Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Barium diboron tetraoxide

EC Number: 237-222-4

CAS Number: 13701-59-2

Index Number: Not assigned

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1 PHYSICAL HAZARDS

Not assessed in the CLH dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 [Study 1] *In vivo* percutaneous absorption of boric acid, borax, and disodium octaborate tetrahydrate in humans (boric acid and borate salts)

Reference	Wester, R. C., Hui, X., Hartway, T., Maibach, H. I., Bell, K., Schell, M. J Northington DJ and Culver, B. D. (1998a). In vivo percutaneous absorption of boric acid, borax, and disodium octaborate tetrahydrate in humans compared to in vitro absorption in human skin from infinite and finite doses. Toxicological Sciences, 45(1), 42-51.
Guideline	No guideline followed
Reliability	Klimisch 1: reliable without restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)
Species / strain	Human
Test material	Boric acid, borax, and disodium octaborate tetrahydrate (DOT) Purity: unknown
Study design	Twenty-four normal, healthy, males or females, aged from 22 to 50 years were selected for this study and divided into three groups. Groups I, II and III received two separate topical applications of 5% boric acid, 5% borax, and 10% disodium octaborate tetrahydrate solutions on their back skin, respectively, for 13 days. One dose was applied on day 5 under normal skin conditions and the other dose was applied on day 12 under potentially irritated skin conditions created by a 24-h application of 2% sodium lauryl sulfate (SLS) solution. Background urine was collected for 4 days. After topical application, the dosed area was allowed to air dry and then the volunteer was dressed in a commercial white T-shirt for 24 h. The volunteers were requested not to touch or wash the dosed area for 24 h. Twenty-four hours after second dosing (day 13), the T-shirt was carefully removed and the dosed site was washed using gauze pads and liquid soap and water (50/50). T-shirts and skin washes were analyzed for boron content.
Findings	The absorbed dose of boric acid was 0.226 ± 0.125, with flux and permeability constants calculated at 0.0094 μg/cm^2/h and 1.9 x 10^{-7} cm/h, respectively. Borax (disodium tetraborate decahydrate) percent dose absorbed was 0.210 ± 0.194, with flux and permeability constants calculated at 0.00875 μg/cm^2/h and 1.8 x 10^{-7} cm/h, respectively. Disodium octaborate tetrahydrate absorbed dose was 0.122 ± 0.108, with flux and permeability constants calculated at 0.010 μg/cm^2/h and 1.0 x 10^{-7} cm/h, respectively.
Conclusion	*In vivo* absorption of boron applied for 24h to human skin as boric acid, borax, or disodium octaborate was in the range of 0.12-09.23% and did not vary significantly from one borate to the other. This is equivalent to 0.7 mg of absorbed boron for a person entirely immersed in a saturated boric acid solution for 24 h. For comparison, 0.7 mg B is significantly less than the average daily dietary intake. In the replication of the study with the pretreatment of skin with SLS prior to the application of borate, there was no detectable erythema, change in transepidermal water loss or effect on boron skin absorption. Thus the replication study using SLS provided an opportunity for confirmation of the results of the initial borate applications in which absorption ranged from 0.11 to 0.24%. This very low boron skin absorption makes it apparent that, for borates tested that have low human toxicity, the use of gloves to prevent systemic uptake is unnecessary. However, the findings of this study do not apply to abraded or otherwise damaged skin.

2.1.2 [Study 2] *In vitro* percutaneous absorption of boric acid, borax, and disodium octaborate tetrahydrate in humans (boric acid and borate salts)

Reference	Wester, R. C., Hartway, T., Maibach, H. I., Schell, M. J., Northington, D. J., Culver, B. D., and

Strong, P. L. (1998b). In vitro percutaneous absorption of boron as boric acid, borax, and disodium octaborate tetrahydrate in human skin. Biological trace element research, 66(1-3), 111-120.

Guideline No guideline followed

Reliability Klimisch 1: reliable without restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Human

Test material Boric acid, borax, and disodium octaborate tetrahydrate (DOT)

Purity: unknown

Study design *In vitro* diffusion from aqueous solution was determined in receptor fluid accumulation over a 24h period. Human cadaver skin (dermatomed) was clamped onto an AMIE Systems in-line cell in a flow-through apparatus, with 1 cm^2 surface area of skin exposed. Receptor fluid was pumped at a rate of 3 mL/h and collected every 4h to 24h. After 24h the skin surface was washed. Boric acid (enriched) was applied at 0.05 %, 0.5 % and 5 % and either an infinite dose of 1000 mL/ cm^2 or a finite dose of 2 mL/ cm^2. Changes in boron isotope ratios by IPCMS (Inductively Coupled Plasma-Mass Spectrometry) were used to measure absorption.

Findings The absorbed doses of boric acid were 1.2 for 0.005 % dose, 0.28 for 0.5 % dose and 0.70 % for 5 % dose. These absorption amounts translated into flux values of 0.25, 0.58 and 14.58 mg/cm^2/h and permeability constants (Kp) of 5.0 x 10-^{4}, 1.2 x 10^{-4} and 2.9 x 10^{-4} /cm/h. The above doses were at a standard 1000 µL/cm^2 dosing solutions. When the 5 % dose was applied at 2 µL/cm^2 (*in vivo* dosing volume), flux decreased some 200-fold to 0.07 mg/cm^2/h and Kp of 1.4 x 10^{-6} cm/h.

Borax (disodium tetraborate decahydrate) dosed at 5 %/1000 µL/cm^2 had 0.41 % dose absorbed. Skin surface wash recovery was 87.7 ± 5.9 % dose. Flux was 8.5 µg/cm^2/h, and Kp was 1.7 x 10^{-4} cm/h.

Disodium octaborate tetrahydrate dosed at 10 % /1000 µL/cm^2 was 0.19 % dose absorbed. Skin surface wash recovery was 91.3 ± 25.2 % dose. Flux was 0.8 x 10^{-4} cm/h. These *in vitro* results from infinite dose (1000 µL) were several magnitudes higher than those obtained *in vivo*. The results from the finite dose (2 µL) were closer to the *in vivo* results (also 2 µL).

Conclusion The corresponding *in vivo* study (Wester et al. 1998a) was done using a 2 µL/cm^2 dose. Because of the known absorption variability in skin samples both *in vitro* and *in vivo*, this *in vitro* study was done with skin sources from 6 different human cadavers in order to include individual variability. The variability seen here is within the expected range of *in vitro* studies. Boric acid was dosed at 0.05, 0.5, and 5% at 1 mL/cm^2. The flux would be expected to increase with increased dose, and this occurred with fluxes of 0.25, 5.8, and 14.58 µg/cm^2/h. These results were obtained with an infinite dose (1 mL/cm^2), so extrapolation to a finite dose must be done with caution. The permeability constant Kp normalizes absorption to a constant, and this occurred, ranging from 1.2 to 5.0 x 10^{-4} cm/h. The average Kp of 3 x 10^{-4} cm/h would best represent boric acid human skin absorption from an infinite dose.

Boric acid at 5% concentration was also administered at a 2 µL/cm^2 finite dose. This finite dose has less mass than the infinite dose, and flux was less, Kp was less than that with the infinite dose.

2.1.3 [Study 3] Dermal absorption of boron in infants treated with a boric acid ointment

Reference Friis-Hansen, B., Aggerbeck, B., & Jansen, J. A. (1982). Unaffected blood boron levels in newborn infants treated with a boric acid ointment. Food and Chemical Toxicology, 20(4), 451-454.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Human

Test material Boric acid

Purity: unknown

Study design The plasma boron content in 22 newborn infants was assessed, following repeated daily applications of a water-emulsifying ointment containing the equivalent of 3 % boric acid to the napkin region; 3 g ointment administered in total to each infant, corresponding to 90 mg boric acid (equivalent to 15.7

mg boron).

Findings The mean plasma-boron concentration decreased over a 5 days period, from a pre-treatment value of 0.49 to 0.29 mg/L, the corresponding values in ten untreated neonates being 0.62 and 0.21 mg/L, respectively.

Conclusion Repeated topical application of the water-emulsifying ointment natusan, which contains 3% boric acid, was shown to cause no increase in the natural boric acid level in the blood of newborn infants. Topical application of such preparations is safe, irrespective of the condition of the skin.

2.1.4 [Study 4] Literature review of published and proprietary data (boric acid and borate salts)

Reference ATSDR (2010) Toxicological profile of boron.

Species / strain Human

Test material The report considered human exposure to equivalent boron doses calculated from compounds such as boric acid, boron oxide, borate salts (e.g. calcium borate) and various hydration states of sodium borate salts (anhydrous, pentahydrate, decahydrate).

Study design Literature review

Findings Absorption: inhaled boron is absorbed and systemically distributed, almost complete gastrointestinal absorption following oral exposure.

Distribution: widely distributed throughout the body including reproductive tissues, but has a low affinity for fat. At high doses, boron accumulates in the bone.

Metabolism: being an inorganic element, boron is not metabolised by humans, but the parent borate is recovered in the blood, tissues and urine.

Elimination and excretion: excretion primarily through renal elimination; over 93% of the inhaled and ingested dose is excreted in the urine; a calculated mean half-life of 13.4 h (range 4 – 27.8 h) in nine cases of boric acid poisoning.

Conclusion The ADME profile of boron was described based on human data.

2.1.5 [Study 5] *In vivo* human excretion of boron (renal clearence)

Reference Pahl MV, Culver BD, Strong PL, Murray FJ and Vaziri ND (2001). The effect of pregnancy on renal clearance of boron in humans: A study based on normal dietary intake of boron. Toxicological Sciences 60, 252 - 256.

Guideline No guideline followed

Reliability Klimisch 1: reliable without restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Human (pregnant women)

Test material The source of boron used for the measurement of renal boron clearance was the dietary boron normally present in human food (present in high amounts especially in fruits and vegetables).

Study design Sixteen pregnant women in the 2nd trimester (14 – 28 weeks) and 15 nonpregnant women (designated as age-matched references) between the ages 18 – 40 participated in the study. Blood samples for boron, creatinine and urea were collected at the start, at 2h and 24h. Urine was collected during the first 2h in the Clinical Research Centre and during 22 h outside the centre for measurement of volume, boron and creatinine. The resultant plasma concentrations and timed urinary boron excretion amounts were used to calculate clearance, using the relationship that clearance equals the amount of boron excreted in the urine over the measured time interval divided by the average plasma boron concentration over that time interval.

Findings The pregnant and non-pregnant boron intake was 1.35 mg boron/24h and 1.31 mg boron/24h,

respectively. Renal boron clearance measured over the initial 2h was 68.30 ± 35.0 mL/min/1.73 m^2 for pregnant subjects and 54.31 ± 19.35 mL/min/1.73 m^2 for non-pregnant subjects based on surface area. Based on body weights, the renal clearances were 1.02 ± 0.55 mL/min/kg and 0.8 ± 0.31 mL/min/kg for pregnant and nonpregnant subjects respectively.

The renal clearance was 61.04 ± 36.7 mL/min/1.73 m^2 for pregnant subjects and 43.85 ± 21.59 mL/min/1.73 m^2 for nonpregnant subjects based on surface area. Based on body weights, the renal clearances were 0.92 ± 0.59 mL/min/kg and 0.64 ± 0.4 mL/min/kg for pregnant and nonpregnant subjects, respectively.

The baseline plasma levels of boron were 0.022 ± 0.013 and 0.023 ± 0.015 mg B/mL for nonpregnant and pregnant subjects respectively. At 2h and 24h, the levels were as follows: 2 hours: 0.024 ± 0.015 and 0.018 ± 0.011 mg B/mL for non-pregnant and pregnant subjects respectively; 24 hours: 0.027 ± 0.018 and 0.013 ± 0.006 mg B/mL for non-pregnant and pregnant subjects respectively.

Differences in the serum creatinine clearances indicated that urine collection had not been complete over the entire 24 h collection period.

Conclusion Comparison of renal boron clearance with creatinine clearance indicated that tubular reabsorption of boron occurred in both pregnant and non-pregnant women.

2.1.6 [Study 6] Neutron activation analysis-electrothermal atomic absorption spectroscopy (ETA-AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis

Reference Minoia C, Sabbioni E, Apostoli P, Pietra R, Gallorini M, Nicolaou G, Alessio L and Capdoaglio E (1990). Trace element reference values in tissues from inhabitants of the European Community I. A study of 46 elements in urine, blood and serum of Italian subjects. The Science of the Total Environment 95, 89 - 105.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Human

Test material Enviromental exposure to boron

Study design Forty-six elements from urine, blood and serum of unexposed Italian subjects living in the same region, were determined. The subjects were considered representative of five subgroups resident in urban, suburban, rural and low and high hill areas.

A questionnaire supplied detailed information on age, sex, area of residence, occupation, smoking habits, body weight, alimentary habits, socioeconomic and ethnic factors as well as on the elemental composition of the drinking water from the municipal supply and mineral water used.

Multielement analysis, using neutron activation, was carried out on urine and blood by instrumental (INAA) and/or radiochemical (RNAA) procedures. Dried samples in sealed polyethylene or quartz vials were irradiated for 10 h in the Triga Mark II reactor, University of Pavia (thermal neutron flux of the order of l0 is neutrons $cm^{-2} s^{-1}$), or for 40h in the HFR reactor at the JRC, Petten (The Netherlands) (2×10^{14} neutrons $cm^{-2} s^{-1}$). The irradiated samples were then counted by computer-based,gamma-ray spectroscopy using a Ge(Li) detector and/or submitted to radiochemical

separation involving: mineralization in a Teflon bomb, chromatography on a set of ion-exchangers or ion-exchange resins, including TDO (tin dioxide, C. Erba, Milan), Dowex l-X8 resin (Bio Rad) and CuS (copper sulphide, C. Erba), and counting of the fractions by gamma-ray spectroscopy.

Findings Boron was present in the urine of 119 subjects. The mean concentration ± standard deviation was 1890 ± 126 μg/L; with an experimental range of 470 – 7800 μg/L.

The reference values were 9490 - 3290 μg/L and range of uncertainty was > 3290 – 7800 μg/L.

The upper limit form metabolic anomalies was > 7800 μg/L.

Conclusion Boron was not present in the blood or serum of healthy Italian subjects.

A "range of uncertainty" and an upper limit above which metabolic abnormalities could be expected were defined for boron on the basis of s statistical considerations.

2.1.7 [Study 7] Inhalative exposure to soluble barium compounds

Reference	Zschiesche, W., Schaller, K. H. and Weltle, D. (1992). Exposure to soluble barium compounds: an interventional study in arc welders. International archives of occupational and environmental health, 64(1), 13-23.
Guideline	No guideline followed
Species / strain	Human
Test material	Occupational exposure to boron (Barium-containing stick electrodes or barium-containing self-shielded flux cored wires)
Study design	Eighteen workers (welders) exposed for one week to welding fumes containing 31 – 37% barium, were investigated. The workers were divided in three groups (A, B, C) based on the type of electrodes used, where the first two groups had no ventilation system at the working site, as opposed to group C. The workers did not use Ba-containing consumables minimum 10 days before the study was performed. On average, the welders worked for about 4 h per day with a mean arc time of about 80 %. The investigation included measurements of the external exposure to total welding fumes and soluble Ba in the breathing zone behind the welding shields and helmets, assessment of internal exposure to Ba by biological monitoring of plasma and urine spot samples, medical history taking, thorough clinical and neurological investigations, ECG (limb and precordial leads), continuous 24h ECG (two channels), and measurement of plasma electrolytes (sodium, potassium, magnesium, and total and ionized calcium). Whole blood was checked for pH, standard bicarbonate, and base excess The activities of tubular renal enzymes [N-acetyl P-D-glucosaminidase (NAG) and alanine aminopeptidase (AAP)] were measured in urine spot samples.
Findings	Excretion Group A: increased renal excretion of Ba, with median concentrations of 101.7 µg/L urine and 89.1 µg/g creatinine. The highest concentrations in the individual spot samples were 407.7 µg/L urine and 370.6 µg/g creatinine. Group B: increased renal excretion of Ba, with median concentrations of 113.1 µg/L urine and 77.3 µg/g creatinine. The maximum individual values were 313.8 µg/L urine and 287.9 µg/g creatinine. Group C: slightly increased renal excretion of Ba, with median concentrations of 44.3 µg/L urine and 49.2 µg/g creatinine. The highest individual concentrations were 4.1 µg/L urine and 3.1 µg/g creatinine. Plasma levels Group A: marked increase in Ba plasma levels, with a median concentration of 24.7 µg/L. The individual post shift concentrations were in the range of 4.1 – 63.4 µg/L. Group B: marked increase in Ba plasma levels, with a median concentration of 16.6 µg/L. The individual postshift concentrations were in the range of 4.5 – 74.0 µg/L. Group C: slightly increased Ba plasma levels, with a median concentration of 4.4 µg/L. The individual postshift concentrations were in the range of 1.2 – 7.9 µg/L.
Conclusion	The results show that airborne barium was absorbed either after mucociliary clearance from the gastrointestinal tract or through the respiratory system. The biological half-life time of Ba was calculated based on both urine and plasma and found to be 10 – 18h.

2.1.8 [Study 8] Emission spectrography analysis of barium in human tissues

Reference	Schroeder, H. A., Tipton, I. H., and Nason, A. P. (1972). Trace metals in man: strontium and barium. Journal of Chronic Diseases, 25(9), 491-517.
Guideline	No guideline followed
Species / strain	Human

Test material	The source of Ba used for the measurements performed by this study is represented by dietary Ba normally present in food and water.
Study design	A number of approx. 400 subjects (between 0 – 80 years of age) from the United States, Africa, Switzerland, Near Est and Far East were investigated. Strontium and barium in human tissues and in collected diets were measured by emission spectrography. The limits of detection were 1.0 ppm for strontium and 0.3 ppm for barium, in terms of ash, or about 0.01 and 0.003 ppm respectively, wet weight, depending on ash content of the sample. Coefficients of variation of the method were 8 per cent in both cases.
Findings	The mean plasma concentration of Ba was found to be 79 µg/L, and the calculated renal plasma clearance of Ba was 330 mL/day. The urinary excretion of Ba was found to be 3% of the total ingested amount.
Conclusion	Ba was also found in the testis in 75% of the collected samples, and in the ovaries in 87% of the collected samples. The authors also conclude that Ba crosses the placental barrier based upon the fact that Ba is found in infants and children in the first decade of life and even in the stillborn.

2.1.9 [Study 9] Literature review of published and proprietary data (barium and barium compounds)

Reference	ATSDR (2007) Toxicological profile of barium and barium compounds. CICAD WHO (2001) Barium and barium compounds.
Species / strain	Human
Test material	The reports considered human exposure to barite and barium salts (e.g. barium chloride, barium nitrate, barium hydroxide, barium sulphate, barium carbonate), which occurred environmentally, occupationally, intentionally or accidentally.
Study design	Literature review
Findings	Absorption: gastrointestinal absorption was 20% in adults, 30% for 1-15 year old children, 60% for infants. Airborne Ba can either be absorbed by the respiratory system or from the gastrointestinal tract (after mucociliary clearence). Distribution: 90% was detected in the bone where Ba was primarily deposited in active bone growth areas; 1-2% of the total body burden was found in muscle, adipose, skin and connective tissue. Metabolism: barium is not metabolised in the body, but it can be transported or incorporated into complexes or tissues. Elimination and excretion: primarily through faecal excretion (approx. 90%) and only 2 – 3% through urine.
Conclusion	The ADME profile of barium was described based on human data.

2.1.10 [Study 10] The effects of pregnancy on renal clearance of boron in rats given boric acid orally

Reference	Vaziri, N. D., Oveisi, F., Culver, B. D., Pahl, M. V., Andersen, M. E., Strong, P. L. and Murray, F. J. (2001). The effect of pregnancy on renal clearance of boron in rats given boric acid orally. Toxicological Sciences, 60(2), 257-263.
Guideline	No guideline followed
Reliability	Klimisch 1: reliable without restrictions (reliability according to publically disseminated REACH Registration dossier for boric acid)
Species / strain	Rat / Sprague-Dawley (Charles River)
Test material	Boric acid Purity: >99%

Study design

Test Animals

Sex: Female

Age/weight at study initiation: At the time of administration of the test substance, females were within the bodyweight range of 200 -250 g. The animals were about 36 weeks old at the time of initation of the study.

Number of animals per group: 10 non-pregnant/group and 10 pregnant/group for the renal clearance investigation and 6 non-pregnant/group and 6 pregnant/group for the half-life study.

Control animals: No

Administration/Exposure: Oral gavage

Exposure period: single administration, at GD 16

Concentration: 0.3, 3.0 or 30 mg boric acid/kg bw (equivalent to 0.052, 0.52 and 5.2 mg boron /kg bw, respectively) for the renal clearance study and 30 mg boric acid/kg bw/day for the half-life study (equivalent to 5.24 mg B/kg bw).

Vehicle: ultrapure water

Total volume applied: 10 ml/kg bw

Examinations

Body weight of all rats were determined daily for 7 days prior to and on the day of administration, in both clearance and half-life study. Food and water consumption were measured throughout the study.

Renal clearance study:
Two blood samples were drawn from each rat, the first after approximately 3 h after administration; the second approximately 12 h after the first.
A 12 h urine sample was collected from each rat the clearance study during the period between the first and second blood samples being taken.

Plasma half-life study:
Six blood samples were drawn from each animal during a 12 h period starting 3 h after dosing on Day 8 of the study.

Statistics:

Reanal clearance was expressed as mean ± standard deviation. Two way analysis of variance, multiple range test (Student-Newman-Keuts Method) was used as appropriate. For all statistical analyses p values < 0.05 were considered statistically significant.

Findings

RESULTS

Details on absorption: Plasma half-life evaluation

Gavage administration resulted in plasma levels of 1.82 ± 0.32 and 1.78 ± 0.32 μg B/mL among non-pregnant and pregnant rats in the first blood sample which was taken 3 h after dosing. This was followed by a monophasic decline in plasma boron concentrations in both pregnant and non-pregnant rats; the plasm levels were consistent with a compartmental model. There was no evidence of saturation kinetics. The estimated half-lives of boric acid in non-pregnant and pregnant rats were 2.93 ± 0.24 and 3.23 ± 0.28 h respectively. This difference was not statistically significant.

Details on excretion:

The urinary concentration of boron was significantly higher in pregnant compared to non-pregnant rats at the high dose, but not at the mid or low dose. The concentration of boron in the urine during the 12 h collection period in the urine of non-pregnant rats was 1.67 ± 0.62, 10.12 ± 8.16 and 66.82 ± 47.00 μg B/mL at the low, mid and high doses respectively. In pregnant rats the corresponding urine boron concentrations were 1.62 ± 0.49, 12.30 ± 5.12 and 121.45 ± 47.09 μg B/mL, respectively. The amount of boron excreted in the urine increased proportionately with increasing dose. The percentage of the administered dose recovered in the urine was significantly higher in the low dose group compared to the mid and high dose groups. No significant dose-

related differences in boric acid clearance were observed in either non-pregnant or pregnant rats.

Toxicokinetic parameters:

half-life 1st: The plasma half-life of boric acid in non-pregnant and pregnant rats given boric acid by gavage was 2.93 ± 0.24 and 3.23 ± 0.28 hours, respectively.

Conclusions APPLICANT'S SUMMARY AND CONCLUSION

Gavage administration resulted in plasma levels of 1.82 ± 0.32 and 1.78 ± 0.32 µg B/mL among non-pregnant and pregnant rats in the first blood sample which was taken 3 h after dosing. This was followed by a monophasic decline in plasma boron concentrations in both pregnant and non-pregnant rats; the plasma levels were consistent with a compartmental model. There wsa no evidence of saturation kinetics. The estimated half-lives of boric acid in non-pregnant and pregnant rats were 2.93 ± 0.24 and 3.23 ± 0.28 h respectively. This difference was not statistically significant.
The urinary concentration of boron was significantly higher in pregnant compared to non-pregnant rats at the high dose, but not at the mid or low dose. The concentration of boron in the urine during the 12 h collection period in the urine of non-pregnant rats was 1.67 ± 0.62, 10.12 ± 8.16 and 66.82 ± 47.00 µg B/mL at the low, mid and high doses respectively. In pregnant rats the corresponding urine boron concentrations were 1.62 ± 0.49, 12.30 ± 5.12 and 121.45 ± 47.09 µg B/mL, respectively.

Conclusion: The amount of boron excreted in the urine increased proportionately with increasing dose. The percentage of the administered dose recovered in the urine was significantly higher in the low dose group compared to the mid and high dose groups. No significant dose-related differences in boric acid clearance were observed in either non-pregnant or pregnant rats.

2.1.11 [Study 11] Testicular toixicity of boric acid in rats

Reference Ku, W. W., Chapin, R. E., Wine, R. N. and Gladen, B. C. (1993). Testicular toxicity of boric acid (BA): relationship of dose to lesion development and recovery in the F344 rat. Reproductive toxicology, 7(4), 305-319.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (reliability according to publically disseminated REACH Registration dossier for boric acid)

Species / strain Rat / Fisher 344

Test material Boric acid

Purity: 99.99%

Study design **Test Animals**

Sex: Male

Age/weight at study initiation: At the time of administration of the test substance, females were within the bodyweight range of 200 -220 g. The animals were about 60-70 days old at the time of initiation.

Number of animals per group: 6/dose group

Control animals: Yes

Administration/Exposure: Oral via feed

Exposure period: 9 weeks via feed, *ad libitum*

Concentration: 0, 3000, 4500, 6000 and 9000 ppm boric acid, equivalent to 0, 545, 788, 1050 and 1575 ppm boron (< 0, 0.2, 26, 38, 52, 68 mg B/kg bw/day), respectively.

Examinations

To estimate daily B intake, feed consumption was monitored gravimetrically during both weeks 6 and 7 of exposure. Feed spillage was negligible. A technical error in feeding was discovered at week 2: control and 3000 ppm BA feed were switched. Thus, during week 2 only, both groups received dose feed somewhere between 0 and 3000 ppm BA. This was verified by B analysis of dose feed. No significant changes occurred at this time point for the low dose, and the data were included for control and 3000 ppm at week 2.

At weekly intervals for 9 weeks, 6 rats from each group (control and 4 dose groups) were examined. Rats were weighed and briefly anesthetized with CO2, blood was collected by cardiac puncture, and then they were euthanatized by CO2 asphyxiation.

The following tissues were removed: left testis for histology; right testis for weight and then for subdivision for B analysis and testicular spermatid head count (TSHC); right epididymis for weight and epididymal sperm count (ESC). Serum was separated from clotted whole blood, and sera and tissues were stored at -70 °C prior to processing and analysis.

Recovery

Rats in control and 4500, 6000, and 9000 ppm BA dose groups (n = 96, above) were placed on control NIH-31 pelleted feed after 9 weeks of exposure, and recovery was assessed at 8-week intervals for up to 32 weeks post treatment. Rats were given NIH-31 pelleted feed during the post-treatment period to avoid dental malocclusion problems. At each 8-week posttreatment interval, 6 rats from each group (control and 3 dose groups) were examined as described above.

Testis histology

The left testis was fixed in 4% buffered paraformaldehyde. Cut 2- to 3-mm transverse sections of testis were rinsed with phosphate-buffered saline, dehydrated through a graded series of ethanol, and embedded in glycol methacrylate (JB-4® Plus Embedding Kit, Polysciences, Inc., Warrington, PA). Sections (2- to 3-/zm) were cut and stained with periodic acid Schiff's reagent (PAS)/hematoxylin (Harris type). Seminiferous tubules were staged. A total of 200 to 300 tubules per animal was examined for lesions and recovery unblinded. The number of spermatogonia per 100 Sertoli cells was determined in atrophic tubules by counting a minimum of 1000 Sertoli cell nuclei and using the morphologic criteria for spermatogonia according to Clermont and Bustos-Obregon.

Boron (B) analysis

Serum, urine, and testis samples were prepared for B analysis using the microwave acid digestion procedure (19). B levels were measured by inductively coupled plasma emission spectrometry (Research Triangle Institute, Research Triangle Park, NC) using appropriate matrix standard curves (correlation coefficients = 0.9999). The estimated detection limits for B in serum and testis were <4/ µg/ mL and 0.4 µg/g wet weight, respectively. B recoveries for all samples were greater than 90%.

Statistics

For most endpoints, analysis of variance was used to assess the effects of week, dose, and their interaction. For terminal body weight, analysis of covariance was used instead; prestudy body weight was added as a covariate while studying the effects of week, dose, and their interaction. For both the analyses of variance and of covariance, F tests were used to assess overall effects, and t-tests with pooled error terms were used to compare each dosed group to the control for that week. Differences were considered significant at $p < 0.05$.

Findings

Distribution: mean (± SD) testis B levels over the 9-week period were 5.6 ± 0.8, 8.8 ±0.7, 11.9 ± 1.4 and 15.1 ± 1.9 µg/g for 3000, 4500, 6000 and 9000 ppm boric acid, respectively.

Mean (± SD) serum B levels (weeks 1, 4 and 9) were 6.7 ± 1.0, 10.3 ± 0.6, 13.3 ± 0.7 and 17.3 ± 2.2 µg/g for 3000, 4500, 6000 and 9000 ppm boric acid, respectively.

Identified metabolites: no, boric acid is not metabolised.

Control testis and serum B levels were at or near the limits of detection (0.4/µg/g testis; <4 tµg/mL serum) throughout the 9-week period. Aside from the dosing error at week 2 there was a consistent relationship between BA dose and testis B levels.

Conclusions There was no B accumulation in testis over serum levels throughout the 9-week period, with mean testis/plasma ratios of less than one for all doses.

2.1.12 [Study 12] Tissue disposition of boron in male rats (boric acid)

Reference Ku, W. W., Chapin, R. E., Moseman, R. F., Brink, R. E., Pierce, K. D. and Adams, K. Y. (1991). Tissue disposition of boron in male Fischer rats. Toxicology and applied pharmacology, 111(1), 145-151.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (reliability according to publically disseminated REACH Registration dossier for boric acid)

Species / strain Rat / Fisher 344

Test material Boric acid

Purity: unknown

Study design **Test Animals**

Sex: Male

Age/weight at study initiation: At the time of administration of the test substance, females were within the bodyweight range of 200 -220 g. The animals were about 60-70 days old at the time of initiation.

Number of animals per group: 30/dose group

Control animals: Yes

Administration/Exposure: Oral via feed

Exposure period: 7 days via feed, *ad libitum*

Concentration: 0 and 9000 ppm (0 and 1575 ppm boron), equivalent to 0 and 94 mg B/kg bw/day.

Examinations

Plasma and tissue collection

Six rats from both control and treated groups were sacrificed by decapitation without anesthesia at 1, 2, 3, 4, and 7 days after the start of exposure. Blood from the severed jugular vein was collected in polypropylene tubes containing EDTA and placed on ice. Selected tissues were removed, washed of adhering blood with 0.9% saline, blotted, and placed in polypropylene tubes on ice. Plasma was separated from whole blood by centrifugation at 2500 rpm for 10 min at 4°C in a Beckman tabletop centrifuge. Plasma and tissues were stored at -20°C until they could be analyzed for tissue boron. Since animals in the control group were exposed to only trace amounts of boron in the control diet (<20 ppm), plasma and tissue boron levels in controls were only determined for Day 1 of the exposure. In the treated group, plasma and tissue boron levels were determined at 1, 2, 3, 4, and 7 days after the start of exposure.

The number of sample replicates for boron determination was dependent on the quantity of various tissues available for analysis and the sample requirements for adequate analytical detection. For plasma, liver, kidney, adipose tissue, muscle, brain (minus hypothalamus), and bone, samples of plasma and tissue from each of three animals were analyzed. Muscle was sampled from the abdominal wall and adipose tissue was from the peritoneum.

Cleaned marrow-filled tibia/fibula from both hind legs served as the sample of bone. Three samples of large intestine, seminal vesicles devoid of secretions, and epididymis were analyzed; each sample represented a pool of tissue from two animals. For large intestine, a 4-cm section was washed free of the luminal contents with 0.9% saline.

For adrenal glands, prostate gland, seminal vesicle secretions, and hypothalamus, a single sample of each tissue was analyzed, representing a pool from six animals. Approximately 0.8-1.0 mL seminal vesicle secretion from six animals was collected in a polypropylene tube on ice.

Tissue boron analysis

Plasma and tissue samples were thawed and prepared for boron analysis using the microwave acid digestion procedure as described previously (Moseman *et al.*, 1991). Briefly, 5 ml of concentrated nitric acid (Baker Analyzed) was added to 0.5-1.0 g of tissue in pressure relief vessels (PRV; CEM Corp., Indian Trail, NC) and heated by alternating on/off cycles in a 600-W microwave oven (Model MDS 81D, CEM Corp.) for 18 min. After cooling and venting, 0.75 ml of 30% hydrogen peroxide was added and samples were digested for an additional 13 min. Samples were cooled and vented and filtered through Whatman 541 filters. The PRVs and filters were rinsed with deionized water and brought to a final volume of 15 ml. Samples were analyzed on a Thermo Jarrell Ash Model 61 inductively coupled argon plasma emission spectrometer (ICAP). Serial dilutions of a boric acid reference solution (Fisher Scientific, Pittsburgh, PA) served as the working standard curve (0-10 µg/ml). The standard curves had correlation coefficients of at least 0.995. The samples were not blank corrected since blanks showed little or no boron (<0.05 ug/ml). Analysis of matrix spikes for plasma, kidney, liver, epididymis, and testis showed recoveries ranging from 95-109%.

Findings

Control boron levels in plasma and all tissues examined were below 4 µg/g (range 0.66-3.69 µg/g), with the exception of adrenal glands (7.99 µg/g). There was a rapid increase in plasma and tissue boron levels 1 day after the start of exposure to boric acid (range: 2- to 20-fold), with the exception of adipose tissue (1.3-fold). On Day 1, bone showed the greatest increase at 20-fold control levels. Hypothalamus, rest of brain, liver, and kidney showed 12- to 15-fold increases. Testis, epididymis, seminal vesicles, seminal vesicle secretions, and prostate showed 7- to 11-fold increases after the first day of exposure. Plasma, adrenal glands, large intestine, and muscle showed only a 2- to 6-fold increase.

Plasma and all soft tissues examined, including the testis, epididymis, prostate, seminal vesicles and secretions, hypothalamus, and rest of brain, appeared to reach steady state boron levels (range 12-30 µg/g) by 3-4 days, with the exception of bone and adipose tissue. Bone boron levels continued to increase up to the termination at 7 days (40-50 µg/g by Day 7). All of the soft tissues examined, including the epididymis and accessory sex organs (Table 1), as well as the testis, hypothalamus, and rest of brain (Fig. 1), appeared to show no appreciable accumulation of boron over plasma levels, with a mean tissue/plasma ratio of 1.11 + 0.05 (mean + SE) at both Days 4 and 7, excluding bone and adipose tissue.

TABLE 1

TISSUE BORON LEVELS IN MALE FISCHER RATS AT VARIOUS TIMES FOLLOWING EXPOSURE TO 9000 ppm BORIC ACID (1575 ppm BORON) IN THE DIET

Tissue	Day of treatment					
	Control	1	2	3	4	7
	µg boron/g tissue					
Plasma	1.94 ± 0.17	10.82 ± 0.50	14.40 ± 0.35	14.03 ± 0.42	16.37 ± 1.42	16.00 ± 0.71
Liver	0.66 ± 0.10	10.09 ± 0.60	11.97 ± 0.38	12.60 ± 0.50	12.33 ± 0.37	13.13 ± 0.54
Kidney	1.55 ± 0.03	19.53 ± 1.62	24.10 ± 1.60	21.80 ± 0.72	19.77 ± 1.60	19.80 ± 1.65
Adipose	1.71 ± 0.17	2.14 ± 0.08	2.33 ± 0.19	2.55 ± 0.08	3.45 ± 0.22	3.78 ± 0.13
Muscle	3.69 ± 0.54	13.73 ± 0.97	12.00 ± 0.86	11.00 ± 1.06	13.20 ± 0.99	14.23 ± 0.19
Bone	1.17 ± 0.19	23.57 ± 1.19	31.23 ± 0.78	30.90 ± 0.97	39.77 ± 0.44	47.40 ± 1.14
Large intestine[a]	3.08 ± 0.17	10.87 ± 0.72	14.43 ± 0.35	14.70 ± 0.67	16.43 ± 0.94	14.90 ± 0.7
Brain	0.76 ± 0.02	11.20 ± 0.47	11.60 ± 0.32	13.20 ± 0.55	14.27 ± 0.51	13.50 ± 0.86
Hypothalamus[b]	0.91	10.90	13.40	13.40	14.80	14.30
Testis	0.97 ± 0.10	10.41 ± 0.78	13.23 ± 0.43	14.33 ± 1.24	14.50 ± 1.71	16.00 ± 1.19
Epididymis[a]	0.81 ± 0.15	8.89 ± 1.10	9.93 ± 0.68	18.97 ± 1.32	19.40 ± 1.46	16.81 ± 3.7
Seminal vesicles[a]	1.64 ± 0.23	14.40 ± 3.87	13.53 ± 0.88	24.47 ± 9.93	27.87 ± 9.80	23.70 ± 6.56
Seminal vesicle fluid[b]	2.05	14.90	15.10	21.00	24.70	19.20
Adrenals[b]	7.99	17.40	19.20	20.20	22.30	21.90
Prostate[b]	1.20	13.90	12.90	12.20	19.10	14.80

Note. Values are means ± SE; $n = 3$ animals, unless indicated otherwise by footnote.
[a] Mean ± SE, $n = 3$ samples, each sample represents a pool of tissue from two animals.
[b] A single sample was analyzed, representing a pool from six animals.

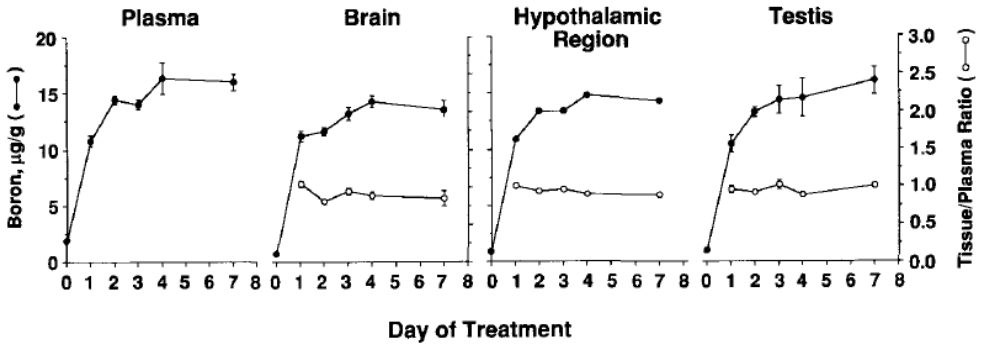


FIG. 1. Boron levels and tissue/plasma ratios in brain, hypothalamus, and testis in male Fischer rats at various times following dietary exposure to 9000 ppm boric acid (1575 ppm boron). Animals were killed at 1, 2, 3, 4, and 7 days after the start of exposure to 9000 ppm boric acid (1575 ppm boron) in the diet. Plasma and tissues were collected and analyzed for boron. For plasma, brain, and testis, values are the mean microgram boron per gram tissue ± SE for three animals. For hypothalamus, values represent a pool from six animals. Boron levels at Day 0 represent control values.

