate that there may be a treatment related reduction in body weight *gain* at concentrations higher than 1000 mg boric acid/kg fd. Body weight gain in the post-exposure period is comparable with the control, indicating that effects are reversible. Applicant states that actual intake of boron might have been higher due to boron being present in the basal diet and that the absence of analytical measurements is thus considered a deficiency. Dietary analysis is not required for the 8-days test. The endpoint is expressed as added boric acid and there is no mortality, so any background boron in the diet is not considered to have influenced the outcome. Based on average body weight of 15.5 g (day 0 – 5) and food consumption of 9 g/bird.d, LC_{50} is equivalent to > 3263 mg boric acid/kg bw.d (> 571 mg B/kg bw.d)
Conclusion	The dietary LC_{50} of boric acid for *Colinus viginianus* is > 5620 mg boric acid/kg fd, equivalent to > 983 mg B/kg bw. Expressed as daily dose, the LC_{50} is > 571 mg B/kg bw.d.
Reliability	1
Acceptability	Acceptable, the LC_{50} of > 983 mg B/kg fd, equivalent to > 571 mg B/kg bw.d, will be included in the risk assessment.
Remarks	Supplied by applicant under Section 7.5.3.1.1 Acute oral toxicity on birds, appropriate section is 7.5.3.1.2: Short-term toxicity on birds
	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_3_1_2-1: Method of administration of the test substance

Carrier / Vehicle	Details
Water	No
Organic carrier	No
Concentration of the carrier [% v/v]	
Other vehicle	Mixed with basal diet
Function of the carrier / vehicle	

Table A7_5_3_1_2-2: Test animals

Criteria	Details
Species/strain	Bobwhite quail (Colinus virginianus)
Source	Sand Prairie Quail Farm, Maquoketa, Iowa
Age (in weeks), sex and initial body weight (bw)	Age of 11 days at start of study
Breeding population	Day-old chicks shipped
Amount of food	Ad libitum
Age at time of first dosing	Day 11
Health condition / medication	No antibiotics or other medication given

Table A7_5_3_1_2-3: Test system**Criteria**	**Details**
Test location	Wildlife International Avian toxicology facility
Holding pens	Beacon battery brooders (Model B735Q) of 72 x 90 x 23 cm dimension
Number of animals	50 control, 50 in treatment groups
Number of animals per pen [cm²/bird]	10 per battery brooder, [648 cm^2 per bird]
Number of animals per dose	50 control, 10 per other dose
Pre-treatment / acclimation	Upon receipt until day 10, maintained as during test
Diet during test	Basal Diet | | Pounds per Ton | KG/ | Corn meal, No. 2, yellow 933 466 Oats, heavy, pulverized 200 100 Wheat, standard middlings 300 150 Soybean oil meal, 50% protein, 250 125 Fish meal, 60% protein 160 80 Fish solubles, dried basis 10 5 Dried brewers yeast, 40% protein 20 10 Whey, dried product, 55% lactose 20 10 Alfalfa meal, dehydrated, 17% 60 30 Dicalcium phosphate 10 5 Calcium carbonate, ground 30 15 Salt, iodized 5 2.5 Manganese sulfate, feed grade 0.5 0.25 Copper sulfate 0.5 0.25 Zinc carbonate 0.25 0.12 DL-methionine (hydroxyanalog) 1 0.50 Vitamin supplement in Basal Diet Stabilized vitamin A 10000000 USP/ton Vitamin D3 1500000 ICU/ton Vitamin E 5000 IU/ton Riboflavin 3 gram/ton Choline chloride 112 gram/ton Niacin, pure 40 gram/ton Calcium 6 gram/ton Vitamin K 4 gram/ton Vitamin B12 6 mg/ton ANALYSIS Protein, % 20

	Fat, % 6.5 Fiber, % 4 Calcium, % 1.2 Phosphorus, % 0.7
Dosage levels (of test substance)	Diet prepared by adding specified amount of boric acid to 65 ml corn oil + 2938.3 g basal diet (starter ration) 562 ppm Treatment - 1.6877 g boric acid 1000 ppm Treatment - 3.0030 g boric acid 1780 ppm Treatment - 5.3453 g boric acid 3160 ppm Treatment - 9.4895 g boric acid 5620 ppm Treatment - 16.8769 g boric acid
Replicate/dosage level	Five replicates for control, one replicate per other dosage level
Feed dosing method	Added to diet, available ad libitum
Dosing volume per application	
Frequency, duration and method of animal monitoring after dosing	Average weight (grams) of bobwhite chicks Group Day 0 Day 5 Day 8 Control 1 15 24 29 Control 2 14 21 25 Control 3 15 22 27 Control 4 14 21 27 Control 5 14 21 26 562 ppm 15 22 27 1000 14 22 28 1780 14 20 26 3160 14 17 21 5620 14 17 23
Time and intervals of body weight determination	Body weights measured at day 0, day 5 and day 8.

