Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (2 year) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier

11 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (2 year) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier

11.1	**Materials and methods**	This study was conducted in the mid-1960s before OECD guidelines were established.
11.2	**Results and discussion**	Groups of 4 male and 4 female beagle dogs were fed diets containing 0, 0.051, 0.103, or 0.309% borax to provided doses of 0, 1.9, 3.6 or9.6 mg B/kg/day. No toxicity attributable to borax was discerned at these doses. Since no toxicity was observed at doses up to 9.6 mg B/kg/day, additional groups of 4 male and 4 female dogs were fed diets containing 0 or 1.03% borax to provide doses of 0 or 38 mg B/kg/day for 38 weeks. Testicular effects (i.e., decreased testes weight, decreased sperm count, and histological evidence of reduced spermatogenesis) were observed at 26 weeks. According to the authors, "Apart from possible testicle changes, ingestion of borax at a dietary level of 1.03% for 36 or 38 weeks did not produce any adverse effects. Although degenerative changes of unknown cause were noted in the testes of the control animals sacrificed at 38 weeks, and only a small number of animals were employed in this study, the severe degree of testicle atrophy and spermatogenic arrest in the two test animals sacrificed at 26 weeks seems to be related to the ingestion of borax."
11.3	**Conclusion**	The testes is a key target organ for borax in the dog, as it was in rodent species. Adverse effects on the testes were observed in dogs at a dose level of 38 mg B/kg/day (1.03% borax in the diet), a dose level that did not produce other toxic effects.
11.3.1	LO(A)EL	The study authors considered 1.03% borax in the diet (338 mg borax/kg/day or 38 mg B/kg/day) to be the LOAEL based on the presence of testicular effects.
11.3.2	NO(A)EL	The study authors considered 0.309% borax in the diet (84.7 mg borax/kg/day or 9.6 mg B/kg/day) to be the NOAEL.
11.3.3	Other	
11.3.4	Reliability	4 This study was reliable for its intended purpose, which was to identify target organs and toxicological endpoints in the dog (a non-rodent species). This study is not considered suitable for purposes of quantitative risk assessment. The International Programme on Chemical Safety (IPCS, 1998) evaluated this study, as well as the 2-year study of boric acid in dogs, and concluded: "Confidence in this study is low, and it was considered not suitable for inclusion into the [quantitative] risk assessment because of (1) small and variable numbers of dogs, (2) variable background lesions in controls leading to uncertainty regarding the strength of the response to treatment, (3) lack of GLP, and (4) other, more recent studies of greater scientific quality with findings at a similar or lower intake level of boron (Ku et al., 1993; Price et al., 1994)."—IPCS (1998) Environmental Health Criteria 204. Boron. P. 86.
11.3.5	Deficiencies	Yes. In a study of this age, it is not surprising that there are deficiencies. These include: • The number of dogs used was low, with only four dogs per

dose level; these dogs were sacrificed at three different time intervals, resulting in variable group sizes of 1-2 males/group/sacrifice interval.

- In the 38-week study, there were testicular lesions, including testicular degeneration in 3 out of 4 control dogs, and the fourth control dog had a low sperm count. Although a compound-related effect is apparent at the highest dose, firm conclusions about the NOAEL cannot be drawn.
- There is a large gap between the NOAEL and LOAEL, and the NOAEL and LOAEL were determined from portions of the study performed at different times. In addition, there is evidence that the effect at the LOAEL may be reversible which indicates that the LOAEL may be close to the NOAEL. This is consistent with findings in rats.
- Lack of statistical analysis
- Dogs were from unknown source
- Age unknown
- Disease and dietary history of dogs is unknown
- Previous exposure to drugs, pesticides, chemicals unknown
- Dogs sacrificed at different time intervals (12, 24, 27 months; 26, 38, 41 weeks), sometimes with no concurrent control
- The dog is not the most appropriate species for type of study for reasons such as seasonal breeding performance, inbreeding factors, and insufficient historical background data.
- Limited analysis of test material
- No testing to determine homogeneity of diet
- Some dogs were housed in metabolism cages for part of the study
- Some dogs were catheterized; others were not.
- Background level of boron in the dog chow was never measured.

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

Section A6.4.1	**Subchronic toxicity**
Annex Point IIA6.4	*Section A6.4.1 Subchronic (2 year) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier*

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

Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (90 Day) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1966) 90 Day Dietary Feeding -- Dogs. Borax. [redacted] This study was published in summary form in Weir RJ and Fisher RS. (1973) Toxicological studies on borax and boric acid. Toxicol Appl Pharmacol. 23(3):351-64. Unfortunately, the published version does not always accurately reflect the original study reports. Thus, it is necessary to evaluate the original study reports to appreciate the limitations of these studies.
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2		
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I

2 GUIDELINES AND QUALITY ASSURANCE

Section A6.4.1	**Subchronic toxicity**
Annex Point IIA6.4	*Section A6.4.1 Subchronic (90 Day) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier*

2.1	**Guideline study**	No. No guidelines available at the time the study was conducted.
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	No guidelines available at the time the study was conducted.
	3 MATERIALS AND METHODS	
3.1	**Test material**	Borax was provided by [redacted].
3.1.1	Lot/Batch number	Not stated.
3.1.2	Specification	Not stated.
3.1.2.1	**Description**	"soft, fine, white powder without noticeable odor"
3.1.2.2	**Purity**	"Chemical analysis revealed the boron content to be 104% of the theoretical value as shipped."
3.1.2.3	**Stability**	Test material expected to be stable.
3.2	**Test Animals**	
3.2.1	Species	Dog
3.2.2	Strain	Purebred Beagle
3.2.3	Source	Unknown
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Body weights of the dogs ranged from 4.2-10.6 kg during the first week of the study. The dogs were described as "young," but no specific age was provided.
3.2.6	Number of animals per group	5 males and 5 females per group
3.2.7	Control animals	Yes. The control group was employed as a common control with a 90-day study of boric acid.
3.3	**Administration/ Exposure**	Oral (diet)
3.3.1	Duration of treatment	90 days.
3.3.2	Frequency of exposure	Seven days per week
3.3.3	Postexposure period	No.
3.3.4	**Oral**	

Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (90 Day) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier

3.3.4.1 Type	In food
3.3.4.2 Concentration	0, 0.0154, 0.154, 1.54% borax in the diet These concentrations provided doses of 0, 0.4, 4.1, and 38 mg B/kg/day, based on the actual body weight and food consumption data in the study. Others in the scientific literature have described these doses as 0, 0.4, 4.4, and 44 mg B/kg/day, but these calculations are based on standard assumptions regarding body weight and food consumption, not on the actual data from this study. Food consumption was *ad libitum*.
3.3.4.3 Vehicle	
3.3.4.4 Concentration in vehicle	
3.3.4.5 Total volume applied	
3.3.4.6 Controls	Plain dry diet (Wayne Dog Feed) for seven days per week. The dry diet for each dog was supplemented with a 100-gram ration of canned meat (Hill Packing Company) five days per week. Neither diet was analyzed for the background level of boron. Boron is an essential plant element, and plant-derived foods (such as fruits and vegetables) are significant dietary sources of boron. The background level of boron in the control diet was not considered in the calculations of the dose levels.
3.4 Examinations	
3.4.1 Observations	
3.4.1.1 Clinical signs	Yes. The dogs were observed daily for appearance, behavior, and gross signs of systemic toxicity or pharmacological effects.
3.4.1.2 Mortality	Yes. Daily throughout the study.
3.4.2 Body weight	Yes. Weekly.
3.4.3 Food consumption	Yes. Weekly.
3.4.4 Water consumption	No.
3.4.5 Ophthalmoscopic examination	No.
3.4.6 Haematology	Yes. All dogs initially and at 2, 4, and 13 weeks. Parameters: Haematocrit, haemoglobin concentration, total and differential leukocyte count, sedimentation rate.
3.4.7 Clinical Chemisty	Yes. All dogs initially and at 2, 4, and 13 weeks. Parameters: glucose, blood urea nitrogen.