Bone accumulated the most boron (two- to threefold over plasma levels) (Fig. 2) while levels in adipose tissue were 20% of plasma levels during the 7-day exposure period.

Compared to control levels, by Days 4 and 7, bone showed the greatest increase in boron concentration (37-fold). Epididymis, liver, hypothalamus, rest of brain, testis, seminal vesicles, and prostate showed 15- to 22-fold increases over controls. Plasma, kidney, and seminal vesicle secretions showed 8- to 13-fold increases. Adrenals, muscle, and large intestine, all showing high control boron concentrations (>3 µg/g), showed only 3- to 5-fold increases, while adipose tissue showed only a 2-fold increase over control levels.

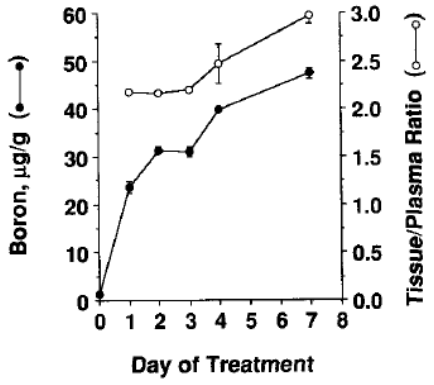


FIG. 2. Boron levels and tissue/plasma ratios in bone in male rats at various times following dietary exposure to 9000 ppm boric acid (1575 ppm boron). Animals were killed as described. Two cleaned marrow-filled tibia/fibula from each rat were collected, pooled, and analyzed for boron. Values are the mean microgram boron per gram tissue ± SE for three animals. Boron level at Day 0 represents control value.

Conclusions The tissue disposition of boron in reproductive, accessory sex organs, and other selected tissues was examined in adult male rats fed 9000 ppm boric acid (1575 ppm boron) for a period of up to 7 days. No differences in daily feed consumption between control and treated groups were noted; both groups consumed 15-16 g/rat/day.

The data showed a rapid increase in plasma and tissue boron levels 1 day after the start of exposure to boric acid, with the exception of adipose tissue. All tissues examined, except bone and adipose tissue, appeared to reach steady state boron levels by 3-4 days. Bone boron levels continued to

increase up to the termination at 7 days.

The greatest increase in boron concentration was observed in bone. All other tissues, including the testis, epididymis, accessory sex organs, hypothalamus, and rest of brain, did not show an appreciable accumulation of boron over plasma levels.

2.1.13 [Study 13] Literature review of published and proprietary data (boric acid and borate salts)

Reference	ATSDR (2010) Toxicological profile of boron.
Species	Rats, rabbits, mice
Test material	The report considered experimental animal exposure to equivalent boron doses calculated from compounds such as boric acid, boron oxide, borate salts (e.g. calcium borate) and various hydration states of sodium borate salts (anhydrous, pentahydrate, decahydrate), which occurred through various routes of exposure (i.e. inhalation, oral, dermal, intravenous and intra-tympanic).
Study design	Literature review
Findings	Absorption: oral absorption fraction in rats was found at 95%. Boron is readily absorbed through damaged skin in rabbits. Distribution: in male rats, boron is evenly distributed to liver, kidney, brain, muscle, adrenals, epididymis, testes, seminal vesicles, and blood, but not fat, following 61 mg boron/kg/day as boric acid for 28 days. In rats, boron accumulates in the bone, reaching 3-fold higher levels than in the soft tissue. Metabolism: being an inorganic element, boron is not metabolised by animals, but the parent borate is recovered in the blood, tissues and urine. Elimination and excretion: excretion primarily through renal elimination, with a renal clearance value of 163 mg/kg/ hour in rats.
Conclusion	The ADME profile of boron was described based on animal data.

2.1.14 [Study 14] Absorption of barium from the gastro-intestinal tract of rats (non-guideline)

Reference	Taylor, D. M., Bligh, P. H. and Duggan, M. H. (1962). The absorption of calcium, strontium, barium and radium from the gastrointestinal tract of the rat. Biochemical journal, 83(1), 25.
Guideline	No guideline followed
Reliability	-
Species / strain	Rat /brown-hooded August (female)
Test material	Barium chloride, strontium chloride, radium chloride, calcium chloride Purity: unknown

Study design

Animals: The animals used were aged between 14 days and 70 weeks. Animals less than 28 days old were suckled by the mother up to the start of each experiment; all other animals were maintained on M.R.C. diet no. 41B, both food and water being allowed ad libitum.
This diet contains about 0-8% by wt. of calcium.

Radioactive materials: Solutions of 45CaC12, 140BaC12 and 226RaCl2 in dilute HCI were obtained from The Radiochemical Centre, Amersham, Bucks. The specific activity of the 45Ca was from 5 to 10 mc/mg. of Ca. The 140Ba was carrier-free; 85Sr was prepared as a carrier-free solution of 55SrC12 from RbCl bombarded with deuterons in a cyclotron (Overstreet, Jacobson, Scott & Hamilton (1951)). The activity administered, as a single dose, to each animal was approx. 1 ,uC of 45Ca or 85Sr, 10,uC of 140Ba and approx. 0-01 µ. of 226Ra.

Measurement of absorption: Appropriate amounts of the pair of isotopes being studied, contained in 0-2-0-5 mL of 0-01 N-HCI, or in some experiments in cow's milk, were administered by intragastric intubation. The activity was always administered between 9.00 a.m. and 9.45 a.m., and the animals were killed approx. 7 hr. later. The entire gastrointestinal tract was carefully dissected out, and the radioactivity in the gastrointestinal tract and the remainder of the carcass determined in the manner described below. In the three younger age groups the urinary excretion
of the administered material was negligible. The older animals, however, excrete a few per cent of the administered activity in the urine during the experimental period; these animals were therefore kept in individual metabolism cages during the experiment and the urine was collected on filter paper.
'Absorption' has been calculated as the sum of the percentages of the administered material remaining in the carcass plus that in the urine at the end of the 7 hr. period, less that in the gastrointestinal tract. In some experiments the animals were deprived of food, but not water, for 18 hr. before the administration. In all other cases the animals were allowed free access to food and water up to the commencement of each experiment.

Preparation of samples and measurement of radioactivity: All samples were ashed overnight in a muffle furnace at 5500; the gut and dried urine samples were ashed in silica crucibles and the whole carcasses in large porcelain dishes. With the exception of the samples containing 85,Sr and
226Ra, the resulting ash was dissolved in 3 N-HCI and diluted to a suitable volume. The ash from the samples containing 85Sr and 226Ra was weighed and ground to a fine powder.
The radioactivity in the various samples was then determined in one of the following ways:

(a) 45Ca and85Sr: for the assay of 45Ca the total calcium was precipitated as calcium oxalate from samples of the ash solutions containing about 5 mg. of calcium. The precipitated calcium oxalate was mounted on a tared polythene planchet, dried in air, weighed and counted in an
end-window Geiger-Miiller counting assembly. The resulting counting rates were corrected for background counts, self-absorption and for the resolving time of the counter. 85Sr was determined by counting a suitable fraction,usually 5 ml., of the ash solutions in a well-type sodium iodide
crystal-scintillation counter. Since 45Ca emits only particles of 0-25 Mev this isotope does not interfere with the assay of 85Sr. Under the conditions used here the 0-51 Mev gamma rays from the 85Sr made only a negligible contribution to the observed counting rate of the 45Ca samples.

(b)85Sr andL40Ba: the decay of 140Ba gives rise to a radioactive daughter product 140La, and it is necessary to allow the 140Ba-140La mixture to build up to equilibrium before counting the samples. A period of 12 days, the half-life of 140Ba, was found to be sufficient to allow equilibrium to be re-established and all samples were stored for this period before counting. The 140Ba-140La equilibrium mixture emits a number of ,B particles of varying energy and y rays
of energies from 0-03 to 2-57 Mev. The samples containing 85Sr and the 140Ba-14'La mixture were assayed in a welltype scintillation counter which was coupled to a singlechannel pulse-height analyser. The counting rate was measured in the region of the 0-51 Mev gamma peak of85Sr and again in the region of the 1-60 Mev gamma peak of 140Ba-'40La. The counting rate in the 1-60 Mev region is a direct measure of the activity due to 140Ba-14°La. The activity due to85Sr was obtained by subtracting the counting rate due to '40Ba-'40La in the 0-5 Mev region from the total counting rate in that region. The ratio of the counting rate due to 40Ba-40La in the 0-5 and 1-60 Mev region was determined by counting a pure sample of the 140Ba-140La
equilibrium mixture with each batch of samples. The contribution to the counting rate in the 0-5 Mev region due to140Ba-140La for any sample was calculated from the counting rate of that sample in the 1-60 Mev region.

(c) 85Sr and 226Ra: For the measurement of 85Sr weighed portions of the ash were dissolved in 5 ml. of 3N-HCI and counted in the scintillation counter. The counting rate was determined in the region of the 0-5 Mev peak only. Since the quantity of 226Ra used was only 1 % of the activity of 85Sr the contribution from the 226Ra gamma rays to the counting rate in the 05 Mev region could be ignored. The 226Ra content of the finely ground ash was determined by ascintillation counting with the method of sample preparation and counting described by Turner, Mayneord & Radley (1958).
In all cases standards consisting of suitable dilutions of the solutions administered to the animals were prepared and assayed with each batch of samples. All results are expressed as a percentage of the administered activity.

Findings

Absorption:
- At 14 – 18 days of age, approx. 80% of Ba was absorbed;
- For 6 – 8 weeks of age, the absorption of Ba decreased to approx. 7%;
- For 60 – 70 weeks of age, the absorption of Ba was approx. 7.5%.

The absorption of Ba was markedly increased by food deprivation before exposure:
- At 6 – 8 weeks of age, approx. 20% of Ba was absorbed;
- At 60 – 70 weeks of age, approx. 19% of Ba was absorbed.

The administration of cow milk had no effect on the absorption of Ba.

Table: Effect of age, deprivation offood and administration of the metal in cow's milk on the absorption of calcium, strontium, barium and radium from the gastrointestinal tract of the rat

Results are expressed as percentages of the administered dose ± S.E.M. with numbers of animals used given in parentheses.

Age of animal	Fed rats				Starved rats				Milk administration to fed rats			
	Ca	Sr	Ba	Ra	Ca	Sr	Ba	Ra	Ca	Sr	Ba	Ra
14–18 days	97·5 ±0·8 (9)	95·2 ±0·4 (31)	84·6 ±2·4 (10)	78·6 ±3·1 (12)	—	—	—	—	—	—	—	—
22 days	—	74·4 ±2·4 (5)	63·0 ±4·1 (5)	—	—	—	—	—	—	—	—	—
6–8 weeks	63·0 ±2·6 (15)	24·6 ±1·0 (45)	6·8 ±0·3 (5)	11·3 ±1·5 (20)	53·7 ±2·4 (5)	24·2 ±2·4 (19)	20·0 ±2·7 (5)	17·5 ±2·6 (9)	71·5 ±4·6 (10)	31·9 ±1·0 (25)	7·2 ±1·4 (5)	12·7 ±4·1 (10)
60–70 weeks	31·6 ±2·0 (10)	11·1 ±0·8 (24)	7·5 ±1·9 (10)	3·2 ±0·7 (4)	24·8 ±1·9 (10)	16·7 ±1·8 (18)	19·9 ±6·8 (5)	8·4 ±1·3 (3)	30·2 ±3·4 (5)	9·9 ±0·9 (5)	—	—

Table: Absorption of strontium, barium and radium relative to that of calciu

Results are expressed as fractions of amount of ^{45}Ca absorbed by the fed animals in each age group.

Age group	Fed rats				Starved rats				Milk administration			
	Ca	Sr	Ba	Ra	Ca	Sr	Ba	Ra	Ca	Sr	Ba	Ra
14–18 days	1·00	0·97	0·87	0·80	—	—	—	—	—	—	—	—
6–8 weeks	1·00	0·39	0·11	0·18	0·85	0·38	0·32	0·28	1·13	0·51	0·11	0·20
60–70 weeks	1·00	0·35	0·24	0·10	0·79	0·53	0·63	0·27	0·96	0·31	—	—

Conclusions

Absorption of barium from the gastro-intestinal tract of the rat was characterised.

2.1.15 [Study 15] Literature review of publish and proprietary data (barium and barium compounds)

Reference	ATSDR (2007) Toxicological profile of barium and barium compounds. CICAD WHO (2001) Barium and barium compounds.
Species / strain	Rat, mice, rabbits, dogs
Test material	The reports considered experimental animal exposure to barium salts (e.g. barium chloride, barium barium sulphate, barium carbonate), which occurred through various routes of exposure (i.e. inhalation, oral, dermal and intravenous).
Study design	Literature review
Findings	Absorption: rapid absorption following inhalation or nasal deposition with more efficient clearance in the upper respiratory tract than in the trachea (0.41, 0.145, 0.044, and 0.043% retained ^{133}Ba in the trachea one week after administration for rats, rabbits, dogs, and monkeys, respectively). Gastrointestinal absorption was approx. 50% in dogs, compared to 30% in rats and mice. Younger rats absorbed approx. 10 times more (i.e. 63-84%) barium from the gastrointestinal tract than older rats (approx. 7%). Distribution: predominantly in the bone, with the following non-skeletal distribution 24h after ingestion in rats: heart > eye > skeletal muscle > kidney > blood > liver. Metabolism: Ba is not metabolised in the body, but it can be transported or incorporated into complexes or tissues. Elimination and excretion: faecal excretion exceeds urinary excretion in the case of rats, dogs and rabbits. A biological half-life time of 12.8 days following inhalation exposure, was estimated in dogs.
Conclusion	The ADME profile of barium was described based on animal data.

2.1.16 [Study 16] Comparative review of the kinetics of boric acid I rodents and humans

Reference	Murray, F. J. (1998). A comparative review of the pharmacokinetics of boric acid in rodents and humans. Biological trace element research, 66(1-3), 331-341.
Species / strain	Animal and human data
Test material	The review considered both human and experimental animal exposure to boric acid, which occurred through various routes of exposure (i.e. oral, dermal, intravenous).
Study design	Literature review
Findings	Absorption: rapid absorption following inhalation or nasal deposition with more efficient clearance in the upper respiratory tract than in the trachea (0.41, 0.145, 0.044, and 0.043% retained ^{133}Ba in the trachea one week after administration for rats, rabbits, dogs, and monkeys, respectively). Gastrointestinal absorption was approx. 50% in dogs, compared to 30% in rats and mice. Younger rats absorbed approx. 10 times more (i.e. 63-84%) barium from the gastrointestinal tract than older rats (approx. 7%).

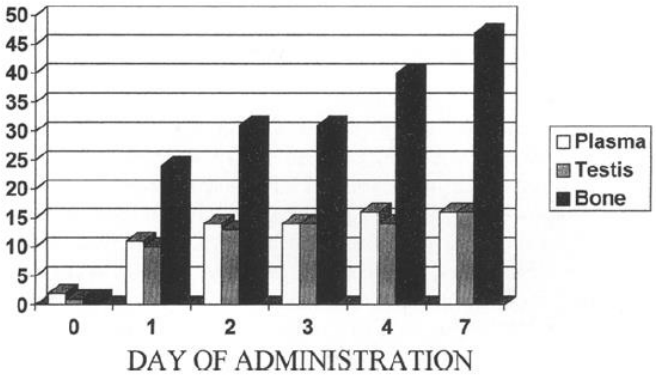


Fig. B levels (mcg B/g) in plasma and tissues of male rats given BA in the diet (adapted from Ku et al. 1991)

Distribution: predominantly in the bone, with the following non-skeletal distribution 24h after ingestion in rats: heart > eye > skeletal muscle > kidney > blood > liver.

Metabolism: Ba is not metabolised in the body, but it can be transported or incorporated into complexes or tissues.

Elimination and excretion: faecal excretion exceeds urinary excretion in the case of rats, dogs and rabbits.

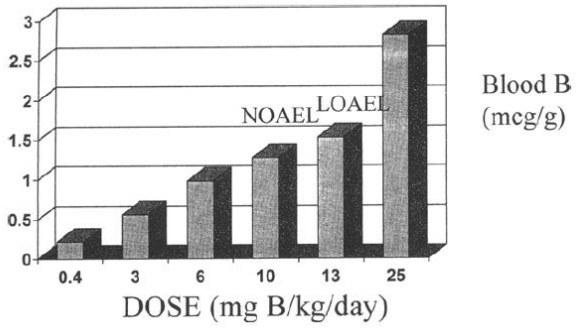


Fig. Blood B (mcg/g) in pregnant rats given various doses of BA in the diet (adapted from Price et al. 1997)

A biological half-life time of 12.8 days following inhalation exposure, was estimated in dogs.

Conclusion Comparative review of the kinetics of boric acid based on human and animal data.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

3.1.1 [Study 1] Acute oral toxicity study (barium diboron tetraoxide)

Reference Study report (1979a). Acute oral toxicity of Busan 11-M1 in rats (as summarised in the publically disseminated REACH Registration dossier of barium diboron tetraoxide)

Guideline No guideline followed (similar to OECD 401, but no information on the purity of the test sample, limited information on the animals and the testing was provided)

Reliability	Klimisch 2: reliable with restrictions (reliability according to publically disseminated REACH Registration dossier of barium diboron tetraoxide)
Species / strain	Rat / Sprague-Dawley
Test material	Busan 11-M1 (barium metaborate monohydrate) Purity: unknown
Study design	**Test Animals** Sex: Male/female Number of animals per group: 8/sex/dose group Control animals: No **Administration/Exposure: Oral via gavage** Exposure period: singal oral dose, 14 days post-exposure observation period Doses: 0.34, 0.50, 0.73, 1.07, 2.31, 5.0 g/kg Frequency of observations and weighing: Weight was measured at study initiation, day 7 and study termination. Pharmacotoxic signs were observed at hours 1, 2, 5 and daily thereafter.
Findings	**Mortality**: No animals died in the 0.34 dosage group 1 male and 6 females died in the 0.5 dosage group 3 males and 6 females died in the 0.73 dosage group 6 males and all females died in the 1.07 dosage group All animals died in the 2.31 dosage group All animals died in the 5.0 dosage group. **Clinical signs**: 0.34 dosage group: all animals appeared normal with no clinical signs 0.5 dosage group - 5.0 dosage group: Animals showed a range of symptoms including diarrhea, hypoactivity, ataxia, decrease limb tone, bradypnea, piloerection, absence of grasping reflex, hypothermic to touch, fasciculations, prostration, bradycardia, loss of pain reflex, loss of placing reflex, flaccidity. **Body weight**: 0.34 dosage group: average male body weight was 304 day 7 and 334 at termination, while average female body weight was 246 day 7 and 253 at termination 0.5 dosage group: average male body weight was 263 day 7 and 297 at termination, while average female body weight was 255 day 7 and 268 at termination 0.73 dosage group: average male body weight was 321 day 7 and 343 at termination, while average female body weight was 244 day 7 and 245 at termination 1.07 dosage group: average male body weight was 313 day 7 and 348 at termination, while all females died prior to reaching the day 7 weighing point 2.31 dosage group: All animals died prior to reaching the day 7 weighing point 5.0 dosage group: All animals died prior to reaching the day 7 weighing point. **Gross pathology**:No significant visible lesions were observed.
Conclusions	The oral LD_{50} was found to be 850 mg/kg bw in males and 530 mg/kg bw in females.

3.2 Acute toxicity - dermal route

3.2.1 [Study 1] Acute dermal toxicity study (barium diboron tetraoxide)

Reference	Study report (1979b). Acute dermal toxicity of Busan 11-M1 in rats (as summarised in the publically disseminated REACH Registration dossier of barium diboron tetraoxide)

Guideline	No guideline followed (similar to OECD 402, but no information on the purity of the test substance, limited information on the animals and the testing conditions (such as on temperature and humidity), no information on the size of test site was provided. The study was conducted on abraded skin and the animals were immobilised)
Reliability	Klimisch 2: reliable with restrictions (reliability according to publically disseminated REACH Registration dossier of barium diboron tetraoxide)
Species / strain	Rabbit / New Zealand White
Test material	Busan 11-M1 (barium metaborate monohydrate) Purity: unknown
Study design	**Test Animals** Sex: Male/female Age at study initiation: approximately 14 weeks Weight at study initiation: 2-3 kg Housing: Individually, in screen bottom cages Diet (e.g. ad libitum): continuous access to commercial laboratory feed Water (e.g. ad libitum): continuous access to water Acclimation period: 7 days Number of animals per group: 5/sex/dose group Control animals: Not specified **Administration/Exposure: dermal via occlusive coverage** Exposure period: singal dermal dose (animals were imbobilised during exposure), 14 days post-exposure observation period Doses: 2 g/kg Frequency of observations and weighing: observations were made hourly for 5 hours after treatment initiation and twice daily for the remainder of the observation period.
Findings	**Mortality**: One female died on day 2. **Clinical signs**: None observed and no signs of skin irritiation. **Body weight**: All animals gained weight during the study. **Gross pathology**: The liver of one male had white areas, possible tapeworm migration scars. No visible lesions were found in any of the other male test animals. The liver of one female was slightly pale with a nodule inguinal region (1.5 cm diameter) and another had tapeworm cysts in omentum and white area on liver, probably tapeworm migration scars. No other visible lesions were found in any of the other female animals.
Conclusions	The dermal LD_{50} was found to be > 2000 mg/kg bw.

3.3 Acute toxicity - inhalation route

3.3.1 [Study 1] Acute inhalation toxicity study (barium diboron tetraoxide)

Reference	Study report (1983). Acute inhalation toxicity of Busan 11-M1 in rats (as summarised in the publically disseminated REACH Registration dossier of barium diboron tetraoxide)

Guideline No guideline followed (similar to OECD 403, but no information on the purity of the test sample, or on the conditions of exposure was provided. The study was carried out at the maximum attainable concentration)

Reliability Klimisch 2: reliable with restrictions (reliability according to publically disseminated REACH Registration dossier of barium diboron tetraoxide)

Species / strain Rat / Sprague-Dawley

Test material Busan 11-M1 (barium metaborate monohydrate)

Purity: unknown

Study design

Test Animals

Sex: Male/female

Age at study initiation: males 45 - 53 days, females 71 - 85 days (upon arrival)
Weight at study initiation: Males mean group weight range 233.6 - 241.0 g, Females mean group 221.4 - 241.6
Housing: Individually in stainless steel wire-mesh cages
Acclimation period: at least 7 days

Number of animals per group: 5/sex/dose group

Control animals: yes

Administration/Exposure: whole-body inhalation as dust

Exposure period: 4h exposure duration (single exposure), 14 days post-exposure observation period

Doses: Nominal concentration: 14.52 mg/L and 21.70 mg/L. Mean gravimetric concentration: 2.98 mg/L and 3.54 mg/L

Particle size:
MMAD (Mass median aerodynamic diameter): Group 2: Mean = 3.4, S.D. = 0.28; Group 3: Mean 2.8, S.D = 0.14
- GSD (Geometric st. dev.): Group 2: Mean = 1.8, S.D. = 0.13; Group 3: Mean 1.9, S.D = 0.05

Frequency of observations and weighing: The animals were observed prior to exposure, at the start of exposure and at 30 minute intervals during exposure. The animals were observed twice daily for 14 days post exposure. Body weights of all animals were recorded immediately prior to exposure and on Days 2, 3, 4, 7 and 14 post exposure.

Other examinations performed: Special attention was paid to lungs and respiratory tract during necropsy. The lungs, lever, kidneys and any other organs exhibiting gross pathologic changes were removed and fixed for histopathology.

Findings

Mortality:
One Group 2 male was found dead on Day 2 and one Group 3 female was found dead on Day 1 of exposure.

Clinical signs:
During exposure, all Group 2 and 3 animals appeared languid from 30 min through 4 hours. Group 2 animals showed slight dyspnea from hour 2 through 4. Group 2 animals showed rhinorrhea from hour 1 through 4. Dust covered the fur of all Group 2 and 3 animals.
On day 1 post exposure Group 3 animals showed the following clinical signs: 3 males and 1 female appeared languid, blood crusts were observed around the nose of 1 male and 1 female and polypnea and wheezing were observed in 1 male and 1 female. All remaining Group 3 animals appeared normal from Day 2 through termination.

Body weight:
Mean body weights of the Group 1 (control) females decrease slightly on days 2 and 4 post exposure. Mean body weights of both sexes of test groups decrease markedly on Day 2 and increased steadily thereafter with the exception of a decrease in the Group 2 female mean body weight on day 14.

Gross pathology:

7 Group 1 (control) animals, 7 of the Group 2 animals and 8 of the group 3 animals had no gross pathology observations.
The lungs of one Group 1 (control) animal showed scattered pinpoint red areas. The lobes of the lung of the Group 2 animal found dead were dark red, and those of the Group 3 animal found dead failed to collapse when thoracic cavity was opened. One or both renal pelves were dilated in one Group 1 (control) female, one Group 2 female and one Group 2 male. The uterine horns of two Group 1 (control) females and one group 3 females were distended with clear fluid.

Conclusions The inhalation LD_{50} was found to be > 3.54mg/L air (analytical), i.e. the maximum attainable concentration.

3.4 Skin corrosion/irritation

Not assessed in the CLH dossier.

3.5 Serious eye damage/eye irritation

Not assessed in the CLH dossier.

3.6 Respiratory sensitisation

Not assessed in the CLH dossier.

3.7 Skin sensitisation

Not assessed in the CLH dossier.

3.8 Germ cell mutagenicity

Not assessed in the CLH dossier.

3.9 Carcinogenicity

Not assessed in the CLH dossier.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 [Study 1] 90-day oral repeated dose toxicity study in rats (barium metaborate monohydrate)

Reference Study report (1993a) 90-Day Oral Toxicity of Busan 11-M1.

Guideline EPA OPP 82-1 (90-Day Oral Toxicity) and
EPA OPP 82-7 (Neurotoxicity)
Carried out under GLP and conducted according to US EPA guideline 82-1 and 82-7 but follows OECD TG 408 with the exception of the following organ weights: epididymides, uterus, thymus, spleen.

Reliability Klimisch 1: reliable without restriction (reliability according to publically disseminated REACH Registration dossier for barium diboron tetraoxide)

Species / strain Rat, Sprague-Dawley (male/female)

Test material Busan 11-M1 (barium metaborate monohydrate)
Purity: 94.3%
Form: powder

Study design

Materials and methods

Sixty males and 60 females were allocated to the various groups using a computer randomization procedure (based on body weight; variation did not exceed ± 20% of group mean for each sex). At randomization, 15 males and 15 females were placed into each group. Ten animals/sex/group were allocated to the subchronic toxicity evaluation phase of the study, and the remaining 5/sex/group were allocated to the subchronic neurotoxicity evaluation phase of the study. Additionally, 5 rats/sex/group from the subchronic toxicity portion of the study were combined with the rats in the neurotoxicity portion of the study for the purposes of conducting the Functional Observational Battery (FOB) and Locomotor Activity (LA) assessments. fter randomization into study groups, these 10 rats/sex/group were then randomized into four study replicates to allow sufficient time for the reasonable conduct of the FOB and LA assessments.

Route of administration: oral, feed

Exposure: 91, 92, 93 or 94 consecutive days prior to necropsy

Doses / Concentrations:

0, 1000, 5000, 10000 ppm, equivalent to 0, 70, 349 and 707 mg/kg bw in males and 0, 80, 406, and 794 mg/kg bw in females, equivalent to 0, 6.3, 31.4 and 0, 63.6 mg B/kg bw in males and 0, 7.2, 36.5 and 71.4 mg B/kg bw in females, respectively.

Test diets were prepared weekly and stored at room temperature. The appropriate amount of Busan 1l-M1 was mixed with the feed. The diet preparations were analyzed for homogeneity (prior to study), 14-day stability (prior to study), and test material concentration (samples collected for weeks 0 1, 2 3, 7, and 11).

No. of animals: 10 animals/sex/dose group

The rats were housed individually during the study and were fed Purina® Certified Rodent Chow® #5002 ad libitum, except during the period of fasting prior to blood collection. Water was available ad libitum.

Clinical Observations: The rats were observed twice daily for mortality and/or moribundity, and detailed clinical observations were recorded on a daily basis. Individual body weights were recorded weekly (from one week prior to study initiation), on treatment days when FOB and LA evaluations were performed, and prior to the scheduled sacrifice. Food consumption (individual) was recorded weekly (from one week prior to study initiation). The mean amounts of Busan 11-M1 consumed (mg/kg/day) by each group and sex were calculated from the mean food consumption (g/kg/day) and the appropriate concentration of Busan 11-M1 in the food (ppm).

Statistics: Body weights/gains, food consumption, clinical pathology values, absolute/relative organ weights brain dimensions: one-way analysis of variance (ANOVA); if significant, Dunnett's test was used to compare the control and treated groups. Histopathological findings: one-tailed Kolmogorov-Smirnov test. The above tests were performed by a Digital® MicroVAX 3400 computer with appropriate programming.

Findings Survival and Clinical Observations: Two low-dose males were euthanized in extremis; one was hypoactive and unkempt displaying whole body tremors, lacrimation, constricted/ dilated pupils, soft stool, decreased urination and clear material around mouth and neck-on day of sacrifice (during week 7). The other sacrifice was due to an apparent mechanical trauma. Neither death was attributed to treatment since no deaths occurred at either the 5000 or 10000 ppm dose level. All rats survived to study termination.

There were no clinical signs observed that could be attributed to treatment.

Boody Weight and Food Consumption: The high-dose rats of both sexes displayed significant reductions in body weight compared to their respective controls throughout most of the study (females from week 5 on and males from

week 2 on) . Mid-dose females displayed a significant decrease in body weight compared to the control during weeks 8 and 12 (see Table 2, below) . Body-weight gains were significantly reduced throughout the study at the high-dose level for both sexes compared to their respective control values. Mid-dose females also displayed decreased body-weight gains, although statistical significance was reported only for the week 0-8 interval see Table 3, below) .

Food consumption on a g/rat/day basis was decreased (1-4 grams) at the high-dose level for both sexes throughout the study, although statistical significance was not always attained. Mid-dose females also displayed a slight decrease. On a g/kg/day basis, consumption was comparable among the male groups, but the high-dose females displayed reduced consumption during the first weeks of the study.

Table: Body weight (% of controls)

Week/Dose (ppm)	1000	5000	10000
FEMALES			
-1	99	99	100
0	102	97	101
1	101	95	97
2	102	96	96
3	101	95	95
5	100	92	92*
6	98	93	91*
8	98	92*	91*
11	99	92	90**
12	99	92*	90*
13	100	92	90*
MALES			
-1	101	101	101
0	99	101	101
1	101	101	93
2	100	100	90*
3	100	100	91*
4	99	99	89**
6	99	100	89**
9	100	100	89*
10	102	100	90*
13	101	98	88**

* p <0.05; ** p <0.01

Table: bodyweight gains (% of controls)

Interval/Dose (ppm)	0	1000	5000	10000
FEMALES				
0-1	23	21	19 (83%)	15** (65%)
0-2	44	44	40 (91%)	35* (80%)
0-3	62	60	56 (90%)	49** (79%)
0-5	91	88	77 (85%)	70** (77%)
0-6	101	93	87 (86%)	76** (75%)
0-8	114	105	98* (86%)	88** (77%)
0-12	140	134	121* (86%)	109** (78%)
0-13	141	137	122 (87%)	110** (78%)
MALES				
0-1	49	52	49	30** (61%)
0-2	104	105	103	74** (71%)
0-3	140	141	139	108** (77%)
0-5	198	200	198	160** (81%)
0-6	225	223	225	176** (78%)
0-13	323	325	312	238** (83%)

* p<0.05; ** p<0.01

Clinical pathology:

Both sexes of the high-dose group displayed decreased meimn red blood cell count, hemoglobin, and hematocrit values, which may be treatment-related since similar decreases were reported at 15000 ppm in the range-finding study. High-dose females also displayed concomitant decreases in MCV and MCH.

Gross-necropsy: The low-dose male that displayed hypoactivity and was euthanized had a reddened cervical lymph node and reddened lacrimal glands. The other low-dose male euthanized showed a compound fracture of the maxilla, an intramuscular hemorrhage associated with the fracture, red foamy contents in the trachea, dark red contents in the stomach and duodenum, and a white area on the liver. At terminal sacrifice, 9 out of ten high-dose males displayed small testes and 7 displayed soft testes. Enlarged lymph nodes were observed in 1 low- and mid-and 3 high-dose males and in 2 low- and high-dose females. These låtter findings and others are not considered to be treatment-related.

Organ Weights: High-dose males displayed significant decreases in absolute and relative liver and testes weights, compared to the control values. Mid-dose females and high-dose rats of both sexes displayed increased relative brain weights compared to their respective controls. High-dose males displayed a significant decrease in relative (to brain weight) kidney weights compared to the control value, and high-dose females displayed increased relative (to body weight) kidney weights.

Table: Organ weights

Organ/Group/Dose	0 ppm	1000 ppm	5000 ppm	10000 ppm
MALES - FBW♦	480	491	477	433*
Liver				
absolute♦	15.54	16.00	14.19	12.14**
relative-body♥	3.232	3.277	2.981	2.805**
relative-brain♥	767.8	772.7	656.7**	581.2**
Testes				
absolute	3.54	3.48	3.58	1.39**
relative-body	0.743	0.712	0.754	0.317**
relative-brain	174.4	167.6	165.6	66.4**
Kidneys				
absolute	3.67	3.66	3.73	3.29
relative-body	0.765	0.750	0.782	0.760
relative-brain	180.6	176.4	172.6	157.5*
Adrenals				
absolute	0.0661	0.0701	0.0747	0.0742
relative-body	0.014	0.014	0.016	0.017*
relative-brain	3.252	3.373	3.453	3.562
Brain				
absolute	2.03	2.08	2.16**	2.09
relative-body	0.427	0.426	0.455	0.485**
FEMALES - FBW♦	271	262	243*	243*
Liver				
absolute	8.94	7.93	7.54*	8.02
relative-body	3.303	3.031	3.111	3.293
relative-brain	470.6	406.7*	393.4*	411.9
Kidneys				
absolute	2.19	2.12	2.05	2.15
relative-body	0.810	0.812	0.847	0.886*
relative-brain	115.4	108.9	106.9	110.4
Brain				
absolute	1.91	1.95	1.92	1.95
relative-body	0.710	0.749	0.794*	0.805**

♦ grams; ♥ grams/100 grams; * p<0.05; ** p<0.01; ♦ FBW=final body weight

Histopahtology results:

Treatment-related changes were observed in the testes and epididymides of the high-dose males, which consisted of aspermatogenesis in the testes of 10/10 males (one mild/9 severe), and no spermatocytes were present in the tubules of the epididymides in 9 of the 10 males examined. There were no other lesions observed that could be attributed to treatment.

Discussion

No adverse effectswere observed following the administration of Busan 11-M1 to rats for 91 days at dose levels of 0 1000, 5000, and 10.000 ppin with respect to survival, clinical signs, and ocular lesions, and no apparent differences were noted between treated and control rats of either sex with respect to the locomotor activity evaluations. Additionally, no differences were displayed in brain weight or brain dimensions in the rats perfused at necropsy, and there were no treatment-related neuropathological lesions observed between the high-dose and control rats at the microscopic examination of perfused tissues. One parameter (forelimb grip strength) in the functional observational battery appeared to be affected by treatment; males at the high-dose level displayed a significant decrease (84% of the control value) in forelimb grip strength at the 3-week interval, and the mid-dose -males also displayed a decrease (11% of control value), although statistical significance was not attained. An examination of the individual -data suggests that the effect is real, based on the fact that the decrease was displayed by most of the rats in the two groups and is not due to outliers. The significance of this effect is lessened in light of the fact that it occurred in only one sex at one time point and was not accompanied by any other changes in the FOB or any microscopic lesions, but forelimbs were not examined. Additionally, at week 3, a small but genuine stimulatory effect was observed in the mid-dose females and in both sexes at the high-dose level.

With regard to the subchronic phase of the study, there was a treatment-related decrease in body weight and body-weight gain at the mid-(females) and high-dose (both sexes) levels throughout the study, and food consumption (on a

g/animal/day basis) was decreased in these same groups. At the high-dose level, decreases in RBC, hemoglobin, and hematocrit were displayed by both sexes, and mid- and high-dose males displayed decreased total protein, globulin, and cholesterol values. Males at the high-dose level displayed decreased liver (absolute and relative to lbrain/ body), testes (absolute and relative to brain/body), and kidney (relative to brain) weights. Relative, but not absolute, brain weight was increased in both sexes at the high-dose level and in raid-dose females (dose-related), and relative (to body) kidney weight was increased in the high-dose females. Macroscopically, small and/or soft testes were observed in 9 out of 10 high-dose males. Aspermatogenesis was displayed in the testes of all 10 high-dose males, and there was an absence of spermatocytes in the epididymal tubules of 9 out of 10 high-dose males. The author stated that similar microscopic changes in the testes and epididymides have been observed in studies with boron, a component of Busan 11-Ml. No microscopic changes were observed in the liver, kidney, or brain to correlate with the organ weight findings.

Conclusion Under the conditions of the study, administration of Busan 11-M1 to rats at dose levels of 0, 1000, 5000, and 10000 ppm for at least 91 days resulted in reduced body weight/gains in rats of both sexes at the high-dose level throughout the study and to some extent in females at the mid-dose level, with concomitant decreases in food consumption. Other findings include decreases in several hematology [RBC, HGB, HCT; high dose level (both sexes)) and clinical chemistry [total protein, cholesterol, globulin; mid- and high-dose males] parameters, decreased liver and testes (absolute and relative) weights and relative (to brain) kidney weight in the high-dose males, increased relative brain weight in females at the mid-dose level and in rats of both sexes at the high-dose level, increased relative (to body) kidney weight in the high-dose females, and small and/or soft testes with aspermatogenesis in males at the high dose. Additionally, there was an absence of spermatocytes in the epididymal tubules at this dose level.