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	Details
Test temperature	99 ± 2 degrees F (37 ± 1degrees C)
Shielding of the animals	Not reported
Ventilation	Not reported
Relative humidity	Not reported
Photoperiod and lighting	14 hr /10 hr

Table A7_5_3_1_2-5: Mortality data after test termination

Test substance dosage level [mg/kg feed]	Mortality after test termination (5 + 3 days)									
	Total number per dose level					Percentage per dose level				
	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5
Control	1/10*	1/10	1/10	0/10	0/10	10%	10%	10%	0%	0%
562 ppm	1/10**					10%				
1000 ppm		1/10**					10%			
1780 ppm			0/10					0%		
3160 ppm				0/10					0%	
5620 ppm					0/10					0%
Temperature [°C]	37 ± 1									
Relative humidity										

*Control mortalities on day 1, day 6, day 7
**Mortality in 565 ppm group on day 8, in 1000 ppm group on day 6

Table A7_5_3_1_2-7: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	Not fulfilled
Mortality of control animals <10%	X	

Section A7.5.5	**Bioconcentration in terrestrial organisms**	
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		Official use only
	38 REFERENCE	
Reference	Pendleton, G.W., M.R. Whitworth, G.H. Olsen. 1995. "Accumulation and loss of arsenic and boron, alone and in combination, in mallard ducks." Environ Toxicol Chem 14(8): 1357-1364 Authors affiliation: US National Biological Service	
Data protection	No	
Data owner	Publication	
Criteria for data protection	No data protection claimed	
	GUIDELINES AND QUALITY ASSURANCE	
Guideline study	No – no guideline available	
GLP	No – GLP not compulsory. Quality assurance protocols used.	
Deviations	Not applicable	
	MATERIALS AND METHODS	

Section A7.5.5	**Bioconcentration in terrestrial organisms**
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Test material	Boric acid
Lot/Batch number	
Specification	
Purity	
Further relevant properties	
Radiolabelling	
Method of analysis	Perchloric acid/nitric acid digestion of tissue and food samples, followed by inductively coupled plasma emission spectroscopy (ICP-ES)
Reference substance	No
Method of analysis for reference substance	
Testing/estimation procedure	Non-entry field
Test system/ performance	Adult male mallard ducks (Anas platyrhynchos) obtained from commercial game bird farm were randomly assigned to pens (2.5 x 6.5 m) with 17 birds per pen. During 2-week acclimation period, birds were fed commercial duck developer mash. At test initiation, 3 pens assigned as controls, 4 pens assigned to each treatment. Treatments were addition of boric acid (1600 ppm boron) or sodium arsenate (300 ppm arsenic) or both. (Arsenic results not included here). On days 1, 2, 4, 8, 16, 32 and 48 after test initiation, one bird from each pen was sacrificed for analyses. After 48 days on treated diets, all remaining birds were fed control diet. On days 1, 2, 4, 8, 16, 32, and 48 after starting this depuration phase, one bird from each pen was sacrificed for analyses. Liver and blood samples were taken from each bird. Brain samples were also taken on days 4 and 32. Limits of detection for boron were 2 ppm. Average recovery of boron from spiked samples was 91%. Accumulation of boron was described using the exponential equation: $C = A \times (1 - e^{-Bt})$, where C = concentration of boron in ppm (micrograms/gram, dry weight) A = concentration at equilibrium B = first-order rate constant T = number of days on treated diet Losses were to be described using a similar exponential equation, but

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loss was so rapid (<1 day) that parameters could not be determined. Parameter estimates were generated using nonlinear least-squares (Draper and Smith, 1980).

Reference

Draper N.R., and H Smith, 1981. Applied Regressions Analysis, 2nd Ed. John Wiley & Sons, New York.

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Estimation of bioconcentration

The authors did not estimate bioconcentration factors (BCF) per se.

See section 4.1. for data. See section 5.2 for estimation of BCF.

RESULTS

Experimental data

Non-entry field

Mortality/behaviour

No mortalities were observed

Lipid content

Not reported

Concentrations of test material during test

Concentrations of boron in the control and amended diet are shown in table below.