Section A6.4.1 — **Subchronic toxicity**

Annex Point IIA6.4 — *Section A6.4.1 Subchronic (90 Day) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier*

3.4.8	Urinalysis	Yes. All dogs initially and at 2, 4, and 13 weeks. Parameters: appearance, specific gravity, pH, protein, glucose, acetone, bilirubin, blood, and microscopic findings.
3.5	**Sacrifice and pathology**	All dogs were sacrificed after 13 weeks.
3.5.1	Organ Weights	Yes. All dose groups. Organs: liver, kidneys, adrenals, testes, spleen, brain, thyroid
3.5.2	Gross and histopathology	Yes. All dose groups. Organs: brain, thyroid, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, lungs, testes, pituitary, urinary bladder, bone, bone marrow
3.5.3	Other examinations	The liver and spleen of two control dogs and three dogs that received borax were stained for ferric iron by Perl's method.
3.5.4	Statistics	Yes, but statistical methods were not described. Level of significance (p value) not stated.
3.6	**Further remarks**	This study was conducted in a parallel with a 90 day study of boric acid in dogs. The studies were identical in design, and they employed a common control group.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Normal appearance, behaviour, and elimination throughout the study, except for one high dose male dog. This dog showed depression and diarrhea on the 68th day of the study; later that day, the condition progressed to severe lethargy and death.
4.1.2	Mortality	One death was observed in a high dose male dog. "The animal which died apparently suffered from an acute enteritis of unknown etiology." No other deaths occurred.
4.2	**Body weight gain**	The authors stated: "Body weights showed some fluctuation during the study but held generally to within $\pm$1.0 kg of the starting weights."
4.3	**Food consumption and compound intake**	According to the authors, there was variable acceptance of the diet containing the test material and complete acceptance of the meat ration.
4.4	**Ophtalmoscopic examination**	
4.5	**Blood analysis**	
4.5.1	Haematology	A decrease in hematocrit and haemoglobin values were observed in two male and three female dogs at the high dose (1.54%). Otherwise, all values were within normal limits.
4.5.2	Clinical chemistry	No effects.

Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (90 Day) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier

4.5.3	Urinalysis	No effects.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	At the high dose (1.54%), the authors reported a statistically significant decrease in testes and thyroid weights relative to body and brain weights in the male dogs. Similar effects were noted in male dogs in a 90-day study given the same high dose of boron in the form of boric acid. In female dogs, a significant increase in mean brain weight was observed at the high dose. At the middle dose (0.154%), no significant effects on organ weights or ratios were observed. At the low dose (0.0154%), the mean spleen/body weight ratio was significantly decreased, but not at the middle or high dose.
4.6.2	Gross and histopathology	Treatment-related histological changes were clearly evident at the high dose (1.54%), but not at lower doses. Complete or partial testicular atrophy was reported in 5 out of 5 high dose dogs. Signs of red blood cell destruction, as indicated by the presence of hemosiderin in reticular cells of the liver and spleen and the proximal tubule of the kidney, was somewhat greater at the high dose. At the high dose, the thyroid gland of the males "presented a slightly greater proportion of solid epithelial nests and minute follicles than was found in the control animals." In high dose female dogs, there was a tendency for the zona reticularis in the adrenal glands to be increased in width.
4.7	**Other**	Staining for ferric iron confirmed the presence of an increased amount of hemosiderosis in the liver and spleen of the dogs ingesting borax. .

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (90 Day) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier

5.1	**Materials and methods**	This study was conducted in the early 1960s before OECD guidelines were established.
5.2	**Results and discussion**	Groups of 5 male and 5 female beagle dogs were fed diets containing 0, 0.0154, 0.154, or 1.54% borax for 90 days to provided doses of 0, 0.4, 4.1 or 38 mg B/kg/day, respectively. There were no clinical signs of toxicity attributable to borax at any dose, with the possible exception of a single high dose male dog (diarrhea, depression, lethargy, death). There was variable acceptance of the diet containing the test material. Yet, body weights showed some fluctuation during the study but were within $\pm$ 1.0 kg of the starting weights. Roughly half of the high dose dogs of both sexes exhibited a decrease in hematocrit and haemoglobin values during the study. The most significant indication of toxicity was a decrease in testicular weight and histological evidence of testicular atrophy in 5 out of 5 male dogs at the high dose (1.54%). Unfortunately, the report did not include the raw data on the histological evaluation. The reason that this is important is that other studies in beagle dogs in the same laboratory showed an unusually high incidence of testicular lesions, as high as 3 out of 4 controls in one study. However, it is highly likely that the high dose in this study produced a treatment-related adverse effect on the testes. Similar effects were seen in a subsequent study of dogs given 1.03% borax in the diet for 26-38 weeks. At the mid-dose (0.1%), sporadic and minor histological changes were reported. It is not clear whether any changes were treatment-related. Therefore, the mid-dose (0.1%) is considered a NOAEL in this study.
5.3	**Conclusion**	The testes is a key target organ for borax in the dog, as it was in rodent species. Adverse effects on the testes were observed in dogs given 1.54% borax in the diet (38 mg B/kg/day) for 13 weeks. These findings are consistent with a subsequent study by the same team of investigators that reported similar testicular effects in dogs administered a diet containing 1.03% borax (38 mg B/kg/day) for 26 or 38 weeks.
5.3.1	LO(A)EL	1.54% borax in the diet (341 mg borax/kg/day or 38 mg B/kg/day) based on the presence of testicular effects.
5.3.2	NO(A)EL	0.154% borax in the diet (36 mg borax/kg/day or 4.1 mg B/kg/day).
5.3.3	Other	
5.3.4	Reliability	4 This study is considered reliable for its intended purpose, which was (1) to identify target organs and toxicological endpoints in the dog (a non-rodent species) and (2) to identify appropriate dose levels for a subsequent 2-year study in dogs. This study is not considered appropriate for purposes of quantitative risk assessment.
5.3.5	Deficiencies	Yes. In a study of this age, it is not surprising that there are deficiencies. These include: • The number of dogs used was small (5 males and 5 females per group)

Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (90 Day) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier

- In other dog studies conducted around the same time in the same laboratory, an unusually high incidence of testicular lesions were reported among control animals. In fact, in a 38-week study of borax, testicular lesions were observed in 3 out of 4 control dogs, and the fourth control dog had a low sperm count. As a result, firm conclusions about the NOAEL cannot be drawn.
- The statistical methods were not described.
- Dogs were from unknown source
- Age of the dogs is unknown
- Disease and dietary history of dogs is unknown
- Previous exposure to drugs, pesticides, chemicals unknown
- Limited analysis of test material
- No testing to determine homogeneity of diet
- Background level of boron in the dog chow was never measured.

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

Section A6.4.1

Annex Point IIA6.4

Subchronic toxicity

Section A6.4.1 Subchronic (90 Day) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	5 April 2005
Materials and Methods	The applicant's version is acceptable.
Results and discussion	Recalculation of the dose levels on the basis of the original disodium tetraborate decahydrate consumption data yielded daily intake levels of 3.5 (0.4), 42 (4.7) and 374 (42)mg/kg bw/day for the 0.0154, 0.154 and 1.54% groups respectively. Data between brackets indicate intake of boron/kg bw/day. In addition to the effects at the highest dose as described by the applicant, treatment-related effects (weight and histopathology) on testes were already observed at the other dose levels. Testes weight

treatment	testes weight (g)	ratio to body weight (%)
control	17.2	0.20
0.0154 %	14.4	0.16
0.154 %	15.8	0.17
1.54 %	9.6	0.10

Compared to the control animals absolute testes weights of the 0.0154, 0.154 and 1.54 % groups are reduced by 16, 8 and 44 %, respectively. Relative testes weight at 0.0154, 0.154 and 1.54% were reduced by 20, 15 and 50% respectively. The reductions in absolute and relative testes weight at 0.0154and 0.154% were not statistically significant. At 0.154% histological examination revealed that the spermatogenic epithelium was intact and active. However, at this dose of disodium tetraborate decahydrate, in the testes of the males histological changes, described as 'artifactual distortion of the tubules in the outer one-third of the glands' were observed. Although these changes are described as artifactual, it is striking that they were found in all males at this dose, but not in males of the control or the low dose groups. A similar effect was found in the 90-day dog study with boric acid at equimolar boron levels. Therefore these histological changes observed at the mid-dose are considered to be a consequences of a boron-related alteration of the structure of the testes. Since at this dose also the testes weights were reduced, the histological changes are considered to be toxicologically relevant. At 1.54%, testes revealed complete atrophy in 4 of 5 animals and partial atrophy in the fifth animal. At 1.54% extramedullary haematopoiesis and the presence of hemosiderin in reticular cells of the liver and spleen and the proximal tubule of the kidney indicate increased red blood cell destruction.