The NOAEL for general toxicity was set at 1000 ppm (70 mg/kg bw for males and 80 mg/kg bw for females).

3.10.1.2 [Study 2] 90-day oral repeated dose toxicity and three-generation reproductive toxicity studies in rats and dogs (boric acid or borax, non-guideline)

Reference Weir Jr, R. J., and Fisher, R. S. (1972). Toxicologic studies on borax and boric acid. Toxicology and applied pharmacology, 23(3), 351-364 (Study 1, 2 and 3).

Weir, R. J. (1967). Two-year dietary feeding study-albino rats. Boric acid. Final Report. Hazleton Laboratories Inc., Falls Church, VA, July 8th, 1966 and Addendum to Final Report. Unpublished report to US Borax Research Corporation (Study 4).

Guideline No guideline followed for 90-day oral repeated dose toxicity studies.

No guideline specified for the reproductive toxicity study, but conforms to the standard three-generation, 2 litters per generation multi-generation studies normally used at the time.

Reliability Klimisch 2: reliable with restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Rat, Sprague-Dawley (male/female)

Beagle dogs (male/female)

Test material Boric acid or borax

Purity: unknown

Study design

Materials and methods

<u>90-day oral repeated dose toxicity study in rats and dogs (Study 1 and 2)</u>

Male and female rats er group were placed for a period of 90 days on dietary concentrations of borax or boric acid at 52.5, 175, 525, 1750 and 5250 ppm as boron equivalent added with thorough mixing to the basal diet on a w/w basis. In addition, five young male and five female beagle dogs per group were placed for a period of 90 days on dietary concentrations of borax or boric acid at 17.5, 175 and 1750 ppm as boron equivalent added to the laboratory diet.4 In both studies all animals were individually caged.

<u>Route of administration</u>: oral, feed

<u>Exposure</u>: 90 days

<u>Doses / Concentrations</u>:

- in rats: 0, 52.5, 175, 525, 1750 and 5250 ppm boron, equivalent to 0, 4.7, 15.7, 47.2, 157.5 and 472.5 mg B/kg bw/day, respectively

- in dogs: 0, 17.5, 175, and 1750 ppm boron, equivalent to 0, 0.4, 4.3 and 43.7 mg B/kg bw/day, respectively

No. of animals: 10 rats/sex/dose group and 5 dogs/sex/dose group

Body weights: Body weights and food consumption were measured at weekly intervals.

Clinical observations: Hematologic studies included packed cell volume, hemoglobin, erythrocyte count, total and differential leukocyte counts on all dogs initially, at 2 and 4 wk and at termination. Biochemical studies including blood urea nitrogen, blood sugar, serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase were performed at the same time. Urine samples were analyzed for specific gravity, pH, protein, sugar, bilirubin, acetone and sediment at similar intervals. Survivors were sacrificed after 90 days on the diet.

Necropsy evaluation: the weights of brain, thyroid, liver, spleen, kidney, adrenals and testes were recorded.The tissues preserved in buffered formalin and studied histopathologically were brain. pituitary, thyroids, lung, heart, liver, spleen, kidneys, adrenals, pancreas, small and large intestines, urinary bladder, testes, ovary (for rat only), bone and bone marrow.

Statistics: Numerical deviation from the control observations were evaluated by conventional statistical tests using $P < 0.05$ as the fiducial limit (Snedecor,1956).

Reproductive toxicity study (Study 3)

Prior to initiation of the first breeding phase, the male and female rats were maintained in individual cages and fed their respective diets for 14 wk. After the 14 wk feeding period, 1 male and 2 females were placed in each breeding cage. At 24 hr after birth, the litters were reduced to a maximum of 8 progeny to be raised. The first filial generation (F1A) was carried through weaning and discarded. The parental generation (P1) was rebred to produce their second litter (F1B). At the time of weaning, 16 females and 8 males each from the control and test groups were selected at random and designated the second parental generation (P2) for continuation of the reproduction study. These animals were bred to produce the F2A and F2B litters as before. The F2B litter became the P3 generation and were bred to produce the F3A and F3B litters.

Route of administration: oral, feed

Exposure: from the beginning of the study (14 weeks pre-mating exposure) until sacrifice of parents P1, and from weaning until sacrifice of the F1- and F2-generations

Doses / Concentrations: 0, 117, 350 and 1170 ppm boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day

No. of animals: 8 males/dose group and 16 females/dose group

Body weights: Body weight and food consumption were recorded weekly.

Necropsy evaluation: With the exception ofthe P1, P2 and P3, control and test groups, necropsies

were performed on all rats.

Statistics: Numerical deviation from the control observations were evaluated by conventional statistical tests using $P < 0.05$ as the fiducial limit (Snedecor,1956).

Two-year feeding study with boric acid in rats (Study 4)

The control group of 70 male and 70 female weanling rats (Sprague-Dawley strain) received the basal diet. Test groups of 35 male and 35 female weanling rats each received a diet containing borax or boric acid at 117, 350 and 1170 ppm as boron equivalent, for a period of 2 yr. All animals were individually housed and provided with free access to the diet and drinking water.

Route of administration: oral, feed

Exposure: 2 years

Doses / Concentrations: 0, 117, 350 and 1170 ppm boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day

No. of animals: 70/sex/dose group for controls and 35/sex/dose group for the treatnebt

Body weights: Data on body weight, food consumption and toxic signs were recorded regularly (interval not specified).

Clinical parameters: Biochemical studies and urine analyses (as described in study 1 above) were carried out on all dogs at similar intervals. Pooled urine samples from 5 rats each were analyzed at 6,18 and 24 mo. Samples of blood for hematologic studies were taken from representative rats in each group at seven intervals during the 2 yr feeding period.

Organ weights: Organ weights (organs involved were described in study 1 above) were recorded and organ weight/body weight rations were calculated.

Histopathology examination: Sections of tissues involved (as described in Stuyd 1 above) were examined for

histopathologic alteration.

Necropsy evaluation: Five rats of both sexes from each group at 6 and 12 mo, and all survivors at 2 yr were sacrificed and necropsied.

Statistics: Numerical deviation from the control observations were evaluated by conventional statistical tests using $P < 0.05$ as the fiducial limit (Snedecor, 1956).

Findings **90-day oral repeated dose toxicity study in rats and dogs (Study 1 and 2)**

Rats

Clinical observations: the physical appearance of the rats receiving either borax or boric acid at levels at and below 525 ppm boron were generally comparable to those of the controls throughout the study. Rats fed 1750 and 5250 ppm of boron as borax or boric acid had a rapid respiration, inflamed eyes, swollen paws, and desquamated skin on the paws and tails. These animals appeared excited when handled. All males had a shrunken scrotum during the last weeks of the study.

Mortality: One rat each at 52.5 and 1750 ppm of boron (in borax) died during the study. At 5250 ppm of boron, both borax and boric acid killed all rats within 3 to 6 wk.

Feed consumption: Growth and food utilization efficiency were significantly reduced for males fed borax at 1750 ppm boron content and for both males and females at 5250 ppm boron. Boric acid at 525 ppm (or less) as boron equivalent did not affect the growth, food consumption and food efficiency. At 1750 ppm boron levels, boric acid reduced growth and food consumption in both males and females.

Organ weights: Borax at 52.5 ppm as boron equivalent caused an increase in the weight of brain, spleen, kidneys and ovaries in female rats, while boric acid at the same level caused an increase in liver weight; no changes of organ weights occurred in male rats. Increase in kidney weight was observed in males fed borax at 175 ppm boron content. Rats which received either borax or boric acid at 525 ppm of boron showed organ weights comparable to those of the controls. Male rats fed boron compounds at 1750 ppm boron content had a significant decrease in body weight and the weights of liver, spleen, kidneys and testes; borax at this level also caused a reduction in brain weight, while boric acid lowered adrenal weight.

Table: Changes in body weights in male rats administered 1750 ppm boron

	Control	Borax	Boric acid
Body	477 + 31	215 + 90[b]	268 ± 44[b]
Brain	2.13 ± 0.17	1.86 ± 0.11[b]	1.97 ± 0.15
Thyroids	0.019 ± 0.004	0.015 ± 0.002	0.016 ± 0.004
Liver	16.85 ± 1.35	7.04 ± 1.42[b]	7.41 ± 1.61[b]
Spleen	0.78 ± 0.12	0.34 ± 0.09[b]	0.40 ± 0.11[b]
Kidneys	3.08 ± 0.34	1.92 ± 0.32[b]	1.89 ± 0.33[b]
Adrenals	0.046 ± 0.006	0.039 ± 0.008	0.037 ± 0.009[b]
Testes	3.50 ± 0.26	0.79 ± 0.17[b]	0.83 ± 0.11[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

Female rats which received the same dose levels of either borax or boric acid had decreases in body weight and weights of liver, spleen and ovaries; in addition, boric acid caused a fall in adrenal weight.

Table: Changes in body weights in female rats administered 1750 ppm boron

	Control	Borax	Boric acid
Body	247 ± 21	222 ± 28[b]	216 ± 28[b]
Brain	1.91 ± 0.09	1.91 ± 0.13	1.91 ± 0.17
Thyroids	0.015 ± 0.003	0.015 ± 0.003	0.018 ± 0.006
Liver	7.90 ± 1.20	6.40 ± 1.46[b]	6.38 ± 1.16[b]
Spleen	0.52 ± 0.12	0.39 ± 0.11[b]	0.38 ± 0.06[b]
Kidneys	1.88 ± 0.15	1.75 ± 0.28	1.73 ± 0.19
Adrenals	0.05 ± 0.01	0.040 ± 0.009[b]	0.047 ± 0.007
Ovaries	0.124 ± 0.02	0.071 ± 0.025[b]	0.090 ± 0.030[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

An increase in brain/body weight ratio occurred in female rats fed borax at 52.5 ppm, while boric acid at the same

dose level was accompanied by a decrease in brain/body weight ratio in male rats. Borax caused an increase in kidney/body weight ratio at 525 ppm.Both boron compounds, when fed to rats at 1750 ppm, caused increases in brain, thyroids and adrenal/body weight ratios in the males.

Table: Changes in body weight: body weight ratios (%) for male rats administered 1750 ppm boron

	Control	Borax	Boric acid
Brain	0.447 ± 0.003	0.81 ± 0.20[c]	0.75 ± 0.11[c]
Thyroids	0.814 ± 0.002	0.007 ± 0.002[c]	0.006 ± 0.002[c]
Liver	3.54 ± 0.27	2.96 ± 0.23[b]	2.77 + 0.34[b]
Spleen	0.17 ± 0.02	0.14 ± 0.02	0.15 ± 0.04
Kidneys	0.65 ± 0.08	0.81 ± 0.08[c]	0.71 ± 0.09
Adrenals	0.010 ± 0.001	0.015 ± 0.002[c]	0.014 ± 0.003[c]
Testes	0.73 ± 0.06	0.34 ± 0.04[b]	0.32 ± 0.07[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.
[c] Significantly higher than controls, $p < 0.05$.

In addition to these findings, borax also increased kidney/body weight ratio. There was an increase in brain/body weight ratios in female rats receiving either borax or boric acid at 1750 ppm boron content.

Table: Changes in organ weight: body weight ratios (%) for female rats administered 1750 ppm boron

	Control	Borax	Boric acid
Brain	0.78 ± 0.07	0.90 ± 0.12[c]	0.90 ± 0.14[c]
Thyroids	0.006 ± 0.001	0.007 ± 0.001	0.008 ± 0.003[c]
Liver	3.19 ± 0.21	3.06 ± 0.26	2.95 ± 0.22[b]
Spleen	0.20 ± 0.03	0.18 ± 0.03	0.18 ± 0.04
Kidneys	0.75 ± 0.05	0.79 ± 0.06	0.81 ± 0.05
Adrenals	0.022 ± 0.002	0.019 ± 0.005	0.022 ± 0.002
Ovaries	0.05 ± 0.01	0.034 ± 0.010[b]	0.042 ± 0.013

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.
[c] Significantly higher than control, $p < 0.05$.

Furthermore, borax decreased ovaries/body weight ratio: boric acid increased thyroids and decreased liver/body weight ratios. There were no changes of organ/brain weight ratios in the rats fed either borax or boric acid at 52.5, 175 and 525 ppm as boron equivalent. Both borax and boric acid at 1750 ppm boron content caused decreases in liver, spleen, kidneys and testes/brain weight ratios in male rats.

Table: Organ: brain weight ratios (%) for male rats administered 1750 ppm boron

	Control	Borax	Boric acid
Thyroids	0.88 ± 0.22	0.81 ± 0.16	0.79 ± 0.21
Liver	795 ± 68	378 ± 72[b]	373 ± 58[b]
Spleen	37.0 ± 5.0	18.0 ± 4.0[b]	20.0 ± 5.0[b]
Kidneys	146 ± 20	103 ± 16[b]	95.2 ± 11.1[b]
Adrenals	2.2 ± 0.3	2.1 ± 0.4	1.9 ± 0.4
Testes	165 ± 14	42.4 ± 7.7[b]	42.4 ± 5.8[b]

[a] All values are expressed as mean ± SD for 9 rats. All lower levels are comparable to controls.
[b] Significantly lower than control, $p < 0.05$.

Female rats receiving boron compounds at the Furthermore, borax at 1750 ppm boron content also caused a decrease in adrenals/brain weight ratio in female rats.

Necropsy examination: Necropsies performed on the animals that died (one each from 52.5 and 1750 ppm boron levels of borax and all rats at 5250 ppm boron level of borax and boric acid) showed congestion of liver and kidneys, bright red lungs and in several animals a swollen appearance of the brain, small gonads and a thickened pancreas.

Microscopic examination of the tissues revealed complete atrophy of testes in all males fed either borax or boric acid at 1750 ppm as boron equivalent, partial atrophy in 4 males at 525 ppm of borax and in 1 at 525 ppm of boric acid. Spermatogenic arrest was found in 1 male at 525 ppm of borax. The adrenals of the majority of the males and several females at 1750 ppm boron equivalent of borax revealed a slight to moderate increase in lipid content and the size of the cells in the zona reticularis; the adrenals of 4 males at 1750 ppm boron content of boric acid had similar changes but to a lesser degree.

Dogs

Clinical observations: dogs, with one exception, fed both borax and boric acid at 17.5, 175 and

1750 ppm as boron equivalent were essentially normal in appearance, behavior,

elimination, body weights and food consumption.

Mortality: One male dog at 1750 ppm level of boron as borax died of diarrhea on day 68 of the study and showed congested kidneys and severe congestion of the mucosa of small and large intestines. Clinical parameters: Hematologic, biochemical and urine values were within normal limits except for 2 male and 3 female dogs in the high borax level group (1750 ppm boron content). These animals had decreased packed cell volume and hemoglobin values during the study.

Organ weights: The spleen/body weight ratio in male dogs at 17.5 ppm level of boron as borax was

significantly lower than that of the controls. At 175 ppm boron content, as boric acid, a decrease in testes/body weight ratio was observed. Both borax and boric acid caused significant decreases in thyroids and testes/body weight ratios in dogs at 1750 ppm boron content.

Table: Mean body weights, organ weights and organ:body weight ratios (%) of male and female dogs administered 1750 ppm boron

	Control		Borax		Boric acid	
	Weight	Ratio	Weight	Ratio	Weight	Ratio
Male						
Body weight	8.5 ± 1.9		9.3 ± 0.8		8.6 ± 1.0	
Thyroids	0.77 ± 0.14	0.009 ± 0.001	0.59 ± 0.13	0.006 ± 0.001[b]	0.48 ± 0.18	0.006 ± 0.002[b]
Testes	1.72 ± 3.3	0.20 ± 0.03	9.6 ± 3.4	0.10 ± 0.03[b]	10.5 ± 1.5	0.12 ± 0.02[b]
Female						
Body weight	6.2 ± 2.0		7.7 ± 1.2		9.0 ± 2.3	
Brain	68.7 ± 7.1	1.1 ± 0.4	80.3 ± 3.1[c]	1.10 ± 0.15	72.3 ± 2.7	0.85 ± 0.23
Liver	190.0 ± 47.0	2.8 ± 0.5	257 ± 47	3.3 ± 0.5	345 ± 49	4.1 ± 1.2[c]

[a] All numbers are expressed as mean ± SD for four male and five female dogs. All lower levels are comparable to controls.
[b] Significantly lower than control at $p < 0.05$
[c] Significantly higher than control at $p < 0.05$.

Other organs including spleen, liver, kidneys and adrenals were found to be within normal limits. Neither borax nor boric acid at 17.5 and 175 ppm boron content levels produced any changes in organ weights and organ/body weight ratios in female dogs. Increases in brain/body weight ratio and liver/body weight ratio occurred in dogs fed 1750 ppm boron content levels of borax and boric acid, respectively.

Histopathology examination: No histologic alterations were seen in dogs fed 175 ppm (or less) of boron in boric acid. Both borax and boric acid at the 1750 ppm boron level produced severe testicular atrophy in all male dogs. Degeneration of the spermatogenic epithelium was generally complete. Red blood cell destruction, as indicated by the presence of hemosiderin in the reticular cells of the liver and spleen and the proximal tubules of the kidney, was somewhat greater in the animals that received borax than in those that received boric acid. The thyroid gland of the borax treated males presented a slightly greater proportion of solid epithelial nests and minute follicles than was found in the control animals, In the adrenal gland, the zona reticularis was consistently increased in width in borax fed dogs and only in boric acid treated female dogs. The high level of boric acid (1750 ppm boron content) also increased the width of the zona glomerulosa in the adrenals of the female dogs. The zona fasciculata was, in general, somewhat decreased in width. The thyroids of the two females were infiltrated by lymphoid tissue, and one was rather markedly atrophied.

Reproductive toxicity study (Study 3)

There were no adverse effects on the reproduction of rats receiving a diet containing either borax or boric acid at 117 and 350 ppm as boron equivalent. Litter size, weights of progeny and appearance were normal compared with those of the controls. The overall fertility indices for the two test compounds at levels of 177 and 350 ppm boron were significantly higher than those of the controls. Live birth indices were within normal limits in the test groups.

Table: Fertility indices for F1, F2 and F3 filial generations of rats (5.9 and 17.5 mg B/kg bw/day administered as boric acid or borax)

Index	Control	5.9 mg B/kg bw/day	17.5 mg B/kg bw/day	Control	5.9 mg B/kg bw/day	17.5 mg B/kg bw/day
Borax						
Fertility index [a]	P1-F1A			P1-F1B		
	62.5	68.8	75	60	62.5	75
	P2-F2A			P2-F2B		
	81.3	81.3	100	80	75	93.8
	P3-F3A			P3-F3B		
	68.8	87.5	100[b]	68.8	87.5	100[b]
	Boric acid					
	P1-F1A			P1-F1B		
	62.5	87.5	81.3	60	87.5	75
	P2-F2A			P2-F2B		
	81.3	93.8	93.8	80	93.8	93.8
	P3-F3A			P3-F3B		
	68.8	100[b]	87.5	68.8	93.8	93.8

[a] Fertility index: number of pregnancies/number of matings x 100.

[b] Significantly higher than controls.

Histopathological examination: No gross abnormalities were observed in the organs examined from either parents or weanlings. Evidence was also found of decreased ovulation in the majority of the ovaries examined from the same level females sacrificed following the reproduction study (data not shown).

Mating: The high level test groups fed both borax and boric acid at 1170 ppm as boron equivalent were found to be sterile. An attempt to obtain litters by mating the treated females with the males fed only the basal diet was not successful. Microscopic examination revealed the lack of viable sperm in the atrophied testes of all males at the 1170 ppm boron equivalent level of both borax and boric acid.

For all filial generations (i.e. F1, F2 and F3), for both low- and mid-dose groups, the litter size, weights of progeny and appearance were not statistically significantly different from controls (data not shown). No other information on maternal toxicity is reported.
At 58.5 mg/kg bw/day there were no offspring produced from P1 animals.

Table: Live birth indices for F1, F2 and F3 filial generations of rats (5.9 and 17.5 mg B/kg bw/day administered as boric acid or borax)

Index	Control	5.9 mg B/kg bw/day	17.5 mg B/kg bw/day	Control	5.9 mg B/kg bw/day	17.5 mg B/kg bw/day
Borax						
Live birth index[a]	P1-F1A			P1-F1B		
	98.4	98.4	100	99.1	99.2	99.4
	P2-F2A			P2-F2B		
	97.8	99.4	96.9	98.6	92.4	98.8
	P3-F3A			P3-F3B		

	100	100	99.4	100	100	100
	Boric acid					
	P1-F1A			P1-F1B		
	98.4	96	97.2	99.1	99.4	100
	P2-F2A			P2-F2B		
	97.8	100	99.4	98.6	99.4	97.9
	P3-F3A			P3-F3B		
	100	99.5	97.9	100	99	98.8

[a] Live birth index = number of pups born alive/number of born pups x 100.

Effects on or via lactation

Significantly higher ($p<0.05$) lactation indices were observed at 5.9 and 17.5 mg B/kg bw/day, for both boric acid and borax treatments, and at 17.5 mg B/kg bw/day, the P3-F3A generation administered borax showed a significantly ($p<0.05$) lower lactation index than controls.

Table: Lactation indices for F1, F2 and F3 filial generations of rats (5.9 and 17.5 mg B/kg bw/day administered as boric acid or borax)

Index	**Control**	**5.9 mg B/kg bw/day**	**17.5 mg B/kg bw/day**	**Control**	**5.9 mg B/kg bw/day**	**17.5 mg B/kg bw/day**
Borax						
Lactation index[a]	P1-F1A			P1-F1B		
	56.3	63.6	82.3[b]	58.8	60	74.2
	P2-F2A			P2-F2B		
	48.3	79.8b	82.7[b]	92.1	93.2	95.5
	P3-F3A			P3-F3B		
	91.5	81.1	79.1[c]	89.7	91.8	95.9
	Boric acid					
	P1-F1A			P1-F1B		
	56.3	96.2	70.3[b]	58.8	85.6b	80[b]
	P2-F2A			P2-F2B		
	48.3	79.2[b]	83.1[b]	92.1	81	98
	P3-F3A			P3-F3B		
	91.5	82.5	86.5	89.7	86.7	87.9

[a] Lactation index = number of weaned pups/number left to nurse x 100.
[b] Significantly higher than controls.
[c] Significantly lower than controls.

Two-year feeding study in rats (Study 4)

Clinical observations: The appearance and behavior of the rats fed both borax and boric acid at 117 and 350 ppm as boron equivalent in the diets were generally comparable with those of the controls. The following gross signs were observed among the rats at the highest level (1170 ppm boron content): coarse hair coats, scaly tails, a hunched position, swelling and desquamation of the pads of the paws, abnormally long toenails, shrunken appearance of the scrotum of the males, inflamed eyelids and bloody discharge of the eyes. Onset of these signs was at the beginning of the second month. They became more frequent and pronounced by the end of the first year, but remained relatively unchanged during the second year.

Feed consumption: Both borax and boric acid at 1170 ppm as boron equivalent lowered food consumption during the first 13 wk and suppressed growth in rats throughout the 2 yr study.

Clinical parameters: Low packed cell volume and hemoglobin values found at many intervals during the study are considered to be significant in male and female rats fed borax at 1170 ppm as boron equivalent, and in female rats which received the same level of boric acid. Biochemical values and urine analyses were found to be within normal limits in rats which received different levels of both boron compounds.

Organ weights: The testes weights and testes/body weight ratios were significantly lower, whereas the brain and thyroid/body weight ratios were significantly higher than those of the controls (data not shown).

Histopathology examination: There were no histologic alterations in the organs of rats fed either borax or boric acid at 117 and 350 ppm levels as boron equivalent for 2 yr. Atrophic testes were found in all males receiving 1170 ppm boron in both borax and boric acid at 6, 12 and 24 mo. Microscopic examination revealed atrophied seminiferous epithelium and decreased tubular size in the testes.

Table: Testes atrophy was observed at 24 months:

Dose level (mg B/kg bw/day)	**0**	**5.9**	**17.5**	**58.5**
No. of animals	3/10	1/10	4/10	10/10

Conclusion Rats exposed to the high dose of 336 mg/kg bw boric acid (corresponding to a level of 58.5 mg B/kg bw) were sterile. Microscopic examination of the atrophied testes of all males in this group showed no viable sperm. The authors also reported evidence of decreased ovulation in about half of the ovaries examined from the females exposed to 58.5 mg B/kg bw and only 1/16 matings produced a litter from these high dose females when mated with control male animals. There were no adverse effects on reproduction reported at exposures of 34 and 100 mg/kg bw boric acid (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 examined from either parents or weanlings from these dose groups.

3.10.1.3 [Study 3] Nine-week oral repeated dose toxicity study in rats (boric acid, non-guideline)

Reference Ku, W. W., Chapin, R. E., Wine, R. N. and Gladen, B. C. (1993). Testicular toxicity of boric acid (BA): relationship of dose to lesion development and recovery in the F344 rat. Reproductive toxicology, 7(4), 305-319.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Rat, Fischer 344 (male)

Test material Boric acid

Purity: 99.99%

Study design

Materials and methods

Adult male Fischer 344 rats (60 to 70 days old, 200 to 220 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC) and acclimated for 10 days to the NIEHS animal facility. Animals were housed three per polycarbonate cage with 12:12 h light/dark cycles, 50% -+ 10% humidity, and an ambient temperature of 20 + 1 °C.

To estimate daily B intake, feed consumption was monitored gravimetrically during both weeks 6 and 7 of exposure. Feed spillage was negligible. A technical error in feeding was discovered at week 2: control and 3000 ppm BA feed were switched. Thus, during week 2 only, both groups received dose feed somewhere between 0 and 3000 ppm BA. This was verified by B analysis of dose feed. No significant changes occurred at this time point for the low dose, and the data were included for control and 3000 ppm at week 2.

Route of administration: oral, feed

Exposure: 9 weeks

Number of animals per group: 6/dose group

Doses / Concentrations: 0, 3000, 4500, 6000 and 9000 ppm boric acid, equivalent to 0, 545, 788, 1050 and 1575 ppm boron (< 0, 0.2, 26, 38, 52, 68 mg B/kg bw/day), respectively.

Body weights: Body weights and food consumption were measured at weekly intervals.

Histology examination: At weekly intervals for 9 weeks, 6 rats from each group (control and 4 dose groups) were

examined. Rats were weighed and briefly anesthetized with CO2, blood was collected by cardiac puncture, and then they were euthanatized by CO2 asphyxiation. The following tissues were removed: left testis for histology; right testis for weight and then for subdivision for B analysis and testicular spermatid head count (TSHC); right epididymis for weight and epididymal sperm count (ESC). Serum was separated from clotted whole blood, and sera and tissues were stored at -70 °C prior to processing and analysis.

Recovery: Rats in control and 4500, 6000, and 9000 ppm BA dose groups (n = 96, above) were placed on control NIH-31 pelleted feed after 9 weeks of exposure, and recovery was assessed at 8-week intervals for up to 32 weeks post treatment. Rats were given NIH-31 pelleted feed during the post-treatment period to avoid dental malocclusion problems. At each 8-week posttreatment interval, 6 rats from each group (control and 3 dose groups) were examined as described above.

Testis histology: The left testis was fixed in 4% buffered paraformaldehyde. Cut 2- to 3-mm transverse sections of testis were rinsed with phosphate-buffered saline, dehydrated through a graded series of ethanol, and embedded in glycol methacrylate (JB-4® Plus Embedding Kit, Polysciences, Inc., Warrington, PA). Sections (2- to 3-/zm) were cut and stained with periodic acid Schiff's reagent (PAS)/hematoxylin (Harris type). Seminiferous tubules were staged. A total of 200 to 300 tubules per animal was examined for lesions and recovery unblinded. The number of spermatogonia per 100 Sertoli cells was determined in atrophic tubules by counting a minimum of 1000 Sertoli cell nuclei and using the morphologic criteria for spermatogonia according to Clermont and Bustos-Obregon.

Boron (B) analysis: Serum, urine, and testis samples were prepared for B analysis using the microwave acid digestion procedure. B levels were measured by inductively coupled plasma emission spectrometry (Research Triangle Institute, Research Triangle Park, NC) using appropriate matrix standard curves (correlation coefficients = 0.9999). The estimated detection limits for B in serum and testis were <4/ µg/ mL and 0.4 µg/g wet weight, respectively. B recoveries for all samples were greater than 90%.

Serum FSH/LH: Serum LH and FSH were measured by doubleantibody radioimmunoassay (RIA). Purified LH and

FSH standards and antisera were supplied by the Rat Pituitary Hormone Distribution Program, NIAMD. Rat LH and FSH were iodinated using the method of Greenwood and colleagues . The intra- and interassay coefficients of variation for LH were 4.8% and 14.5%, respectively. For FSH, the values were 8.2% and 5.5%, respectively.

Statistics: For most endpoints, analysis of variance was used to assess the effects of week, dose, and their interaction. For terminal body weight, analysis of covariance was used instead; prestudy body weight was added as a covariate while studying the effects of week, dose, and their interaction. For both the analyses of variance and of covariance, F tests were used to assess overall effects, and t-tests with pooled error terms were used to compare each dosed group to the control for that week. Differences were considered significant at $p < 0.05$.

Findings

Feed consumption and estimated daily boron (B) intake

An immediate and lasting decrease in body weight gain for the 9000 ppm dose group was observed (data not shown), and by week 9 this group weighed approximately 16% less than controls (controls = 323 ± 6 [SD] g; 9000 ppm = 270 ± 5 g). No changes in body weight gain were observed for the other dose groups. Mean (± SD) feed consumptions estimated during weeks 6 and 7 were 49.3 - 1.0, 50.2 ± 0.3, 49.2 ± 2.6, 49.2 -+ 1.6, and 44.0 ± 2.1

g/kg body weight/day for 0 (Control), 3000, 4500, 6000, and 9000 ppm BA dose groups, respectively, with the 9000 ppm dose group consuming approximately 11% less feed than controls. Based on feed consumption and body weights, the estimated daily intakes of B were <0.2, 26, 38, 52, and 68 mg B/kg body weight/day for control and the respective dose groups.

Testis lesion development: Testes from control rats showed stages with the normal cell associations consistent with intact spermatogenesis. Rats fed various BA doses during the 9-week period developed testicular lesions ranging from mildly inhibited spermiation to varying degrees of degenerative changes leading to atrophy. Atrophy was defined as the complete absence of postspermatogonial germ cells. The inhibited spermiation was characterized

by the aberrant retention of step 19 spermatids in stage 1X/X tubules, which, in more severe cases, involved stage XI/XII tubules. Varying degrees of degenerative changes, ranging from epithelial disorganization and germ cell loss

to eventual atrophy were also observed. Histologically intact spermatogonia were present in atrophic tubules at a

frequency of 2.6 to 2.9 spermatogonia per 100 Sertoli cell nuclei. The Leydig cells appeared histologically normal at the light microscopic level.

To assess testis lesion development over time for each dose group, lesions were assigned a numeric score between 0 and 6, depending on both the lesion characteristics and percentage of tubules affected:

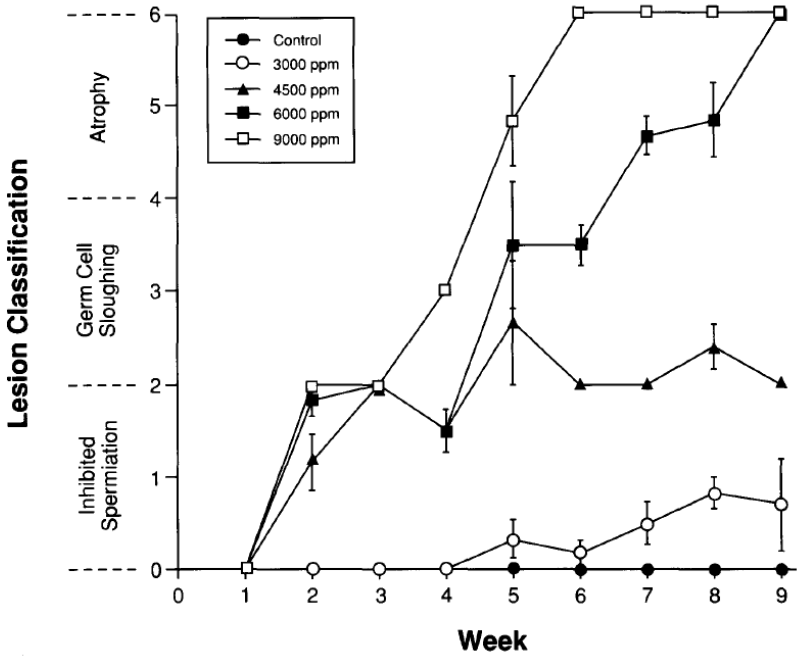
- rats fed 3000 ppm BA showed only mildly inhibited spermiation (Grade 1) by week 5, which continued variably to week 9. Rats fed 4500 ppm BA showed severe and widespread inhibition of spermiation (Grade 2) by week 2 that was maintained out to week 9. At week 9, germ cell exfoliation (<5% of tubules) was also observed in this dose group, but overall, the lesion was relatively mild and limited.
- rats fed 6000 ppm and 9000 ppm BA showed initially severe inhibition of spermiation by week 2, followed by

progression to Grade 6 atrophy. The progression to atrophy was dose- and time-dependent, with 6000 ppm reaching atrophy by week 9 and 9000 ppm by week 6. This difference was due to the sustained presence of postspermatogonial germ cells in tubules of the 6000 ppm dose group between weeks 6 and 9.

Table: histologic grading scheme for testicular lesions

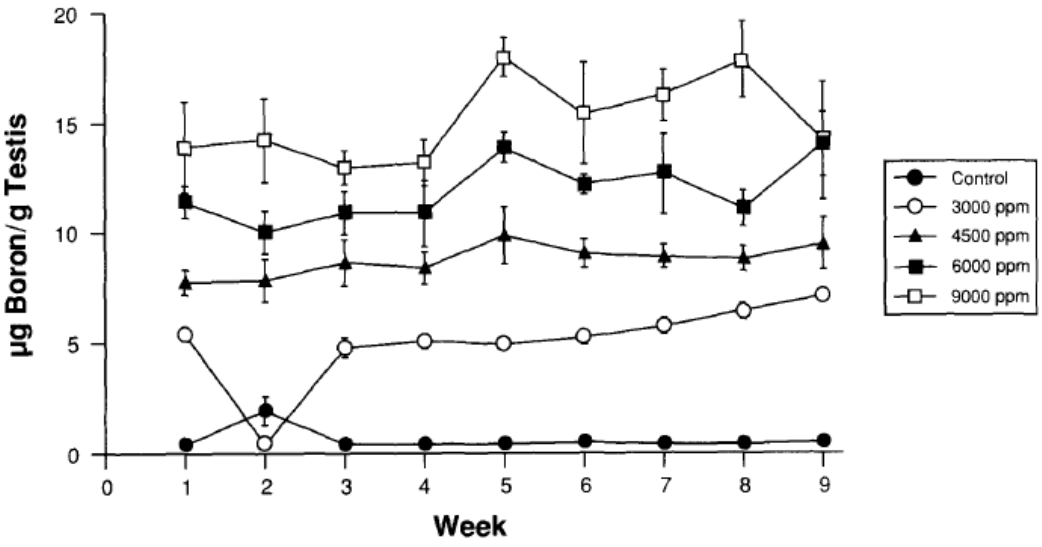
Lesion grade	0	1	2	3	4	5	6
% Tubules at stages below the inhibited spermiation	<5	25–50	>50	>50			
Stages with retained spermatids		IX	X,XI	XI,XII			
% Tubules with germ cell exfoliation			<5	10–25	>75	<25	<5
% Atrophic tubules					<25	>75	>95

Figure: Testis lesion development in BA dose groups over time.



Testis/Serum B concentration during lesion development: Control testis and serum B levels were at or near the limits of detection (0.4/µg/g testis; <4 µg/mL serum) throughout the 9-week period. Aside from the dosing error at week 2 there was a consistent relationship between BA dose and testis B levels. Mean (±SD) testis B levels over the 9-week period were 5.6 -+ 0.8 (minus week 2 data), 8.8 ± 0.7, 11.9 -+ 1.4, and 15.1 ± 1.9/~g/g for 3000, 4500, 6000, and 9000 ppm BA, respectively. Mean (± SD) serum B levels (weeks 1, 4, and 9) were 6.7 ± 1.0, 10.3 ± 0.6, 13.3 ± 0.7, and 17.3 + 2.2 µg/mL for these doses (data not shown). There was no B accumulation in testis over serum levels throughout the 9-week period, with mean testis/plasma ratios of less than one for all doses.

Figure: Levels of testis boron over time



Testis B concentrations in BA dose groups over time. Values are the mean µg B/g testis ± SE for 6 rats/group/week. Control testis B levels over the 9-week period were at or near the limits of detection (0.4 µg/g). The significance levels for comparing each week's dose to that week's control were all $P < 0.05$. Week 2 data were not analyzed as a result of dose feed error.

The relationship between estimated B intake, testis B levels, and the characteristics of testis lesions over the 9-week period is summarized in Table 2. Testis B levels of 5 to 6 /xg/g were associated with mildly inhibited spermiation appearing by week 5, which variably continued up to week 9. Levels of 8 to 9/µg/g were associated with severely inhibited spermiation by week 2, which continued up to week 9. Levels of 11 to 12 /xg/g were associated with severely inhibited spermiation (week 2) followed by progression to atrophy by week 9, and 15 to 16 µg/g with inhibited spermiation (week 2) followed by atrophy by week 6.

Table: Testis B levels and testicular lesions

BA dose		Testis B[b]	Lesion characteristics
ppm	approximate mg B/kg/day[a]	(µg/g)	(week first detected)
3000	26	5.6 ± 0.8	Mild IS[c] (5)
4500	38	8.8 ± 0.7	Severe IS (2)
6000	52	11.9 ± 1.4	IS (2)/atrophy (9)
9000	68	15.1 ± 1.9	IS (2)/atrophy (6)

[a] Calculations based on average feed consumption and body weight data during weeks 6 and 7.
[b] Mean ± SD for each dose over the 9-week exposure period.
[c] IS = inhibited spermiation.