Boron in feed	mg-B/kg diet
Control mash	9.0 (standard error = 1.3)
1600 ppm mash	1615 (standard error = 14.9)

The fitted exponential equations for liver and blood are shown below:

Boron accumulation	A	B	days to 95%
Liver - added boron diet	25.59	1.053	2.8
Liver - added B+As diet	25.59	0.246	12.2
Blood - added boron diet	52.07	1.83	1.6
Blood - added B+As diet	52.07	0.206	14.5

(A = concentration at equilibrium, B = first-order rate constant) The combination of arsenic plus boron reduced the rate that equilibrium concentrations were obtained as shown by the number of days estimated to reach 95% of equilibrium levels.

The mean levels of boron appeared to reach a maxima at day 32. These are shown in the table below.

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	Mean tissue level, day 32	
Liver	29.75	mg/kg
Brain	37.5	mg/kg
Blood	67.57	mg/kg

Depuration was begun on day 48. Boron was detected in only two blood samples and no liver samples at 1 day after depuration was begun. Consequently, no loss equations could be estimated.

The following graphs were in the publication. The legend appears to be in error: Points labeled "AS" should be "B".

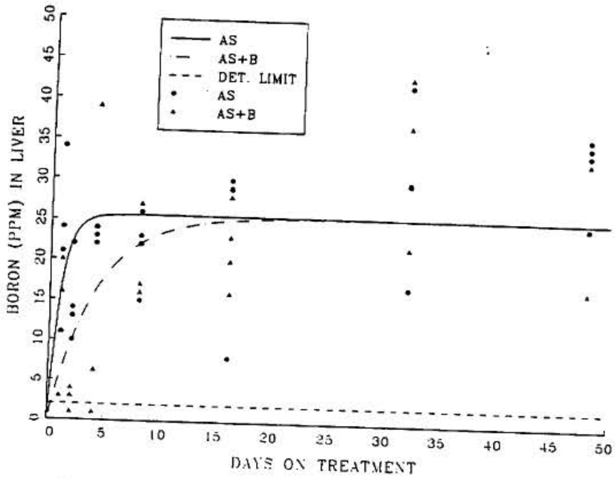

Fig. 3. Accumulation curves and data points for boron in the liver.

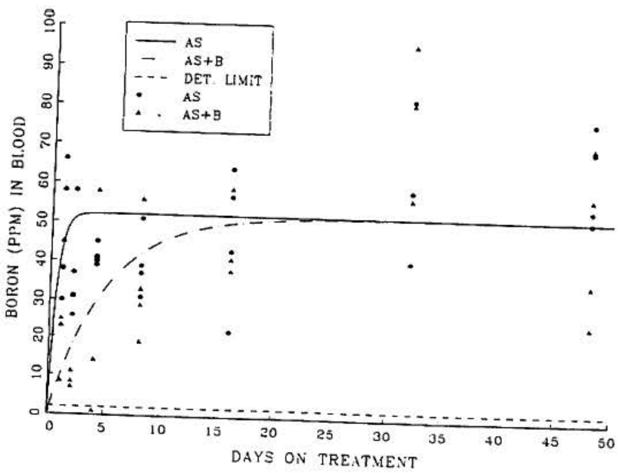

Fig. 4. Accumulation curves and data points for boron in the blood.

Section A7.5.5 **Bioconcentration in terrestrial organisms**

Annex Point IIA7.5 Section A7.5.5

Bioconcentration factor (BCF)

The authors did not calculate BCF values.

Uptake and depuration rate constants

The parameters for the fitted exponential model are given in section 4.1.3 above. Time to 95% of equilibrium concentration was estimated as 1.6 days (blood) or 2.8 days (liver). When the diet contained both As and B, rates were slower.

Depuration time

Boron was detected in only two blood and one liver sample within 1 day after the depuration phase was begun. No half-life was estimated.

Metabolites

None

Other Observations

Estimation of bioconcentration

The authors concluded that both accumulation and loss of boron were rapid. Consequently, tissue levels would be elevated only at locations where the birds are exposed to very high levels at contaminated sites. Transient animals would not be greatly affected, and the substance would disappear rapidly if exposed birds more to uncontaminated locations.

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

The study added boron (as boric acid) to a commercial feed at 1600 mg/kg-feed. Groups of male mallard ducks were fed the commercial feed un-altered or with the added boron. Tissue samples (liver, blood, brain) from sacrificed animals were analysed for boron over the 48 day exposure, and over a 48 day depuration period. Concentrations of boron in the un-altered and amended feed were measured. The authors used the data to estimate parameters to a one-compartment exponential model. (A two-compartment model was attempted, but estimation procedures failed to converge to a solution; in addition, the one-compartment model showed good fit.) The parameters of the model involved the asymptote – the equilibrium concentration – and a rate constant.