Section A6.4.1	**Subchronic toxicity**
Annex Point IIA6.4	*Section A6.4.1 Subchronic (90 Day) – Oral Dogs Borax (Sodium Tetraborate Decahydrate. Also submitted in Boric Acid Dossier*

Conclusion	LO(A)EL: 0.154% disodium tetraborate decahydrate, equal to 42 mg disodium tetraborate decahydrate/kg bw/day or 4.7 mg B/kg/day) based on the 15% reduction in relative testes weight, accompanied by distortion of the tubules in the outer one-third of the testes. NOAEL 0.0154 % disodium tetraborate decahydrate, equal to 3.5 mg/kg bw/day or 0.4 mg B/kg bw/day. (see also dose-response modeling at the end of this document and the justification of the choice of critical endpint and overall NOAEL)
Reliability	3
Acceptability	acceptable
Remarks	Although there are a number of deficiencies in the study, in repeated dose studies with borates in different species, the testes are consistently identified as a major target organ for toxicity. The present adverse effects on testes with disodium tetrborate decahydrate are confirmed by a 90 days study in which dogs were treated with boric acid. Therefore the present study is acceptable for risk assessment purposes.

	COMMENTS FROM TMIIIO7
Date	TM 12th of July 2007
Materials and Methods	Although the RMS presented a complete toxicological profile of the substance, including the 90 day dog studies with dose modelling and a justification of the choice of critical endpoint and overall NOAEL, to demonstrate the scientific bases of the NOAEL based on the 90 day dog studies, the TM replied that there are deficiencies in the 90 day dog study compared to the teratogenicity study and that the NOAEL is not reliable and as in other regulatory programms not be used for risk assessment purposes. It was observed that by choosing the NOAEL from the 90 day dog study the AOEL derived was lower than the general background exposure
Results and discussion	No reliable NOAEL is set.
Conclusion	The study is not acceptable for risk assessment purposes. The justification is not valid anymore.
Reliability	4
Acceptability	Not acceptable
Remarks	

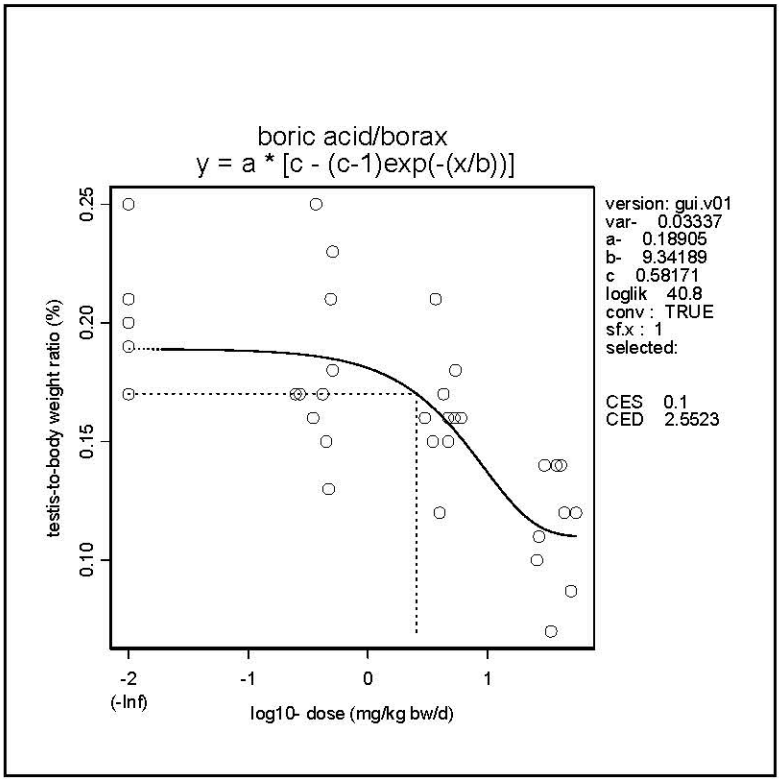
Dose-response modeling performed by CA

It was noted that in this 90-day dog study and in the 90-day dog study with disodium tetraborate decahydrate it was not mentioned which statistical tests were used to determine whether changes in testicular weight were significant, which could be a reason for criticism on the study. We therefore subjected the individual animal data (boron consumption, testes weight and testes/body weight ratio) of the present study and the disodium tetraborate dechaydrate to a dose-response modeling, in order to

provide a Bench Mark type approach using all the individual data in this study. For this a program developed by RIVM (PROAST) was used. PROAST is a widely accepted dose-response modeling program used also in international evaluations (e.g. within the WHO/FAO JECFA committee and EMEA/CVMP). Our analysis indicates that no statistical difference could be found between the studies with boric acid and disodium tetraborate decahydrate. Therefore, the data are allowed to be pooled providing a control group of n=5 and a treatment group with n=9-10/dose. This analysis indicated that the critical effect dose for a 5 or 10 % change in testicular weight (critical effect size) was 1.2 (90% confidence interval (CI) = 0.59-3.01) mg B/kg bw/day or 2.6 (90% CI =1.27-6.39) mg B/kg bw/day respectively. These values of the critical effect doses are in between the NOAEL and LOAEL determined from the boric acid study, with the lower confidence limits close to the NOAEL of 0.46 mg/kg bw/day. This dose response analysis strongly supports the choice of the NOAEL being 0.46 mg/kg bw/day.

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

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



Critical Effect Size	Critical Effect Dose (90% C.I.) in mg/kg bw/d
-5%	1.189 (0.593 – 3.013)

-10%	2.552 (1.269 – 6.388)

JUSTIFICATION OF THE CHOICE OF CRITICAL ENDPOINT AND OVERALL NOAEL

Introduction

For the notification of the use of simple borates as biocidal products, the toxicology of boric acid, boric oxide, disodium tetraborate decahydrate (borax), disodium tetraborate pentahydrate, anhydrous borax and disodium octaborate tetrahydrate was evaluated on the basis of original study reports and data from published literature.

The purpose of the evaluation is to set the acceptable operator exposure level (AOEL) and establish whether occupational exposure may lead to health effects. This process involves 3 separate/independent steps :

hazard identification (determination of the most adequate Point of Departure)

determination of appropriate assessment factors,

Risk characterization

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

We noticed that the Point of Departure (or critical effect) we used for the present evaluation differs from the Point of Departure used in evaluations performed within other frameworks.

In the present document a justification of our choice of the Point of Departure is given and a possible explanation for the apparent discrepancy between the present evaluation and the evaluations within other frameworks is given.

On the basis of the available data we considered that the most sensitive effect induced by borates was testes weight reduction and atrophy. The most sensitive species for testicular effects appears to be the dog; the LOAEL for testicular effects in two 90-day feeding studies in dogs with boric acid and borax was 4.2 mg Boron/kg bw/day. The overall NOAEL in these studies was 0.46 mg Boron/kg bw/day. We noticed that the NOAEL and LOAEL are respectively 15 - 20 and 140 - 183 times higher than the human average daily intake of 0.023 - 0.03 mg/kg bw/day.