Comparison of reproductive toxicity indices during lesion development: Mildy inhibited spermiation (3000 ppm BA) was detected histologically, but with no consistent changes in any parameter. Severe and widespread inhibition of spermiation (4500 ppm BA) was reflected by variable increases in TSHC (24% to 62%, detected initially and most significantly at week 2), with no significant changes in testis weight. This was followed by decreases in epididymis weight (10% to 29%) and profound decreases in ESC (72% to 97%) during weeks 4 to 9. Severely inhibited spermiation and progression to atrophy (6000 and 9000 ppm BA) were represented initially by increased TSHC (31% to 51%), reflecting the inhibited spermiation at week 2, followed by progressive and profound decreases in testis weight (12% to 68%), TSHC (16% to 99%), epididymis weight (12% to 57%), and ESC (78% to 99%), reflecting the progression to atrophy during weeks 3 to 9.

Serum FSH/LH: Serum FSH was significantly increased (1.4 to 1.9-fold) in all BA dose groups. Serum LH showed apparent increases (1.8 to 3.3-fold) in all BA dose groups evaluated during post-treatment, but was significant only for the 9000 ppm dose group.

Table: Serum FSH and LH concentrations at 24 weeks post-treatment

BA dose group (ppm)	(ng/mL[a]) FSH	(pg/mL[a]) LH
0 (Control)[b]	10.8 ± 1.0	723 ± 125
4500	15.5 ± 1.5*	1330 ± 404
6000	20.7 ± 1.5*	1492 ± 222
9000	20.8 ± 1.0*	2378 ± 722*

[a] Mean ± SEM, $N = 6$
[b] Controls are same age animals given control feed during the 9-week BA exposure period.
* Significant difference from control, $P < 0.05$.

Conclusion In conclusion, the observed effects on fertility were considered treatment-related. These findings showed that (i) inhibited spermiation did not appear exclusively at high doses and it was expressed at different testicular levels of B than testicular atrophy, (ii) the progression to testicular atrophy was dose-dependent and (iii) a relationship between dietary and testis levels of boron could be established.

3.10.1.4 [Study 4] 28-day oral repeated dose toxicity study in male rats (boric acid, non-guideline)

Reference Treinen, K. A., and Chapin, R. E. (1991). Development of testicular lesions in F344 rats after treatment with boric acid. Toxicology and applied pharmacology, 107(2), 325-335.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Rat, Fischer 344 (male)

Test material Boric acid
Purity: unknown

Study design

Materials and methods

Mature (120 day) Fischer 344 rats were obtained from the Raleigh, North Carolina facility of Charles River Breeding Laboratories and allowed 2 weeks to acclimate to the NIEHS animal facility. Animals were housed three/polycarbonate cage with 12: 12-hr light/ dark cycles, 50 f 10% humidity, and ambient temperature of 20 ± 1 °C. The rats were computer randomized by body weight into control and treated groups (total n = 30 and 36, respectively). A pair-fed control group was also included for the 28-day animals to control for effects of possible body weight loss. Treated animals in this group were housed individually and allowed to eat boric acid-treated feed ad libitum, and their feed cups weighed daily. Each treated animal was weight-matched to a pair-fed control which received as much food as its treated pair animal ate the previous day. If residual feed in the cup of the pair-fed controls was present, it was discarded daily and the correct amount of control rat chow added.

Route of administration: oral, feed

Exposure: 28 days

Doses / Concentrations: 0 and 9000 ppm w/w boric acid, equivalent to 0 and 1575 ppm B (0 and 189 mg B/kg bw/day), respectively. UV spectrophotometric analysis (Radian Corp., Morrisville, NC) of the feed determined that the final concentration of boric acid was 97-99% of the target 9000 ppm.

No. of animals: 6/time-point (36 male rats in total) for administration of boric acid, and 5/time-point (30 male rats in

total) as controls.

Histopathology examination: For the histology study, the animals were euthanized after 4. 7, 10, 14, 21 and 28 days of dosing. Animals (six treated and four controls per time point) were deeply anesthetized with methoxyfluorane and perfused through the ascending aorta with 0.1% procaine HCl in Ringer's balanced salts prior to perfusion with 5% glutaraldehyde/4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4 (Karnovsky, 1965). Right testis, kidney, and a section of liver were removed and placed in Kamovsky's fixative for 24-48 hr, and then transferred to 0.1 M sodium phosphate, pH 7.4, until further processing. For light microscopic studies, a 2-mm transverse section of tissue was excised and dehydrated through graded alcohols to 95% ethanol. The tissues were then embedded in 2-hydroxyethyl methacrylate and 2-pm sections were cut on a Leitz 1512 microtome. The sections were stained with periodic acid and Schiff's stain (PAS) and counterstained with hematoxylin. Tubules with an axial ratio < 2 were staged according to Leblond and Clermont (1952). Transmission electron microscopy was used to identify any ultrastructural changes that preceded those visible with the light microscope. Thus. only tissues from animals treated for 7 and 10 days were examined by electron microscopy. These tissues were embedded in Epon and Araldite. stained with uranyl acetate and lead citrate and then examined on a Philips 400 electron microscope.

Serum testosterone unalysis: For the subsequent hormone study, 10 control and 10 treated (9000 ppm boric acid in feed) animals per time group were orbitally bled under 70% CO,/30% O2 anesthesia for basal testosterone levels on specific days after the initiation of dosing. On Days 4, 7. 10, and 28, or days 10 and 28, 100 IU of hCG (Pregnyl. Organon Pharmaceutics, W. Orange, NJ) or 100 ng LHRH (Penninsula Laboratories, Inc., Belmont. CA), respectively, was injected ip into each animal, and after 90 min the animal was killed and blood collected for secretagogue- stimulated testosterone levels. Testicular histology was evaluated on randomly selected animals at each termination time to ascertain lesion development. Serum testosterone concentrations were determined using a radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA).

Boron analysis: For the tissue boron concentrations, blood, liver, kidney, epididymis, and testis were removed from the hormone study animals and the tissue samples (0.5-l g) were heat digested in 5 ml concentrated nitric acid by alternating on/off cycles in a 600-watt microwave (Microwave Digestion System, Model MDS-8 1 D, CEM Corp., Indian Trail, NC) for 18 min. Hydrogen peroxide (0.75 ml of 30%) was added. and the samples were further digested for 13 min. Digestates were filtered through Whatman 541 filter paper, and brought to 15 ml with distilled water. Samples were analyzed on a Thermo Jarrell Ash inductively coupled argon plasma spectrometer Model 6 I.

Statistical analysis: Statistical data for each treatment group were compared for normality of distribution. ABP data were compared by ANOVA, followed by pairwise comparisons using Student's t test. Testosterone data were compared pairwise by Student's t-test, and adjusted for unequal variances where appropriate. The significance level was set at $p < 0.05$.

Findings

Body weights: Over the 28-day study period, the rats consumed approximately 348.3 ± 66.8 mg/kg/day boric acid (mean +- SD). At this concentration, the treated animals gained less weight, so that by Day 28, the treated animals weighed 8% less than the controls (controls = 288 + 4 g; boric acid = 265 + 14 g, $p < 0.05$ by t-test adjusted for unequal variances).

Light microscopy results: The testes from the control rats displayed no abnormalities; all stages displayed the cell associations consistent with uninterrupted spermatogenesis. No abnormalities were detected after 4 days of dosing. By Day 7 of boric acid treatment, half of the animals exhibited an inhibition in spermiation in approximately 10-30% of stage IX tubules, whereas step 19 spermatids had been released in stage VIII tubules from control animals. By Day 10, all the treated animals showed inhibited spermiation in all stage IX and X tubules. Additionally, varying percentages of stage X, XI, and XII tubules (100, 83, and 31%, respectively) contained greater than or equal to four condensed spermatid nuclei near the Sertoli cell basement membranes. Spermatocytes and round spermatids were also seen in the lumina of approximately 10% of all the tubules in four of the six 10-day-treated animals. Since Day 10 was the first time point observed where all animals showed a boric acid-related lesion, the number of tubules in each stage from each treated and control animal at this time point was determined.

Table: Testicular effects at 9000 ppm boric acid after 28-day treatment.

Effects	Days of exposure						Pair-fed
	4	7	10	14	21	28	28
Inhibited spermiation	−	+	+++	+++	+++	+++	−
Peripheral spermatid nuclei	−	+	++	+++	+++	+++	−
Cell sloughing/epithelial disorganization	−	+	+	+	++	+++	−
Occluded lumina	−	−	−	−	++	+++	−
Abnormal residual bodies	−	−	−	+	++	+	−
Cell death	−	−	−	−	+	++	−
Number of animals affected	0/5[a]	3/6	6/6	6/6	6/6	6/6	0/6

Note. Table represents the percentage of all tubules of relevant stages demonstrating the listed effects; +++, >60%; ++, 30–60%; +, 5–30%; −, <5%.

[a] One animal had severely disrupted spermatogenesis and no epididymal sperm; this rat was not included in analyses.

On Day 14, in addition to the changes described, large, abnormal residual bodies were seen in several stage IX and X tubules. By Day 21, sloughed germ cells occluded the lumina in approximately 20-50% of all tubules in these animals. In addition, the number of stage IX/XII tubules displaying abnormal residual bodies had increased. Spermatid and spermatocyte cell death, as determined by pyknotic nuclei and an increased staining intensity, was also present in approximately 5-10% of stage VII and XIV tubules. After 28 days of dosing, there was advanced epithelial disorganization, cell exfoliation (found in 70-90% of all tubules), luminal occlusion (60-80% of tubules), and cell death (30-50% of tubules). As a result, there was a significant loss of spermatocytes and spermatids from all stage tubules in the treated animals. Multinucleated giant cells and "Sertoli cell only" tubules were infrequently seen by Day 28. Sections of Day 10 and 28 liver and kidney from all treated and control rats from the testicular histology study were examined, and no difference was found between the treatment groups at the light microscopic level (not shown). The histology of the 28-day pair-fed rats was indistinguishable from that of the ad libitum controls.

Electron microscopy results: In the seminiferous tubule, the Sertoli cells in stages VII and VIII of treated and control animals appeared indistinguishable at Days 7 and 10. All groups contained irregular nuclei with one or two nucleoli, Golgi, condensed circular or dumbell-shaped mitochondria, and circular smooth endoplasmic reticula in the basal parts of the cells. Microtubules were visible along the central-peripheral axis. Sertoli tight junctions were clearly visible along the lateral aspects of the cells in both treated and control animals. Tubulobulbar complexes were infrequently seen in association with step 19 spermatids in both treated and control animals. Residual bodies were found in all groups of stage VIII tubules, containing organelle debris, "vacuoles" with flocculent inclusions, degenerate mitochondria, and fat. The only detectable difference between boric acid treated and control rats was the presence, on Day 10, of condensed spermatid nuclei undergoing degradation near the basement membrane. Germ cells were also indistinguishable between groups; there were no observable differences in organelle or membrane structure or placement caused by boric acid treatment.

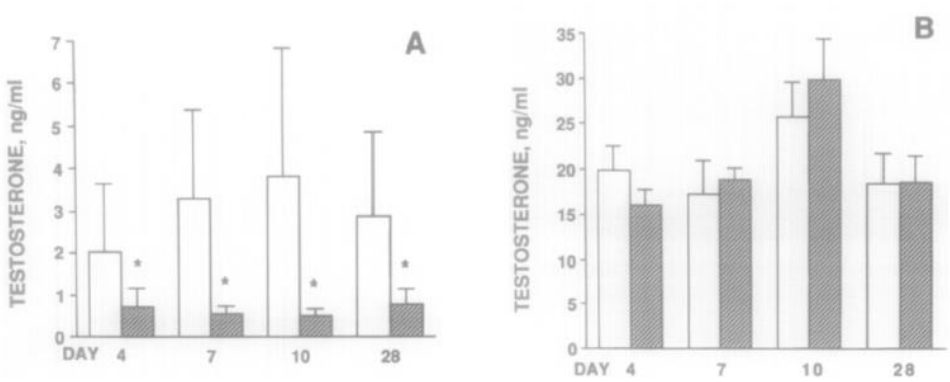
Testosterone analyses: boron concentrations in blood, testis, epididymis, liver, and kidney at

selected times during boric acid treatment are given below (see table). These data show that no tissue examined concentrated boron from the blood, as all boron consistent with a change in androgen status concentrations are approximately equal. In addition, steady-state levels were apparently reached by Day 4 of administration, as continued exposure did not alter boron levels in any of these tissues.

Table: Tissue boron levels

Days of treatment	Tissue				
	Kidney	Liver	Epididymis	Testis	Blood
4	14.6 ± 3.4	13.8 ± 1.5	10.6 ± 1.3	13.6 ± 0.6	10.1 ± 0.4
7	14.1 ± 1.2	14.4 ± 1.3	11.2 ± 1.7	14.3 ± 1.2	12.7 ± 1.4
10	13.3 ± 0.8	11.2 ± 1.1	10.2 ± 1.0	13.3 ± 1.3	10.5 ± 1.4
28	18.5 ± 2.7	12.2 ± 2.0	9.9 ± 0.8	13.7 ± 1.5	11.7 ± 2.1

[a] Boron levels are corrected for tissue blanks. Data are expressed as μg/g tissue from five to eight animals (mean ± SD). Control values (mean ± SD) and percentage of recovery for each tissue are as follows: Kidney = −0.10 ± 0.22, 80%; Liver = 0.03 ± 0.16; 81%, Epididymis = 0.06 ± 0.16; 80%; Testis = 0.08 ± 0.11, 83%; and Blood = 0.16 ± 0.18, 83%.



Serum testosterone data from control (open bars) and boric acid-treated animals (cross-hatched bars). (A) Basal serum testosterone values (mean ± SD, $n = 10$) from animals after the indicated duration of treatment; note the boric acid effect at all time points examined. An asterisk denotes values significantly different from control at $p < 0.05$. (B) hCG-stimulated testosterone levels (mean ± SD, $n = 10$). There were no differences between treated and control animals, showing that Leydig cell response was not adversely affected by boric acid exposure.

Figure: Serum testosterone levels of rats administered 9000 ppm boric acid for 28 days

Conclusion In conclusion, the histopathological examinations for all time-points contribute to the characterisation of the boron-mediated adverse effects on the male reproductive system. Even if only one dose level and a low number of animals were used and no reproductive organ weights were reported, there were no indications that the adverse effects on the male reproductive organs were secondary to general toxicity.

3.10.1.5 [Study 5] Reproductive assessment by continuous breeding in mice (boric acid)

Reference Fail, P. A., George, J. D., Seely, J. C., Grizzle, T. B., and Heindel, J. J. (1991). Reproductive toxicity of boric acid in Swiss (CD-1) mice: assessment using the continuous breeding protocol. Fundamental and Applied Toxicology, 17(2), 225-239.

Guideline Performed according to the NTP's Reproductive Assessment by Continuous Breeding Protocol.

Reliability Klimisch 2: reliable with restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Mouse/Swiss (male/female)

Test material Boric acid (Lot No. 872703, CAS No. 10043-35-3)

Purity: >99%

Study design

Materials and methods

Route of administration: oral, feed

Exposure: 27 weeks

Doses / Concentrations: 0, 1000 ppm, 4500 ppm or 9000 ppm equivalent to 0, 152, 636 and 1262 mg boric acid/kg bw, equivalent to 0, 26.6, 111.3 and 221 mg B/kg bw, respectively amount of ground NIH-07 diet (Zeigler Brothers). This was then added to a preweighed portion of feed and mixed in a Patterson-Kelly, 1-quart blender for 15 min, with the intensifier bar in operation for the first 5 min. Dose formulations were prepared every 2 weeks and stored at

4°C until added to feed jars at weekly intervals. Reference aliquots or selected dose formulations were within 90 to 100% of the nominal concentration for all weeks. In addition, the bulk chemical was reanalyzed for purity three times during the study period and proved to be 98 to 100% of a reference standard.

No. of animals: 19/sex/dose groups

Albino Swiss mice were purchased from Charles River Breeding Laboratories, Inc. (Raleigh, NC), at 9 weeks of age. After a 2-week quarantine period, the study animals were individually identified by ear tag and randomly assigned to treatment groups using a stratified randomization procedure based on body weights. Mice were 11 weeks of age at the start of the continuous breeding phase of these studies. Upon receipt, two males and two females were sacrificed and their sera evaluated for antibodies against 11 mouse viruses (Microbiological Associates, Inc., Bethesda, MD). All sera were negative for viral antibodies. After the study, sera from four randomly selected study animals were positive for only one antibody-mouse hepatitis virus. However, no study animal had clinical symptoms,

and it is unlikely that the virus afhected study results.

Male and female CD- 1 mice were group-housed by sex during quarantine and the l-week premating period in solid-bottom, polycarbonate cages with stainless-steel wirelids. The animals were subsequently housed as breeding pairs or individually. Ad-Sorb-Dri (Laboratory Products, Inc., Garfield. NJ) bedding was used in all cages. Deionized filtered water and ground rodent chow (NIH-07, Zeigler Brothers, Gardnets, PA) were provided ad libitum. Ground feed was made available by placing glass flat-bottom feeder jars, with stainless steel grid tops (Wahmann Manufacturing, Timonium, MD), into the animal's cage. Deionized filtered water was made available in either propylene or polycarbonate 500-ml bottles, fitted with butyl rubber stoppers (Girton Manufacturing, Millville, PA) and stainless steel sipper tubes (Ancare Corp., Manhasset, NY). Cages were sanitized weekly using detergent and 180°F water. All animal care procedures conformed to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication 85.23).

Histopathology: Selected nonreproductive tissues to be examined microscopically were fixed in neutral buffered 10% formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin according to standard

procedures. Ovaries were fixed in Bouin's and processed as above. Male reproductive tissues were embedded in glyco1 methacrylate plastic and stained with hematoxylin and eosin after a reaction with the periodic acidSchilI's reagent (Bio-Tek, Research Triangle Park, NC).

Evaluation of testicular tissue: Testicular tissue was evaluated by four criteria: (1) the presence of histological lesions (qualitative), (2) the Spermatogenic Index, a rating of spermatogenic activity (Whitsett et al.. 1984) (semiqualitative), (3) measurement of seminiferous tubule diameter (quantitative), and (4) an estimate of the number

of spermatids per testis (quantitative). Histological lesions were rated in one of three categories: degeneration. atrophy, or normal. Testicular degeneration was diagnosed when one or more of a spectrum of histological lesions, including disruption of the normal progression of spermatogenesis, vacuolization of the germinal epithelium layer, increased nuclear basophilia and pyknosis of germ cells, increased sloughing of germ cells, the presence of multinucleated giant cells, and/or an overall loss of spermatids, were observed. The severity of degeneration was evaluated by the proportion of seminiferous tubules affected and the degree of damage within those affected tubules. Testicular atrophy, considered an endpoint ofdegeneration, was characterized by collapsed seminiferous tubules lined by an apparent single Sertoli cell layer. Atrophic tubules were completely devoid of any spermatogenic activity. Because of the biological continuum between degeneration and atrophy, any testis could theoretically contain both lesions. In one cross section per animal, all tubules were evaluated for the presence of spermatogonia, spermatocytes and spermatids. In 10 circular sections of tubules, each from a different region of the cross section and each homogeneous throughout with respect to cell associations, we measured tubular diameters using a Bausch and Lomb Labophot microscope with an ocular micrometer and counted the number of "late" spermatids present. This designation was applied to a spermatid with an elongated nucleus, similar to Steps 1 l- 16 of spermiogenesis in house mice (Oakberg, 1956), or that appeared to be a mature spermatozoon still attached to the luminal edge of the epithelium. Late spermatids were observed in tubules resembling cycle Stages I-VIII and XI-XII in mice (Oakberg, 1956: Clermont and Trott, 1969). A testis was rated for its spermatogenic potential (spermatogenic index) on a scale of 1 to 6. The index was based on the appearance of the spermatogenic cells throughout the testis and included number of cell layers, types of cells, and the presence of late spermatids in the seminiferous tubules (Whitsett et al.. 1984). The above parameters were quantified in the left testis. The right testis was stored frozen and later homogenized for determination of spermatid numbers (Almquist and Amann, 1961; Hafs et al., 1974). As the nuclear chromatin condenses, it becomes resistant to disruption by homogenization. As a result. the number of elongating spermatids (Step 13 to 16) can be counted using a hemocytometer.

Statistics: Most hypotheses were tested using the nonparametric multiple comparison procedures of Dunn (1964) or Shirley (1977), as modified by Williams (1986). Shirley's test is designed to detect treatment-related differences when the response to treatment consistently increases (or decreases) with increasing dose. Although the test employs a smoothing algorithm to adjust for dose-response inversions, Dunn's test is more appropriate if the departure from monotonicity is severe. Jonckheere's test (1954) was used to ascertain if there was sufficient evidence of a dose-

related response to apply Shirley's test. If the p value from Jonckheere's test was less than 0.10, then Shirley's test was used; otherwise, Dunn's test was applied. In the crossover mating trial (Task 3) the Kruskal- Wallis test (Kruskal and Wallis, 1952) was used to assess equality of response among dose groups, while multiple comparison tests use the method of Dunn. Because Task 4 was performed with only one dose group and the control group, Wilcoxon's test (Conover, 197 1) was used. For data expressed as a proportion, such as number fertile/number cohabited, the Cochran-Armitage test (Armitage, 1971) was used to test for a dose-related trend, and pairwise comparisons were performed using a x2 test (Conover, 1971). In Task 3, where dose groups did not represent increasing dose levels, a x2 test was used to test simultaneous equality of response between groups and for pairwise comparisons. Since the number of pups in a litter may influence the average pup weight in that litter, a parametric analysis of covariance (Neter and Wasserman, 1974) was used to test overall equality in average pup weight after adjustment for average litter size. Pairwise comparisons were performed using Dunnett's test. For evaluation of the cauda epididymal sperm parameters, the following conditions were applied: (1) the concentration was computed from all observations, including preparations having no observed spermatozoa that were recorded as zeros, and (2) only samples having sufficient spermatozoa were used in dose group means and statistical analysis, because motility ratings and percentage abnormal were meaningless in animals with few or no spermatozoa. Observations were omitted from calculation of means for the "motility" ratings if the preparations had fewer than 20 spermatozoa and for "percentage abnormal" if fewer than 100 sperm were observed.

Findings **1000 ppm (equivalent to 26.6 mg B/kg):**

F0: The fertility index for 1 – 4 litters was 100%, and 84% for the fifth litter. The F0 males showed statistically significantly lower sperm motility than controls (i.e. 69 ± 5% for treated mice vs. 78 ± 3% for the controls), in 19/19 males. The histopathological exam did not reveal any significant changes for male mice; no histopathological results reported for F0 female mice.

Table: Body and organ weights in F0 males

Parameter	Dose group			
	0 ppm	*1000 ppm*	*4500 ppm*	*9000 ppm*
Body (g)[b]	42.24 ± 0.80 (39)	42.11 ± 1.16 (19)	40.70 ± 0.88 (20)	35.69 ± 0.89 (15)*
Liver (g)	2.38 ± 0.06 (39)	—	2.24 ± 0.07 (20)	—
Kidney and adrenal (g)	0.91 ± 0.03 (39)	—	0.82 ± 0.02 (20)[c,*]	—
Seminal vesicles (mg)[d]	422 ± 0.26 (39)	—	385 ± 32 (20)	—
Right testis (mg)	140 ± 3 (39)	140 ± 4 (19)	69 ± 5 (20)*	20 ± 1 (15)*
Right cauda epididymis (mg)	18.44 ± 0.69 (39)	20.11 ± 1.12 (19)	16.17 ± 1.00 (20)	14.99 ± 0.72 (15)*
Right caput and corpus epididymis (mg)	40.65 ± 1.03 (39)	42.92 ± 0.95 (19)	32.10 ± 1.44 (20)*	27.01 ± 1.82 (15)*
Prostate (mg)	48.32 ± 3.60 (39)	—	38.73 ± 2.91 (20)*	—

[a] Mean weight (g) ± standard error (number of animals). Animals were exposed to boric acid for 27 weeks. All significant comparisons are reported as $*p < .05$.

[b] Only body, testis, and epididymis weight data were collected at necropsy from 1000 and 9000 ppm males.

[c] Relative weights were not different.

[d] Seminal vesicle weight includes coagulating gland. Glandular secretions were not removed.

Table: Body and organ weights in F0 females

Parameter	Dose group	
	0 ppm	4500 ppm
Body (g)	38.98 ± 0.53 (39)	37.78 ± 0.63 (20)
Liver (g)	2.36 ± 0.04 (39)	2.15 ± 0.06 (20)*,b
Kidneys/adrenals (mg)	687 ± 13 (39)	633 ± 18 (20)*
Uterus (mg)	340 ± 17 (39)	345 ± 28 (20)

[a] Mean absolute weight ± standard error (number of animals). Animals were expoxed to boric acid for 27 weeks; *$p < 0.05$.

[b] Relative weights were also significantly different ($p < 0.05$).

4500 ppm (equivalent to 111.3 mg B/kg):

F0: The number of females producing litters decreased from 95% for the production of the first litter, to 85% for the second litter, to 30% for the third litter, to 5% for the fourth and fifth litter. In the female mice, there were no statistically significant changes on body weight, absolute or relative uterus weight; and vaginal cytology revealed normal cyclicity.

In the male mice, the following statistically significant (p<0.05%) effects were reported, as compared to controls:

- decreased mean sperm concentration (by approx. 72%);
- decreased mean percentage of motile sperm (by approx. 32%);
- increased mean percentage of abnormal sperm (by approx. 439%);
- decreased seminiferous tubular diameter (by approx. 32%);
- decreased number of spermatids in stages VII and VIII/tubule (by approx. 50%);
- decreased spermatogenic index (by approx. 28%);
- decreased absolute testis weight (by approx. 51%);
- decreased absolute epididymis weight (by approx. 21%);
- decreased prostate absolute weight (by approx. 20%).

No statistically significant changes on body weight were observed.The histopathological exam performed in F0 male mice revealed degenerative changes in the majority of the tubules, fewer germ cells that were not organised into the layered epithelium and few mature spermatozoa were observed (incidence not reported).

Table: Reproductive performance of F0 fertile pairs

Reproductive parameter	Dose group			
	0 ppm	1000 ppm	4500 ppm	9000 ppm
Initial fertility index[b]	38/38 (100%)	19/19 (100%)	19/20 (95%)	0/20 (0%)
Progressive fertility index[c]	38/38 (95%)	19/19 (100%)	1/20 (5%)	0/0 (0%)[d]
Litters per pair	4.71 ± 0.12 (38)	4.84 ± 0.09 (19)	2.32 ± 0.20 (19)*	—[d]
Live pups per litter	13.52 ± 0.38 (38)	13.31 ± 0.43 (19)	8.67 ± 0.76 (19)*	—
Proportion of pups born alive	0.99 ± 0.01 (38)	0.97 ± 0.02 (19)	0.88 ± 0.04 (19)*	—
Sex of pups born alive (males/total)	0.52 ± 0.01 (38)	0.53 ± 0.01 (19)	0.52 ± 0.02 (19)	—
Live pup weight (g)	1.61 ± 0.01 (38)	1.62 ± 0.03 (19)	1.43 ± 0.03 (19)*	—
Adjusted live pup weight (g)[e]	1.62 ± 0.02 (38)	1.64 ± 0.02 (19)	1.39 ± 0.03 (19)*	—

[a] Only pairs surviving to the end of Task 2 were included for statistical analysis of data. Figures are means ± standard error (number of fertile pairs). Figures are averages of individual means across litters. Statistical analysis was appropriate comparisons to controls (see Methods). All significant comparisons are reported as $*p < .05$.
[b] Number of cohabited pairs with first litter/number cohabited (percentage).
[c] Number of females having four litters/number of pairs having pregnancies (percentage).
[d] No litters were born to 9000 ppm pairs.
[e] Least-squares estimate of the mean of the average pup weight from each fertile pair, adjusted for average litter size ± standard error (number of fertile pairs producing live pups).

Table: Evaluation of cauda epididymal spermatozoa and seminiferous epithelium from F0 males

Endpoint	Dose group			
	0 ppm	1000 ppm	4500 ppm	9000 ppm
Cauda epidiymal spermatozoa				
Concentration[b]	518.64 ± 35.77 (39)	532.37 ± 40.92 (19)	146.90 ± 26.55 (20)*	2.80 ± 1.68 (15)*[c]
Percentage motile	78.13 ± 2.98 (38)	68.96 ± 4.50 (19)*	53.26 ± 8.18 (17)*	42.90 (1)
Percentage abnormal	11.34 ± 0.91 (39)	6.43 ± 0.77 (19)	61.17 ± 5.25 (20)*	6.83 ± 1.49 (6)
Testicular morphometrics[d]				
Seminiferous tubular diameter (μm)	265 ± 4.4	234 ± 6.2	180 ± 9.4*	98 ± 5.3*
Spermatids/tubule (stages VII and VIII)	118 ± 3.1	90.8 ± 5.7	58.8 ± 7.8*	0 ± 0*
Spermatogenic index[e]	6.0 ± 0.0	5.5 ± 0.1	4.3 ± 0.4*	0 ± 0*
Testicular homogenates				
Spermatids/testis ($\times 10^4$)[f]	8.2 ± 0.39 (39)	7.4 ± 0.53 (19)	7.9 ± 0.95 (20)	2.9 ± 0.10 (15)*

[a] Endpoint mean ± standard error (number of animals). All significant comparisons are reported as $*p < .05$.
[b] Concentration expressed as sperm per mg caudal tissue $\times 10^3$.
[c] Twelve of 15 observed males had no sperm.
[d] Testicular morphometrics were done for 10 tubules per testes in 39, 19, 20, and 15 animals for the controls, low-, mid-, and high-dose groups, respectively.
[e] Whitsett *et al.* (1984), a semiquantitative rating of cell types present.
[f] Spermatids per gram of testis ($\times 10^4$), a quantitative assessment of the number of late spermatid per testis (Hafs *et al.*, 1974; Almquist and Amann, 1961).

9000 ppm (equivalent to 221 mg B/kg):

F0: None of the F0 pairs was fertile.

In the male mice, the following statistically significant (p<0.05%) effects were reported, as compared to controls:

- decreased mean sperm concentration (by approx. 95%), 12/15 males had no sperm;
- decreased seminiferous tubular diameter (by approx. 63%);
- no stage VII and VII spermatids/tubule (incidence not reported);

- decreased number of spermatids/testis (x 10^4) by approx. 65%;
- decreased absolute testis (by approx. 86%);
- decreased absolute epididymis weights (by approx. 34%).

Histologic examination revealed marked seminiferous tubular atrophy with many tubules per testis characterised by an end-stage, Sertoli cell-only appearance in male rats (100% incidence).

No histopathological results reported for F0 female mice.The males presented significantly decreased absolute body weight (by approx. 16%; $p<0.05$). The average body weight gain was significantly decreased as compared to controls for both males and females (data not shown).

Table: Mating Fertility and reproductive performance of F0 pairs

	Dose group		
Reproductive parameter	Control male × control female	4500 ppm bora male × control female	Control male × 4500 bora female
Mating index[b]	15/19 (79)	6/20 (30)*	14/20 (70)
Fertility index[c]	14/19 (74)	1/20 (5)*	13/20 (65)
Live pups per litter[d]	11.5 ± 1.0 (14)	3.00 (1)	9.6 ± 0.8 (13)
Proportion of pups born alive	0.97 ± 0.03 (14)	1.00 (1)	0.91 ± 0.04 (13)
Males/litter (%)	49 ± 5 (14)	67 (1)	50 ± 2 (13)
Live pup weight (g)	1.69 ± 0.05 (14)	2.28 (1)	1.57 ± 0.04 (13)
Adjusted live pup weight (g)	1.71 ± 0.03 (14)	—	1.55 ± 0.03 (13)*
Dam weight (g)	47.22 ± 0.74 (14)	36.60 (1)	43.97 ± 1.04 (13)*
Number days to litter[e]	19.08 ± 0.19 (12)	—	20.18 ± 0.12 (11)*

[a] Numbers are percentages or means ± SEM (females with litters). Statistical analysis was appropriate comparisons to control (see Methods). All significant comparisons are reported as $*p < .05$.
[b] Number of females with copulatory plugs/mated pairs (percentage).
[c] Number of females with observed litters/mated pairs (percentage).
[d] Number of live pups per total pups observed (number of litters observed).
[e] Only dams with both a vaginal copulatory plug date and litter date were used for this parameter.

Maternal effects

The females in the high dose group (221 mg B/kg bw/day) presented statistically significantly decreased body weight (a 10% difference was observed between post-partum controls and barren females).

Effects on the offspring

1000 ppm (equivalent to 26.6 mg B/kg/day):

F1 pups: no statistically significant changes were observed.

F2 pups: statistically significantly ($p<0.05$) decreased adjusted live pup weight (by approx. 3% compared to control).

4500 ppm (equivalent to 111.3 mg B/kg/day):

F1 pups: statistically significant decreased parameters compared to controls:

- adjusted live pup weight by approx. 14%;
- number of litters/pair by approx. 51%;
- live birth index by approx. 11%.

Only 1/19 F1 dams had 5 litters and all her pups in the 4th litter were born dead.

9000 ppm (equivalent to 221 mg B/kg/day):

F0: No litters were born to F0 animals.

Table: Average number of F1 pups/litter during continuous breeding

Litter	Dose group			
	0 ppm	1000 ppm	4500 ppm	9000 ppm
1	12.7 ± 0.4 (38)	12.1 ± 0.9 (19)	9.7 ± 0.9 (19)*	—
2	13.8 ± 0.5 (38)	13.9 ± 0.8 (19)	8.2 ± 1.0 (17)*	—
3	14.2 ± 0.5 (36)	14.3 ± 0.7 (19)	4.2 ± 1.2 (6)*	—
4	13.5 ± 0.7 (36)	13.5 ± 0.9 (19)	— (1)[b]	—
5	13.7 ± 0.7 (31)	13.5 ± 0.6 (15)[c]	3.00 (1)	—
Combined[d]	13.5 ± 0.4 (38)	13.3 ± 0.4 (19)	8.7 ± 0.8 (19)*	—

[a] Mean ± standard error (number of fertile pairs). All significant comparisons are reported as $*p < .05$.
[b] One dam had five litters. All pups in her fourth litter were born dead.
[c] One litter is excluded from these data as it was counted incorrectly.
[d] Mean of the average number of live pups produced by each fertile pair (number of pairs having one or more litters).

Table: Mating fertility and reproductive performance of F1 breeding pairs

Reproductive parameter	Dose group	
	0 ppm	1000 ppm
Mating index (%)[b]	27/40 (68)	17/20 (85)
Fertility index (%)[c]	36/40 (90)	18/20 (90)
Live F_2 pups per litter	12.64 ± 0.35 (36)	12.94 ± 0.62 (17)
Proportion of F_2 pups born alive	0.99 ± .004 (36)	0.99 ± .007 (17)
F_2 males/litter (%)	0.47 ± 0.02 (36)	0.51 ± 0.02 (17)
Live F_2 pup weight (g)	1.55 ± 0.01 (36)	1.50 ± 0.03 (17)
Adjusted live F_2 pup weight	1.55 ± 0.01 (36)	1.50 ± 0.02 (17)*
Average number days to litter	18.74 ± 0.09 (27)	18.80 ± 0.11 (15)
Average postpartum dam weight (g)[d]	35.87 ± 0.40 (36)	35.88 ± 0.83 (18)

[a] Only pairs surviving to the end of Task 3 were included for statistical analysis of data using appropriate tests (see Methods). All significant comparisons are reported as $*p < .05$.
[b] Number of females with copulatory plugs/mated pairs (percentage).
[c] Number of females with observed litters/mated pairs (percentage).
[d] Only dams with both a vaginal copulatory plug date and litter date were used for this parameter.

Conclusion Dose-dependent effects were observed in F0 male mice mainly expressed as decreased sperm motility at both 26.6 and 111.3 mg B/kg bw/day, in the absence of general toxicity. The decreased testis, epididymis and prostate absolute weights observed in the absence of general toxicity at 111.3 mg B/kg bw/day are considered a direct effect of the treatment and thus, as supportive data for classification. The decreased absolute testis and epididymis weights at the highest dose level (221 mg B/kg bw/day) were seen in the presence of general significantly decreased body weight gain and body weight and might be subsequent effects of general toxicity.

3.10.1.6 [Study 6] 30-day and 60-day oral repeated dose toxicity studies in rats (borax, non-guideline)

Reference Lee, I. P., Sherins, R. J. and Dixon, R. L. (1978). Evidence for induction of germinal aplasia in male rats by environmental exposure to boron. Toxicology and applied pharmacology, 45(2), 577-590.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Rat, Sprague-Dawley (male)

Test material Borax
Purity: unknown

Study design **Materials and methods**
Each experimental group consisted of 18 male rats (200-250 g) and was exposed to dietary boron at concentrations of 0, 500, 1000, and 2000 ppm for 30 and 60 days. Borax (Fisher Scientific Co.) was added to the basal diet (Wayne

Sterilizable rat feed) on a weight to weight basis and mixed thoroughly. Throughout the study, animals were housed two per cage and provided free access to food and drinking water. At the end of 30 and 60 days, five male rats from each experimental group were serially mated to assess fertility, 10 rats were used to assess plasma concentrations of FSH, LH, and testosterone, and only three rats were killed to evaluate selected specific enzyme activities, histology, and the weights of testes, epididymides, prostate, liver spleen, kidneys, heart, and lungs. For determination of hormone concentrations, 6 ml of heparinized blood was obtained from the jugular vein under ether anesthesia.

Route of administration: oral, feed

Exposure: 30 or 60 days

Doses / Concentrations: 0, 500, 1000 and 2000 ppm borax, equivalent to 0, 50, 100 and 200 mg B/kg bw/day, respectively.

No. of animals: 18 males/dose group

Body weights: Body weights and food consumption were determined at weekly intervals.

Testicular and epididymal histology: Testes and epididymides were removed and fixed in Bouin's solution, washed, dehydrated, and embedded in paraffin for sectioning. Slides were stained with periodic acid-Schiff and counterstained with Harris' hematoxylin. Seminiferous tubular diameters were measured with a Leitz ocular micrometer. All other organs were fixed in 10% neutral formalin, and the tissue sections were stained with hematoxylin and eosin.

Boron determinations in plasma and testes. To determine the boron concentrations in both plasma and testes, the tissue samples were ashed in the presence of sodium carbonate. After fusion of the ash, the boron concentration was determined directly in the residue by reaction with curcumin (Pinta, 1971). Absorbance was measured at 555 nm with a Spectronic-20 spectrophotometer. The limit of detection was 0.05 pg. Plasma FSH, LH, and testosterone concentrations. Reagents to measure FSH and LH were supplied by the National Institute of Arthritis and Metabolic Disease, Hormone Distribution Program. Rat FSH-RP-1 and LH-RP-1 were used as the reference preparations. Assay sensitivity is 20 rig/ml for FSH and 2 rig/ml for LH. Intra-assay variation is less than 10% (Krueger et al., 1974). Plasma testosterone was measured in plasma pooled from 10 rats and assayed according to the method of Nieschlag and Loriaux (1972).