The intent of the study was to evaluate the kinetics of boron and arsenic uptake and depuration, and to relate different tissue concentrations to each other and to exposure history. This presumably would be applicable to the sponsoring agency (U.S. National Biological Service, formerly the U.S. Fish and Wildlife Service) responsibilities for evaluating contaminated sites.

Results and discussion

The dietary exposure led to rapid uptake in the mallard, with less than 3 days required to reach 95% of the estimated equilibrium concentration. When exposure stopped, depuration was equally rapid, with few detectable boron concentrations found one day or more after the exposure was stopped. The depuration was too rapid to permit quantitative estimate of a depuration rate constant. Based on the rapid disappearance, a DT_{50} can be estimated as $\leq$ 1 day.

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Annex Point IIA7.5	Section A7.5.5

The equilibrium liver concentration was estimated as 25.6 mg/kg (dry weight). In another study from the same research group (Stanley et al., 1996), the liver concentration was 27 mg/kg after feeding 900 ppm-B for >90 days.

The evidence that boron concentration reaches an asymptotic or equilibrium concentration and that it is rapidly eliminated suggests that boron is not bioconcentrated. The ratio of concentrations in the diet (1615 mg-B/kg) to the tissues (26 mg-B/kg in liver, 52 mg-B/kg in blood) are much less than zero, making the estimated BCF <<1.

Stanley et al. (1996) found that mallards fed 900 ppm boron in the diet showed decreased haemoglobin concentration, egg weight and egg fertility in adults, and reduced duckling growth and production. Consequently, the Pendleton et al. study was conducted at exposures that may have had adverse effects on the test animals.

Reference

T.R. Stanley, Jr., G.J. Smith, D.J. Hoffman, G.H. Heinz, R Rosscoe, 1996. "Effects of boron and selenium on mallard reproduction and duckling growth and survival." Environ Toxicol Chem 15(7): 1124-1132.

Conclusion

No guideline exists for determining BCF in avian wildlife. However, the procedures used are straight-forward and consistent with procedures for avian toxicity studies. The tissue sampling established that an equilibrium concentration of boron was attained in blood and liver within 2 to 15 days and that depuration was very rapid. These data clearly support the conclusion that boron should not be considered a bioconcentrating substance.

Reliability

2 – valid with restrictions. Limitations are noted in section 5.3.2. Published in a peer-reviewed journal.

Deficiencies

Yes - Exposures were higher than shown to have adverse effects in another study. Data from controls not provided. Only males were tested. Single exposure level was tested.

	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
Materials and Methods	Applicant's summary is acceptable
Results and discussion	id.
Conclusion	Annex point IIA VII 7.5 refers to bioconcentration in earthworms and/or plants as a potential exposure route for worm-eating birds and mammals or grazing non-target organisms. In the present study, the bioconcentration in birds is investigated and is not useful for risk assessment. Although the study itself is reliable, it is not relevant for the present risk assessment
Reliability	4
Acceptability	not acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
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 A.7.5.6-01 **Terrestrial Arthropod – Chronic Toxicity Test**

Annex point IIA.7.5 **Section A7.5.6**

Reference: ESG International, Inc. and Aquaterra Environmental Consulting, Ltd. (2003) Assessment of the Biological Test Methods for Terrestrial Arthropods: Further Refinement of the Collembola Test Method using *Onychiurus folsomi*. Prepared for: Method Development and Applications Section, Environmental Technology Centre, Environment Canada. Unpublished report 99096.

Data Protection: No

Data Owner: Environment Canada

GUIDELINES AND QUALITY ASSURANCE

Guideline study: No. Project was to develop standard method for Env Canada.

GLP Study: No

METHODS

Test Material: Boric acid

Lot/batch No; not provided. Obtained from Fisher Scientific, Catalog No. A74-500

Purity 98.29%

Composition powder

TESTING PROCEDURE

Preparation of test substance: Powder mixed with deionized water to volume, solution added to soil to achieve TS concentration and desired soil moisture.

Application of TS: Toxicant solution and additional deionized water added to dry soil, mixed for about 3 minutes with hand-held mechanical mixer. Criterion for homogeneity was visible uniformity of colour and texture.

Test organisms: *Onychiurus folsomi* (Collembola, springtails)

Test system: Two test soils were used. Artificial soil prepared from 70% silica sand, 20% kaolinite clay, 10% Sphagnum peat and 10-30 g calcium carbonate per kg-peat. Clay loam reference soil was field collected from Alberta, Canada, classified as a Delacour Orthic Black Chernozem, a fine loam soil with organic matter content of 12.8%. Soil was collected in May 1995, air-dried to 10-20% moisture content, sieved 2-6 mm, then kept in cold storage (4°C) until used. Physicol chemical characteristics of the control soils are shown in Table A.1 below. Soil pH and conductivity measurements are shown in Table C.1 below.