5.4 Justification of the choice of critical endpoint and overall NOAEL

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

Testicular effects in mouse, rat and dog

Compound	Route	duration of study	Species Strain	Results	NOAEL (mg B/kg bw/d)	LOAEL (mg B/kg bw/d)	Reference
Boric acid	Oral in diet	13 weeks	Mouse, B6C3F1	Degeneration and atrophy of the seminiferous tubules.	71	142	National Toxicology Program (NTP) Technical Report Series No. 324, 1987
Boric acid	Oral	2 year	Mouse,	Testicular atrophy, loss	48	96	National

Compound	Route	duration of study	Species Strain	Results	NOAEL (mg B/kg bw/d)	LOAEL (mg B/kg bw/d)	Reference
	in diet		B6C3F1	of spermatogenia. and various stages of spermatogenesis of the seminiferous tubules			Toxicology Program (NTP) Technical Report Series No. 324, 1987
Boric acid	Oral in diet	2 year, interim kills at 6 and 12 months	Rat Sprague Dawley	testicular atrophy	17.5	58.5	Weir, 1966a
Borax	Oral in diet	2 year, interim kills at 6 and 12 months	Rat Sprague Dawley	testicular atrophy	17.5	58.5	Weir, 1966b
Boric acid	Oral in diet	90 days	Dog Beagle	reduction in testes weight, histopathological changes	0.46	4.2	Paynter, 1963a
Borax	Oral in diet	90 days	Dog Beagle	reduction in testes weight, histopathological changes	0.39	4.7	Paynter, 1963b

The lowest NOAELs for effects on the testes were observed in two concurrent 90-day dietary studies in the dog, performed with boric acid and borax (O. E. Paynter, 1963a,b). Group size was 5 dogs per dose group. In these studies, at a dose of about 4 mg Boron/kg bw/day, reductions in relative testes weight were observed. In the study with boric acid a statistically significant 25% reduction in relative testis weight was observed at a dose level of 24 mg boric acid/kg bw/day, equal to 4.2 mg/B/kg bw/day. In the study with borax a 15% reduction (not statistically significant) in relative testis weight was observed at a dose level of 42 mg borax/kg bw/day, equal to 4.7 mg B/kg bw/day. Furthermore, at these doses of both boric acid and borax, in the testes of the males histological changes, described as 'artifactual distortion of the tubules in the outer one-third of the glands' were observed. Although these changes are described as artifactual, it is striking that they were found in all males at this dose, but not in males of the control or the low dose groups. Therefore these histological changes observed at the mid-dose are considered by the present evaluators to be a consequences of a boron-related alteration of the structure of the testes. Since at this dose also the testes weights were reduced, the histological changes are considered to be toxicologically relevant. The NOAELs, expressed as boron equivalents, in the boric acid and borax studies were 0.46 and 0.39 mg B/kg bw/day, respectively. Based on these two studies the overall NOAEL for this effect is considered to be 0.46 mg/kg bw/day. The original study reports of these two 90-day studies in the dog were provided by the notifier.

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

values of the critical effect doses are in between the NOAEL and LOAEL determined from the boric acid study, with the lower confidence limits close to the NOAEL of 0.46 mg/kg bw/day. This dose response analysis strongly supports the choice of the NOAEL being 0.46 mg/kg bw/day. At present, there is no general agreement on the choice for a CES for changes in testicular weight. Therefore, the NOAEL of 0.46 mg B/kg bw/day is used as a Point of Departure for setting the AOEL.

In addition, for the present evaluation two 2-year dietary studies in the dog with boric acid and disodium tetraborate decahydrate were available (Weir, 1966c,d; 1967a,b). In these studies the testes were identified as a major targer organ. However, since these studies had a number of deficiencies, they were considered by the present evaluators not acceptable for use in risk assessment.

Also two multigeneration reproduction toxicity studies in the rat with boric acid (Weir, 1966c) and disodium tetraborate decahydrate (Weir, 1966d) were available. In these studies again the testes were identified as a major target organ. However, since these studies had a number of deficiencies, they were considered by the present evaluators not acceptable for use in risk assessment.

Weir and Fisher, Toxicol. Appl. Pharmacol 23 (1972)pp 351-364.

It appears that a number of evaluations performed within other frameworks are partly based on a description of the dog studies in an article in published literature by Weir and Fisher. In this article a very concise, and not completely accurate description of the 90-day and 2-year dog studies was presented. With respect to the 90-day dog study, the Weir and Fisher article reports that the mid dose (25.1 mg/kg bw/day) boric acid induced a reduction in testes/body weight ratio, but no histological change in the testes. No further information on the mid-dose groups of dogs treated with boric acid or borax is presented. The article further reports that in the 90-day study both boric acid and borax induced a significant reduction in testes/body weight ratio, and testicular atrophy at the high dose. For the high-dose groups data on testes weight and testes/body weight ratio are presented.

With respect to the 2-year dog study, it is reported that boric acid and borax did not cause any effects up to dose levels 62.4 and 84.7 mg/kg bw/day respectively, and that a 38 weeks exposure to boric acid or borax at levels of 233 and 338 mg/kg bw/day respectively causes decreased testes weight, testicular atrophy and spermatogenic arrest. However, the authors fail to discussion some serious flaws in the 2-year study, e.g. that conclusion are drawn upon data from 1-2 animals/dose group and that testicular atrophy was observed in some control dogs.

Since for the present evaluation of borates within the framework of biocides, the original study reports were available, we based our assessment on these study reports rather than on the publication of Weir and Fisher.

5.5 Discrepancy with evaluations within other frameworks

The toxicology of simple borates has also been evaluated within other frameworks. We noticed that in these other evaluation in general the overall NOAEL was based on developmental effects observed in a developmental toxicity study in the rat (Price et al., 1994). In this study the NOAEL was 55 mg boric acid/kg bw/day (equal to 9.7 mg B/kg bw/day) based on reduced fetal bodyweight and increased incidence of short rib XIII at 76 mg boric acid/kg bw/day (equal to 13.3 mg B/kg bw/day).

In order to explain the reason for the apparent discrepancy in the choice of the critical endpoint between our evaluation for biocides and the other evaluations, we looked into the data base of the other evaluatons upon which the overall NOAEL was based, and to the justification for choosing the developmental effects in the rat rather than the testis effects in the dog as the critical endpoint.

Below, a number of toxicological evaluations of borates are discussed.

Environmental Health Criteria 204 (IPCS, WHO, 1998)

The evaluation of the WHO task group on Environmental Health Criteria for Boron appears to be based upon data from published literature.

The 90-day dog studies, as described by Weir and Fisher (1972) are mentioned in the EHC204 document. On page 75 it is mentioned that indeed in the 90-day study with boric acid in the dog a statistically significant reduction of 25 and 40% in testes weight was observed in the mid and high-dose group. However, the information concerning the reduction in testes weight in the mid-dose animals is not presented in the summarising table on page 79. In addition the data from the 2-year dietary studies in the dogs, also as described by Weir and Fisher (1972) were discussed. On the basis of the deficiencies in the 2-year dog studies, EHC204 concluded that these 2-year studies were considered not suitable for inclusion into the risk assessment.

No further mention of the 90-day dog studies was made, probably since the Weir and Fisher article presents only limited data on these studies. But it was not stated that this particular experiment neither the species used (dogs) would be irrelevant. In the EHC204 document the study on the developmental effects in the rat is considered the provide the overall NOAEL of 9.6 mg B/kg bw/day.

US-EPA. Toxicological review of boron and compounds, June 2004

US-EPA describes the 90-day dog studies with boric acid and borax. In addition to the testicular effects in the high dose groups, it is stated that "decreased testes:body weight ratio was also observed in one dog from the mid-dose boric acid group". It is not clear why the US-EPA came to the latter conclusion (in fact 4 out of 5 dogs of the mid-dose group have a testes/body weight ratio outside the range of the control group). The 2-year studies in the dog were considered not to be adequate for establishment of a defensible NOAEL. It is not clear whether the original studies were available for the US-EPA evaluation, or whether it is based on the Weir and Fisher publication of 1972, although the remark about just one dog from the mid-dose group having a decreased testes: body weight ratio may suggest that the original study report was available to the US-EPA.

The study on the developmental effects in the rat is considered the provide the overall NOAEL of 9.6 mg B/kg bw/day.