Serial mating (*in vivo* assessment of fertility): After 30 and 60 days of boron exposure, five rats from each group were selected for serial mating studies to assess the time of onset of infertility (Jackson et al., 1961). Each rat was maintained in an individual cage and placed on normal laboratory diet with free access to drinking water. Each male rat was housed singly with a virgin female for a period of 7 days. After each 7-day period, the female rats were removed from the males and replaced with other virgin females. Females were examined daily for vaginal plugs to assess copulation. Breeding studies were terminated at the end of 12 weeks. Pregnant rats were allowed to litter, and the first filial generation (F,) was scored and examined to determine whether any gross congenital abnormalities had occurred.

Statistics: The Fisher nonparametric test (Siegel, 1956) was used to analyse fertility data. The statistical differences between control and experimental groups in other studies were calculated using the Student t-test (Snedecor and Cochran, 1967).

Findings **After 30 days of exposure:**

500 ppm borax (equivalent to 50 mg B/kg bw/day): No statistically significant changes in the body, epididymis or testis absolute weight, and no morphological changes observed at the testicular histology examination.

1000 ppm borax (equivalent to 100 mg B/kg bw/day): Statistically significant ($p<0.05$) decreased absolute epididymis weight (by approx. 19%), marked reduction of spermatocytes, spermatids and mature spermatozoa (incidence not reported).

2000 ppm borax (equivalent to 200 mg B/kg bw/day): Statistically significant ($p<0.05$) decreased absolute

epididymis weight (by approx. 30%), severe loss of germinal elements and non-statistically significant loss in tubular diameter (by approx. 15%).

Serial mating: no statistically significant changes were observed at 50 mg B/kg bw/day. At 100 mg B/kg bw/day, the pregnancy rates were significantly reduced during the first 3 weeks post-treatment (by 33%; $p<0.05$).

At 200 mg B/kg bw/day, the pregnancy rate was statistically significantly ($p<0.05$) reduced (by 100 %) up to 8 weeks after the termination of exposure, with a partial recovery observed up to week 10 post-treatment.

Table: Changes in body weight and organ weight for male rats exposed to borax 30 and 60 days

			Boron concentration (ppm)		
	Days	Control	500	1000	2000
Body weight	30	405 ± 28	416 ± 37	431 ± 28	381 ± 55
	60	464 ± 45	439 ± 42	394 ± 40	401 ± 63
Epididymis	30	0.64 ± 0.03	0.55 ± 0.04	0.52 ± 0.05[b]	0.45 ± 0.04[b]
	60	1.23 ± 0.06	1.23 ± 0.09	0.77 ± 0.41[b]	0.81 ± 0.34[b]
Testis	30	1.64 ± 0.05	1.73 ± 0.18	1.54 ± 0.18	1.54 ± 0.19
	60	1.81 ± 0.06	1.76 ± 0.19	0.68 ± 0.16[b]	0.63 ± 0.01[b]
Prostate	30	0.26 ± 0.13	0.31 ± 0.08	0.38 ± 0.06	0.25 ± 0.14
	60	0.36 ± 0.05	0.59 ± 0.12	0.42 ± 0.34	0.47 ± 0.39
Liver	30	13.44 ± 1.59	11.12 ± 1.00	13.51 ± 2.14	10.49 ± 2.6
	60	13.79 ± 0.79	16.09 ± 0.69	11.15 ± 1.03[b]	10.41 ± 1.28[b]
Spleen	30	0.65 ± 0.04	0.69 ± 0.04	0.69 ± 0.07	0.70 ± 0.13
	60	0.64 ± 0.04	0.74 ± 0.05	0.71 ± 0.02	0.60 ± 0.06
Kidney	30	2.83 ± 0.39	2.80 ± 0.10	3.05 ± 0.31	2.77 ± 0.69
	60	1.33 ± 0.11	1.60 ± 0.18	1.23 ± 0.18	1.40 ± 0.18
Heart	30	1.34 ± 0.07	1.11 ± 0.13	1.10 ± 0.05	1.00 ± 0.15
	60	1.20 ± 0.04	1.38 ± 0.10	1.07 ± 0.07	1.08 ± 0.13
Lung	30	2.04 ± 0.12	1.92 ± 0.25	2.10 ± 0.24	2.06 ± 0.47
	60	1.56 ± 0.15	1.73 ± 0.25	'.86 ± 0.13	1.89 ± 0.22

[a] All values are expressed in grams as mean ± SD for three rats.
[b] Significantly different from control ($p < 0.05$).

After 60 days of exposure:

500 ppm borax (equivalent to 50 mg B/kg bw/day):

No statistically significant changes in the body, epididymis or testis absolute weight. A statistically significant ($p<0.05$) decrease (by approx. 16%) in seminiferous tubular diameter was observed, but no morphological changes were observed at the testicular histology examination.

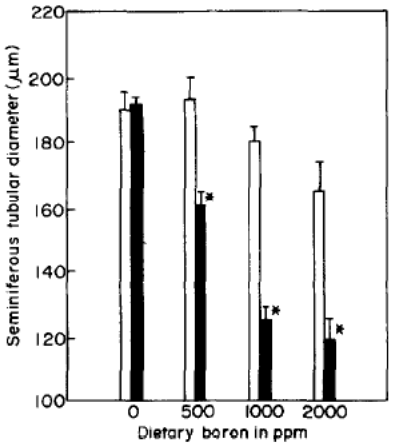
1000 ppm borax (equivalent to 100 mg B/kg bw/day): Statistically significantly ($p<0.05$) decreased absolute testis weight (by approx. 62%) and absolute epididymis weight (by approx. 37%); most germinal elements were absent (incidence not reported) and a statistically significant decrease (by approx. 34%) in seminiferous tubular diameter was observed.

2000 ppm borax (equivalent to 200 mg B/kg bw/day): Statistically significantly ($p<0.05$) decreased absolute testis (by approx. 65%) and absolute epididymis weight (by approx. 34%), a statistically significant decrease (by approx. 38%) in seminiferous tubular diameter, and complete germinal aplasia (incidence not reported) were observed.

Testicular histology examination 32 weeks post-treatment showed persistent germinal aplasia (incidence not reported).

A significant decrease in absolute liver weight at 100 and 200 mg B/kg bw/day as compared to controls, with no liver histological changes was observed (by approx. 19% and 25%, respectively; $p<0.05$).

A statistically significant ($p<0.05$) dose-dependent increase in the mean plasma FSH concentration by 139%, 175% and 236% for the 500 ppm, 1000 ppm and 2000 ppm dose groups, respectively, was observed after 60 days exposure.



Seminiferous tubular diameter of rats receiving dietary borax (0, 500, 1000, and 2000 ppm as boron equivalent) for 30 (open column) and 60 days (closed column). All values are expressed as mean ± SD of 60 seminiferous tubules from each of three rats. Asterisks denote values significantly different than controls ($p < 0.05$).

Figure: Seminiferous tubular diameter of rats administered borax for 30 and 60 days

Serial mating: the pregnancy rates at the mid-dose level were significantly low during weeks 2 – 4 post-treatment (by approx. 80 – 100%), and the males from the highest dose groups were infertile throughout 12 weeks post-treatment (and additional 20 weeks) of serial mating. No statistically significant changes were observed at 50 mg/kg bw/day.

Table: Effects of borax on male rat fertility (30 days treatment)

Weeks following exposure	Percentage pregnant/vaginal plugs				Average litter size			
	0	500	1000	2000	0	500	1000	2000
1	100/80	100/100	20/60[b]	0/60[b]	10.4	9.6	9.0	—
2	80/80	100/100	20/80	0/80[b]	10.8	9.6	2.0	—
3	80/100	80/100	0/80[b]	0/100[b]	11.0	10.5	—	—
4	100/100	80/100	100/80	0/100[b]	10.6	12.5	9.8	—
5	100/100	100/100	60/100	0/100[b]	8.0	10.4	11.3	—
6	100/100	100/100	100/100	0/100[b]	10.5	9.2	9.8	—
7	100/100	100/100	100/100	25/100[b]	10.4	10.6	9.8	—
8	40/100	100/100	80/100	0/100	11.0	12.4	12.0	—
9	100/100	100/100	80/100	50/100	10.8	10.6	11.2	12.5
10	100/100	100/100	80/100	50/100	13.0	12.5	10.8	6.0
Overall	88	94	66	10	10.6	10.7	10.3	9.2

[a] Each treatment group was composed of five males with the exception of the 2000-ppm dose which had four.
[b] Significantly different from control ($p < 0.05$).

Table: Effects of borax on male rat fertility (60 days treatment)

Weeks following exposure	Percentage pregnant/vaginal plugs				Average litter size			
	0	500	1000	2000	0	500	1000	2000
1	80/80	60/60	20/60	0/60[b]	11.0	12.3	4.0[c]	—
2	20/20	60/60	0/60	0/60	12.0[c]	9.0	—	—
3	100/100	100/100	0/80[b]	0/80[b]	10.2	9.2	—	—
4	100/80	80/100	20/80[b]	0/100[b]	11.8	13.0	3.0[c]	—
5	80/100	100/100	60/100	0/100[b]	12.0	10.8	13.0	—
6	100/100	100/100	80/100	0/100[b]	12.0	10.8	12.0	—
7	100/100	100/100	80/100	0/100[b]	10.2	11.4	11.2	—
8	100/100	100/100	80/100	0/100[b]	9.6	10.0	10.8	—
9	100/100	100/100	80/100	0/100[b]	11.4	9.2	11.8	—
10	100/100	100/100	80/100	0/100[b]	10.2	10.4	11.5	—
11	80/100	—	80/100	0/100[b]	10.8	—	10.2	—
12	80/100	—	60/100	0/100[b]	12.5	—	11.3	—
Overall	87	90	52	0	11.0	10.6	10.9	—

[a] Each treatment group was composed of five males.
[b] Significantly different from control ($p < 0.05$).
[c] Based on one animal.

Conclusion The reported effects on fertility were observed in the absence of general toxicity (body weight or clinical observations). The dose-dependent germinal aplasia, complete and partially reversible infertility in male rats (at 200 mg B/kg bw/day for 60- and 30-day treatments, respectively) are considered treatment-related. Since the body weights of the males were comparable to those of controls, the significantly decreased epididymis weights (at 100 and 200 mg B/kg bw/day for both treatments) are considered a direct effect of boron treatment and not a subsequent effect of general toxicity. The significantly decreased absolute liver weight after 60 days of treatment is difficult to explain or attribute to treatment since it was reported in the absence of any histological liver changes.

3.10.1.7 [Study 7] 60-day oral repeated dose toxicity study (boric acid, non-guideline)

Reference Marat, I., Arstan, M., Galymzhan, Y., Timur, J., Yerbolat, I. and Almasbek, Y. (2018). Impact of chromium and boron compounds on the reproductive function in rats. Toxicology and industrial health, 34(6), 365-374.

Guideline No guideline followed (conforms to Rodent Dominant Lethal Test)

Reliability -

Species / strain Rats/white, outbred (male)

Test material Boric acid

Purity: unknown

Study design

Materials and methods

Route of administration: oral gavage

Exposure: 60 days (Males were administered test substance during the entire spermatogenesis cycle)

Doses / Concentrations: 0, 1 and 10 mg B/kg bw/day

No. of animals: 6 males/dose group

Statistics: In all statistical analysis procedures, significance level was set at $p< 0.05$. The arithmetic mean values of quantitative indices were calculated. Statistical data processing was carried out with the Statistica 10.0 (StatSoft, Inc., USA). The null hypothesis in the absence of differences between the discovered distribution of the attribute and the theo- retically expected normal distribution was tested with the Shapiro–Wilk *W*-test. Differences between samples were assessed with the Student's *t*-test in the case of normal distribution of paired variables, and with the Mann–Whitney *U* test in the absence of a normal distribution and in the cases of paired inde-pendent sets.

At the end of the exposure period, the males were mated with untreated females at a 1:1 ratio. Gestation was terminated at day 20 and number of implantation sites, resorptions, and embryos on the uterine horns and the corpus luteum count in the ovaries were investigated.The fertility index (FI) was calculated as a ratio of the number of

pregnant females to the number of mated females.

In a parallel series of experiments, the ability of the test substance to induce mutations in germ and somatic cells was investigated after i.p administration of male rats and frequencies of dominant lethal mutations were also investigated using sequential mating intervals.

The experiment was conducted on two groups of animals. The first group was used to assess the ability of potassium dichromate ($K_2Cr_2O_7$) and boric acid to induce mutations in germ and somatic cells under the isolated and combined administration (single dose). Mutagenic and antimutagenic actions of $K_2Cr_2O_7$ and H_3BO_3 (separately and in combination) in somatic cells were assessed with the micronuclei (MN) test in the polychromatophilic erythrocytes (PCEs) of the bone marrow of rats (*n* 24) *in vivo*. This method was recommended as the main one for screening mutagens and antimutagens among phar- macological and chemical compounds (Kolmakova et al., 2003) and was included as a mandatory research method in European Economic Community member states and Japan (Durnev et al., 2005). Dominant lethal mutations (DLMs) test (Iztleuov et al., 2015) was used to identify mutations in germ- inal cells and assess the antimutagenic activity of $K_2Cr_2O_7$ and H_3BO_3 with a single use. This test indi- cates the genetic alterations and aberrations of chro- mosomes in the germ cells of parent animals (first generation of offspring). DLMs increase the rate of embryonic loss and, consequently, reduce the survi- vability of embryos and fertility. DLM frequency is registered according to the increase in the embryonic mortality rate among intact pregnant females that mated with experimental males.

In the second series of experiments, the effect of $K_2Cr_2O_7$ and H_3BO_3 on the reproductive function was tested by dividing male rats (*n* 36, *m* 160–180 g) into six groups. The first (control) group included intact animals. The second group included animals with simulated chromium-induced microelementosis ("Cr" group) that was conducted via daily intragastric administration of $K_2Cr_2O_7$ at 1.0 mg of chromium per 1 kg of BM during the spermatogenesis cycle (60 days). The third and fourth groups included animals with simulated boron-induced microelementosis ("B-1" and "B-10" groups) that was conducted via daily intragastric administration of H_3BO_3 at 1.0 and 10 mg of boron per 1 kg of BM, respectively, throughout the entire spermatogenesis cycle. Animals of the fifth and sixth groups were administered a combination of $K_2Cr_2O_7$ and H_3BO_3 intragastrically on a daily basis at 1 mg of chromium per 1 kg of BM 1 mg of boron per 1 kg of BM ("Cr B-1" group) and 1 kg of BM 10 mg of boron per 1 kg of BM ("Cr B-10" group), respectively. Intact male rats (*n*=6) were administered distilled water on an identical basis and at iden- tical volumes. At the end of the exposure period, test males were mated with intact females at a 1:1 ratio with a subsequent analysis of the embryonic material of females on the 20th day of pregnancy under brief ether anesthesia. We have counted the number of implantation sites, resorptions, and embryos on the uterine horns and the corpus luteum count in the ovar- ies. Based on the obtained data, we have calculated the indices of pre and postimplantation mortality (PrIM and PtIM), the DLM frequency, and mating effectiveness.

Findings No information on general toxicity was available for any of the dose groups.

1 mg B/kg bw /day

The fertility index was not different from control (86% versus 89% in controls).

10 mg B/kg bw/day

Reduced fertility index (62.5% compared to 89% in controls, unclear if statistically significantly different). Increased pre-implantation loss (23.81% compared to 2.69% in control, $p \leq 0.05$).

Table: DLMs induced by co-exposure to chromium and boron compounds

Spermatogenesis stage	Exposure type	FI (%)	Living embryo (per 1 female)	Dead embryo (per 1 female)	PtIM (%)	DLM frequency (F_1)
Mature spermatozoids	Control	90	10.22 ± 0.55	0.67 ± 0.41	6.92 ± 2.02	
	Boron (B)	83	6.6 ± 0.80[a]	2.0 ± 0.50[a]	23.6 ± 3.3[a]	0.35
	Chromium (Cr)	71.4	4.3 ± 0.45[a]	4.0 ± 0.48[a]	45.98 ± 5.34[a]	0.57
	B + Cr	88.0	3.3 ± 0.33[a]b	5.3 ± 1.4[a]b	61.4 ± 11.6[a]b	0.68
Late spermatids	Control	92.3	9.85 ± 0.67	0.71 ± 0.48	6.91 ± 1.24	
	Boron (B)	86	7.1 ± 0.90	1.9 ± 0.30[a]	21.3 ± 2.7[a]	0.20
	Chromium (Cr)	69.2	4.29 ± 0.48[a]	3.71 ± 0.48[a]	46.4 ± 4.75[a]	0.56
	B + Cr	89	3.57 ± 0.46[a]b	4.4 ± 0.48[a]b	55.3 ± 6.66[a]b	0.640
Early spermatids	Control	100	9.9 ± 0.50	0.70 ± 0.35	6.48 ± 2.19[a]	
	Boron (B)	87	5.9 ± 0.33[a]	2.0 ± 0.43[a]	25.3 ± 3.60[a]	0.40
	Chromium (Cr)	73.3	4.55 ± 0.55[a]	3.9 ± 0.53[a]	46.24 ± 5.17[a]	0.55
	B + Cr	87	3.71 ± 0.43[a]b	4.5 ± 0.50[a]b	55.1 ± 6.24[a]b	0.63

PtIM: postimplantation mortality; DLM: dominant lethal mutation; FI: fertility index.
[a]$p \leq 0.05$ versus $K_2Cr_2O_7$.
[b]$p \leq 0.05$ versus the control.

Table: Repoductive performance of male rats for separate and co-exposure to chromium and boron compounds

Indicators group	FI (%)	Living embryo (per 1 female)	Dead embryo (per 1 female)	Corpus luteum (per 1 female)	PrIM (%)	PtIM (%)	DLM frequency (F_1)
Control	89	9.71 ± 0.33	0.714 ± 0.45	10.7 ± 0.30	2.69 ± 1.49	6.92 ± 1.67	
PDC (Cr) (1 mg/kg of BM)	87.9	3.25 ± 0.22[a]	6.5 ± 0.43[a]	10.6 ± 0.76	7.62 ± 1.64[a]	68.6 ± 4.2[a]	0.665
B-1 (1 mg/kg of BM)	86	8.5 ± 0.62[b]	1.3 ± 0.35[b]	10.2 ± 0.63	4.21 ± 2.1	13.62 ± 5.1[b]	0.125
B-10 (10 mg/kg of BM)	62.5	6.0 ± 0.61[a,b]	1.3 ± 0.25[b]	9.5 ± 0.72	23.81 ± 4.3[a,b]	18.0 ± 6.1[a,b]	0.382
Cr + B-1 (1 mg/kg + 1 mg/kg)	88	7.0 ± 0.71[a,b]	3.5 ± 0.27[a,b]	11.4 ± 0.80	8.1 ± 3.6	33.3 ± 7.0[a,b]	0.279
Cr + B-10 (1 mg/kg + 10 mg/kg)	90	3.0 ± 0.43[a]	6.87 ± 1.36[a]	12.0 ± 0.71	18.5 ± 3.5[a,b]	70.0 ± 4.84[a]	0.691

BM: body mass; DLM: dominant lethal mutation; PDC: potassium dichromate; PrIM: preimplantation mortality; PtIM: postimplantation mortality; FI: fertility index.
[a] $p \leq 0.05$ versus $K_2Cr_2O_7$.
[b] $p \leq 0.05$ versus the control.

Table: Cytogenetic effects of chromium and boron compounds under separate and combined exposure.

Experiment conditions	Number of analyzed cells	Cell count with MN (‰)	Antimutagenic effect (%)
Control	3000	3.2 ± 0.60	
PDC ($K_2Cr_2O_7$)	3000	12.6 ± 1.50[a]	
BA (H_3BO_3)	3000	3.0 ± 0.43	
PDC + BA	3000	8.1 ± 1.2[a,b]	35.7–36

$K_2Cr_2O_7$: potassium dichromate; MN: micronuclei.; H_3BO_3: boric acid; PDC: potassium dichromate; BA: boric acid.
[a] $p \leq 0.05$ versus the control.
[b] $p \leq 0.05$ versus $K_2Cr_2O_7$.

Conclusion There is no information available on clinical conditions, body weights or body weight gains of the animals, and it is therefore not possible to conclude that the observed findings are not a secondary consequence of general toxicity.

3.10.1.8 [Study 8] Prenatal developmental toxicity study in rabbits (barium metaborate monohydrate)

Reference Study report (1993b) Prenatal Developmental toxicity of Busan 11-M1.

Guideline EPA OPP 83-3 (Prenatal Developmental Toxicity Study)
GLP guideline

Reliability Klimisch 1: reliable without restrictions (reliability according to the publically disseminated REACH Registration dossier of barium diboron tetraoxide)

Species / strain Rabbits / New Zealand (female)

Test material Busan 11-M1 (barium metaborate monohydrate)
Purity: 94.3%
Form: powder

Study design

Materials and methods

Route of administration: oral gavage

Exposure: gestation days 7-19

Doses / Concentrations: 0, 2, 10, 20 mg/kg bw/day, equivalent to 0, 0.18, 0.9 and 1.8 mg B/kg bw/day, respectively

PREPARATION OF DOSING SOLUTIONS:
An appropriate amount of the test material was weighed for each group and transferred to a mortar. The test material was triturated with a small amount of the vehicle until a slurry was formed. This was then transferred to a graduated cylinder and a sufficient amount of vehicle was added to attain an appropriate volume for mixing. The suspension was mixed on a mixer for approximately 5 minutes to reduce any large particles.
A magnetic stir bar was added and the mixture was stirred continuously throughout the sampling and dosing procedures. Dosing preparations were dispensed on a daily basis for dose administration. Dosing preparations were prepared twice during the study period and were stored at room temperature.

VEHICLE
- Justification for use and choice of vehicle (if other than water): No data
- Concentration in vehicle: Each litre of vehicle was prepared by heating 1000 ml of deionised water to approximately 70 °C and gradually adding 5.0 g of the control material powder. The mixture was stirred until clear. The vehicle was prepared approximately once each week and stored refrigerated between periods of use.
- Amount of vehicle (if gavage): Dosage volume 1 ml/kg
- Lot/batch no. (if required): 50H0209
- Purity: 0.5% aquous methyl cellulose

Details on analytical verification of doses or concentrations:
Prior to initiation of dosing, duplicate aliquots taken from the top, middle and bottom of the preparations were tested for homogeneity. Two duplicate sets of aliquots were collected for the middle of the preparations for concentration and 14-day stability analysis. Duplicate sets of sample aliquots were collected form the middle of the dosing solutions at dosing initiation for concentration analysis. Analysis was carried out at EPL BioAnalytical Services, Inc. The dosing preparations were homogenous, contained the amounts of test material specified by the protocol and were stable for 14 days.

No. of animals: 20/dose group

Body weights: The bodyweights were not measured with the frequency recommended in the guideline.

Details on test animals and environmental conditions:

TEST ANIMALS
- Source: Hazleton Research Products, Inc., Denver, Pennsylvania
- Age at study initiation: approximately 5 months
- Weight at study initiation: 2.344 - 3.710 kg
- Fasting period before study:
- Housing: Individually, stainless steel wire bottom cages suspended above a cage-board
- Diet (e.g. ad libitum): Purina Certified Rabbit Chow #5322 ad libitum
- Water (e.g. ad libitum): Municipal water ad libitum
- Acclimation period: approximately 6 weeks

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20 - 22.2
- Humidity (%): 28-60%
- Air changes (per hr): 10 fresh air changes per hour
- Photoperiod (hrs dark / hrs light): 12 hours light/dark cycle

Details on mating procedure:

Impregnation procedure: artificial insemination
- A 0.25 – 0.5 ml aliquot of each diluted semen sample (collected from 10 resident males of the same strain and obtained from the same supplier as the females) was deposited into the anterior vagina of each female with a glass insemination pipette. Immediately following insemination each doe was administered an intravenous injection of human chorionic gonadotropin to ensure ovulation. The day of insemination was designated gestation day.

Details on study design:

Dose selection rationale: based on the results of a preliminary range-finding study
Five groups of 7 pregnant females were dosed with 20, 55, 90, 125, 160 mg/kg/day Busan 11-M1 daily from gestation day 7-19 by oral gavage. The animals were then observed until gestation day 29 at which point they were sacrificed and gross necropsy examination was performed. Maternal toxicity was exhibited at dose levels of 20 mg/kg/day and greater by mortalities and changes in the general clinical condition of the animals. No developmental toxicity was expressed at any dose level available for evaluation 20, 55, 90 mg/kg/day. Based on these results, the dose levels 2, 10, and 20 mg/kg/day were selected for the definitive developmental toxicity study.

- Rationale for animal assignment (if not random): computer randomisation procedure

Maternal examinations:

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: twice daily
- Cage side observations included: mortality and moribundity

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: On the first day of dosing, animals were observed one hour following dosing. On the second day of dosing, animals were observed one, two and four hours following dosing. For the remainder of the test, animals were observed one hour following dosing.

BODY WEIGHT: Yes
- Time schedule for examinations: recorded individually on gestation days 0, 7-20, 24 and 29

FOOD CONSUMPTION: Yes
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/animal/day and g food/kg body weight/day: Yes

POST-MORTEM EXAMINATIONS: Yes
- Sacrifice on gestation day 29
- Post mortem findings were correlated with ante mortem clinical finding as and any abnormalities were recorded.

OTHER:
Gross necropsy was performed on females which aborted or died during the course of the study. Maternal tissues were retained for possible future histopathological examination. The number and location of implantation sites and corpora lutea were recorded. Foetal finding for females which aborted were not included in any tabulation or statistical analysis.

Ovaries and uterine content:
The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
Gravid uterine weight, net body weight (excluding the weight of the uterus and contents) and net body weight change were presented for each gravid female.
- Number of corpora lutea: Yes, in each ovary
- Number of implantations: Yes
- Number of early resorptions: Yes
- Number of late resorptions: Yes
- Other: Uteri with no macroscopic evidence of nidation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss.

Fetal examinations:
- External examinations: Yes: all per litter, including but not limited to eyes, palate and external orifices. Crown-rump measurements for late resorptions. Sex was determined.
- Soft tissue examinations: Yes: all per litter, heart and major vessels, kidneys
- Skeletal examinations: Yes: all per litter
- Head examinations: Yes: all per litter, brain was examined by a mid-coronal slice

Statistics:
All statistical tests were performed by a Digital MicroVAX 3400 computer with appropriate programming. All analyses were conducted using two-tailed tests for a minimum significance level of 5% comparing each treated group to the vehicle control group. Each mean was presented with the standard deviation and the number of animals used.

Foetal sex ratios: Chi-square test with Yate's correction factor
Malformations and variations: Fishers Exact test
Early and late resorptions, dead foetuses, postimplantation losses: Mann-Whitney U-test
Corpora Lutea, total implantations, viable foetuses, foetal bodyweights, maternal bodyweights and weight changes, maternal net bodyweight changes and gravid uterine weights, maternal food consumption: ANOVA with Dennett's test
Litter proportions of intrauterine data: Kruskal-Wallis test

Findings **Details on maternal toxic effects:**

Maternal toxicity
At 20 mg /kg bw/day, 1 dam died on GD 16, at necropsy 3 normally developing implantations and 5 early resorptions were observed *in utero* (no clinical signs were noted in this female during the study). At 20 mg/kg bw/day, 1 dam aborted on GD 22 (this dam was hypoactive on GD 20-21) 7 late resorptions and 2 early resorptions; No statistically significant changes in mean body weight and body weight, mean gravid uterine weights and food consumption were reported for any of the dose groups.

Effect levels (foetuses)

NOAEL: 20 mg/kg bw/day (actual dose received)

The number of foetuses (litters) available for morphological examination were: 124(20), 129(19), 119(19) and 103(16) at 0, 2, 10 and 20 mg/kg bw, respectively.

External malformations were observed in 3, 0, 0, and 1 foetuses at 0, 2, 10 and 20 mg/kg bw, respectively. In the control group, 2 foetuses with short tail and 1 foetus with omphalocele were reported. One foetus with carpal flexure on the right front limb was reported at 1.8 mg B/kg bw.

Soft tissue malformations were observed in 1, 0, 0 and 3 foetuses at 0, 2, 10 and 20 mg/kg bw, respectively. At 20 mg/kg bw, one foetus with diaphragmatic hernia was observed. Hydrocephaly (consisting of increased cavitation of the lateral ventricles) was seen in 2 foetuses from separate litters at 20 mg/kg bw. The incidence of foetuses within the same dose group (1.9%) with hydrocephaly was within the historical data range (2.9%), whereas the incidence of litters was over the value in historical control data (12.5% vs. 10.5%). Due to the historical control data showing an increased incidence of hydrocephaly (in studies performed before 1990, in 13/71 data sets, one or more foetuses had hydrocephaly; in studies performed 1990-1993, 4/17 data sets contained one or more foetuses with hydrocephaly), the study director concluded that this developmental effect could not have been conclusively ascribed to the treatment.

Skeletal malformations were observed in 7, 1, 5 and 3 foetuses at 0, 2, 10 and 20 mg/kg bw, respectively.These malformations consisted of:

- in the control group, vertebral anomalies with/without associated rib anomaly in 6 foetuses, and severely malaligned sternebrae with a rib anomaly in 1 foetus;
- an extra site of ossification anterior to sternebra no. 1 was observed in 1 foetus at 2 mg/kg bw;
- at 10 mg/kg bw, vertebral anomalies with/without associated rib anomaly in 4 foetuses, and costal cartilage anomaly with fused sternebrae in 1 foetus;
- at 20 mg/kg bw/day, vertebral anomalies with/without associated rib anomaly in 2 foetuses, and an extra site of ossification anterior to sternebra no. 1 in 1 foetus.

Conclusion **Conclusions:**
No evidence of teratogenicity or developmental toxicity was seen in this study in the rabbit at dose levels sufficient to cause maternal toxicity.

3.10.1.9 [Study 9] Prenatal developmental toxicity of boric acid in rats (non-guideline)

Reference Price, C. J., Strong, P. L., Marr, M. C., Myers, C. B. and Murray, F. J. (1996a). Developmental toxicity NOAEL and postnatal recovery in rats fed boric acid during gestation. Fundamental and applied toxicology, 32(2), 179-193.

Guideline No test guideline followed
GLP guideline

Reliability Klimisch 1: reliable without restriction (according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / Rat, Sprague-Dawley (female)

strain

Test material

Boric acid

Purity: 98%

Study design

Materials and methods

Route of administration: oral, diet

Exposure phase I: days 0 - 20 post mating

Exposure phase II: days 0 - 20 post mating, then on normal diet until termination on day 21 postpartum

Doses / Concentrations: 0, 250, 500, 750, 1000, 2000 ppm boric acid equivalent to 0, 19, 36, 55, 76 and 143 mg boric acid/kg bw/day, respectively (equivalent to 0, 3.3, 6.3, 9.6, 13.3 and 25 mg B/kg bw/day)

No. of animals: groups of 14 -17 females/dose group/phase

Assessment of prenatal development (Phase I): Timed-mated females were weighed on gd 0, 3, 6, 9, 12, 15, 18, 19, and 20. Clinical signs were recorded daily (gd 0-20). Maternal food and water intake were measured from gd 0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, 18 to 19, and 19 to 20. On gd 20, timed-mated females were terminated by CO2 asphyxiation. At termination of each timed-mated female, pregnancy was confirmed by uterine examination, and the body, liver, right kidney, and uterus were weighed. Uteri with no visible implantation sites were stained with 10% ammonium sulfide to visualize very early resorption sites (Salewski, 1964). The numbers of ovarian corpora lutea, uterine implantation sites, resorptions, dead fetuses, and live fetuses were recorded. Fetuses were dissected from the uterus and anesthesia was induced by hypothermia (Lumb and Jones, 1973; Blair, 1979). Individual fetuses were weighed and live fetuses examined for external malformations (including cleft palate) and variations. For ~50% of the fetuses, sex was determined by external examination prior to evisceration under terminal cold anesthesia. Remaining fetuses were decapitated and examined internally using a fresh tissue dissection method (Staples, 1974; Stuckhardt and Poppe, 1984). Fetal heads were fixed in Bouin's solution, sectioned free-hand, and examined for malformations and variations (Wilson, 1965). All fetal carcasses (~50% without heads) were macerated, stained with alcian blue/alizarin red S (Marr *et al.,* 1988), and evaluated for skeletal malformations and variations.

Assessment of postnatal development (Phase II): Timed-mated females were weighed periodically during gestation (see Phase I, above), and dams with litters were weighed on postnatal days (pnd) 0, 4, 7, 14, and 21. Clinical signs were recorded daily (gd 0-pnd 21). Maternal food and water consumption were measured periodically during gestation (see Phase I, above) and also from pnd 0 to 4, 4 to 7, 7 to 14, and 14 to 21. Litters were delivered naturally and reared to weaning age (pnd 21). Sex was determined by anal/genital distance on pnd 0 and 4. On pnd 4, pups were identified within litters by applying tattoo ink to the paws. Clinical signs were recorded each morning, and morbidity/mortality checks were conducted each afternoon. On pnd 0, 4, 7, 14, and 21, the number of live or dead pups/litter was recorded, pups were weighed and examined externally.

Timed-mated females which delivered a litter were terminated by CO2 asphyxiation upon death of the entire litter or on pnd 21. Maternal liver and right kidney were weighed, and uterine implantation sites were counted. If no implantation sites were visible, the uterus was stained (see Phase I, above). Pups were terminated by CO2 asphyxiation on pnd 21, and —50% were examined internally for morphological anomalies and gross pathology of the viscera; heads from these pups were decapitated, fixed in Bouin's solution, sectioned free-hand, and examined for soft-tissue malformations and variations. All pup carcasses were processed for evaluation of skeletal malformations and variations (see Phase I, above). Severely debilitated or moribund animals were humanely terminated by CO2 asphyxiation (>14 days of age) or by ip injection of euthanasia solution (<14 days of age). Pups which died or were sacrificed *in extremis* during the study were examined by the same procedures described above for pups on pnd 21, condition of the specimen permitting.

Statistics: Maternal and developmental end points from each study phase were analyzed separately, and the litter was the experimental unit for all developmental measures. Statistical procedures were based on SAS software (SAS, 1989a,b, 1990a,b,c, 1992). In conjunction with analysis of variance (ANOVA), Bartlett's test for homogeneity of variance (a = 0.001) was applied to all data, and an arcsine-square root transformation was performed on litter-derived percentage data (Winer, 1962). ANOVA determined the significance of dose effects, replicate effects and dose x replicate interactions (a = 0.05). Dunnett's test (one-tailed) and Williams' test were used to compare BA-treated groups to the control group, except that a two-tailed Dunnett's test was used for maternal organ and body weight parameters, maternal food and water consumption, fetal or pup body weight,and percentage males/litter (Dunnett, 1955, 1964; Williams, 1971, 1972). A test for linear trend determined the significance of dose-response relationships. For nonparametric analyses, the x^2 test for Independence determined differences among treatment groups, the Cochran-Armitage test evaluated linear trends on proportional data, and Fisher's exact test (one-tailed) compared individual BA-treated groups to controls (a = 0.05; Cochran, 1954; Armitage, 1955; Agresti, 1990; SAS,

1992).

Findings **Maternal toxiicty**

Mortality: No maternal deaths and no treatment-related signs of toxicity were associated with BA exposure (data not shown). Pregnancy was confirmed in 25-30 dams (88-100%)/group/phase.

Body weight: Maternal body weight did not differ among groups during gestation or lactation, and weight gain was similarly unaffected (data not shown). Decreasing trends for maternal body weight or weight gain after gd 15 (data not shown), or for the gestational period as a whole, appeared to be secondary to the slight reduction in gravid uterine weight at the high dose. Thus, maternal corrected weight gain and *postpartum* body weight (data not shown) were not affected. Absolute (g) or relative (% body weight) maternal liver weight did not differ among groups on gd 20 or pnd 21. Relative, but not absolute, maternal right kidney weight was elevated in the 0.200% BA group on gd 20, but no effect was observed on pnd 21.

Food intake and water consumption: Marginally detectable, transient decreases in maternal food intake were observed during the first 3 days of exposure. The maximum decrease was 11% at the high dose. Otherwise, treatment did not appear to affect maternal food intake (data not shown). Average daily intake of BA (mg/ kg body weight/day) was calculated from maternal body weight, measured food intake, and nominal concentration of BA in feed. Boron (B) intake was calculated as 17.5% of BA intake. Relative maternal water consumption (g/kg/day) was transiently elevated at 0.05, 0.075, and 0.1% BA from gd 15 to 18 and also at 0.2% BA from gd 15 to 20 (Phase I only; data not shown), but was otherwise affected.

The percentage of dams delivering after gd 22 increased slightly with BA exposure, but differences among groups were not statistically significant. The range of delivery days (gd 21-23) was within the historical control range (Charles River, 1993).