Test units contained 30 g ww of test soil in 125 mL glass wide-mouth mason jar. Ten individuals were added per test unit, 4 replicates per

Section A.7.5.6-01	**Terrestrial Arthropod – Chronic Toxicity Test**
Annex point IIA.7.5	**Section A7.5.6**
	treatment for acute (7- and 14-day) tests, and 10 replicates per treatment for chronic (35-day) tests. Cover was metal lid with rubber seal and screw ring, although lids were not tightly screwed on.
Test Conditions:	Static, temperature 20±2°C, photoperiod 16hr/8hr light/dark.
Test duration	Chronic – 35 days Acute 7- and 14 days
Test parameters	Chronic – adult survival and reproduction, mean number of juveniles produced per treatment, adult fecundity Acute – adult survival Physical-chemical: pH, electrical conductivity, soil moisture content
Examination	Physical and chemical measurements made at beginning and end of test. TS analyses were made on days 0, 7, 14 and 35. Biological measurements were made at the end of the test. Collembolans were evaluated by floatation: deionized water was added to the jar, the resulting slurry stirred and collembolans removed as they floated to surface (their hydrophobic outer integument causes floatation). Any trapped in air bubbles were freed and removed. Juveniles were distinguished by size. Missing individuals were assumed to be dead (and decomposed).
Monitoring of test Substance concentration:	Samples collected on days 0, 7, 14 and 35 from 0, 75, 500 and 1500 mg/kg soil dw for chemical analysis. Analysis was for total boric acid via ICAP/AES using protocol of OMEE (1996) and back-calculating to boric acid concentration. Corrections were made for recovery efficiency of the extraction and detection procedures. OMEE (Ontario Ministry of Environment and Energy) (1996) Guidance on sampling and analytical methods for use at contaminated sites in Ontario – Section 8.3.7 Inorganics. Version 1.31, ISBN 0-07778-4056-1, December 1996. Queen's Printer, Toronto.
Statistics	Linear or non-linear regression procedures were applied to chronic test results. ANOVA procedures were applied to chronic data, in combination with Dunnett's and Fisher protected Least Significant Difference pairwise comparison tests.
	RESULTS
Initial concentrations	Nominal concentrations were: 0, 50, 75, 125, 250, 500, 650, 850, 1000, 1500 mg-boric acid/kg soil-dw Equivalent to boron concentrations of 0, 8.8, 13, 22, 44, 88, 114, 149, 175, 263 mg-B/kg-soil dw.
Concentrations	Measured concentrations (as boric acid) are shown in Table B.1 below. Data were not corrected for recovery efficiency. Apparent transposition of

Section A.7.5.6-01 **Terrestrial Arthropod – Chronic Toxicity Test**

Annex point IIA.7.5 **Section A7.5.6**

values on Day 0 (clay loam soil) and clearly anomalous results on Day 14 (clay loam soil) indicate poor reliability of measured results. Nominal concentrations are used in report.

Effects

Individual data values not reported. Acute test results shown in Table C.2 below (not addressed further). Graphs showing values and means from chronic tests are included below (Figure C.3, C.4).

	NOEC		LOEC	
	mg-BA/kg soil	mg-B/kg soil	mg-BA/kg soil	mg-B/kg soil
Artificial Soil:				
Adult survival:	1000	175	1500	263
Adult fecundity:	500	88	650	114
Juvenile reproduction	250	44	500	88
Clay-loam Soil:				
Adult survival:	650	114	850	149
Adult fecundity:	125	22	250	44
Juvenile reproduction	125	22	250	44

Results of controls

Data not provided. From graphs, appears that control mortality averaged about 15%, fecundity averaged about 17 to 20 offspring per initial adult, and juvenile reproduction was about 125 to 160 per test unit.

Test with reference substance:

Not performed

APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods:

Test was part of development program to use boric acid an 4-nitrophenol as reference toxicants.

Results and discussion:

Lowest NOEC values were for juvenile reproduction, 22 and 44 mg-B/kg-soil dw for the artificial and reference clay-loam soils, respectively. NOEC for adult fecundity in clay-loam soil also 22 mg-B/kg-soil dw (although this uses same data as juvenile reproduction, so not an independent variable). These were based on Dunnett's pairwise comparison procedure and data were not transformed. All estimates were based on nominal (added) concentrations.