ECETOC Technical report No. 63 (1995)

The reproductive and general toxicology of borates has been evaluated by an expert group on behalf of ECETOC. In the bibliography of the ECETOC Technical report reference is made to *"Weir and Fisher, 1961-1967. Full toxicologic study reports on borax and boric acid. Volume 1-8, prepared for US Borax."* It is not clear whether the 90-day dog studies are described in the Weir and Fisher 1961-1967 report. Furthermore, for the ECETOC report the Weir and Fisher article (1972) was also available. Nevertheless, in the ECETOC report the 90-day dog studies are not mentioned at all. The 2-year studies in dogs as described by Weir and Fisher, are discussed. Due to their deficiencies these studies ECETOC considers the 2-year studies not suitable for use in Risk Assessment

In the ECETOC document the study on the developmental effects in the rat is considered the provide the overall NOAEL of 9.6 mg B/kg bw/day.

EFSA: Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Boron (Sodium Borate and Boric Acid). Request No EFSA-Q-2003-018 (July 2004)

The evaluation of the EFSA Scientific panel is based upon information from published literature. Again it is mentioned that in the 90-day dog study (as described by Weir and Fisher, 1972) at the mid- and high dose significantly lower testes weights were observed. The 90-day studies are however not further mentioned in the document. So no reason was provided for neglecting the study or the animal species. The 2-year dog studies were considered equivocal. The study on the developmental effects in the rat is considered the provide the overall NOAEL of 9.6 mg B/kg bw/day.

WHO guideline for drinking-water quality. Addendum to Volume 1. WHO 1998

Evaluation based upon EHC204 (1998). The testes are recognized as a target in mice, rats and dogs. The drinking water limit is based upon the NOAEL of 9.6 mg/kg bw/day from the developmental toxicity study in the rat. Apart from the developmental study no individual studies are described at all.

US-Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile: Boron

The ATSDR mentions that in animals, boron affects gonads in dogs, rats and mice. It is stated that the data suggest that dogs are more sensitive than rats or mice. The evaluation is only based on data from published literature (among others Weir and Fisher, 1972). In the summarizing table 2-2, 'Levels of significant exposure to Boron and compounds-oral' composed by ATSDR, the NOAEL and LOAEL for the testes effects in the dog are set at 4.4 and 44 mg Boron/kg bw/day, respectively. In view of the lack of information on the testes effects at 4.4 mg Boron/kg bw/day, provided in the Weir and Fisher article it is easy to understand why ATSDR concluded this was the NOAEL.

Commission Working Group of Specialized Experts in the fields of Reprotoxicity. Summary record 1.a: Boric acid and Borates. ECBI/132/04 Rev2, 2004

The reproduction and developmental effects of boric acid and the borates were discussed by the Working Group of Specialized Experts. A representative from the industry maintained that reprotoxic properties were not disputed, but did not merit classification. Among others, the representative referred to a dog study of very poor quality. The Expert Working Group concluded that boric acid and borates have an adverse effect on fertility in rat, mouse and dog, and development in rat, mouse and rabbit. The experts recommend to classify boric acid and the borates with Repr. Cat. 2; R60-61.

Remark: The original 90-day dog studies were not discussed. The reference of the representative from the industry to the dog study of poor quality presumably concerns the 2-year study in the dog. It should be noted that the Expert Working Group identifies the hazards of the substance and does not determine overall NOAELs, ADIs, AOELs etcetera.

5.6 Discussion

The toxicology of borates has been evaluated within many frameworks. Although it is recognized that the testes is a target organ in mice, rats and dogs, and that in this respect the dog may be more sensitive than rats and mice, the dog data are not used to set the NOAEL. However, many, if not all of these evaluations appear to be based upon data from published literature. In published literature (i.e. Weir and Fisher, 1972) the description of the dog data is not completely adequate and very concise. In particular the description of the 90-day studies in the dog is very limited. Since the 2-year studies in the dog, also described in the same publication, have serious deficiencies, these studies are generally discarded as being inadequate to include in risk assessment. None of these evaluations discarded the dog as an irrelevant or hypersensitive species (compared to humans). None of these evaluations provided specific reasons for not including the 90-day dog studies. Apparently, the limited description of the 90-day studies by Weir and Fisher and the poor quality of the 2-year studies, led to the exclusion of these data in the risk assessment.

The original study reports from the 90-days studies in the dog were available for the present evaluation. Although the studies are old, not according to GLP, and have limitations, they were considered by the present evaluators to be acceptable for use in risk assessment. The effects of boron on the testes of the dogs are in line with findings in other species and are marked (relative testes weight reduction: 25 and 40% for boric acid mid- and high-dose groups, 15 and 50% for borax mid- and high-dose groups). Dose response modelling supports the choice of the NOAEL.

The discrepancy in the choice of the overall NOAEL between the present evaluation and evaluations performed within other frameworks may be caused by the different data sets available for the evaluations.

5.7 Justification of the used assessment factors

For the present evaluation the default assessment factor of 100 was used. With respect to toxicodynamics of borates, no data on human intraspecies variability or relative sensitivity of humans compared to other species are available. With respect to toxicokinetics of borates, little information is present. The available data do indicate however that substantial differences in toxicokinetic exist (e.g. elimination half-lives of 1h in the mouse, 3h in the rat and 21h in humans are reported). In view of this, we considered that there was insufficient evidence to deviated from the default assessment factors.

EHC 204

EHC 204 (1998) considered it not appropiate to deviate from the inter- and intraspecies factor of 2.5 and 3.2 for toxicodynamics. With respect to toxicokinetics EHC204 considered that an interspecies factor of 1.25 (default =4) was appropriate since it was argued that no marked interspecies were observed in the extent of oral absorption, metabolism, distribution and excretion. With respect to intra-human variability in toxicokinetics it was argued that a reduction of the default value of 3.2 to 2.5 was justified in view of the lack of boron metabolism.

Based on the reasons given above, EHC204 considered a total assessment factor of 2.5 x 3.2 x 1.25x 2.5= 25

WHO Guidelines for drinking water quality

The evaluation of The Working group on Chemical Substances in Drinking Water was based on the EHC204 (1998). The Working group took note of the lower uncertainty factor proposed in EHC204 by decided to use a more conservative uncertainty factor. The Working group considered it not appropiate to deviate from the default inter- and intraspecies assessment factors for toxicodynamics, and the interspecies default assessment factor for toxicokinetics. With respect to intraspecies variability it was noted that an increased glomerular filtration rate (GFR) is a recognized physiological adaptation in pregnancy. Accordingly it can be assumed that renal clearance of boron is increased in pregnant women. Based on data on human mean GFR in pregnancy and intraspecies variation of this factor it was concluded that the standard intraspecies factor of 3.2 could be reduced to 1.8, resulting in a total uncertainty factor of 60. This was considered appropriate since the Working Group considered that developmental toxicity in the rat was the critical endpoint for borates. A detailed description can be found in Dourson et al., Biological Trace Element Research, V66 (1998) pp. 453 - 463.

5.8 Discussion

The general view within different frameworks appears to be that there are no data available to justify a deviation from the default assessment factor for toxicodynamics. Both EHC204 and the WHO Working Group for Drinking Water considered it appropriate to decrease the assessment factor for toxicokinetics. We recognize that borates are simple compound that, in the body, will be predominantly be present as boric acid, which will not under go further metabolism. Accordingly, the default assessment factor for toxicokinetics of borates may be conservative. However, we consider to have no actual data to justify a reduction of the standard assessment factor.

Should the developmental toxicity in rats be considered as the critical endpoint for borates, then the increased glomerular filtration rate during pregnancy could be used as an argument to reduce the intraspecies assessmentfactor for toxicokinetics. However, since we considered the testes effects to be the critical endpoint, the increased renal clearance during pregnancy does not justify a reduction of the standard assessment factor.

5.9 Discussion of the risk assessment of naturally occurring substances

The toxicological data base reveals that a major target for toxicity of borates is the testes. Based on the testes effects in the 90 days feeding studies with boric acid and borax in dogs an overall NOAEL of 0.46 mg B/kg bw/day was established.