Table: Maternal toxicity on GD 0-20, phase I and II

	0.0	0.025	0.050	0.075	0.100	0.200
Subjects (Dams)						
No. treated (Total)	60	60	60	60	60	60
No. removed (Total)[b]	1	0	0	0	1	1
No. dead or euthanized (Total)	0	0	0	0	0	0
No. (%) pregnant						
Phase I (teratology study)	27 (96)	29 (91)	27 (96)	29 (100)	30 (97)	27 (90)
Phase II (postnatal study)	29 (94)	27 (96)	28 (88)	29 (94)	25 (89)	28 (97)
Maternal food consumption (g/day)[c]						
Phase I (gd 0 to 20)	23.8 ± 0.4	24.0 ± 0.5	23.6 ± 0.5	23.7 ± 0.4	24.2 ± 0.5	22.8 ± 0.5
Phase II (gd 0 to 20)	23 3 ± 0 5	23.6 ± 0.5	24.0 ± 0.5	24.1 ± 0.5	23.7 ± 0.5	23.2 ± 0.4
Maternal relative food consumption (g/kg/day)[c]						
Phase I (gd 0 to 20)	72.0 ± 0.9	74.5 ± 1.2	72.4 ± 1.1	73.4 ± 0.9	75.9 ± 1 0*	71.5 ± 1.2
Phase II (gd 0 to 20)	72.2 ± 1.2	73 8 ± 1.3	74 4 ± 1.3	74.3 ± 1.0	74.0 ± 1.3	72.3 ± 1.3
Calculated chemical dose (mg/kg/day)[c]						
Boric Acid[d]						
Phase I (gd 0 to 20)		18.6 ± 0.3	36.2 ± 0.6	55.1 ± 0.7	75.9 ± 1.0	142.9 ± 2.5
Phase II (gd 0 to 20)		18.5 ± 0.3	37.2 ± 0.7	55.7 ± 0.8	74.0 ± 1.3	144.6 ± 2.6
Boron[e]						
Phase I (gd 0 to 20)		3.3 ± 0.1	6.3 ± 0.1	9.6 ± 0.1	13.3 ± 0.2	25.0 ± 0.4
Phase II (gd 0 to 20)		3.2 ± 0.1	6.5 ± 0.1	9.7 ± 0.1	12.9 ± 0.2	25.3 ± 0.4
Maternal weight change (g)[c]						
Treatment (gestational)[f]						
Phase I (gd 0 to 20)§	150 ± 5	157 ± 4	153 ± 4	146 ± 5	150 ± 4	140 ± 5
Phase II (gd 0 to 20)	162 ± 3	159 ± 4	163 ± 4	165 ± 4	157 ± 5	156 ± 4
Corrected gestational[f,g]						
Phase I	62 ± 2	67 ± 3	62 ± 2	61 ± 3	64 ± 3	61 ± 3
Maternal organ weights[c]						
Absolute maternal organ weights (g)						
Gravid uterine weight						
Phase I (gd 20)§	88 ± 4	90 ± 3	91 ± 3	85 ± 4	86 ± 4	79 ± 3
Liver Weight						
Phase I (gd 20)	17 ± 0 3	18 ± 0.4	17 ± 0.3	17 ± 0.3	17 ± 0.3	17 ± 0.4
Phase II (pnd 21)	16 ± 0.4	15 ± 0.4	16 ± 0.4	17 ± 0.5	16 ± 0 5	15 ± 0.3
Right kidney weight						
Phase I (gd 20)	1.17 ± 0.02	1.25 ± 0.03	1 21 ± 0.03	1.21 ± 0.03	1.21 ± 0.02	1.26 ± 0.03
Phase II (pnd 21)	1.42 ± 0.03	1.42 ± 0.03	1.46 ± 0.05	1.53 ± 0.06	1.41 ± 0.03	1.44 ± 0.04
Relative maternal organ weights (% body weight)[f]						
Liver weight						
Phase I (gd 20)	4.08 ± 0.03	4.26 ± 0.06	4.14 ± 0.06	4.17 ± 0.06	4.21 ± 0.06	4.19 ± 0.06
Phase II (pnd 21)	5.11 ± 0.10	5 00 ± 0.10	5.13 ± 0.07	5.26 ± 0.11	5.06 ± 0.10	5.03 ± 0.09
Right kidney weight						
Phase I (gd 20)§	0 29 ± 0.01	0.31 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0 32 ± 0.01*
Phase II (pnd 21)	0 46 ± 0.01	0.46 ± 0.01	0 47 ± 0.01	0.49 ± 0.02	0.45 ± 0.01	0 48 ± 0.01

[a] Phase I, teratologic evaluation; Phase II, postnatal evaluation.
[b] Each dam was removed for one of the following reasons: postnatal day (pnd) 0 not determined (Phase II), early delivery of a litter (Phase I), or sipper tube malfunction (Phase I).
[c] Mean ± SEM.
[d] Food consumption (g/kg/day) × 1000 mg/g × (% boric acid in feed/100)
[e] Boric acid intake (mg/kg/day) × 0.175.
[f] Maternal gestational and corrected weight gains and relative organ weights (% body weight or % adjusted weight) were calculated using body weight at the time of sacrifice (gd 20) for animals in Phase I. "Adjusted weight" is defined as the maternal body weight at sacrifice on gd 20 minus the gravid uterine weight. Relative organ weights (% body weight) were calculated using body weight at the time of sacrifice (pnd 21) for animals in Phase II.
[g] Weight gain during gestation minus gravid uterine weight.
§ $p < 0.05$; test for linear trend.
* $p < 0.05$; Dunnett's test or Williams' test; pairwise comparison to the concurrent vehicle control (0.0%) group.

Embryo/fetal and pup effects

Pup viability: On gd 20, the number of ovarian corpora lutea/dam, number of implantation sites/ litter, percentage preimplantation loss/litter, and live litter size were equivalent among groups. Likewise, the percentages of resorptions or late fetal deaths were not affected. Spurious decreases in the percentage of litters with at least one resorption were not dose-related. The control incidence of resorptions was higher than expected, but the incidence among BA-exposed groups was similar to historical data for this species and strain (RTI, unpublished; Charles River, 1993). During lactation, the number and percentage of pup deaths/litter exhibited increasing trends, but the number of implantation sites/litter, cumulative offspring mortality (i.e., percentage of implantation sites) and number of live pups/litter did not differ among groups. Thus, there was no definitive evidence for an adverse effect on offspring viability from conception through weaning.

Foetal body weight: On gd 20, fetal body weight was significantly decreased by 6 and 12% at 0.1 and 0.2% BA, respectively. Fetal body weight deficits on gd 20 did not persist into the postnatal period.

Table: Developmental toxicity following maternal exposure to boric acid during GD 0-20

		Boric acid (% in feed)					
		0.0	0.025	0.050	0.075	0.100	0.200
No. confirmed pregnancies	(gd 20)	27	29	27	29	30	27
	(pnd 0)	29	27	28	29	25	28
No. (%) dams delivering,[b,c]	(≤gd 22)§	26 (90)	22 (81)	22 (79)	25 (86)	16 (64)	19 (68)
	(gd 23)	3 (10)	5 (10)	6 (21)	4 (14)	9 (36)	9 (32)
No. corpora lutea/dam	(gd 20)	19.4 ± 0.7	19.3 ± 0.3	19.4 ± 0.7	17.9 ± 0.6	19.0 ± 0.6	19.0 ± 0.7
No. implantation sites/dam	(gd 20)	16.4 ± 0.7	16.5 ± 0.6	16.6 ± 0.7	15.8 ± 0.7	16.4 ± 0.7	16.0 ± 0.7
	(pnd 0–21)	16.6 ± 0.5	15.5 ± 0.6	16.0 ± 0.6	15.9 ± 0.6	14.8 ± 0.9	16.5 ± 0.5
% Preimplantation loss	(gd 20)	13.8 ± 4.1	13.5 ± 2.7	13.2 ± 3.5	12.8 ± 3.7	11.5 ± 4.0	14.1 ± 4.1
% Resorptions/litter[d]	(gd 20)	9.5 ± 3.6	3.3 ± 1.0	2.6 ± 0.8	3.9 ± 0.8	6.7 ± 3.4	4.8 ± 1.1
% Litters with ≥1 resorption	(gd 20)	70	34*	33*	52	37*	48
% Late fetal deaths/litter	(gd 20)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
% Litters with ≥1 late fetal death	(gd 20)	0	0	0	7	0	0
% Litters with 100% nonlive[e]	(gd 20)	4	0	0	0	3	0
	(pnd 0)	0	0	4	0	0	4
% Postimplantation mortality/litter[e]	(gd 20)	9.5 ± 3.6	3.3 ± 1.0	2.6 ± 0.8	4.7 ± 1.0	6.7 ± 3.4	4.8 ± 1.1
	(pnd 0)	7.6 ± 1.4	6.2 ± 1.4	11.2 ± 3.7	2.5 ± 0.9	5.3 ± 1.6	9.9 ± 3.7
	(pnd 21)	8.3 ± 1.4	7.7 ± 1.5	13.4 ± 3.8	3.9 ± 1.1	7.3 ± 1.8	12.5 ± 3.6
% Postnatal mortality/litter[f]	(pnd 0 to 4)§	0.55 ± 0.31	1.17 ± 0.49	1.80 ± 1.02	1.35 ± 0.51	1.83 ± 0.71	2.80 ± 0.76
	(pnd 4 to 21)	0.38 ± 0.27	1.14 ± 0.47	0.84 ± 0.49	0.76 ± 0.45	1.00 ± 0.48	0.45 ± 0.31
	(pnd 0 to 21)§	0.93 ± 0.39	2.28 ± 0.74	2.64 ± 1.08	2.08 ± 0.72	2.83 ± 0.77	3.25 ± 0.76
No. live litters	(gd 20)	26	29	27	29	29	27
	(pnd 0)	29	27	27	29	25	27
No. live fetuses or pups/litter[g]	(gd 20)	16.0 ± 0.4	15.9 ± 0.6	16.2 ± 0.7	15.1 ± 0.7	16.2 ± 0.6	15.2 ± 0.7
	(pnd 0)	15.7 ± 0.6	14.7 ± 0.6	15.2 ± 0.6	16.0 ± 0.4	14.4 ± 0.9	15.7 ± 0.4
	(pnd 21)	15.5 ± 0.5	14.3 ± 0.6	14.7 ± 0.6	15.6 ± 0.4	14.0 ± 0.9	15.2 ± 0.4
Offspring body weight/litter (g)							
Male	(gd 20)§	3.71 ± 0.05	3.64 ± 0.05	3.62 ± 0.05	3.60 ± 0.07	3.48 ± 0.05*	3.23 ± 0.06*
	(pnd 0)	6.61 ± 0.10	6.79 ± 0.13	6.68 ± 0.13	6.53 ± 0.11	6.49 ± 0.13	6.59 ± 0.11
	(pnd 21)	43.25 ± 1.71	47.82 ± 1.91	44.29 ± 1.70	41.99 ± 1.39	43.76 ± 1.16	42.56 ± 1.19
Female	(gd 20)§	3.52 ± 0.05	3.47 ± 0.04	3.45 ± 0.06	3.38 ± 0.06	3.27 ± 0.05*	3.04 ± 0.05*
	(pnd 0)	6.21 ± 0.11	6.34 ± 0.09	6.26 ± 0.15	6.18 ± 0.10	6.29 ± 0.16	6.20 ± 0.10
	(pnd 21)	41.22 ± 1.61	44.88 ± 1.57	42.20 ± 1.77	40.27 ± 1.21	43.94 ± 1.84	40.45 ± 1.09

[a] Includes dams confirmed pregnant by uterine examination or uterine stain at necropsy on gd 20 (Phase I), confirmed pregnant on pnd 0 by the presence of a litter (Phase II), or confirmed pregnant by uterine stain at necropsy (Phase II).

[b] Includes all dams in Phase II which delivered a litter.

[c] The overall χ^2 test for differences among groups for gestational Days 21, 22, and 23 was not significant ($p = 0.315$). When the proportions were expressed as ≤gd 22 vs gd 23, the overall χ^2 test was also not significant ($p = 0.144$), but the Cochran–Armitage Trend Test was $p = 0.0200$.

[d] Litter size = No. implantations sites/dam; mean ± SEM.

[e] On gd 20, "nonlive" = late fetal deaths plus resorptions. For pnd 0, "nonlive" = late fetal deaths, plus resorptions plus dead pups. There were no full litter losses after pnd 0.

[f] Calculations exclude pregnant dams which had 100% *prenatal* mortality, but includes dams with dead pups found on pnd 4.

[g] Includes all dams with live fetuses (gd 20) or live pups (pnd 0); litter size = No. live fetuses on gd 20 or live pups on pnd 0/dam; mean ± SEM.

§ $p < 0.05$; test for linear trend or Cochran–Armitage trend test.

* $p < 0.05$; Dunnett's test, Williams' test, or Fisher exact test pairwise comparison to the concurrent vehicle control (0.0%) group.

Malformations: On gd 20 and pnd 21, external malformations and variations occurred with low incidences (<0.5% fetuses or pups/ group) which showed no apparent dose-response relationships. Visceral malformations and variations also failed to show dose-related increases following BA exposure. Among the visceral findings on gd 20, enlarged lateral ventricles, hydronephrosis, hydroureter, and distended ureter are relatively common findings for this species and strain in our laboratory.

Table: Morphological defects in rat foetuses following maternal exposure to boric acid on GD 0-20 (Phase I)

	Boric acid (% in the feed)					
	0.000	0.025	0.050	0.075	0.100	0.200
Total No. fetuses examined[b]						
External or skeletal	417	461	437	437	471	411
Visceral	211	226	220	221	236	209
Total No. litters examined[c]						
External, skeletal, or visceral	26	29	27	29	29	27
Any malformations						
No. of fetuses with any malformations[d]	92	94	103	96	112	117
% Fetuses with any malformations	22.1	20.4	23.6	22.0	23.8	28.5
No. of litters with any malformations[e]	20	24	23	27	24	26
% Litters with any malformations	76.9	82.8	85.2	93.1	82.8	96.3
External malformations						
No. of fetuses with external malformations[d]	2	0	2	2	0	1
% Fetuses with external malformations	0.5	0.0	0.5	0.5	0.0	0.2
No. of litters with external malformations[e]	2	0	1	2	0	1
% Litters with external malformations	7.7	0.0	3.7	6.9	0.0	3.7
Agnathia	1 (1)					
Anasarca	1 (1)			2 (2)		1 (1)
Fused digits: hindpaw	1 (1)					
Microdactyly: forepaw			2 (1)			
Visceral malformations						
No. of fetuses with visceral malformations[d]	84	91	97	86	100	99
% Fetuses with visceral malformations	39.8	40.3	44.1	38.9	42.4	47.4
No. of litters with visceral malformations[e]	20	23	23	24	23	22
% Litters with visceral malformations	76.9	79.3	85.2	82.8	79.3	81.5
Hydrocephaly: mild		1 (1)				1 (1)
Enlarged lateral ventricle(s): partial, half, full[f]	69 (19)	74 (21)	77 (20)	71 (17)	78 (20)	84 (19)
Hydronephrosis: right		1 (1)	1 (1)			
Hydroureter: bilateral, right, or left[f]	11 (3)	16 (4)	18 (10)	14 (13)	16 (7)	8 (4)
Skeletal malformations						
No. of fetuses with skeletal malformations[d]	9	4	8	11	17	19
% Fetuses with skeletal malformations	2.2	0.9	1.8	2.5	3.6	4.6
No. of litters with skeletal malformations[e]	7	3	6	10	9	8
% Litters with skeletal malformations	26.9	10.3	22.2	34.5	31.0	29.6
Cleft sternum	1 (1)					
Fused sternebrae		1 (1)				
Abnormally arched rib	1 (1)					
Agenesis of Rib XIII: bilateral or right[f]	1 (1)				1 (1)	
Fused ribs or fused rib cartilage[f]	3 (3)			2 (2)	1 (1)	
Rib cartilage not attached to sternum: ribs I–VII		1 (1)			2 (1)	1 (1)
Severe delay of normal ossification of rib, cartilage normal				1 (1)	3 (2)	1 (1)
Short rib: XIII			1 (1)	3 (2)	7 (4)	14 (6)
Agenesis of the vertebra: lumbar					1 (1)	
Agenesis of the centrum: lumbar	1 (1)					
Bipartite cartilage: normal or dumbbell ossification center						
Thoracic centrum	3 (3)	2 (2)	3 (3)	2 (2)		2 (2)
Bipartite cartilage: bipartite, unilateral or unossified ossification center						
Thoracic centrum	4 (4)	1 (1)	5 (4)	3 (3)	4 (3)	1 (1)
Fused cartilage: thoracic centrum	1 (1)					
Agenesis of the arch: lumbar	1 (1)					
Fused arches: cervical					1 (1)	1 (1)
Misaligned arch	1 (1)					
Any variations						
No. of fetuses with any variations[d]	43	20	33	24	34	52
% Fetuses with any variations	10.3	4.3	7.6	5.5	7.2	12.7
No. of litters with any variations[e]	19	12	17	12	17	18
% Litters with any variations	73.1	41.4	63.0	41.4	58.6	66.7
External variations						
No. of fetuses with external variations[d]	1	0	0	2	0	0
% Fetuses with external variations	0.2	0.0	0.0	0.5	0.0	0.0
No. of litters with external variations[e]	1	0	0	2	0	0
% Litters with external variations	3.8	0.0	0.0	6.9	0.0	0.0
Hematoma: head	1 (1)					
Clubbed limb without bone change				2 (2)		
Visceral variations						
No. of fetuses with visceral variations[d]	3	4	6	0	3	1
% Fetuses with visceral variations	1.4	1.8	2.7	0.0	1.3	0.5
No. of litters with visceral variations[e]	3	3	4	0	3	1
% Litters with visceral variations	11.5	10.3	14.8	0.0	10.3	3.7
Distended ureter, bilateral, left or right	3 (3)	4 (3)	6 (5)		3 (3)	1 (1)
Skeletal variations						
No. of fetuses with skeletal variations[d]	41	16	28	23	31	51
% Fetuses with skeletal variations	9.8	3.5	6.4	5.3	6.6	12.4
No. of litters with skeletal variations[e]	18	10	14	12	16	17
% Litters with skeletal variations	69.2	34.5	51.9	41.4	55.2	63.0
Misaligned sternebrae	1 (1)		1 (1)	1 (1)		2 (2)
Rib abnormally thickened					1 (1)	
Full rib on lumbar I: bilateral, left, or right[f]	2 (2)		3 (2)	1 (1)	2 (2)	
Rudimentary rib on lumbar I: bilateral, left, or right[f]	12 (7)	6 (4)	11 (5)	4 (3)	5 (5)	1 (1)
Wavy rib or wavy rib cartilage[f]		2 (1)		4 (2)	10 (6)	41 (13)
Normal cartilage, bipartite ossification center: thoracic centrum	18 (13)	6 (4)	13 (7)	7 (7)	10 (4)	4 (4)
No ossification center: thoracic centrum	4 (2)	1 (1)				
Dumbbell cartilage: normal ossification center: thoracic centrum	1 (1)					
Dumbbell ossification center, thoracic centrum	4 (4)		2 (1)	6 (3)	3 (3)	3 (1)
Bipartite ossification center: thoracic centrum	3 (2)	2 (2)		2 (2)		1 (1)
Normal cartilage: bipartite ossification center: lumbar centrum	2 (1)					
Unilateral ossification center: lumbar centrum	1 (1)					
Normal cartilage, no ossification center: sacral centrum	1 (1)					
Incomplete ossification, ischium and ilium						1 (1)
Extra ossification site: sternebrae I and II					1 (1)	

[a] The incidence of individual defects is expressed as the number of individual fetuses exhibiting that defect. Thus, a single fetus may be represented more than once in listing individual defects. Data indicate number of fetuses (with number of litters shown in parentheses). Statistical analyses of these data are presented in Table 5.

[b] Only live fetuses were examined.

[c] Includes only litters with live fetuses.

[d] Fetuses with one or more malformations/variations.

[e] Litters with one or more malformed/variant fetuses.

[f] Summarized incidence for all subclassifications of this finding.

Table: Morphological Defects in 21 Day Old Rat Pups Following Maternal Exposure to Boric Acid on Gestational Days 0 to 20: Listing by Defect Type (Phase II)

	Boric acid (% in the feed)					
	0.000	0.025	0.050	0.075	0.100	0.200
Total No. fetuses examined[b]						
External or skeletal	450	386	398	452	349	410
Visceral	236	204	214	241	188	218
Total No. litters examined[c]						
External, skeletal or visceral	29	27	27	29	25	27
Any malformations						
No. of pups with any malformations[d]	38	40	17	22	23	24
% Pups with any malformations	8.4	10.4	4.3	4.9	6.6	5.9
No. of Litters with any malformations[e]	10	17	12	14	13	11
% Litters with any malformations	34.5	63.0	44.4	48.3	52.0	40.7
External malformations						
No. of pups with external malformations[d]	0	1	0	0	0	0
% Pups with external malformations	0.0	0.3	0.0	0.0	0.0	0.0
No. of litters with external malformations[e]	0	1	0	0	0	0
% Litters with external malformations	0.0	3.7	0.0	0.0	0.0	0.0
Anophthalmia: bilateral		1 (1)				
Visceral malformations						
No. of pups with visceral malformations[d]	38	32	15	21	19	8
% Pups with visceral malformations	16.1	15.7	7.0	8.7	10.1	3.7
No. of litters with visceral malformations[e]	10	11	11	14	11	6
% Litters with visceral malformations	34.5	40.7	40.7	48.3	44.0	22.2
Porencephaly (enlarged anterior horn of the lateral ventricle on the left side with communication to the subdural space)				1 (1)		
Enlarged third ventricle: mild	6 (4)					
Enlarged third ventricle, severe (round cavity with evidence of hemorrhaging)			1 (1)			
Enlarged lateral ventricle(s): partial, half, or full[f]	34 (9)	31 (11)	14 (11)	20 (13)	19 (11)	8 (6)
Ventral abdominal hernia		1 (1)				
Skeletal malformations						
No. of pups with skeletal malformations[d]	0	7	2	1	5	16
% Pups with skeletal malformations	0.0	1.8	0.5	0.2	1.4	3.9
No. of litters with skeletal malformations[e]	0	6	1	1	3	6
% Litters with skeletal malformations	0.0	22.2	3.7	3.4	12.0	22.2
Agenesis of rib XIII, right						2 (2)
Fused sternebrae		2 (1)			2 (1)	
Short rib, XIII		5 (5)	2 (1)	1 (1)	3 (2)	16 (6)
Any variations						
No. of pups with any variations[d]	30	35	20	23	17	20
% Pups with any variations	6.7	9.1	5.0	5.1	4.9	4.9
No. of litters with any variations[e]	12	14	12	10	9	10
% Litters with any variations	41.4	51.9	44.4	34.5	36.0	37.0
External variations						
No. of pups with external variations[d]	0	0	0	0	0	2
% Pups with external variations	0.0	0.0	0.0	0.0	0.0	0.5
No. of litters with external variations[e]	0	0	0	0	0	1
% Litters with external variations[e]	0.0	0.0	0.0	0.0	0.0	3.7
Dome shaped head and sunken eyes						2 (1)
Visceral variations						
No. of pups with visceral variations[d]	0	0	0	0	0	0
% Pups with visceral variations	0.0	0.0	0.0	0.0	0.0	0.0
No. of litters with visceral variations[e]	0	0	0	0	0	0
% Litters with visceral variations	0.0	0.0	0.0	0.0	0.0	0.0
Skeletal variations						
No. of pups with skeletal variations[d]	30	35	20	23	17	18
% Pups with skeletal variations	6.7	9.1	5.0	5.1	4.9	4.4
No. of litters with skeletal variations[e]	12	14	12	10	9	9
% Litters with skeletal variations	41.4	51.9	44.4	34.5	36.0	33.3
Wavy Rib			1 (1)	1 (1)		
Normal cartilage, bipartite ossification center: lumbar centrum		1 (1)				
Thoracic centrum	16 (8)	18 (9)	9 (5)	16 (7)	11 (5)	11 (6)
Extra ossification site between sternebrae V and VI	15 (6)	19 (8)	10 (8)	6 (5)	7 (5)	7 (6)

[a] The incidence of individual defects is expressed as the number of individual fetuses exhibiting that defect. Thus, a single fetus may be represented more than once in listing individual defects. Data indicate number of fetuses (with number of litters shown in parentheses). Statistical analyses of these data are presented in Table 5.

[b] Only live pups were examined.

[c] Includes only litters with live pups.

[d] Pups with one or more malformations/variations.

[e] Litters with one or more malformed/variant pups.

[f] Summarized incidence for all subclassifications of this finding

On gd 20, the percentage fetuses with skeletal malformations/ litter showed a significant increasing trend, but the overall incidence of skeletal variations was not affected. Short rib XIII (classified as a malformation) and wavy rib (classified as a variation) were each significantly increased in the 0.1 and 0.2% BA groups relative to controls on gd 20. Short rib XIH occurred in 0, 0, 0.2, 0.7, 1.5, and 3.4% of fetuses from the control through high-dose groups, respectively, and wavy rib (or wavy rib cartilage) occurred in 0, 0.4, 0, 0.9, 2.1, and 10% of fetuses. A significant decreasing trend was found for rudimentary (but not full) extra rib on lumbar I (classified as a variation), and a slight, but not statistically significant, decrease in the incidence of this finding was noted in the high-dose group on gd 20. Thus, the incidence of extra rib on lumbar I (full and/or rudimentary) was 3.1, 1.3, 2.8, 0.9, 1.5, and 0.2% of fetuses (gd 20) for the control through high-dose groups, respectively.

On pnd 21, the overall incidence of skeletal malformations, showed a significantly increased incidence in the low and high dose groups relative to the control group. The incidence of short rib was 0, 1.3, 0.5, 0.2, 0.9, and 3.9% of pups examined in the control through highdose groups, respectively. Based upon the absence of a dose-response

relationship for short rib XIII across the nearly 10-fold concentration range between the low and high dose, findings of short rib XIII at concentrations below 0.2% BA in the diet did not appear to be treatment-related. On pnd 21, the incidence of wavy rib (a variation) was low and not dose related, i.e., 0, 0, 0.3, 0.2, 0, and 0% in the control through high dose groups, respectively. On pnd 21, there were no pups in the control or treated groups with extra rib on lumbar I (full or rudimentary).

Table: Summary and analysis of skeletal defects in foetuses (GD 20) and pupd (PND 21)

	Boric acid (% in feed)					
	0.0	0.025	0.050	0.075	0.100	0.200
% Offspring with skeletal malformations/litter						
gd 20§	2.0 ± 0.7	0.9 ± 0.6	1.6 ± 0.6	2.5 ± 0.7	3.5 ± 1.2	4.3 ± 1.5
pnd 21§	0.0 ± 0.0	2.0 ± 0.8*	0.6 ± 0.6	0.2 ± 0.2	1.3 ± 0.8	3.9 ± 1.8*
% Litters with skeletal malformations						
gd 20	27	10	22	34	31	30
pnd 21	0	22*	4	3	12	22*
% Offspring with skeletal variations/litter						
gd 20	10.0 ± 2.0	3.4 ± 1.0	6.5 ± 1.8	5.3 ± 1.4	7.4 ± 2.1	12.1 ± 3.0
pnd 21	6.8 ± 1.9	9.6 ± 2.4	4.9 ± 1.3	4.9 ± 1.5	4.4 ± 1.6	4.8 ± 1.9
% Litters with skeletal variations						
gd 20	69	34	52	41	55	63
pnd 21	41	52	44	34	36	33
% Offspring with short rib XIII/ litter						
gd 20§	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.6 ± 0.5	1.4 ± 0.7*	3.2 ± 1.2*
pnd 21§	0.0 ± 0.0	1.5 ± 0.6	0.6 ± 0.6	0.2 ± 0.2	0.6 ± 0.5	3.9 ± 1.8*
% Litters with short rib XIII						
gd 20§	0	0	4	7	14	22*
pnd 21	0	19*	4	3	8	22*
% Offspring with wavy rib/litter						
gd 20§	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.8 ± 0.7	2.1 ± 0.9*	9.9 ± 3.0*
pnd 21	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
% Litters with wavy rib						
gd 20§	0	3	0	7	21*	48*
pnd 21	0	0	4	3	0	0
% Offspring with rudimentary lumbar I rib/litter[b]						
gd 20§	3.0 ± 1.3	1.4 ± 0.8	3.0 ± 1.6	1.3 ± 0.8	1.0 ± 0.4	0.2 ± 0.2
pnd 21	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
% Litters with rudimentary lumbar I rib						
gd 20§	27	14	19	10	17	4
pnd 21	0	0	0	0	0	0

[a] Includes all dams with live fetuses (gd 20) or live pups (pnd 21): litter size = No. live fetuses on gd 20 or live pups on pnd 21/dam; mean ± SEM.
[b] The average incidence/group or full lumbar I rib ranged from 0 to 1.1% fetuses/litter on gd 20, but the incidence was not dose-related. On pnd 21, there were no pups with full rib on lumbar I.
§ $p < 0.05$; test for linear trend or linear trend or proportions.
* $p < 0.05$; Dunnett's test, Williams' test, or Fisher exact test pairwise comparison to the concurrent vehicle control (0.0%) group.

Conclusion In conclusion, the NOAELs for developmental toxicity in the prenatal and postnatal phases of this study were 0.075% BA (55 mg/kg/day) and 0.1% BA (74 mg/kg/day) in feed, respectively.

3.10.1.10 [Study 10] Assessment of blood boron concentrations in pregnant rats (boric acid)

Reference Price, C. J., Strong, P. L., Murray, F. J., & Goldberg, M. M. (1997). Blood boron concentrations in pregnant rats fed boric acid throughout gestation. Reproductive Toxicology, 11(6), 833-842.

Guideline No test guideline followed
GLP guideline

Reliability Klimisch 1: reliable without restriction (according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Rat, Sprague-Dawley (female)

Test material Boric acid
Purity: 98%

Study design

Materials and methods

Route of administration: oral, diet

Exposure phase I: days 0 - 20 post mating

Exposure phase II: days 0 - 20 post mating, then on normal diet until termination on day 21 postpartum

Doses / Concentrations: 0, 250, 500, 750, 1000, 2000 ppm boric acid equivalent to 0, 19, 36, 55, 76 and 143 mg boric acid/kg bw/day, respectively (equivalent to 0, 3.3, 6.3, 9.6, 13.3 and 25 mg B/kg bw/day)

No. of animals: groups of 14 -17 females/dose group/phase

Assessment of prenatal development (Phase I): Timed-mated females were weighed on gd 0, 3, 6, 9, 12, 15, 18, 19, and 20. Clinical signs were recorded daily (gd 0-20). Maternal food and water intake were measured from gd 0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, 18 to 19, and 19 to 20. On gd 20, timed-mated females were terminated by CO2 asphyxiation. At termination of each timed-mated female, pregnancy was confirmed by uterine examination, and the body, liver, right kidney, and uterus were weighed. Uteri with no visible implantation sites were stained with 10% ammonium sulfide to visualize very early resorption sites (Salewski, 1964). The numbers of ovarian corpora lutea, uterine implantation sites, resorptions, dead fetuses, and live fetuses were recorded. Fetuses were dissected from the uterus and anesthesia was induced by hypothermia (Lumb and Jones, 1973; Blair, 1979). Individual fetuses were weighed and live fetuses examined for external malformations (including cleft palate) and variations. For ~50% of the fetuses, sex was determined by external examination prior to evisceration under terminal cold anesthesia. Remaining fetuses were decapitated and examined internally using a fresh tissue dissection method (Staples, 1974; Stuckhardt and Poppe, 1984). Fetal heads were fixed in Bouin's solution, sectioned free-hand, and examined for malformations and variations (Wilson, 1965). All fetal carcasses (~50% without heads) were macerated, stained with alcian blue/alizarin red S (Marr *et al.,* 1988), and evaluated for skeletal malformations and variations.

ICP analysis of boron in animal feed and drinking Water: Boron in control feed was below the method quantitation limit (MQL) of 0.00281% BA (c28.1 pg BA/g feed). Feed for the other experimental groups contained boron equivalent to 91% to 109% of the nominal BA concentrations added to the diet. BA in feed was homogeneous [96.3% (rsd = 2.7%) or 93.8% (rsd = 1.2%) of the nominal concentration] and stable (7 d at room temperature or 65 d under refrigeration), as evidenced by the evaluation of the lowest (0.025%) and highest (0.200%) added concentrations. Deionized (DI)/filtered drinking water sampled at the beginning and end of each study replicate contained CO.30 lug BA/mL of water.

Collection and storage of maternal blood samples: At termination by CO, asphyxiation on GD 20, whole blood samples (5 mL) were drawn from the abdominal vena cava of timed-mated females. Samples were frozen at -20°C in heparin-treated Vacutainerm tubes until ready for homogenization and ashing.

Blood analysis method: The boron content of whole blood from rats was determined by ICP optical emission spectrometry. In brief, a high-temperature alkaline ashing procedure was used to volatilize the organic constituents of whole rat blood, as well as some inorganic compounds with low boiling points. An alkaline barium hydroxide reagent was added to convert boron to a stable barium borate, thus preventing boron volatilization. After ashing, the inorganic salt residue was solubilized in nitric acid and injected into the ICP (Therm0 Jarrel Ash Atomscan 16 ICP spectrometer: wavelength = 249.678 nm). Emission intensity of boron was measured and converted to boron concentration in blood. Boron is a constituent of most laboratory glassware and is leachable in acidic solutions. Thus, plastic containers were used whenever possible in the quantitative analysis of boron. For high temperature ashing, fused silica crucibles were used to minimize the loss of boron to the crucibles. Labware was precleaned with soapy water, and DI water was used for the final rinse. Plasticware and fused silica crucibles were soaked in 20% nitric acid and rinsed well with DI water.

Blood samples of sufficient volume were prepared by weighing 2 g of room temperature, homogenized whole blood into individually tared, fused silica crucibles. For samples with insufficient volume, homogenization was not performed; instead, the entire sample was weighed and used for analysis. Barium hydroxide (l%, 6 mL) was added to each crucible, and the slurry was dried on a hot plate. The crucibles were then placed in a muffle furnace (Brinkman/Thermolyne, Model 6000, F6028C) and the samples ashed overnight at 400°C (minimum of 10 h). Ashed samples were cooled in a desiccator and then removed for warm acid dissolution of the ash and salt residue. The crucibles were placed on a hot plate, and 5 mL of 1% nitric acid was added to each to dissolve all soluble materials. The samples were then filtered through Whatman no. 50 filter papers into plastic graduated cylinders using plastic funnels and prerinsed filters. After filtration, the samples were transferred to plastic autosampler vials, capped, and refrigerated at approximately 4°C until analysis by ICP. For each batch of 12 blood samples, two boron recovery standards and one reagent blank were prepared. Two calibration routines were used for boron. For the purpose of calibrating the instrument and enabling "real-time" automated QC checks during analysis, least squares first order (linear) regression curves were used to relate known boron concentrations to measured emission intensities using ICP instrument software. A second order regression, more appropriate for boron over the range of concentrations used, was applied using spreadsheet software after data collection was completed. Two boron blood matrix curves

were prepared to demonstrate that the ICP responded quantitatively to boron1 in the ashed digestate, and that boron was not lost during sample preparation. For the first curve, a series of boron spikes were added to homogenized, ashed blood. The percentage of nominal boron determined was 102.9% on average, with a range of 93.6% to 1 12.2%, indicating that the ICP responded quantitatively to boron in the ashed blood matrix over the range of boron concentrations in this study. For the second curve, a series of boron spikes were added to homogenized blood before ashing and sample preparation. Recovery of boron from these samples averaged 85% of the nominal values. Recovery correction was used for all blood samples in this study.

Correlation of blood boron concentrations on GD 20 with dietary intake and time of blood collection*:* In order to determine the degree to which calculated maternal boron intake predicted maternal blood boron concentration (µg) on CD 20, a linear regression by least-square estimation was performed using the Regression tool and Regression trcndline in EXCEL. In these cases, boron intake (mg boron/kg/d) for the entire exposure period (CD 0 to 20) or for the final 24 h of the exposure period (GD 19 to 20) represented the regressors, and maternal blood boron µg/g) was the response variable. Time of day for blood collection was also applied as a regressor for blood boron µg/g) within individual treatment groups. Results of these analyses were presented as the R2 value (adjusted for degrees of freedom), which indicates the portion, within the range of 0 to 1, of the corrected total variation attributable to the fit of the model.

For purposes of these regression analyses, an intake value of 0.35 mg boron/kg of body weight/d was assigned to each dam in the control group. This value represents the best estimate of the upper limit for average daily boron intake based on the following considerations: (a) average daily food consumption for the control group was 72 g of feed/kg body weight/d throughout gestation (GD 0 to 20) and (b) the concentration of boron in the feed was below the MQL of 0.0049 mg boron/g of feed. Ingestion of boron from filtered drinking water was considered to be negligible in all groups based on the following: (a) average maternal water consumption per group ranged from 122 to 137 g H2O/kg body weight/day; and (b) concentration of boron in filtered drinking water was below the MQL of 0.05 µg boron/mL. Thus, the average contribution of drinking water to boron intake for treatment groups in this study was <006 to 0.007 mg boron/kg of body weight/d. Maternal body weight and food intake measurements, which formed the basis for calculated boron intake per dam, were completed on the morning of GD 20, followed by termination of access to feed with added BA. Dams were then transferred to the necropsy laboratory for euthanasia, blood collection, and dissection. Due to the large numbers of animals evaluated for maternal and embryo/fetal endpoints on each necropsy day, the time of day for maternal blood collection varied for individual animals between 7: 16 a.m. and 4:44 p.m. Time of day was used as an approximate index for the time lag between last access to feed containing added BA and maternal blood collection.

Correlation of selected maternal and developmental toxicity endpoints with blood boron concentrations on GD 20: Indices of exposure, specifically individual maternal boron intake (mg/kg/d), maternal blood boron concentrations on GD 20, or nominal concentration of BA in feed (%) were correlated with data from selected embryo/fetal toxicity endpoints. In order to determine the degree to which each of these exposure variables predicted toxic response, a linear regression by least-square estimation was performed using the Regres sion tool and Regression treadline in EXCEL. In these cases, the exposure parameters represent the regressors, and the developmental toxicity endpoints are the response variables. Results of these analyses are presented as the R^2 adjusted for degrees of freedom.

Findings

Blood boron concentrations in confirmed-pregnant rats on GD 20: The method detection limit (MDL) was 0.105 µg boron/g of blood based on 3X the SD of reagent blanks. The MQL was 0.350 pg boron/g blood, calculated as 10X the SD of reagent blanks. Thus, almost all (21 of 24) of the boron concentrations in the control group and 2 of 29 in the low-dose group fell between the MDL and the MQL. All remaining samples were above the MQL. Blood samples from timed-mated female rats on GD 20 had boron concentrations that were clearly related to the concentrations of BA fed in the diet. Confirmed-pregnant females from the control through the high-dose groups exhibited average concentrations of 0.229, 0.564, 0.975, 1.27, 1.53, and 2.82 pg boron/g whole blood, respectively. Blood boron concentrations were significantly elevated above controls for all treatment groups. Concentrations of 1.27 2 0.298 *µ*g boron/g (range 0.705 to 1.75 µg/g) or 1.53 & 0.546 µg boron/g (range 0.222 to 2.47 pg boron/g) were associated with the previously reported developmental toxicity NOAEL and LOAEL of 10 and 13 mg boron/ kg/d, respectively.

Correlation of blood boron concentrations on GD 20 with dietary intake and time of blood collection: Maternal blood boron µg/g) concentrations at termination on GD 20 showed a robust positive correlation, with measured boron intake (mg boron/kg body weight/d) expressed as either the average daily intake for the entire exposure period (GD 0 to 20) or for the final 24 h of exposure (GD 19 to 20). Linear regression analysis yielded similar results for either measure of boron intake, but recent intake (GD 19 to 20) provided the highest R2 value (0.724). Due to the similarity of outcome, only the scatter plot for GD 19 to 20 boron intake is presented.