Conclusion:

Reliability

2 (reliable with restrictions) – data not provided

Deficiencies

Although no procedural deficiencies were noted (this test was to define the procedure), raw data were not provided except in graphical form. Analytical results not consistent, therefore not considered reliable, so nominal concentrations used in report.

Evaluation by Competent Authorities

	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13-02-2008
Materials and Methods	Applicant's summary is acceptable
Results and discussion	id.
Conclusion	The geometric mean NOEC 31 mg B/kg dwt of the NOECs 44 and 22 mg B/kg dwt is used for the risk assessment
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
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 A.7.5.6-02 Annex point IIA.7.5	**Terrestrial Arthropod – Chronic Toxicity Test** Section A7.5.6	
Reference:	Becker-van Slooten, K., S Campiche, J Tarradellas, Ecole polytechnique federale de Lausanne (EPFL) (2003)	
	"Research in Support of the Environment Canada Collembolan Toxicity Test Method with Folsomia candida for assessment of Contaminated Soils. Prepared for Method Development and Applications Section, Environmental Technology Centre, Environment Canada. By Laboratory of Environmental Chemstry and Ecotoxicology, EPFL, ENAC-ISTE, Switzerland. Unpublished report	
Data Protection:	No	
Data Owner:	Environment Canada	
	GUIDELINES AND QUALITY ASSURANCE	
Guideline study:	ISO 11267 Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants.	
	International Standards Organization, 1999, Geneva Switzerland.	
GLP Study:	No	
	METHODS	
Test Material:	Boric acid	
Lot/batch No;	not provided. (Fluka 15660)	
Purity	not provided	
Composition	not provided	
	TESTING PROCEDURE	

Section A.7.5.6-02 Annex point IIA.7.5	**Terrestrial Arthropod – Chronic Toxicity Test** Section A7.5.6	
Preparation of	Powder mixed with deionized water to prepare a stock solution, with	
test substance	moderate warming to obtain solution of 50 g/L boric acid.	
Application of TS:	Toxicant solution and additional deionized water added to dry soil and mixed in plastic Petri dish (10 cm diameter, 5 cm height).	
Test organisms:	*Folsomia candida* (Collembola, springtails)	
	Culture maintained in laboratory since 1996, obtained from F. Riepert (Biologische Bundesanstalt für Land-und Forstwirtschaft, Berlin), following ISO standard.	
Test system	Two test soils were used. Artificial soil prepared from 70% quartz sand, 20% kaolinite clay, 10% Sphagnum peat and calcium carbonate sufficient to reach a pH of 6+0.5. Clay loam reference soil obtained from ESG, Canada and had been field collected from Alberta, Canada, classified as a Delacour Orthic Black Chernozem, a fine loam soil with organic matter content of 12.8%. Soil was collected in May 1995, air-dried to 10-20% moisture content, sieved 2-6 mm, then kept in cold storage (4°C) until used. Physico-chemical characteristics of the control soils are shown in Appendix A below. Soil pH in the reproduction tests in artificial soil ranged from pH 6.2 to 6.3 in control and 400 mg/kg soil on day 0, to pH 6.3-6.4 in control and pH 6.2-6.3 in 400 mg/kg soil on day 28. In the clay-loam soil test, all pH values were 5.5 in both control and 400 mg/kg soils. Soil humidity was adjusted to 50-60% of water holding capacity.	
	Test units contained 30 g ww of test soil in glass containers (4.5 cm diameter, 9.5 cm height, with plastic covers closing tightly) with soil depth about 4 cm. Ten individuals (10 to 12 days old) were added per test unit. Five replicates for controls and 3 per treatment were prepared for the chronic (28-day) tests. About 10 mg of dry yeast were added onto the soil at test initiation and on day 14. Units were aerated twice per week.	
Test Conditions:	Static, temperature 20+2°C, photoperiod 16hr/8hr light/dark.	
Test duration	Chronic – 28 days	
Test parameters	Adult survival and number of juveniles produced per test unit	
	Physical-chemical: pH	
Examination	Physical and chemical measurements made at beginning and end of test. TS analyses were made on days 0 and 28.	
	Biological measurements were made at the end of the test. Collembolans were evaluated by floatation: soil was removed to a 500 ml glass beaker and tap water was added. Blue bromophenol was added to improve visual contrast between whitish collembolans and dark soil. Adults were counted by eye. Additional tap water was added, to 500 ml and a picture taken of the surface of the water. Numbers of adults and juveniles were counted from the digital image or a print of the photograph.	
Monitoring of test Substance concentration:	Samples collected on days 0 and 28 from 0 and 400 mg/kg soil dw for chemical analysis. Analysis was for total boric acid via ICP/AES using protocol of Swiss soil ordnance (Ordonnance sur les atteintes portees aux sols (Osol).1998 RS 814.12). Total boron was estimated after 2 hour extraction with 2M nitric acid and "available" boron was	