For the general population the average daily intake of boron through food is about 1.2 mg per day (WHO, 1998). Drinking water on average contains 0.1 - 0.3 mg boron per liter (WHO, 1998), although in some regions much higher concentrations (up to 29 mg/L) have been reported (Sayli, 1998). Based on the intake of boron through food and drinking water, the average daily exposure for the general population will be 1.4 - 1.8 mg, equal to 0.023 - 0.03 mg B/kg bw/day for a person weighing 60 kg. In subpopulations with relatively high food and water intake, such as children, or in populations living in regions with high boron levels in the drinking water exposure may be considerably higher. In view of the LOAEL of 4.2 mg B/kg bw/day and the NOAEL of 0.46 mg B/kg bw/day in the 90-day dog study it can be concluded that the margin between the daily exposure level to boron and the levels at which toxic effects are observed in experimental animals is small.

A small margin between background exposure levels and levels at which toxicity occurs is not uncommon for substances from natural sources (e.g. copper, selenium). It appears that the traditional methodology for risk assessment, based on application of assessment factors to data from animal toxicity studies may be not adequate to perform risk assessment for these naturally occurring substances.

6 REFERENCES

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[redacted] 1966b.

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[redacted] 1967b.

[redacted] [illegible]1966c.

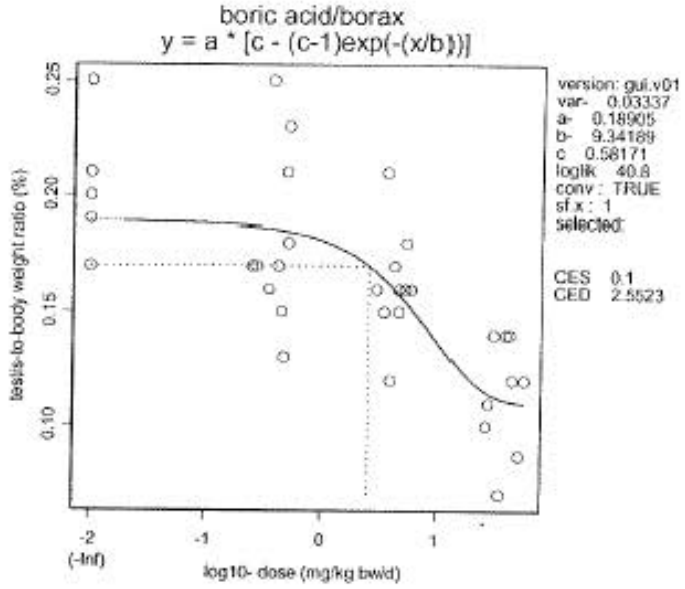
[redacted] 1966d.
WHO. Guidelines for drinking-water quality, Addendum to Volume 1, 1998.

6.1 Appendix a

group	# dog	substance (mg/kg bw/d)	Boron (mg/kg bw/d)	testes weight	rel. testes weight	av. rel. testes weight.
1	4521	0	0	18,6	0,2	
1	4912	0	0	20	0,19	
1	4932	0	0	20	0,21	
1	4939	0	0	14,6	0,25	
1	4970	0	0	12,8	0,17	0,204
2	4595	3	0,53	22	0,25	
2	4800	2,7	0,47	18,5	0,23	
2	4917	2,4	0,42	17,5	0,18	
2	4921	2,1	0,37	21	0,21	
2	4972	2,9	0,51	15	0,17	0,208
		2,62	0,46			
2	4922	20	3,5	17	0,15	
2	4925	30	5,3	14	0,16	
2	4928	31	5,4	17	0,18	
2	4935	23	4	13	0,12	
2	4937	17	3	10,5	0,16	0,154
		24,2	4,24			
2	4798	172	30	13	0,14	
2	4927	153	27	9,5	0,11	
2	4933	150	26	9	0,1	
2	4967	312	55	10,5	0,12	
2	4983	216	38	10,5	0,14	0,122
		200,6	35,2			
3	4879	4,2	0,47	10	0,13	
3	4882	3,1	0,35	14	0,16	
3	4904	3,7	0,42	18	0,17	
3	4920	2,4	0,27	17,5	0,17	
3	4968	4	0,45	12,5	0,15	0,156
		3,48	0,392			
3	4914	33	3,7	24,5	0,21	
3	4936	38	4,3	14	0,17	
3	4971	42	4,7	12	0,15	
3	4973	53	6	14	0,16	
3	4984	42	4,7	14,5	0,16	0,17
		41,6	4,68			
3	4875	302	34	6	0,07	
3	4918	360	41	14	0,14	
3	4977	390	44	10	0,12	
3	4979	443	50	8,4	0,087	0,10425
		373,75	42,25			

Individual animal data from the 90-day dog studies
groups: 1 = control, 2= boric acid, 3= borax

Dose-response modeling (PROAST) of data from 90-day studies with boric acid and borax in the dog



Critical Effect Size (CES)	Critical Effect Dose (CED) (90% Confidence Interval) in mg Boron/kg bw/d
-5%	1.189 (0.593 – 3.013)
-10%	2.552 (1.269 – 6.388)

Chronic toxicity (Repeated Dose)

Section 6.5

Section 6.5; Oral; Rat Sodium tetraborate decahydrate.

Also presented in Boric Acid Dossier

Annex Point IIA6.3 / 6.4 / 6.5

Official use only

7 REFERENCE

7.1	**Reference**	[redacted] *(1966). Two-year dietary feeding study - albino rats. Borax (Sodium tetraborate decahydrate).* [redacted]
7.2	**Data protection**	Yes
7.2.1	**Data owner**	[redacted]
7.2.2	**Companies with letter of access**	**Curent Access** [redacted]
7.2.3	**Criteria for data protection**	*Data on new a.s for first entry to Annex I/IA*

8 GUIDELINES AND QUALITY ASSURANCE

Chronic toxicity (Repeated Dose)

Section 6.5

Section 6.5; Oral; Rat Sodium tetraborate decahydrate.

Annex Point IIA6.3 / 6.4 / 6.5

Also presented in Boric Acid Dossier

8.1	**Guideline study**	No Predates Guidelines, there is another (see 6.7) 2 year study in mice carried out by the US NTP. In addition there are a number of other old studies and literature studies that support the results and NOAELS observed. Therefore in the interests of animal welfare and protecting Laboratory animals no further testing is deemed necessary.
8.2	**GLP**	No Although this is an old, pre GLP study, there is another 2 year study in mice carried out by the US NTP. In addition there are a number of other old studies and literature studies that support the results and NOAELS observed. Therefore in the interests of animal welfare and protecting Laboratory animals no further testing is deemed necessary.
8.3	**Deviations**	
	9 MATERIALS AND METHODS	
9.1	**Test material**	Disodium Tetraborate Decahydrate
9.1.1	Lot/Batch number	not available
9.1.2	Specification	As given in section 2 of boric acid and Sodium Tetraborate
9.1.2.1	**Description**	Fine white powder with no odour
9.1.2.2	**Purity**	> 99%
9.1.2.3	**Stability**	Stable
9.2	**Test Animals**	Non-entry field
9.2.1	Species	Rat
9.2.2	Strain	Sprague Dawley
9.2.3	Source	not specified
9.2.4	Sex	male and female
9.2.5	Age/weight at study initiation	Age not specified; Weight males 93-130g; females 86-128g
9.2.6	Number of animals per group	35 per sex per group for treated and 70 per sex per group for controls
9.2.7	Control animals	Yes
9.3	**Administration/ Exposure**	Oral

Chronic toxicity (Repeated Dose)

Section 6.5

Section 6.5; Oral; Rat Sodium tetraborate decahydrate.