Linear regression indicated a differential effect for time of day on maternal blood boron concentrations within individual treatment groups. Time of day had virtually no impact on maternal blood boron concentrations in the

control and lowest exposure groups (R's between -0.0163 and 0.0356), but appeared to exert a significant influence on maternal blood boron concentrations in the higher dose groups (R' values between 0.101 and 0.381). Scatter plots for these data indicated that maternal blood boron tended to be slightly lower later in the day. This finding is consistent with the use of time of day to estimate the time interval between last access to dosed feed and collection of blood samples (this study), and with a half-life of <24 h reported for rats exposed to levels higher then those encountered in the present study.

Table: Correlation of infividual animal boron intake or time of blood collection with blood boron on GD 20

Regressor	Response variable	R^{2a}
Boron intake (mg/kg/d)[b,c]	Blood boron (μg/g)	
GD 0 to 20	(all groups)	0.693
GD 19 to 20	(all groups)	0.724
Time of day for blood collection (h, min)	Blood boron (μg/g) (by treatment group)	
	Control	−0.0163
	0.025%	0.0356
	0.050%	0.298
	0.075%	0.344
	0.100%	0.101
	0.200%	0.381

[a]R^2 adjusted for degrees of freedom (see text).
[b]Boron intake for the 0.025% to 0.200% BA dose groups was calculated from individual animal food intake data as reported previously (17). Boron intake (mg boron/kg body wt/d) = food consumption (g/kg/d) × 1000 mg/g × (nominal % BA in feed/100) × 0.175.
[c]For these regression analyses, each dam in the control group was assigned an intake value of 0.35 mg boron/kg/d according to the estimated upper limit for boron intake in control feed

Correlation of selected developmental toxicity endpoints with blood boron concentrations on GD 20*:* Examples of affected and unaffected developmental toxicity endpoints were selected as response variables for regression analysis. Three related exposure indices were used as the regressors, specifically nominal concentration of BA in feed (%), individual maternal daily intake (mg boron/kg body weight/d from GD 0 to 20), and individual maternal blood boron concentrations (µg boron/g) on GD 20. The regression analyses for number of live fetuses per litter yielded R2 values close to zero for all three indices of boron exposure. This is consistent with the finding that the number of live fetuses per litter was not affected by ingestion of diets containing 0 to 0.2% BA from GD 0 to 20.

Table: Correlation of maternal blood boron levels with developmental toxicity endpoints

Response variable	Toxic effect	Regressor	R^{2a}
No. live fetuses/litter	No effect	Intake[b]	−0.00554
		Blood[c]	−0.00525
		Dose[d]	−0.00612
Female fetal body wt. (g)/litter	↓[e]	Intake	0.253
		Blood	0.340
		Dose	0.290
Male fetal body wt (g)/litter	↓[e]	Intake	0.239
		Blood	0.343
		Dose	0.273

[a]R^2 adjusted for degrees of freedom (see text).
[b]Boron intake was calculated from individual animal food intake data as reported previously (17). Boron intake (mg boron/kg body wt./d) = food consumption (g/kg/d) × 1000 mg/g × (nominal % BA in feed/100) × 0.175.
[c]Maternal blood boron concentrations (μg boron/g of whole blood) on gd 20.
[d]Nominal concentration of BA (%) added to the diet.
[e]Mean fetal body weights (g/litter) were significantly reduced in treatment groups fed 0.100% or 0.200% BA in the diet

Three embryo/fetal endpoints were affected on GD 20 following exposure to 10.1% BA in the diet from GD 0 to 20, specifically decreased fetal body weight and increased incidences of short rib and wavy rib. Data for the rib endpoints were not suitable for regression analysis, due to the large proportion of litters that exhibited no fetuses

with either of these findings in most of the dose groups. Therefore, fetal body weight was the only affected embryo/fetal endpoint used for regression analysis. Regression analyses for fetal body weight indicated a significant, though not robust, correlation between average male or female body weight per litter and each of the three exposure indices. Because R2 values were similar for male or female fetuses, the scatter plot for the female fetal body weight per litter versus maternal blood boron are provided to demonstrate the outcome of the regression analyses for fetal body weight. These results are consistent with the finding that average fetal body weight per litter on GD 20 (both sexes) was significantly reduced following exposure to 0.1% or 0.2% BA (94% or 88% of control fetal weight, respectively).

Conclusion Increasing dietary concentrations of boric acid were positively associated with whole blood boron concentrations in confirmed pregnant rats: 0.229 ± 0.143, 0.564 ± 0.211, 0.975 +/- 0.261, 1.27 ± 0.298, 1.53 ±- 0.546, or 2.82 ± 0.987 µg B/g whole blood for the control through high-dose groups.

3.10.1.11 [Study 11] Prenatal developmental toxicity study in rabbits (boric acid, non-guideline)

Reference Price, C. J., Marr, M. C., Myers, C. B., Seely, J. C., Heindel, J. J., & Schwetz, B. A. (1996b). The developmental toxicity of boric acid in rabbits. Fundamental and applied toxicology, 34(2), 176-187.

Guideline No test guideline followed (Equivalent or similar to OECD TG 414)

GLP guideline

Reliability Klimisch 1: reliable without restriction (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Rabbit / New Zealand White (female)

Test material Boric acid

Purity: unknown

Study design

Materials and methods

Route of administration: oral gavage

Exposure: days 6 - 19 post-mating.

Doses / Concentrations: 0, 62.5, 125 or 250 mg/kg bw/day boric acid, equivalent to 0, 11, 22 and 44 mg B/kg bw/day, respectively.

Artificially inseminated New Zealand White does were administered BA (62.5, 125, or 250 mg/kg) or vehicle (distilled/deionized water) once daily on the mornings of GD 6-19. Treatments were administered by gavage (po) using a rubber catheter" fitted with a syringe adapter12 and attached to a syringe of appropriate volume. Due to the limited solubility of BA in water (55 mg/ml), the dose volume was 5 ml/kg body wt adjusted daily.

Assays (UV/VIS spectrometry) conducted prior to dosing indicated that all formulations were within a range of 94-106% of the theoretical concentrations. Formulations were used within the period of demonstrated stability.

Dose selection*:* In a previous investigation, all rabbits died following oral administration of 850-1000 mg BA/kg/day for 4 consecutive days. At 800 mg BA/kg, symptoms included anorexia, weight loss, and diarrhea. Minor symptoms (unspecified) were noted at 600-700 mg BA/kg/day, and no toxic signs were reported at <500 mg BA/kg/day (Draize and Kelley,1959). Since published data from rabbits exposed orally to BA were quite limited, preliminary toxicity data were collected in order to select an appropriate dose range for the developmental toxicity study.

In the preliminary toxicity study, nonpregnant female rabbits (2/group) were dosed with BA (0 or 275 mg/kg/day, po) using a dose volume of 5 ml/kg in distilled/deionized water. No deaths or major clinical symptoms occurred following 14 consecutive days of exposure to 275 mg/kg/day. However, average food and water intake (g/animal/day) were depressed during BA exposure (29 and 26% of controls for food and water, respectively).

Average weight loss during exposure was 0.05 kg for control females and -1.0 kg for BA females. Based upon these findings, 250 mg BA/kg/day (GD 6-19) in distilled/deionized water (5 ml/kg) was selected as the high dose for the developmental toxicity study. It was assumed that this dose would produce some maternal toxicity while allowing survival of all treated females through scheduled necropsy (GD 30). Two lower doses (125 and 62.5 mg/kg/day) were selected as one-half and one-fourth of the high dose, respectively, with the assumption that the lowest dose would be at or near the NOAELfor does.

No. of animals: 30 pregnant female rabbits/ treatment group.

The females were sacrificed on GD 30 and the numbers of uterine implantations, resorptions, dead foetuses and live foetuses were examined.

Animals and husbandry: The experimental animals were female New Zealand White rabbits3 approximately 5 months of age. Male rabbits5 of the same strain were maintained as breeding stock. Rabbits were individually identified by eartags. Following a 14-day quarantine, ovulation was induced by injection of Pregnyl6 (0.1 ml/kg, iv) immediately prior to artificial insemination (Bredderman, 1964; Hafez, 1970) on GD 0. The study was performed in two replicates with two consecutive breeding days within each replicate and 34 days between replicates. Females (30/group) were assigned to treatments by stratified randomization so that body weight on GD 0 did not differ among groups within either replicate. The body weight range for individual females on GD 0 was 2690 to 4380 g. Inseminated females were individually housed in stainless steel cages with mesh flooring.7 Feed8 and deionized/filtered water were available *ad libitum* throughout gestation. Environmental conditions were monitored and controlled by computer.9 Lights were on from 0700 to 1900 hr for females and from 0700 to 2100 hr for males. Mean temperature was 65°F (range, 64-79°F) and mean relative humidity was 56% (range, 47-89%).

Evaluations: Inseminated females were weighed on GD 0, 6-19, 25, and 30. Females were observed daily during and after treatment for clinical signs of toxicity. Maternal food consumption was measured at 2- to 3-day intervals throughout gestation. Surviving females were terminated on GD 30 by injection of Beuthanasia-D Special13 into the marginal ear vein. The maternal liver, kidneys, and intact uterus were weighed and corpora lutea were counted. Uteri which presented no visible implantation sites were stained with ammonium sulfide (10%) to detect very early resorptions (Salewski,1964). Maternal kidneys were bisected, fixed in 10% neutral buffered. Formalin, and subsequently sectioned, stained with hematoxylin/eosin, and evaluated histologically. Live fetuses were dissected from the uterus and euthanized with an ip injection of T-61 Euthanasia Solution14 or sodium pentobarbital.15 They were weighed, examined for external morphological abnormalities, including cleft palate, and dissected for visceral examination and determination of sex by a fresh tissue dissection technique (Staples, 1974; Stuckhardt and Poppe, 1984). Half of the fetuses were decapitated after dissection; the heads were fixed in Bouin's solution and then examined by a freehand sectioning technique (Wilson, 1965). All fetal carcasses were skinned, cleared, and stained with Alcian blue/Alizarin red S and examined for skeletal malformations and variations (Kimmel and Trammell, 1981; Marr *et al*, 1988).
Statistics: The doe or litter was considered the experimental unit for all statistical analyses. General Linear Models (GLM) procedures were applied for the analyses of variance (ANOVA) of maternal and fetal parameters (SAS Institute, 1989a,b, 1990a,b,c). Prior to GLM analysis, an arcsinesquare root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) and Bartlett's test for homogeneity of variance was performed on all data to be analyzed by ANOVA (Winer, 1962). GLM analysis determined the significance of dose-response relationships and the significance of dose effects, replicate effects, and dose x replicate interactions. When ANOVA revealed a significant ($p < 0.05$) dose effect, Dunnett's multiple comparison test (Dunnett, 1955, 1964) compared BA-exposed groups to control groups. One-tailed tests were used for all pairwise comparisons except maternal body and organ weights and fetal body weight. Nominal scale measures were analyzed by a x2 test for independence and by a test for linear trend on proportions. When a x2 test showed significant groupwise differences, a one-tailed Fisher's exact probability test was used for pairwise comparisons of control and BA groups.

Findings

Maternal toxicity

Mortality: Two maternal deaths occurred (one doe at 62.5 mg/kg/day on GD 25; one doe at 125 mg/kg/ day on GD 22), but necropsy did not reveal a definitive cause of death. The condition of the dam at the low dose was suggestive of a gavage error affecting the respiratory system, and the dam at the mid dose had evidence of gastric lesions. Since there was no dose-related incidence of maternal deaths, and no deaths in the high-dose group, there appears to be no connection between maternal death and BA exposure in this study. Among 120 females assigned to this study, 22 (3-7 does/group) were removed for cause as follows: 3 for gavage accidents, 1 for accidental injury, 3 for abortion between GD 20 and 23, and 15 for deviation from *ad libitum* access to food and/or water (these cases were generally due to the animal dislodging the food hopper or water bottle from the cage or digging the food out of the food hopper).

Clinical observations: Among the remaining does, the following percentages were confirmed pregnant by uterine examination on GD 30 for the control through high-dose groups: 75% (18/24), 88% (23/26), 87% (20/23), and 96% (22/23). Pregnant does did not exhibit clinical symptoms attributable to BA toxicity except for vaginal bleeding (i.e., fresh or dried blood in the cage pan, on the legs, in the urine, or near the vagina). The incidence of bleeding was noteworthy at 250 mg/kg/day (2-11 does/day on GD 19-30), and all does with this symptom had no live fetuses on GD 30. Bleeding was not observed in any control females, and in only one female/group at 62.5 and 125 mg/kg/day (Days 20 and 22, respectively; 5-7 live fetuses/litter at term).

Food consumption: At 250 mg/kg/day, maternal food consumption was decreased during the first 10 days of treatment (GD 6 to 15), was comparable among groups during the final days of treatment (GD 15 to 19), was increased during for the period immediately following treatment (GD 19 to 25), and was increased in both the 125 and 250 mg/kg/day groups relative to controls during the final days of gestation (GD 25 to 30).

Table: Maternal Toxicity in New Zealand White Rabbits Exposed to Boric Acid on Gestational Days 6 through 19

	Boric acid (mg/kg/day, po)			
	0	62.5	125	250
No. pregnant at euthanization	18	23	20	22
No. of live litters	18	23	20	6
No. of live fetuses	159	175	153	14
Maternal wt. change (g)[a,b]				
Treatment period (GD 6 to 19)	93 ± 30*	132 ± 40	97 ± 51	−137 ± 42**
Gestation period (GD 0 to 30)	357 ± 69*	493 ± 51	543 ± 63**	226 ± 35
Corrected gestation wt. gain[c]	−205 ± 78*	−52 ± 63	40 ± 57**	165 ± 40**
Gravid uterine wt (g)[a]	562 ± 40*	504 ± 26	502 ± 28	62 ± 18**
Maternal liver wt[a,b]				
(% body wt)	2.59 ± 0.13	2.80 ± 0.09	2.78 ± 0.08	2.87 ± 0.09
Maternal kidney wt[a,b]				
(% body wt)	0.46 ± 0.02	0.46 ± 0.01	0.47 ± 0.01	0.51 ± 0.01**
Renal pathology[d]	0/18	2/23	0/20	1/22
Relative food consumption (g/kg/day)[a]				
Pretreatment (GD 0 to 6)	48.1 ± 1.7	48.0 ± 1.8	48.9 ± 2.4	46.4 ± 1.5
Treatment (GD 6 to 19)	38.8 ± 1.7*	40.0 ± 2.0	38.7 ± 2.3	26.6 ± 2.2**
Posttreatment				
GD 19 to 25	36.9 ± 2.5*	37.0 ± 2.6	40.0 ± 3.1	44.9 ± 2.2
GD 25 to 30	24.5 ± 3.0*	30.9 ± 2.1	33.9 ± 1.9**	41.9 ± 1.6**

[a] Includes all dams pregnant at euthanization; mean ± SEM.
[b] Maternal gestational and corrected weight gains and relative organ weights were calculated using body weight at the time of euthanization.
[c] Weight gain during gestation minus gravid uterine weight.
[d] Renal tubular regeneration (minimal). In addition, one dam in the low-dose group showed minimal mineralization of the renal tubules; one dam in the mid-dose group showed mild, multiple subcapsular cysts.
* $p < 0.05$, linear trend.
** $p < 0.05$, Dunnett's test.

Body weight: Maternal body weight (GD 9-30), weight change during treatment, and gravid uterine weight were each decreased at 250 mg/kg/day. Maternal weight change throughout gestation was greater than that of controls at 125 mg/kg/day. Corrected maternal weight change was increased at both 125 and 250 mg/kg/day (Table 1). Maternal relative liver weight was comparable among groups. Relative kidney weight was increased at 250 mg/kg/day, but appeared to be secondary to decreased maternal body weight since absolute kidney weight (data not shown) was comparable across groups. Furthermore, microscopic evaluation of maternal kidney sections failed to provide evidence for any BA-induced renal toxicity.

Embryo/fetal effects

Pup survival: No definitive evidence of developmental toxicity was observed following exposure of pregnant does to either 62.5 or 125 mg/kg/day BA during the period of major organogenesis (GD 6-19). At 250 mg/kg/day, developmental toxicity included a high average rate of resorptions and a high percentage of does with complete prenatal loss. In contrast, the incidence of late fetal deaths was low in all groups (<2.8%/litter) and showed no systematic relationship to BA exposure (data not shown). Average fetal body weight/litter was 92% of controls at the high dose, but this difference did not reach statistical significance, in part due to the small sample size for this parameter (only 6 litters survived to GD 30 at the high dose, compared to 18-23 litters in the other groups).

Table: Developmental Toxicity in New Zealand White Rabbits Following Maternal Exposure to Boric Acid on Gestational Days 6 through 19

	Boric acid (mg/kg/day, po)			
	0	62.5	125	250
No. implantation sites per litter[a]	9.5 ± 0.8	8.4 ± 0.6	8.3 ± 0.5	8.6 ± 0.7
% Resorptions per litter[a]	6.3 ± 2.4*	5.9 ± 1.9	7.7 ± 2.1	89.9 ± 5.0**
% Litters with one or more resorptions	39	39	45	95***
% Litters with 100% resorptions	0	0	0	73***
No. live fetuses per litter[b]	8.8 ± 0.8*	7.6 ± 0.6	7.7 ± 0.5	2.3 ± 0.8**
Average fetal body wt (g) per litter[b]	44.8 ± 1.5	46.5 ± 1.4	45.7 ± 1.2	41.1 ± 2.7
All malformations				
% Fetuses per litter[b]	25.5 ± 5.8*	26.1 ± 3.8	30.4 ± 6.3	80.6 ± 16.3**
%Litters	72	78	75	83
External malformations				
% Fetuses per litter[b]	0.8 ± 0.8*	1.4 ± 1.0	1.0 ± 1.0	11.1 ± 8.2**
% Litters	6	9	5	33
Skeletal malformations				
% Fetuses per litter[b]	19.9 ± 5.4	19.9 ± 4.0	24.3 ± 6.4	38.9 ± 20.0
% Litters	61	65	55	50
Visceral malformations				
% Fetuses per litter[b]	7.3 ± 1.9*	5.9 ± 2.0	7.4 ± 2.0	80.6 ± 16.3**
% Litters	50	35	45	83
Cardiovascular (CV) malformations				
% Fetuses per litter[b]	2.7 ± 1.6*	3.1 ± 1.5	4.2 ± 1.3	72.2 ± 16.5**
% Litters	17*	22	35	83***
All variations				
% Fetuses per litter[b]	67.7 ± 7.2	54.8 ± 5.1	40.4 ± 5.2	86.1 ± 9.0
% Litters	94	100	90	100
Cardiovascular variations				
% Fetuses per litter[b]	10.6 ± 5.5*	5.7 ± 1.8	7.2 ± 2.5	63.9 ± 17.4**
% Litters	44	35	35	83

[a] Includes all dams pregnant at euthanization; litter size is number of implantation sites per dam; mean ± SEM.
[b] Includes only dams with live fetuses; litter size is number of live fetuses per dam; mean ± SEM.
* $p < 0.05$, linear trend.
** $p < 0.05$, Dunnett's test.
*** $p < 0.05$, Fisher's exact test.

Table: Morphological Abnormalities Observed in New Zealand White Rabbit Fetuses Following Maternal Exposure to Boric Acid on Gestational Days 6 through 19: Listing by Defect Type[a]

	Boric acid (mg/kg/day, po)			
	0	62.5	125	250
Number examined				
Fetuses Examined[b]	(159)	(175)	(153)	(14)
Litters Examined[c]	(18)	(23)	(20)	(6)
Any malformations				
Fetuses with any malformations[d]	40	50	53	11
Litters with any malformations[e]	13	18	15	5
External malformations				
No. of fetuses with external malformations[d]	1	2	1	2
No. of litters with external malformations[e]	1	2	1	2
Dome-shaped head	1		1	
Ears lower than normal	1		1	
Micrognathia			1	
Facial cleft				1
Agenesis of the upper lip				1
Cleft lip				1
Microglossia	1		1	
Cleft palate	1		1	2
Spina bifida		1		
Short tail		1		1
Skeletal malformations				
No. of fetuses with skeletal malformations[d]	30	39	44	4
No. of litters with skeletal malformations[e]	11	15	11	3
Cleft sternum	28	36	40	2
Fused sternebrae	2	3		1
Floating (detached) extra rib: lumbar I. right side			1	
Fused ribs		1	5	1
Visceral malformations				
No. of fetuses with visceral malformations[d]	13	11	12	11
No. of litters with visceral malformations[e]	9	8	9	5
Enlarged lateral ventricle of the brain: left	1	1	1	1
Abnormal papillary muscle				
Agenesis of all in right ventricle		1	1	
Bifurcated, left ventricle	1	1	2	1
Bifurcated, right ventricle	4	1	2	
Small, right ventricle		1	1	1
Abnormal tricuspid valve: solid septum			1	
Agenesis of the subclavian artery: right		1		
Common truncus		1	1	
Enlarged aorta		1	1	5
Enlarged heart			1	1
Interventricular septal defect	1	3	2	8
Pulmonary artery and aorta arise from right ventricle				2
Transposition of aorta, and pulmonary artery			1	
Agenesis of the gall bladder			3	2
Enlarged gall bladder	6	4	1	
Agenesis of the spleen			1	
Small pulmonary artery		1		

[a] The incidence of individual defects is expressed as the number of individual fetuses exhibiting that defect. Thus, a single fetus may be represented more than once in listing individual defects.
[b] Only live fetuses were examined.
[c] Fetuses with one or more malformations.
[d] Includes only litters with live fetuses.
[e] Litters with one or more malformed fetuses.

Malformations: The overall incidence of malformed fetuses/litter was increased at 250 mg/kg/day BA, but not at 62.5 or 125 mg/ kg/day. Even though there was an unusually high background incidence of cleft sternum (Table 3), this did not in any way obscure the identification of treatment-related developmental effects in this study. For reference, when cleft sternum was included in the calculations, the overall incidence of malformed fetuses was 25.2% (40/159), 28.6% (50/ 175), 34.6% (53/153), and 78.6% (11/14) for the control through high-dose groups, respectively. When cleft sternum was excluded from the calculations, the overall incidence of malformed fetuses was 8.8% (14/159), 9.7% (17/175), 11.8% (18/153), and 78.6% (11/14). When malformations were analyzed by general class, the percentage fetuses/litter with external or visceral malformations was increased at the high dose, but the incidence of skeletal malformations was comparable to that of controls. External malformations were observed with the following incidence among individual fetuses in the control through high-dose groups, respectively: 0.6% (1/159), 1.1% (2/175), 0.7% (1/153), and 14.3% (2/14). Although the overall incidence of external malformations was increased at the high dose of BA, distinctive dose-response patterns for individual malformations were not observed.

Two fetuses were found to have multiple anatomical defects (major and minor), but in the absence of a dose-response relationship. Thus, one fetus in the control group displayed a domed head, low-set ears, cleft palate, microglossia, enlarged gall bladder, clubbed limb with no underlying bone change, and bilateral full rib on lumbar I.

Another fetus in the mid-dose group showed a similar range of defects, domed head, low-set ears, cleft palate, micrognathia, microglossia, and clubbed limb with no underlying bone change. The incidence of fetuses with visceral malformations was 8.2, 6.3, 7.8, and 78.6% in the control through high-dose groups. Malformations of the cardiovascular (CV) system (great vessels and heart) were observed with the greatest frequency, and their incidence was significantly increased at the high dose. CV malformations with an elevated incidence included interventricular septal defect in 0.6, 1.7, 1.3, and 57% of the fetuses examined (control through high-dose, respectively); enlarged aorta in 0, 0.6, 0.7, and 36% of fetuses examined; papillary muscle malformations in 3, 2, 4, and 14% of fetuses examined; and double outlet right ventricle (pulmonary artery and aorta both arising from the right ventricle) in 0, 0, 0, and 14% of foetuses examined. The gallbladder was the only other visceral organ which displayed changes in malformation incidence potentially associated with BA exposure. The incidence of enlarged gallbladder decreased with increasing dose, while the incidence of agenesis increased.
The incidence of fetuses with skeletal malformations (all types) was comparable across treatment groups, i.e., 19, 22, 29, and 29%. The incidence of skeletal malformations among study controls (19%) was noticeably higher than that for historical controls (36/912 or 4%) from the same species/ strain in our laboratory (see NTP, 1991). This was due primarily to the high incidence of cleft sternum which is presumed to occur due to abnormal midline fusion of the procartilagenous sternal bands. This defect was found in 18% (28/159) of the control fetuses in this study vs 2% (18/912) among historical controls. In subsequently conducted studies using the same species/strain in our laboratory, only 0.31% (4/1280) of control rabbit fetuses exhibited cleft sternum. The unusually high incidence of cleft sternum in this study thus represents a transient phenomenon.

Only two individual skeletal malformations appeared to show an increased incidence in BA-exposed animals: fused sternebrae occurred with an incidence of 1.3, 1.7, 0, and 7%, and fused ribs occurred with an incidence of 0,0, 1.3, and 7% of fetuses examined. At the high dose, each of these findings occurred in only one fetus and the affected fetuses came from separate litters (i.e., neither defect occurred in more than one high-dose fetus). Thus, the association of fused sternebrae or fused rib with BA exposure was considered equivocal.

Anatomical variations: The percentage fetuses/litter with anatomical variations was not significantly elevated above controls in any BAexposed group. The only external variation noted was clubbed limb (without underlying bone change) which never occurred in more than one fetus per group. A wide variety of visceral variations was observed and the two which appeared to show a dose-related incidence involved the same organs that exhibited BA-related malformations. An abnormal number of cardiac papillary muscles was observed in 5, 6, 5, and 50% of fetuses examined, and small gallbladder was observed in 3, 5, 5, and 14% of foetuses examined. Only two skeletal variations were noted and neither of them showed a clear dose-response relationship. Misaligned sternebrae occurred in 1.26, 1.14, 0.65, and 0% of fetuses examined for the control through high-dose groups, respectively. Full extra rib on lumbar I (bilateral or unilateral) occurred in 57, 39, 30, and 43% of fetuses, and rudimentary extra rib on lumbar I (bilateral or unilateral) occurred in 3, 4, 2, and 14% of fetuses examined.

Table: Morphological Variations Observed in New Zealand White Rabbit Fetuses Following Maternal Exposure to Boric Acid on Gestational Days 6 through 19: Listing by Defect Type[a]

	Boric acid (mg/kg/day, po)			
	0	62.5	125	250
Number examined				
No. of fetuses examined[b]	(159)	(175)	(153)	(14)
No. of litters examined[c]	(18)	(23)	(20)	(6)
Any variations				
No. of fetuses with any variations[d]	107	96	64	11
No. of litters with any variations[e]	17	23	18	6
External variations				
No. of fetuses with external variations[d]	1	0	1	1
No. of litters with external variations[e]	1	0	1	1
Clubbed limb (without bone change)	1		1	1
Skeletal variations				
No. of fetuses with skeletal variations[d]	97	75	50	8
No. of litters with skeletal variations[e]	16	21	17	4
Misaligned sternebrae	2	2	1	
Rib on lumbar I				
Full (bilateral, left or right)	91	68	46	6
Rudimentary (bilateral, left or right)	5	7	3	2
Visceral variations				
No. of fetuses with visceral variations[d]	28	39	24	9
No. of litters with visceral variations[e]	12	18	13	6
Abnormal number of papillary muscles	8	10	8	7
Agenesis of the innominate artery	1	1	1	
Left auricular flap of heart $\frac{1}{2}$ normal size			1	
White mucous-like material in stomach	1	2	1	
Liver-like tissue on gall bladder	15	9	5	
Small gall bladder	4	9	8	2
Mottled spleen		1		
Small spleen				1
Pale mottled liver	1			
Yellow liver	3	10	3	
White spot on left lateral liver lobe		1		
Blood-filled kdiney capsule: right			1	
Red area on left kidney		1		
White spot on kidney		1		

[a] The incidence of individual variations is expressed as the number of individual fetuses exhibiting that defect. Thus, a single fetus may be represented more than once in listing individual defects.
[b] Only live fetuses were examined.
[c] Fetuses with one or more variations.
[d] Includes only litters with live fetuses.
[e] Litters with one or more fetuses with variations.

Conclusion Decreased food intake and vaginal bleeding associated with pregnancy loss were the principal manifestations of maternal toxicity in New Zealand. White rabbits exposed to 250 mg/kg/day BA on GD 6-19. The same dose was associated with severe developmental toxicity, including a high rate of prenatal mortality and malformations. Development of the cardiovascular system was particularly sensitive to disruption. At 125 mg/kg/day, increased maternal food intake and weight gain were not considered adverse. Increased food intake was noted in all three species at one or more doses (Heindel *et al.*, 1994), and some of these increases were attributable to posttreatment rebound. However, further studies are needed to determine the cause of increased food intake when it is not preceded by a decrease during the treatment period. No definitive evidence of developmental toxicity was observed at 125 mg/kg/day. The low dose (62.5 mg/kg/day) was clearly nontoxic to both the maternal animal and the developing conceptus. Thus, the maternal and developmental NOAELs for rabbits were considered to be 125 mg/kg/day.

3.10.1.12 [Study 12] Developmental toxicity of boric acid in rats and mice (non-guideline)

Reference Heindel, J. J., Price, C. J., Field, E. A., Marr, M. C., Myers, C. B., Morrissey, R. E., & Schwetz, B. A. (1992). Developmental toxicity of boric acid in mice and rats. Fundamental and applied toxicology, 18(2), 266-277.

Guideline No guideline followed
GLP guideline

Reliability Klimisch 2: reliable with restrictions (reliability according to the CLH dossier of boric acid, assessed by RAC in 2013)

Species / strain Rat, Sprague-Dawley (female)
Swiss albino (CD-l) mice (female)

Test material

Boric acid (CAS No. 10043-35-3)

Purity: 98 – 99%

Study design

Materials and methods

Route of administration: oral, feed

Exposure:

Rats: GD 0 – 20 for the dose levels of 14 up to 58 mg B/kg bw/day;

GD 6 – 15 only for the highest-dose level (i.e. 94 mg B/kg bw/day), with a separate control group with the same exposure

Mice: GD 0 – 17

Timed-mated animals were given BORA in the feed continuously from the morning of GD 0 to the morning of GD 20 for rats or GD 17 for mice (0,O. 1,0.2, or 0.4% groups) or from GD 6 through 15 (0.8% rats only). Each concentration of BORA in ground feed was mixed independently in a Patterson Kelly Liquid-Solids Twin Shell Blender. Stability studies of BORA (0.1% in feed) indicated that formulations were stable when frozen or refrigerated for up to 2 weeks (95 or 97% recovery at 20°C respectively); minimal loss occurred following storage at higher temperatures (89% recovery after 2 weeks at 24 or 45°C). To minimize the effects of temperature on the integrity of dosed feed preparations, feed was stored under refrigeration in light-protected containers and fresh supplies of dosed feed were obtained from refrigerated stock every third day. Batches of dosed feed were prepared independently for each study replicate and used throughout the period of dosing for that replicate. Dosed feed was verified to be within ±10% by UV/VIS spectrometry prior to administration, and to be within ±10% of the predosing value following completion of dosing.

Doses / Concentrations:

Rats: 0, 0.1, 0.2 or 0.4 % and 0.8% equivalent to 0, 78, 163, 330 and 539 mg boric acid (mg B)/kg bw/day, equivalent to 0, 14, 29, 58 and 94 mg B/kg bw/day, respectively

Mice: 0, 0.1, 0.2 or 0.4 % equivalent to 0, 248, 452 and 1003 mg boric acid/ kg bw/day, equivalent to 0, 43, 79 and 175 mg B/kg bw/day, respectively

Dose selection was based on the results of preliminary studies in rats and mice (four to eight confirmed-pregnant animals/dose group). Boric acid was administered to rats in feed from GD 0 to 20 at concentrations of 0, 0.2, 0.4, 0.8, 1.2, and 2.4%, with resulting average daily exposure levels of 0, 162, 3 11, 6 17, 928, and 1704 mg/kg/day. Maternal toxicity (i.e., decreased body weight gain, increased water consumption, and adverse clinical signs) was observed at 30.8% BORA. Pregnancy among BORA-treated groups ranged from 57 to 75% compared to 88.5% for controls, suggesting that treatment from GD 0 to 6 may adversely affect implantation or cause preimplantation loss. Embryo/fetal toxicity was observed at all doses including 78% prenatal mortality per litter after exposure to 0.8% BORA on GD 0 to 20. Doses for the definitive study in rats were chosen to reduce preimplantation loss and early embryo lethality, to maximize the opportunity to identify teratogenic potential. These doses were 0, 0. I, 0.2, and 0.4% (GD 0 to 20) and 0.8% (GD 6 to 15). Boric acid was administered to mice in preliminary studies in feed from GD 0 to 17 at concentrations of 0, 0.2, 0.4, 0.8, 1.2, and 2.4%. Maternal toxicity (i.e., decreased corrected weight gain, increased water consumption, increased relative kidney weight, and adverse clinical signs) was apparent at doses >0.8%. Based on these preliminary results, doses of 0, 0.1, 0.2 and 0.4% BORA in feed were selected for the definitive study in mice; in significant maternal and fetal toxicity was expected to result at the high dose, gradations of toxicity at the intermediate dose, and no adverse effects at the low dose.

No. of animals: 26 – 28 female mice or rats/dose group

Animals and husbandry: Cesarean-originated, barrier-sustained CWDl(ICR) VAF/Plus outbred Swiss albino (CD-l) mice5 and Crl:CD BR VAF/:Plus outbred Sprague-Dawley (CD) rats6 were used in these studies. After a 7-day quarantine period, individual breeding pairs were cohabited overnight. The morning on which vaginal sperm (rats) or vaginal copulatory plugs (mice) were found was designated as GD 0. Female mice weighed 2 1-34 g and female rats weighed 2 13-275 g on GD 0. Animals were individually identified by eartag and assigned to dose groups by stratified randomization so that body weights did not differ among groups within any individual replicate.

Each study was performed in two replicates with 3 or 4 consecutive days of breeding in each replicate, except for one group of rats (0.8%) which was evaluated only in replicate I. The last breeding date for the first replicate and the first breeding date of the second replicate were 25 and 33 days apart for mice and rats, respectively.

Sperm- or plug-positive females were individually housed in solid-bottom polycarbonate cages with stainless-steel wire lids' and Ab-SorbDri cage litter.8 Feed' and deionized/filtered water were available ad libitum throughout gestation. Environmental conditions (lights on 0700 to 1900 hr, average temperature and relative humidity approximately 22°C and 48%, respectively) were monitored and controlled by computer." Air in each animal room was exchanged 12- 14 times per hour. Chemicul. Boric acid (BORA (CAS No. 10043-35-3) was determined to be 98-99% pure upon procurement. Inductively coupled plasma emission spectrometry produced results showing boron

levels at 99% of theoretical values; titration with 0.1 N sodium hydroxide indicated a purity of approximately 98%. Individual impurities were not identified or quantified except that water content was estimated at 0.06%. Subsequent bulk chemical analyses indicated that purity remained stable (92-100%) throughout the period of use relative to frozen reference standards.

Evaluations: Timed-mated females were weighed and food and water intake measurements were taken on the morning of GD 0, 3, 6,9, 12, and 15 plus 17 for mice or 18 and 20 for rats. Animals were observed daily during treatment for clinical signs of toxicity. Animal weights were also recorded immediately following death by cervical dislocation (mice) or CO2 anesthesia followed by cervical dislocation (rats). Maternal body, liver, kidneys, and intact uterus were weighed, and corpora lutea were counted. Uteri that had no visible implantation sites were stained with ammonium sulphide (10%) to detect very early resorptions (Salewski, 1964). Maternal kidneys were fixed in 10% neutral buffered formalin. Randomly selected maternal kidneys (10 dams/group) were sectioned, stained with hematoxylin/eosin, and evaluated microscopically. Live fetuses were dissected from the uterus and anesthetized on ice. They were weighed, and examined by a fresh tissue dissection technique (Staples, 1974; Stuckhardt and Poppe, 1984). Half of the fetuses were decapitated prior to dissection; the heads were fixed in Bouin's solution and then examined by a free-hand sectioning technique (Wilson, 1965). All fetal carcasses were stained with Alcian blue/alizarin red S stain and examined for skeletal malformations (Marr et al., 1988).

Statistics: General Linear Models (GLM) procedures were applied for the analysis of variance (ANOVA) of maternal and fetal parameters (SAS Institute, 1985a,b). Prior to GLM analysis, an arcsine-square root tmnsformation was performed on all litter-derived percentage data (Snedecor and Co&ran, 1967) and Bartlett's test for homogeneity of variance was performed on all data to be analyzed by ANOVA (Winer, 1962). GLM analysis determined the significance of dose-response relationships using a test for linear trend. The significance of dose effects, replicate effects, and dose X replicate interactions was determined by ANOVA. When ANOVA revealed a significant ($p < 0.05$) dose effect, Williams' or Dunnett's multiple comparison tests (Williams, 197 1, 1972; Dunnett, 1955, 1964) compared each BORAexposed group to the concurrent control group. One-tailed tests were used for all pairwise comparisons except maternal body and organ weights, water and feed consumption, and fetal body weight. Nonsignificant ($p > 0.05$) dose X replicate effects on selected fetal parametric measures were considered justification for pooling data across replicates for nonparametric analysis on related measures. When significant ($p < 0.05$) dose X replicate interactions occurred, nominal scale data were analyzed separately for each replicate in the study, as well as for all replicates combined. Nominal scale measures were analyzed by a X2 test for independence and by a test for linear trend on proportions. When the X2 test showed significant studywise differences, a one-tailed Fisher's exact probability test was used for pairwise comparisons of BORA and control groups.
Within replicate I of the rat study design, a group of animals were exposed to 0.8% dietary BORA on GD 6 to 15. Because there were no replicates of this group, statistical analyses were handled separately from the analysis of other dosed groups; control animals from replicate I served as the controls for the 0.8% group. Dependent variables were divided into three classes: (1) continuous variables presumed to come from a normal distribution; (2) continuous (or nearly continuous) variables presumed to come from distributions other than normal; and (3) categorical variables. The analysis of variables in the first class used Student's t test. If a variance ratio F test indicated heteroscedasticity, Satterthwaite's approximation for the degrees of freedom was used to calculate the probability of the f statistic. The analysis of variables in the second class was performed using the Mann-Whitney U test. Variables in the third class were analyzed with Fisher's exact probability test. In addition, average body weight for male and female fetuses per litter and arcsine-square root transformation of percentage malformed fetuses per litter were analyzed in a three-way repeated-measures ANOVA (dose x replicate x sex) with sex as the repeated measure within dams.