Section A.7.5.6-02 Annex point IIA.7.5	**Terrestrial Arthropod – Chronic Toxicity Test** Section A7.5.6	
	estimated after 2 hour extraction with 0.1M sodium nitrate.	
Statistics	Probit transformation was plotted against the log concentration to estimate an IC50 for reproduction of juveniles. The authors did not report NOEC or LOEC values.	
	To determine NOEC and LOEC values, the data were analyzed by ANOVA technique with pairwise comparisons using Tukey's HSD, Student-Newman-Kuels, and Dunnett's tests using XLSTAT 2007.7 with MS Excel software.	
	RESULTS	
Initial concentrations of test substance	Nominal concentrations were: 0, 20, 35, 50, 80, 120, 180, 270, and 400 mg-boric acid/kg soil-dw.	
	Equivalent to boron concentrations of 0, 3.5, 6.1, 8.8, 14, 21, 32 and 47 mg-B/kg-soil dw.	
Concentrations	Measured concentrations (as boric acid) are shown below. Nominal concentrations are used throughout the report.	
Concentrations	Measured concentrations (as boric acid) are shown below. Nominal concentrations are used throughout the report.	

	Nominal Concentration	Measured	Measured
		Day 0	Day 28
Artificial Soil	0 ug/g dw	56.7 ug/g dw	-
Total Boric Acid	80	85.7	91.1 ug/g dw
	400	288.7	315.1
Artificial Soil	0	3.4	-
Available boric acid	80	59.9	54.3
	400	242.7	240.8
	Nominal Concentration	Measured	Measured
		Day 0	Day 28
Clay-loam Soil	0	Non-detect	-
Total Boric Acid	80	105.5	56
	400	334.6	328.5
Clay-loam Soil	0	11.6	-
Available boric acid	80	38.5	56.5
	400	236.1	219.9

Section A.7.5.6-02 Annex point IIA.7.5	Terrestrial Arthropod – Chronic Toxicity Test Section A7.5.6	
	For the total boric acid, concentrations show slight increase over time in the artificial soil, but a slight decrease in the clay-loam soil. On day 0, the control artificial soil showed an unexpectedly high total boron concentration; when re-analyzed, results (not shown) were even higher. No explanation was offered by the authors. Average recovery for total boron concentration was 86 + 15%	
Effects	Individual data values are shown in Tables 1 and 2 below. Graphs showing values and means from chronic tests are included below (Figure 6, Figure 8). The reported 28-day IC50 values were 147 mg-boric acid/kg-soil (artificial soil) and 169 mg-boric acid/kg-soil (clay-loam soil). These are equivalent to 26 and 30 mg-B/kg-soil, respectively. However, the probit analyses did not use all the data – two concentrations (plus the control) were excluded from the estimation of IC50 values.	
	NOEC results from long-term tests are used to determine PNEC-soil (TGD, Table 20), so the NOEC values were calculated from the individual data values. For the artificial soil, the NOEC for juvenile reproduction was found to be 80 mg-boric acid/kg-soil using the SNK, and Dunnetts' procedures, althought the Tukey HSD procedure identified 120 mg-boric acid/kg-soil as the NOEC. For the clay loam soil, all pairwise comparison procedures identified 120 mg-boric acid/kg-soil as the NOEC, as shown on Tables 3 and 4 below.	

	NOEC		LOEC	
	mg-BA/ kg soil	mg-B/ kg soil	mg-BA/ kg soil	mg-B/ kg soil
Artificial Soil:				
Adult survival:	400	70	>400	
Juvenile reproduction	80	14	120	21
	NOEC		LOEC	
	mg-BA/ kg soil	mg-B/ kg soil	mg-BA/ kg soil	mg-B/ kg soil
Clay-loam Soil				
Adult survival:	270	47	400	70
Juvenile reproduction	120	21	180	32

Results of controls	Control adult mortality was 8% in artificial soil and 14% in the clay loam soil, meeting the ISO validity criterion of <20%.	
	Average number of juveniles per test unit was 308.4 for the artificial soil and 337.4 for the clay loam soil. Variability of juvenile reproduction was 19% in the artifical soil and 13% in the clay loam soil, meeting the ISO vailidity criterion of <30%. The authors did note that not all their tests (with other test substances) met these criteria.	
Test with reference substance:	Not performed	
	APPLICANT'S SUMMARY AND CONCLUSION	