Annex Point IIA6.3 / 6.4 / 6.5

Also presented in Boric Acid Dossier

9.3.1	Duration of treatment	2 years (5 per sex per group were killed at 6 and 12 months)
9.3.2	Frequency of exposure	Daily
9.3.3	Postexposure period	None
9.3.4	**Oral**	
9.3.4.1	**Type**	in food
9.3.4.2	**Concentration**	Disodium tetraborate decahydrate: 0, 1030, 3080, 10300 ppm (0; 117; 350; 1170 ppm as boron equivalents) ad libitum equivalent to 0, 52, 155, 516 mg borax/kg/day or 0, 5.9, 17.5 or 58.5 mg B/kg/day ad libitum
9.3.4.3	**Vehicle**	Dry mix with feed
9.3.4.4	**Controls**	plain diet
9.4	**Examinations**	
9.4.1	Observations	
9.4.1.1	**Clinical signs**	yes : recorded weekly for first 52 weeks then 4 weekly
9.4.1.2	**Mortality**	yes: recorded daily
9.4.2	Body weight	yes: recorded weekly for first 52 weeks then 4 weekly
9.4.3	Food consumption	yes : recorded weekly for first 52 weeks then 4 weekly
9.4.4	Water consumption	no: given ad libitum
9.4.5	Ophthalmoscopic examination	no
9.4.6	Haematology	yes at 1, 2, 3, 6, 12, 18 and end of study on 5 per sex per group. Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count.
9.4.7	Clinical Chemisty	yes: At interim sacrifice at 6, 18 and 24 months blood pH, sodium, potassium, chloride, carbon dioxide combining power on 2 rats per sex per group. At 6, 12 and 24 months SGPT and SGOT were determined in 5 rats per sex in the control and high dose group.
9.4.8	Urinalysis	yes: at 6 months on individual samples from 2 rats per sex per group for 5 days; also at 18 and 24 months on pooled samples from 5 per sex per group Parameters: appearance, volume, osmolality, specific gravity, pH, protein, glucose, blood, acetone, bilirubin and microscopy.

Section 6.5

Annex Point IIA6.3 / 6.4 / 6.5

Chronic toxicity (Repeated Dose)

Section 6.5; Oral; Rat Sodium tetraborate decahydrate.

Also presented in Boric Acid Dossier

9.5 Sacrifice and pathology

9.5.1	Organ Weights	yes organs: liver, kidneys, adrenals, testes, thyroid, spleen, brain.
9.5.2	Gross and histopathology	yes: At 6 and 12 months 5 rats per sex per group, all interim deaths and at termination in 10 per sex per group in controls and high dose surviving animals. organs: brain, pituitary, thyroid, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, lungs, gonads, urinary bladder, sternum, rib junction and all unusual lesions. In addition, 10 rats per sex per group from the mid and low dose groups had gonads examined histologically.
9.5.3	Other examinations	samples of blood, brain, liver and kidney were taken at 6, 12 and 24 months and frozen for boron analysis.
9.5.4	Statistics	yes as appropriate
9.6	**Further remarks**	

10 RESULTS AND DISCUSSION

10.1	**Observations**	See Table A6_3-1 and A6_3-2
10.1.1	Clinical signs	No signs in the control and low dose groups. Ccoarse hair coats, hunched position, swollen pads and inflamed bleeding eyes were observed in animals receiving the highest dose of borax
10.1.2	Mortality	Survival at 6, 12 and 24 months was comparable in all groups including controls.
10.2	**Body weight gain**	No difference from controls in the low and mid dose group. Retarded body weight gain in animals receiving the highest dose of borax.
10.3	**Food consumption and compound intake**	No difference from controls in the low and mid dose group. Reduced food intake in the highest dose group during weeks 1-13 in males and in weeks 1-13 and 27-52 in females.
10.4	**Ophtalmoscopic examination**	Not done
10.5	**Blood analysis**	
10.5.1	**Haematology**	No difference from controls in the low and mid dose groups. Significantly decreased red cell volume and haemoglobin were observed in the high dose group males at 2, 12,and 18 months, and in females red cell volume was decreased at2, 3, 12 and 18 months. Results shown in Table A6_3-2 Results quoted from study: 'at each time of determination the cell volume and hemoglobin values for the male and female rats in the high level test group (1.03% ~58.5mgb/kg) were below the normal range for rats or within the low normal range and were lower than those for the corresponding controls'. At the lower doses the values '... were generally within normal limits and comparable with the controls. Values differing from control determined at six and 24 months for the males in the low and intermediate groups were inconsistent and did not reflect a definite

		trend.'
10.5.2	Clinical chemistry	no significant differences between groups.
10.5.3	Urinalysis	no significant differences between groups.
10.6	**Sacrifice and pathology**	
10.6.1	Organ weights	The testes weights and the testes/bodyweight ratios were significantly lower than those of control animals. The brain- and thyroid-to-bodyweight ratios were significantly higher than those of controls. This was thought to relate to the reduced bodyweight of the animals.
10.6.2	Gross and histopathology	Atrophic testes were found in all males exposed to the high dose (516(58.5) mg borax (B)/kg bw) of borax at 6, 12 and 24 months. Microscopic examination of the tissue revealed atrophied seminiferous epithelium and decreased tubular size in the testes. There was no treatment related increase in tissue masses.
10.7	**Other**	none

11 APPLICANT'S SUMMARY AND CONCLUSION

11.1	**Materials and methods**	Non guideline 2 year dietary feeding study in Sprague Dawley rats, 35 per sex per treated group and 70 controls per sex with interim kills of 5/sex/group at 6 and 12 months
11.2	**Results and discussion**	Testicular atrophy and seminiferous tubule degeneration was observed at 6, 12 and 24 months at the highest dose level only. No treatment related effects were observed in the mid and low dose groups.. Data also presented with boric acid data to support the data obtained with boric acid
11.3	**Conclusion**	
11.3.1	LO(A)EL	*In males 516 (58.5) mg borax (B)/kg bw caused testicular atrophy. In females 516 (58.5) mg borax (B)/kg caused reduced bodyweight*
11.3.2	NO(A)EL	*In males and females 155 (17.5)mg borax (B)/kg bw.*
11.3.3	Other	
11.3.4	Reliability	*2*
11.3.5	Deficiencies	*Although an old study, data are clear and acceptable*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	5 April 2005
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	In addition to the description of clinical signs by the applicant, in the high dose group desquamation of the skin of the tail and the pads of the paws and marked respiratory involvement, were observed. In all males of the high dose group the scrotum appeared shrunken. At the high dose the testes weights and the testes/bodyweight ratios were significantly lower than those of control animals. The reduction was already observed at 26 weeks and the extent of the reduction did not increase over the course of the treatment period. No effect on relative testes weight were observed at the other dose groups at 26, 52 or 104 weeks. As compared to the control group, marked reductions in body weight were observed in males of the high dose group (16%), and in females of the low-, mid- and high-dose groups (17, 9 and 33 % reduction, respectively). The reductions in body weight may be the result of a decreased food consumption in these animals. Haematology: In addition to the effects on haematology at the high dose, as presented in table A6_3-2 below, occasionally reductions (not statistically significant) in white blood cell count were observed in the low- and mid-dose groups. Since only 5 animals per group were sampled the statistical power is low.
Conclusion	LO(A)EL: 10300 ppm, equivalent to 516 mg/kg bw/day (58.5mg B/kg bw/day), based on atrophy of the testes and haematological effects in males and females. NO(A)EL:3080 ppm, equivalent to 155 mg/kg bw/day (17.5 mg B/kg bw/day)
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3-1. Results Rat 2 year Study with Borax

Parameter	Control		low dose		medium dose		high dose		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	70	70	35	35	35	35	35	35		
Mortality at 104 weeks	25/60	20/60	6/25	8/25	9/25	10/24	7/25	5/25	N	N
clinical signs*										
body weight gain 0-104 weeks (g)	557	405	546	318	499	359	449	238	Y	Y
food consumption at week 52 (g/kg/day)	33.3	43.7	35.4	42.9	35.3	44.6	39.7	52.7		
clinical chemistry*	no differences									
haematology*	see separate table									
urinalysis*	no differences									
Organ x										
testes weight*(g) at 26 weeks	3.76±0.29		3.67±0.29		3.81±0.14		0.95±0.06 sig low			
testes weight (g) at 104 weeks	3.65±0.84		3.65±0.63		3.30±0.60 @		0.99±0.24 sig low			
microscopic pathology*Testes atrophy at 24 months	3/10		1/10		4/10		10/10			

** specify effects; for different organs give special findings in the order organ weight, gross pathology and microscopic pathology if there are effects*

[a] *give number of animals affected/total number of animals, percentage, or just ↑ or ↓ for increased or decreased*

@ significantly low for absolute weight but not relative weight

Table A6_3-2 Summary of Haematological Haematological Data from 2 Year Rat Study at Highest dose (1.03% ~58.5mgB/kg).