Section A.7.5.6-02 Annex point IIA.7.5	**Terrestrial Arthropod – Chronic Toxicity Test** Section A7.5.6	
Materials and methods:	Test was part of development program to use boric acid an 4-nitrophenol as reference toxicants.	
Results and discussion:	Lowest NOEC values were for juvenile reproduction, 14 and 21 mg-B/kg-soil dw for the artificial and reference clay-loam soils, respectively. All estimates were based on nominal (added) concentrations. The authors estimated IC50 values using probit techniques; however, the probit technique requires each treatment value to be less than the average control value. Because two exposures had more juveniles than controls, these concentrations were excluded from the authors' analysis, limiting the reliability of the IC50 estimate.	
Conclusion:	Based on the values for juvenile reproduction of the collembolan Folsomia candida, the lowest NOEC values are 14 and 21 mg-B/kg-soil dw in this chronic (28 day) study for an artificial and a field-collected soil.	
Reliability	1 (reliable)	
Deficiencies	No procedural deficiencies were noted. Alternative statistical analysis was performed to utilize the complete data set because probit technique excluded 33% of data points. Analytical results not entirely consistent, therefore not considered reliable, so nominal concentrations used in report.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13-02-2008
Materials and Methods	Applicant's summary is acceptable
Results and discussion	In deviation of the notifier's conclusion, statistical analysis (Williams test) showed that the NOEC for clay-loam soils is 14 mg B/kg in stead of 21 mg B/kg.
Conclusion	The geometric mean NOEC 14 mg B/kg dwt of the NOECs 14 and 14 mg B/kg dwt is used for the risk assessment
Reliability	1
Acceptability	Acceptable
Remarks	The ISO guideline recomments for calculating the NOEC: "With normally distributed and homogenious data, an appropriate statistical analysis, e.g. multiple t-tests such as Dunnett or Williams test should be performed". As the data full fill these criteria, the Williams test was performed.

	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 A8
Annex Point IIA8

Measures to be adopted to protect man, animals and the environment

Section B8

Subsection		Official use only
8.1 Handling and Storage	No special handling precautions are required, but dry indoor storage is recommended. Good housekeeping procedures should be followed to minimise dust generation and accumulation. No specific firefighting measures are required since boric acid in not flammable, combustible or explosive. The product is itself a flame retardant.	X
8.2 Combustion Products	Fused borate glass and water	
8.3 Emergency measures in case of accident	**Inhalation:** If symptoms such as nose or throat irritation are observed, remove to fresh air. **In case of contact with Eyes:** Rinse immediately with plenty of clean water or sterile saline solution for at least 15 minutes. If appropriate, remove contact lenses after 5 minutes rinsing. If symptoms persist, seek medical attention. **Skin Contact:** No treatment necessary because non-irritating. **Ingestion:** Swallowing small quantities (one teaspoon) will cause no harm to healthy adults. If larger amounts are swallowed, give two glasses of water to drink and seek medical attention. Avoid creation of dust. Use vacuum cleaners wherever possible.	
8.4 Decontamination	a) **Air:** Borates are non-volatile. As a dust borates rapidly settle from the atmosphere. b) **Water:** Borates are naturally occurring minerals and are present in surface and underground waters. Borates are rapidly dissolved in water and will disperse with dilution. Removal at low concentrations is unnecessary. Where water containing high levels of borates can be captured precipitation with lime can be used to reduce boron levels to the 100ppm range. Treatments with boron specific ion exchange resins and activated carbon are also possible. c) **Soil:** Borates are naturally found in rocks and soil and are an essential micronutrient for all plant growth. Contaminated soil can be leached with water or acid to reduce boron levels.	
8.5 Waste Management	Small quantities can usually be disposed to landfill sites. No special disposal treatment is required, but local authorities should be consulted about any specific local requirements. Tonnage quantities of products are not considered appropriate for landfills. Such products should, if possible, be used for an appropriate application.	

8.6 Unintended side effects Borates are essential micronutrients for all plant life but at high levels they are phytotoxic.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10-Febr-05
Materials and methods	Section 8.1 Information on container material is given in Doc IIIA3.17: Suitable container material: paper, cardboard, plastic (polypropylene, high density polyethylene). Unsuitable container material: base metals.
Conclusion	As indicated by the notifier
Reliability	-
Acceptability	acceptable
Remarks	-
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10-Febr-05
Materials and methods	Section IIIA8.7 (substances causing groundwater polution) Section IIIA8.7 was not addressed by the notifier.
Conclusion	-
Reliability	-
Acceptability	not acceptable Section IIIA8.7 is required.
Remarks	-
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	