Days	Cell Volume (%)				Hb Value (g/100ml)				WBC Count ($x10^3/cm^2$)				RBC Count ($x10^6/cm^2$)			
	Female		Male		Female		Male		Female		Male		Female		Male	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
30	42.1	38.2	42.6	40.1	14.6	13.5	15.5	13.7	19.8	12.5	18.1	10.7*				
60	41.7	38.4*	44.1	38.8*	14.9	15.1	14.7	13.0*	16.6	14.3	19.3	17.0	7.36	7.07	8.2	7.28
90	44.2	41.2*	45.9	42.8	14.0	12.6	15.7	14.2	26.9	21.1	20.9	17.6	5.64	6.65	7.14	6.35
180	43.3	41.1	45.4	42.8	14.5	14.0	15.4	13.2*	14.6	11.6	19.4	14.7				
365	42.8	38.2*	47.3	42.18	12.9	12.4	14.1	12.4*	9.5	8.3	14.2	10.7				
545	43.0	41.3*	47.8	41.8*	14.8	14.0	15.6	13.6*	10.9	11.8	23.4	17.9	6.58	6.47	5.16	5.24
2 Years	46.2	41.4	46.4	43.9	13.7	12.2*	14.7	13.5	21.8	10.8	19.8	13.7	5.05	5.7	7.09	8.28

* Significantly different from controls

Section A6.8.2.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 6.8.2.2 Rat Three Generation Study Sodium tetraborate decahydrate.
Also presented in Boric Acid Dossier

Official use only

1 REFERENCE

1.1 Reference

[redacted] (1966). Three-generation reproductive study - rats. Sodium tetraborate decahydrate. [redacted]

1.2 Data protection Yes

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access **Curent Access** [redacted]

1.2.3 Criteria for data protection Data on new a.s for first entry to Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No but conforms to the standard 3 generation 2 litters per generation MGS normally used at that time.

Predates modern protocols and GLP. Although this study is not to modern day protocols, there are literature data in 3 species that confirm the results seen. No further testing is necessary in the interests of animal welfare and protecting laboratory animals

2.2 GLP No

Although these studies predate modern protocols and GLP, other data available support the results. Further testing is not warranted in the interests of animal welfare.

2.3 Deviations

3 MATERIALS AND METHODS

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

Section A6.8.2.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 6.8.2.2 Rat Three Generation Study Sodium tetraborate decahydrate.
Also presented in Boric Acid Dossier

3.1	**Test material**	As given in section 2 Sodium tetraborate decahydrate (borax)
3.1.1	Lot/Batch number	Not available
3.1.2	Specification	As given in section 2
3.1.2.1	**Description**	Fine white powder without odour.
3.1.2.2	**Purity**	>99%
3.1.2.3	**Stability**	Stable
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Crl:CD Sprague Dawley
3.2.3	Source	Charles River Laboratories, USA
3.2.4	Sex	male and female
3.2.5	Age/weight at study initiation	Weight: males 130-135g; females 110-149g.
3.2.6	Number of animals per group	8 males and 16 females per group
3.2.7	Mating	See table below
3.2.8	Duration of mating	21 days on each occasion, 1 male and 2 females per cage
3.2.9	Deviations from standard protocol	This is a three generation multigeneration study with two matings (two litters) per generation. The F1a, F2a and F3a litters were sacrificed at weaning, and the F1b and F2b litters raised and used for breeding, and the F3b killed at weaning.
3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	Oral Fill in respective route in the following, delete other routes
3.3.1	Animal assignment to dosage groups	By stratified randomisation
3.3.2	Duration of exposure before mating	14 weeks
3.3.3	Duration of exposure in general P, F1, F2 males, females	From beginning of the study until sacrifice of parents P0 , and from weaning till sacrifice for the parents of the F1 and F2-generations. The high dose group P animals were sterile so only controls, low and mid dose groups were taken to the F2 and F3 generations.
		Oral
3.3.4	Type	in food
3.3.5	Concentration	food: 0, 1030, 3080 or 10300ppm borax (0, 117, 350 and 1,170 ppm boron) in the diet, equivalent to 0, 50 (5.9), 155 (17.5) and 518 (58.5) mg borax (mg B)/kg bw respectively food consumption per day ..... ad libitum

Section A6.8.2.2 **Multigeneration Reproduction Toxicity Study**

Annex Point IIA6.8.2 6.8.2.2 Rat Three Generation Study Sodium tetraborate decahydrate.
Also presented in Boric Acid Dossier

3.3.6	Vehicle	dry powdered food
3.3.7	Concentration in vehicle	as above
3.3.8	Total volume applied	food ad libitum
3.3.9	Controls	plain diet
3.4	**Examinations**	
3.4.1	Clinical signs	Yes weekly
3.4.2	Body weight	Yes weekly
3.4.3	Food/water consumption	yes weekly for food; water ad libitum not recorded.
3.4.4	Oestrus cycle	not done
3.4.5	Sperm parameters	not done except in the high dose group in which histology of testes was performed.
3.4.6	Offspring	number and sex of pups stillbirths live births presence of gross anomalies weight gain physical or behavioural abnormalities culled to 8 per litter at 24 hours after deliveery
3.4.7	Organ weights P and F1	Uterus ovaries testes brain liver kidneys spleen thyroid adrenals
3.4.8	Histopathology P, F1, F2 parents	All parental animals were necropsied and a range of tissues preserved in formalin but not examined histologically except for testes, ovaries and uterus of the high dose group only.
3.4.9	Histopathology F1 not selected for mating, F2	5 of each sex from all groups of the F3b litters were necropsied and a range of tissues fixed in formalin but not examined histologically.
3.5	**Further remarks**	

4 RESULTS AND DISCUSSION

See Tables A6_8_2-1 and Table A6_8_2-2.

Section A6.8.2.2	**Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2	6.8.2.2 Rat Three Generation Study Sodium tetraborate decahydrate. Also presented in Boric Acid Dossier
4.1 Effects	Non-entry field
4.1.1 Parent males	Rats exposed to the high dose of 518 mg/kg borax(corresponding to a level of 58.5 mg B/kg bw) had reduced bodyweights though food intake was not affected and they were sterile. Microscopic examination of the atrophied testes of all males in this group showed no viable sperm. There were no adverse effects on reproduction reported at exposures of 5.9 and 17.5 mg B/kg bw. The authors reported no adverse effects on fertility, lactation, litter size, progeny weight or appearance in rats exposed to either 5.9 or 17.5 mg B/kg bw. Also, no gross abnormalities were observed in the organs from these dose groups.
4.1.2 Parent females	The high dose groups of the Po generation had reduced bodyweight without any effect on food intake. Evidence of decreased ovulation in about half of the ovaries examined from the females exposed to 58.5 mg B/kg bw and only two of 16 females produced a litter(one of which was cannibalised within 24h) when mated with control male animals. There were no adverse effects on reproduction and no gross abnormalities were observed in the organs at exposures of 5.9 and 17.5 mg B/kg bw.
4.1.3 F1 males	There were no adverse effects on reproduction and no gross abnormalities were observed in the organs at exposures of 5.9 and 17.5 mg B/kg bw.
4.1.4 F1 females	There were no adverse effects on reproduction and no gross abnormalities were observed in the organs at exposures of 5.9 and 17.5 mg B/kg bw.
4.1.5 F2 males	There were no adverse effects on reproduction and no gross abnormalities were observed in the organs at exposures of 5.9 and 17.5 mg B/kg bw.
4.1.6 F2 females	There were no adverse effects on reproduction and no gross abnormalities were observed in the organs at exposures of 5.9 and 17.5 mg B/kg bw.
4.2 Other	The high dose group (58.5 mgB/kg bw)males and females showed clinical signs of toxicity with rough fur, scaly tails, respiratory distress and inflamed eyelids.
	5 APPLICANT'S SUMMARY AND CONCLUSION