Findings

Observed effects in rats

Maternal effects:

Statistically significant increases compared to control:
-relative liver weight by 5% and by 6%, at 29 and 58 mg B/kg bw/day, respectively;
-relative kidney weight by 11% and by 12% for 29 and 58 mg B/kg bw/day, respectively.

Statistically significantly decreased body weight by 11% and by 35%, at the dose levels of 58 and 94 mg B/kg bw/day, respectively, compared to controls.

Table: Maternal Toxicity in CD Rats Exposed to Boric Acid on Gestational Days 0 to 20 or 6 to 15

	Boric acid (% feed), GD 0–20				Boric acid (% feed), GD 6–15	
	0	0.1	0.2	0.4	0	0.8
Subjects (dams)						
Total Treated	29	29	29	29	14	14
Number (%) pregnant at sacrifice	28 (97)	28 (97)	26 (90)	26 (90)	14 (100)	14 (100)
Maternal weight gain (g)[a]						
Gestation (GD 0–20)	160.6 ± 3.8†	157.5 ± 3.0	156.6 ± 3.6	143.6 ± 3.9*	157.8 ± 6.1	102.5 ± 5.3*
Treatment (GD 6–15)					54.0 ± 2.9	22.9 ± 3.1*
Corrected weight gain[b]	71.2 ± 2.9†	72.1 ± 2.1	74.6 ± 3.1	81.4 ± 2.5*	66.6 ± 4.8	66.2 ± 6.2
Gravid uterine weight	88.4 ± 2.6†	85.3 ± 2.1	82.0 ± 2.0	62.1 ± 3.1*	88.9 ± 3.6	36.2 ± 4.4*
Maternal body weight (g)[a] on GD 20	409 ± 5	405 ± 4	404 ± 4	393 ± 5	417 ± 6	364 ± 5*
Maternal liver weight[a]						
Absolute (g)	17.15 ± 0.25	17.59 ± 0.27	17.86 ± 0.30	17.54 ± 0.35	17.27 ± 0.40	17.12 ± 0.59
Relative (% body wt)	4.20 ± 0.05†	4.35 ± 0.06	4.42 ± 0.07*	4.46 ± 0.07*	4.15 ± 0.07	4.70 ± 0.13*
Maternal right kidney weight[a]						
Absolute (g)[b]	1.23 ± 0.02†	1.25 ± 0.02	1.35 ± 0.06	1.32 ± 0.03	1.21 ± 0.03	1.37 ± 0.04*
Relative (% body wt)	0.302 ± 0.006†	0.309 ± 0.006	0.335 ± 0.016*	0.338 ± 0.007*	0.289 ± 0.009	0.376 ± 0.009*

[a] Includes all dams pregnant at sacrifice; mean ± SEM.
[b] Gestational weight gain minus gravid uterine weight.
* $p < 0.05$ by pairwise comparison to the control group.
† $p < 0.05$, test for linear trend.

Embryo/foetal effects:
Statistically significantly increased prenatal mortality at 94 mg B/kg bw/day (36% resorptions/litter compared to 4% for the controls).

Statistically significantly reduced average foetal body weight for all treated groups compared to controls:
- 7% decrease at 14 mg B/kg bw/day;
- 13 % decrease at 29 mg B/kg bw/day;
- 37 % decrease at 58 mg B/kg bw/day;
- 50 % decrease at 94 mg B/kg bw/day.

Increased incidences of visceral malformations were observed:
- malformations of the eyes (i.e. displaced eye in 7/136 foetuses and convoluted retina in 9/136 foetuses) at 94 mg B/kg bw/day, compared to the and 0/215 in the control group;
- enlarged lateral ventricles of the brain (in 21/386 foetuses at 58 mg B/kg bw/day and in 36/136 foetuses at 94 mg B/kg bw/day), compared to the respective control groups, 0/431 and 0/215;
- agenesis of rib XIII was observed in 24/386 foetuses at 58 mg B/kg bw/day and in 17/136 foetuses at 94 mg B/kg bw/day, compared to the respective control groups, 1/431 and 0/215.

Statistically significantly increased incidence of short rib XIII observed in 39% and 37% of the foetuses at 58 mg B/kg bw/day and 94 mg B/kg bw/day, respectively (compared to their respective control groups, 0.23% and 0.46%).

Table: Developmental Toxicity in CD Rats following Maternal Exposure to Boric Acid in Feed on Gestational Days 0 to 20 or 6 to 15

	Boric acid (% feed), GD 0–20				Boric acid (% feed), GD 6–15	
	0	0.1	0.2	0.4	0	0.8
All litters[a]	28	28	26	26	14	14
Number of implantation sites/litter[b]	15.9 ± 0.3	16.4 ± 0.4	16.2 ± 0.3	16.1 ± 0.4	16.0 ± 0.5	15.8 ± 0.5
% Resorptions/litter[b]	3.5 ± 1.0	5.9 ± 1.2	3.4 ± 0.8	8.6 ± 3.9	4.4 ± 1.9	36.2 ± 8.7*
% Litters with one or more resorptions	39	61	46	46	36	100
% Late fetal deaths/litter[b]	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 1.6*
% Litters with one or more late fetal deaths	0	4	0	0	0	21
% Adversely affected implants/litter[b,e]	5.46 ± 1.35†	8.66 ± 1.90	11.17 ± 2.16*	53.58 ± 5.63*	7.06 ± 2.41	77.71 ± 6.77*
% Litters with one or more adversely affected implants[e]	50†	75*	85*	100*	50	100*
Live litters[c]	28	28	26	25	14	14
Number of live fetuses/litter[b]	15.4 ± 0.4	15.4 ± 0.5	15.7 ± 0.4	15.4 ± 0.5	15.4 ± 0.7	9.7 ± 1.6*
Average fetal body weight (g)/litter[b]						
Male fetuses	3.779 ± 0.061†	3.554 ± 0.051*	3.280 ± 0.053*	2.405 ± 0.059*	3.820 ± 0.068	1.778 ± 0.153*
Female fetuses	3.609 ± 0.059†	3.364 ± 0.046*	3.130 ± 0.050*	2.266 ± 0.046*	3.646 ± 0.064	1.814 ± 0.060*
% Fetuses malformed/litter[b,d]	2.1 ± 0.8†	2.6 ± 1.4	7.8 ± 2.4*	50.2 ± 5.4*	2.8 ± 1.4	72.6 ± 8.1*
% Litters with one or more malformed fetuses						
All malformations	21†	21	50*	100*	29	100*
Gross malformations	4	0	4	4	7	71*
Visceral malformations	7	4	0	36*	14	86*
Skeletal malformations	14†	18	46*	100*	14	100*
% Fetuses with variations/litter[b,d]	21.2 ± 3.2	7.7 ± 1.4*	8.8 ± 1.9*	27.2 ± 4.4	24.2 ± 4.9	59.5 ± 6.8*
% Litters with variations	96	71*	58*	92	93	100

[a] Includes all dams pregnant at sacrifice; litter size = number of implantation sites per dam.
[b] Reported as mean ± SEM.
[c] Includes only dams with live fetuses; litter size = number of live fetuses per dam.
[d] Only live fetuses were examined for malformations and variations.
[e] Adversely affected implants = nonlive implants plus malformed fetuses.
* $p < 0.05$ by pairwise comparison to the control group.
† $p < 0.05$, test for linear trend or test for linear trend on proportions.

Statistically significantly increased incidence (100%) of litters with 1 or more foetuses with a skeletal malformation was reported for both 58 mg B/kg bw/day and 94 mg B/kg bw/day dose levels (24/24 litters and 14/14 litters, respectively compared to their respective control groups, 4/28 and 2/14).

Statistically significantly increased incidence of foetuses with visceral or external malformations for all dose groups compared to controls (8% and 50% compared to 2% for the control group, for 29 and 58 mg B/kg bw/day, respectively). The incidence of foetuses with visceral or external malformations was statistically significantly increased for the highest dose level (i.e. 73% at 94 mg B/kg bw/day as compared to 2.79% in the control group).

Table: Morphological Defects in CD Rat Fetuses Following Maternal Exposure to Boric Acid in Feed on Gestational Days 0 to 20 or 6 to 15[a,b]

	Boric acid (% feed), GD 0–20				Boric acid (% feed), GD 6–15	
	0	0.1	0.2	0.4	0	0.8
All malformations						
Number of litters with malformations[c]/number examined	6/28	6/28	13/26	25/25	4/14	14/14
Number of malformed fetuses[d]/number examined	9/431	12/432	34/408	188/386	6/215	88/136
External malformations						
Number of litters with malformations[c]/number examined	1/28	0/28	1/26	1/25	1/14	10/14
Number of fetuses with malformations[d]/number examined	2/431	0/432	1/408	2/386	2/215	24/136
Malformations of the tail						
Curly tail and/or short tail						15
Craniofacial malformations						
Meningoencephalocele and flat head	1				1	
Anophthalmia[e]	1				1	6
Microphthalmia[e]				1		7
Cleft lip and/or palate	2		1		2	1
Other external malformations						
Umbilical hernia				1		
Anasarca (generalized edema)						2
Polydactyly: hindpaw						1
Visceral malformations						
Number of litters with malformations[c]/number examined	2/28	1/28	0/26	9/25	2/14	13/14
Number of fetuses with malformations[d]/number examined	2/431	1/432	0/408	30/386	2/215	36/136
Internal cephalic malformations						
Enlarged lateral ventricles of the brain[e]				21		24
Assymetrical fusion of the palate						1
Hydrocephaly[f]						1
Displaced eye[e]						7
Convoluted retina[e]						9
Cardiovascular malformations						
Transposition of aorta and pulmonary artery						2
Pulmonary artery and aorta arise from right ventricle						5
Other pulmonary artery malformations						5
Interventricular septal defect						3
Abnormal semilunar valve						1
Enlarged left heart ventricle						2
Urogenital malformations						
Hydroureter[e]	2	1			2	2
Hydronephrosis: bilateral						2
Renal agenesis: right						1
Other visceral malformations						
Enlarged and spotted adrenals				9		
Small spleen						1
Fused liver lobes						2
Skeletal malformations						
Number of litters with malformations[c]/number examined	4/28	5/28	12/26	25/25	2/14	14/14
Number of fetuses with malformations[d]/number examined	5/431	11/432	33/408	168/386	2/215	72/136
Rib malformations						
Agenesis of rib XIII	1	1		24		17
Short rib						
XIII	1	11	28	152	1	50
XII or VI, VII, and XI				2		
Fused ribs						6
Rib cartilage not attached to sternum: Ribs I–VIII						3
Discontinuous rib cartilage			1	4		
Vertebral malformations						
Agenesis of the vertebra						
Thoracic				2		
Lumbar			1			
Agenesis of the arch: thoracic						1
Agenesis of the centrum: thoracic						1
Misaligned centrum: thoracic						1
Unilateral vertebra: thoracic				3		2
Bipartite vertebra: thoracic	2		1		1	4

Bipartite cartilage						
Dumbbell ossification center: thoracic centrum	1					1
Normal ossification center						
Thoracic centrum	1					
Cervical centrum						1
Other skeletal malformations						
Cleft Sternum			4	8		13
Two ossification centers—sternebra III			1			
Oblong orbit (anophthalmic fetus)						1
Variations						
Number of litters with variations[g]/number examined	27/28	20/28	15/26	23/25	13/14	14/14
Number of fetuses with variations[h]/number examined	91/431	34/432	36/408	100/386	52/215	70/136
External variations						
Hematoma	3	1	1	7	1	10
Clubbed limb (without bone change)				8		3
Kinked or curly tail (without bone change)				2		1
Visceral variations						
Distended ureter[e]	7	4	1		6	
Extra tissue growth on liver[i]						2
Skeletal variations						
Incomplete ossification						
Skull		1	1	6		2
Ribs				5		
Centra				4		10
Lumbar arch				1		
Pubis and ischium						1
Extra ossification site: skull bone		1				
Misaligned sternebrae		4	3			1
Extra ossification site: sternebra II					1	
Rib on Lumbar I						
Rudimentary[e]	60	9		2	32	
Full (left) and rudimentary (right)	2				2	
Wavy rib or wavy rib cartilage	1	4	18	54	1	3
Dumbbell-shaped vertebra						
Thoracic	5	3	3		1	1
Lumbar						1
Unilateral ossification[j]						
Cervical centrum						1
Thoracic centrum				9		31
Bipartite ossification[j]						
Cervical centrum						1
Thoracic centrum	20	10	9	25	11	13
Lumbar centrum						1
No ossification[f]						
Thoracic centrum						16
Lumbar centrum						4
Sacral centrum						2

[a] A single fetus may be represented more than once in listing individual defects. Some defects may appear at one or more sites in a single fetus.
[b] Only live fetuses were examined for malformations and variations; the number of litters examined includes only litters with live fetuses.
[c] Litters with one or more malformed fetuses.
[d] Fetuses with one or more malformations.
[e] Incidence of fetuses with unilateral or bilateral defects combined.
[f] Associated malformations included convoluted retina and micropthalmia.
[g] Litters with one or more fetuses with variations.
[h] Fetuses with one or more variations.
[i] Appeared to be liver tissue on gross exam
[j] Normal cartilage present.

Observed effects in mice

Maternal effects:

At 175 mg B/kg bw/day, maternal body weight was statistically significantly reduced (by approx. 25%) during the treatment period. At 175 mg B/kg bw/day, pale kidneys at necropsy were observed in several dams, while 1 dam had kidney fluid accumulation. A dose-related increase in the incidence of renal tubular dilation was observed at microscopic examination. At the dose levels of 43 and 175 mg B/kg bw/day, ovarian cysts were seen in 1 dam of each dose group.

Table: Maternal Toxicity in CD-l Mice Exposed to Boric Acid in Feed on Gestational Days 0 to 17

	Boric acid (% feed)			
	0	0.1	0.2	0.4
Subjects (dams)				
Total treated	29	28	29	28
Number (%) pregnant	27 (93)	27 (96)	27 (93)	26 (93)
Maternal weight gain (g)[a]				
Gestation/treatment period	21.4 ± 0.8†	21.7 ± 0.5	21.1 ± 0.7	16.0 ± 1.1*
Corrected weight gain[b]	4.5 ± 0.3	5.6 ± 0.3	4.9 ± 0.4	4.7 ± 0.5
Gravid uterine weight (g)	16.9 ± 0.7†	16.1 ± 0.5	16.1 ± 0.6	12.1 ± 0.6*
Maternal body weight (g) on GD 17	49.3 ± 1.1†	48.3 ± 0.8	49.0 ± 1.0	43.1 ± 1.1*
Maternal liver weight[a]				
Absolute (g)	2.36 ± 0.04†	2.36 ± 0.04	2.38 ± 0.05	2.15 ± 0.06*
Relative (% body wt)	4.95 ± 0.08	5.02 ± 0.07	5.00 ± 0.09	5.13 ± 0.07
Maternal right kidney weight[a]				
Absolute (g)	0.20 ± 0.01†	0.19 ± 0.01	0.21 ± 0.01	0.22 ± 0.01
Relative (% body wt)	0.41 ± 0.02†	0.41 ± 0.02	0.45 ± 0.02	0.54 ± 0.04*
Renal histopathology				
Renal tubular dilation and/or regeneration[c]	0/10	2/10	8/10	10/10

[a] Includes all dams pregnant at sacrifice, mean ± SEM.
[b] Gestational weight gain minus gravid uterine weight.
[c] Number affected/number examined.
* $p < 0.05$ by pairwise comparison to the control group.
† $p < 0.05$, test for linear trend.

Embryo/foetal effects:

At 175 mg B/kg bw/day, statistically significantly increased resorption (approx. 19% per litter compared to 6% in controls) was observed.

Statistically significantly reduced foetal body weights were observed at 79 and 175 mg B/kg w/day (by approx. 12% and 33%, respectively compared to controls).

At the 175 mg B/kg bw/day, a statistically significantly increased incidence (approx. 8%) in foetuses with malformations as compared to the control groups (approx. 2%) was reported.

Statistically significantly increased incidence of short rib XIII was observed in 10/250 foetuses at 175 mg B/kg bw/day, compared to 0/311 in controls. Agenesis of one or more vertebra (lumbar) was reported for 3/250 foetuses (as compared to 1/311 in controls) for the highest dose level.

Table: Developmental Toxicity in CD-l Mice following Maternal Exposure to Boric Acid in Feed on Gestational Days 0 to 17

	Boric acid (% feed)			
	0	0.1	0.2	0.4
All litters[a]	27	27	27	26
Number of implantation sites/litter[b]	12.4 ± 0.6	12.0 ± 0.4	12.1 ± 0.4	12.1 ± 0.5
% Resorptions/litter[b]	6.1 ± 1.6†	6.2 ± 1.3	4.8 ± 1.4	19.3 ± 4.5*
% Litters with one or more resorptions	44	56	37	73*
% Late fetal deaths/litter[b]	0.9 ± 0.6	2.0 ± 0.9	0.6 ± 0.4	1.6 ± 0.8
% Litters with one or more late fetal deaths	7	19	7	15
% Adversely affected implants/litter[b]	9.5 ± 1.8†	12.4 ± 2.3	6.9 ± 1.5	27.4 ± 4.9*
% Litters with one or more adversely affected implants	70	70	56	85
Live litters[c]				
Number of live fetuses/litter[b]	11.5 ± 0.6	10.9 ± 0.3	11.4 ± 0.4	10.0 ± 0.7
Average fetal body weight (g)/litter[b]				
Male fetuses	1.08 ± 0.02†	1.03 ± 0.03	0.96 ± 0.02*	0.71 ± 0.02*
Female fetuses	1.04 ± 0.02†	0.99 ± 0.02	0.92 ± 0.02*	0.69 ± 0.01*
% Fetuses malformed/litter[b]	2.7 ± 1.2†	4.5 ± 1.9	1.6 ± 0.7	9.1 ± 2.4*
% Litters with one or more malformed fetuses				
All malformations	22	22	19	44
Gross malformations	7	4	4	16
Visceral malformations	4	7	6	4
Skeletal malformations	11	15	15	28
% Fetuses with variations/litter[b]	29.1 ± 3.5	18.8 ± 4.1*	11.9 ± 2.4*	26.3 ± 5.9
% Litters with variations	96	66*	70*	80

[a] Includes all dams pregnant at sacrifice; litter size = number of implantation sites per dam.
[b] Mean ± SEM.
[c] Includes only dams with live fetuses; litter size = number of live fetuses per dam.
* $p < 0.05$, groupwise comparison to control.
† $p < 0.05$, test for linear trend.

Table: Morphological Defects in CD-l Mouse Fetuses following Maternal Exposure to Boric Acid on Gestational Days 0 to 17[a,b]

	Boric Acid (% in feed)			
	0	0.1	0.2	0.4
All malformations				
Number of litters with malformations[c]/number examined	6/27	6/27	5/27	11/25
Number of malformed fetuses[d]/number examined	7/311	14/295	5/309	20/250
External malformations				
Number of litters with malformations[c]/number examined	2/27	1/27	1/27	4/25
Number of fetuses with malformations[d]/number examined	3/311	2/295	1/309	4/250
Meningoencephalocele		2		
Exencephaly	1			
Microphthalmia				1
Cleft palate	2		1	3
Visceral malformations				
Number of litters with malformations[c]/number examined	1/27	2/27	0/27	1/25
Number of fetuses with malformations[d]/number examined	1/311	2/295	0/309	1/250
Enlarged lateral ventricles of the brain		1		
Transposition of aorta and pulmonary artery		1		
Other pulmonary artery malformations				1
Hydronephrosis	1			
Skeletal malformations				
Number of litters with malformations[c]/number examined	3/27	4/27	4/27	7/25
Number of fetuses with malformations[d]/number examined	3/311	11/295	4/309	15/250
Cleft sternum	2	11		
Agenesis of rib				1
Fused ribs				2
Short rib: XIII			2	10
Agenesis of one or more vertebra: lumbar	1		2	3
Fused arches: thoracic				3
Fused cartilage: thoracic centrum				1
Misaligned centrum: thoracic				1
Unilateral vertebra: thoracic				1
Agenesis of the arch: thoracic				1
Variations				
Number of litters with variations[e]/number examined	26/27	18/27	19/27	20/25
Number of fetuses with variations[f]/number examined	87/311	59/295	36/309	59/250
Incomplete Ossification				
Supraoccipital				1
Thoracic, lumbar, and sacral centra				1
Open eye		1		
Hematoma	3	2	2	7
Misaligned sternebra	15	11	15	7
Extra Ossification Site: sternebra VI		1		
Lumbar I rib				
Full	37	24	4	3
Rudimentary	49	21	8	7
Dumbbell cartilage, unilateral ossification center: thoracic centrum				1
Normal cartilage, unilateral ossification center: thoracic centrum			1	1
Pale spleen	1	7	9	34
Distended ureter				1
Kinked tail (without bone change)			1	1

[a] A single fetus may be represented more than once in listing individual defects. Some defects may appear at one or more sites in a single fetus.
[b] Only live fetuses were examined for malformations. The number of litters examined includes only litters with live fetuses.
[c] Litters with one or more malformed fetuses.
[d] Fetuses with one or more malformations.
[e] Litters with one or more fetuses with variations.
[f] Fetuses with one or more variations.

Conclusion

NOAEL (developmental toxicity for rats): < 14 mg B/kg bw/day

LOAEL (developmental toxicity for rats): 14 mg B/kg bw/day, based on statistically significantly reduced average foetal body weight

NOAEL (developmental toxicity for mice): 43 mg B/kg bw/day

LOAEL (developmental toxicity for mice): 79 mg B/kg bw/day, based on statistically significantly reduced foetal body weight and increased incidence of skeletal malformations (i.e. short rib XIII).

3.10.1.13 [Study 13] 90-day oral repeated dose toxicity and reproductive toxicity study in rats and mice (barium chloride, non-guideline)

Reference Dietz, D. D., Elwell, M. R., Davis Jr, W. E. and Meirhenry, E. F. (1992). Subchronic toxicity of barium chloride dihydrate administered to rats and mice in the drinking water. Fundamental and applied toxicology, 19(4), 527-537.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (reliability according to publically disseminated REACH Registration dossier for barium chloride)

Species / strain Rat, Sprague-Dawley (male/female)

Test material Barium chloride
Purity: 99.5%

Study design

Materials and methods

Route of administration: oral, via drinking water

Exposure: 90 days and the males were exposed for 60 days and the females for 30 days, for the reproductive study

Doses / Concentrations:

Sub-chronic oral toxicity study

-for rats: 0, 11.25, 45, 90, 180 and 360 mg/kg bw/day, respectively

-for mice: 0, 18.75, 75, 150, 300 and 600 mg/kg bw/day, respectively

Reproductive study

0, 1000, 2000, and 4000 ppm barium chloride dehydrate for rats, equivalent to 0, 120, 240 and 480 mg/kg bw/day, respectively
0, 500, 1000, and 2000 ppm barium chloride for mice, equivalent to 0, 90, 180 and 360 mg/kg bw/day, respectively

Drinking water contained 0, 125, 500, 1000, 2000 and 4000 ppm BaCl2 + 2H20 and exposures were continuous throughout both the rat and mouse studies. All animals were observed twice daily for clinical signs of toxicity. Body weights were determined weekly and cage water consumptions were measured twice weekly.

No. of animals: n = 10/sex/dose group/species in the sub-chronic oral toxicity study
n = 20/sex/dose group/species in the reproductive toxicity screening study

Animal husbandry: Male and female 32day-old Fischer-344/N rats and B6C3F, mice were obtained from Simonsen Laboratories (Gilroy, CA). The animals were quarantined for 10 to 11 days after arrival, and representatives were necropsied to verify that they were grossly free of disease. Animals were randomly assigned to groups of 10 per dose level after weight-sorting them by sex. During both the quarantine and test phases. the animals were housed five per cage in drawer-type polycarbonate cages. The shelves supporting the cages were covered with filter sheets. The bedding (Ab-Sorb-Dri, Lab Products, Rochelle Park. NJ), cages. and water bottles were changed twice a week. feeders once a week, and racks and filters every other week. During changing the racks were rotated clockwise and the cages, which were in columns by dose. were rotated by moving the bottom cage to the top. Fluorescent lighting in the animal room was on for 12 hr (0630 to 1830) and off for 12 hr. Filtered fresh air (13.5 room vol/hr) was supplied directly to and removed from the animal room. The temperature range in the room was 21 to 24°C. The animals were fed a diet of NIH-07 pellets (Ziegler Brothers, Gardners. PA) and dosed or undosed water on an ad libitum basis for 92 consecutive days.

Reproductive and fertility assessment. The mating trials and fertility cytological evaluations were performed on separate groups of rats and mice than used in the core study. Only four dose levels of Bat&. 2H20 were tested: 0, 1000, 2000, and 4000 ppm in rats and 0, 500, 1000, and 2000 ppm in mice. Each group contained 20 animals of each sex. After 60 days of exposure, the males were placed in individual cages and one female receiving the same dose level (but exposed for 30 days) was cohabited with each male for up to 1 week. Each morning following a day of cohabitation. each female was examined for the presence of a vaginal plug (mice) or microscopic evidence of sperm in a vaginal swab (rats). When evidence of mating was found, the female was separated from the male: after mating determinations were made on the eighth day of cohabitation, all remaining pairs were separated. Females were

weighed when evidence of mating was found and on the day of parturition. Live offspring were weighed, counted, and examined on Day 0 (day of birth) and again on Day 5. Dead pups were recovered from the nest and examined for external abnormalities. All females were terminated on Days 96 and 97; the vagina, cervix, oviducts, and ovaries were grossly examined and the implantation sites in the uteri were counted. An evaluation of sperm morphology, density, and motility, male reproductive organ weights, and vaginal cytology among treated and control groups were performed according to previously described methods (Morrissey et al.. 1988).

Stafistics: Each parameter for which individual values were available was subjected to a linear least squares regression over the dose levels and the direction of the slope and the p value indicating the significance of the deviation of the slope from 0 was determined. Group means and standard deviations or standard errors were calculated for continuous variables. The multiple comparison procedure of Dunnett (1955) was employed for pairwise comparisons of these variables between dosed groups and controls. Fisher's exact test was used to make pairwise comparisons of discrete variables between dosed groups and controls and the Cochran-Armitage test was used to assess the significance of dose-related trends (Armitage,1971; Cart et al. 1979). Temporal and dose-related variations were evaluated using a repeated measures analysis of variance (Winer. 1971). When a collection of measurements were made on each animal, a multivariate analysis of variance (Morrison, 1976) was used to test for the simultaneous equality of measurements across dose levels.

Findings

Sub-chronic exposure results for rats:

No statistically significant changes in absolute body weight were reported for the 11.25, 45, 90 and 180 mg/kg bw/day, for either male or female rats.

4000 ppm (equivalent to 360 mg/kg bw/day):

Three of 10 males and 1 of 10 female rats died during the last week of the study. Body weights of both sexes were statistically significantly ($p < 0.05$) lower (by approx. 12% for males, and approx. 9% for females) than controls. Kidney lesions observed in both males and females (incidence not reported).

Sub-chronic exposure results for mice:

No statistically significant changes in absolute body weight were observed for the 18.75, 75, 150 and 300 mg/kg bw/day dose groups, for either male or female mice.

4000 ppm (equivalent to 600 mg/kg bw/day)

Six males and 7 female mice died on day 13 of the study. Body weights of both sexes were statistically significantly ($p < 0.05$) lower (by approx. 30% for males, and approx. 44% for females) than controls. Mild to marked toxic nephrosis was observed in both males and females (incidence not reported).

Table: Mortality and Body Weights of Animals Treated with Barium Chloride Dihydrate for 13 Weeks

Dose level (ppm)	Sex	Number dead/total	Body weight gain (g)	Final body weight (g ± SD)	Weight difference[a] (%)
			Mice		
0	M	0/10	12.4	38.1 ± 1.9	—
	F	0/10	10.3	29.5 ± 2.6	—
125	M	1/10	12.3	37.7 ± 4.4	−1.0
	F	0/10	8.7	28.5 ± 2.5	−3.4
500	M	0/10	12.7	38.2 ± 3.1	−0.3
	F	0/10	8.5	27.8 ± 3.3	−5.8
1000	M	0/10	10.8	36.1 ± 3.4	−5.2
	F	0/10	9.7	29.1 ± 3.2	−1.4
2000	M	0/10	12.0	37.9 ± 3.3	−0.5
	F	0/10	7.9	27.6 ± 3.2	−6.4
4000	M	6/10	1.9	26.8 ± 4.9[b]	−29.7
	F	7/10	−3.0	16.4 ± 3.4[b]	−44.4
			Rats		
0	M	0/10	210	347.7 ± 25.4	—
	F	0/10	86	189.6 ± 8.2	—
125	M	0/10	222	352.4 ± 24.4	1.3
	F	0/10	86	197.1 ± 12.7	3.9
500	M	0/10	234	359.6 ± 19.8	3.4
	F	0/10	87	190.7 ± 10.8	0.6
1000	M	0/10	220	342.2 ± 16.1	−1.6
	F	0/10	83	186.9 ± 10.0	−1.4
2000	M	0/10	211	339.9 ± 17.4	−2.2
	F	0/10	82	185.6 ± 10.9	−2.1
4000	M	3/10	174	307.1 ± 13.6[b]	11.7
	F	1/10	64	173.3 ± 15.2[b]	−8.6

[a] Weight difference = weight test group − weight control group ÷ weight control group × 100.
[b] Significantly different from control value of the same sex (t test, $p < 0.05$).

Table: Significant Organ Weight Changes[a] Following 13-Weeks of Exposure to Barium Chloride in the Drinking Water

Organ	Control	Barium chloride concentration (ppm) 125	500	1000	2000	4000
Male mouse						
Liver						
Absolute	2062 ± 120	2011 ± 77[b]	1892 ± 37	1716 ± 64	1714 ± 53	1088 ± 86[e,**]
Relative	54.2 ± 3.10	53.9 ± 2.81[b]	49.7 ± 1.10	47.7 ± 1.69	45.3 ± 0.94	40.8 ± 0.98[e,*]
Kidney						
Absolute	306 ± 8.2	301 ± 12.2[b]	302 ± 10.3	286 ± 10.4	291 ± 9.5	235 ± 12.6[e,**]
Relative	8.04 ± 0.15	8.06 ± 0.44[b]	7.95 ± 0.34	7.94 ± 0.22	7.71 ± 0.25	8.98 ± 0.89[e]
Thymus						
Absolute	44.2 ± 3.13	50.8 ± 5.29[b]	46.6 ± 4.06	40.4 ± 3.72	41.7 ± 4.32	21.5 ± 7.44[e,*]
Relative	1.16 ± 0.08	1.33 ± 0.11[b]	1.22 ± 0.10	1.11 ± 0.09	1.09 ± 0.09	0.75 ± 0.22[e,*]
Body weight	38.1 ± 0.61	37.8 ± 1.49[b]	38.2 ± 0.99	36.1 ± 1.09	37.9 ± 1.04	26.8 ± 2.46[e,*]
Female mouse						
Liver						
Absolute	1502 ± 52	1446 ± 69	1375 ± 74	1334 ± 53	1196 ± 37**	753 ± 126[d,**]
Relative	50.9 ± 1.09	50.6 ± 1.40	49.3 ± 1.43	45.8 ± 0.91*	43.5 ± 0.91**	45.5 ± 2.30[d]
Kidney						
Absolute	181 ± 4.1	183 ± 7.5	180 ± 5.6	188 ± 5.9	182 ± 7.9	143 ± 28.5[d]
Relative	6.15 ± 0.14	6.42 ± 0.18	6.51 ± 0.20	6.50 ± 0.21	6.61 ± 0.22	8.61 ± 0.65[d,**]
Thymus						
Absolute	57.2 ± 3.72	51.1 ± 2.98	46.5 ± 2.73	52.2 ± 3.12	47.1 ± 1.98	8.33 ± 5.36[d,**]
Relative	1.94 ± 0.11	1.80 ± 0.11	1.68 ± 0.09	1.79 ± 0.09	1.72 ± 0.07	0.46 ± 0.25[d,**]
Body weight	29.5 ± 0.83	28.5 ± 0.80	27.8 ± 1.05	29.1 ± 1.00	27.6 ± 1.00	16.4 ± 1.98[d,*]
Male rat						
Liver						
Absolute	11956 ± 439	12033 ± 462	12839 ± 252	12572 ± 493	11549 ± 324	10099 ± 296[c,*]
Relative	34.3 ± 0.65	34.1 ± 0.72	35.7 ± 0.36	36.7 ± 1.17	33.9 ± 0.48	32.9 ± 0.62[c]
Kidney						
Absolute	1061 ± 35	1044 ± 31	1097 ± 22	1092 ± 22	1064 ± 25	1050 ± 37[c]
Relative	3.05 ± 0.05	2.96 ± 0.04	3.05 ± 0.05	3.19 ± 0.03	3.13 ± 0.05	3.42 ± 0.10[c,**]
Body weight	348 ± 9.0	352 ± 7.7	360 ± 6.3	342 ± 5.1	340 ± 5.5	304 ± 4.8[c,*]
Female rat						
Liver						
Absolute	5944 ± 156	6439 ± 143	6083 ± 98	5899 ± 192	5858 ± 137	5024 ± 173[b,**]
Relative	31.3 ± 0.58	32.7 ± 0.40	31.9 ± 0.44	31.5 ± 0.68	31.6 ± 0.47	29.0 ± 0.59[b,*]
Kidney						
Absolute	570 ± 11	609 ± 22	591 ± 9	602 ± 13	635 ± 14**	620 ± 18[b]
Relative	3.01 ± 0.04	3.09 ± 0.09	3.10 ± 0.04	3.22 ± 0.04*	3.42 ± 0.05**	3.59 ± 0.09[b,**]
Thymus						
Absolute	237 ± 9.9	234 ± 7.7	254 ± 12.1	253 ± 13.9	229 ± 20.2	185 ± 19.1[b,*]
Relative	1.25 ± 0.05	1.19 ± 0.04	1.34 ± 0.07	1.35 ± 0.07	1.24 ± 0.11	1.05 ± 0.10[b]
Body weight	190 ± 2.6	197 ± 4.0	191 ± 3.4	187 ± 3.2	186 ± 3.5	173 ± 5.1[b,*]

[a] Mean ± standard error (absolute in milligrams, relative in milligrams per gram) for groups of 10 animals or otherwise as indicated.
[b] Nine animals were examined.
[c] Seven animals were examined.
[d] Four animals were examined.
[e] Three animals were examined.
* $p < 0.05$.
** $p < 0.01$.

Table: Histopathologic Lesions in Mice and Rats[a] Exposed to Barium Chloride in Their Drinking Water for 13 Weeks

Site/lesion	Barium chloride concentration (ppm)					
	Control	2000	4000	Control	2000	4000
	Male mice			Female mice		
Kidney						
Toxic nephrosis	0	0	10	0	0	9
Crystals	0	0	8	0	0	9
Spleen/thymus/lymph node						
Lymphoid depletion	0	0	8	0	0	9
	Male rats			Female rats		
Kidney						
Tubular dilatation	0	0	3	0	0	5
Spleen/thymus						
Lymphoid depletion	0	0	2	0	0	1

[a] Ten animals evaluated per group except nine female mice at 4000 ppm.

Reproductive and fertility assessment results for rats:

NOAEL for fertility impairment: 4000 ppm, equivalent to 480 mg/kg bw/day

The pregnancy rate[§] at 4000 ppm was 65% (compared to 40% in control) and the number of implants per pregnant dam was significantly reduced (7.7 ± 0.52 vs. 9.6 ± 1.10 pups in controls, $p<0.05$). One dam from the highest dose group died, the necropsy revealing 7 foetuses and one resorption site.
[§The pregnancy rate was calculated as the number of pregnant females/number of confirmed matings x 100]

No effects were reported on vaginal cytology, epididymal sperm count, sperm motility, sperm morphology, and testis or epididymal weight up to 480 mg/kg bw/day (data not shown).

Reproductive and fertility assessment results for mice:

The pregnancy rates ranged from 55 – 70% (the pregnancy rates for the controls were approx. 55%; data not shown) for all dose levels.

No effects were reported on vaginal cytology, epididymal sperm count, sperm motility, sperm morphology, and testis or epididymal weight up to 360 mg/kg bw/day (data not shown). Maternal weight gain during pregnancy was comparable to controls for all dose groups (data not shown).

Observed effects in rats
Maternal effects:
At 480 mg/kg bw/day, one dam died during the last week of the treatment, the necropsy revealing 7 foetuses and one resorption site. No information is presented about maternal body weight or signs of general toxicity.

Embryo/foetal effects:
The live pup weight at birth was statistically significantly ($p<0.01$) reduced (5.20 ± 0.06 g vs. 5.70 ± 0.09 g in controls).
The average litter size on postpartum day 5 was reduced compared to controls (7.1 ± 0.56 vs. 9.3 ± 1.16 pups in controls). Pup survival until postnatal day 5 was >99% in all treated groups and controls (data not shown).
No external abnormalities were observed in the offspring.

Observed effects in mice:
Maternal effects:
There was no evidence of maternal toxicity in the treated mice: maternal weight gain during pregnancy was comparable to controls for all dose groups (data not shown).

Embryo/foetal effects:
At the 180 mg/kg bw/day, a statistically significant ($p<0.05$) reduction in the average litter size was observed on postnatal day 0 (7.9 ± 1.02 pups vs. 10.7 ± 0.40 pups in the control group) and postnatal day 5 (7.7 ± 0.97 pups vs. 10.8 ± 0.38 pups in the control group).
A few pups (number not reported) were found dead at birth for all dose levels (not specified for controls), and survival from postnatal day 0 to postnatal day 5 ranged between 98 – 100 % (dose level not specified, data not shown).

No statistical differences in live pup body weights and no external anomalies were seen in the offspring.

Conclusion Applicant's summary and conclusion

Taken together all data of this study, there are no indications of a substantial impairment of fertility in rats up to the highest dose tested. Thus, the NOAEL was 4000 ppm (to average doses of 201.5 and 179.5 mg Ba/kg bw/d to male and and female rats, respectively). No-observed-adverse-effect levels (NOAELs) on developmental toxicity for rats of 4000 ppm were derived from this study. However, this NOAEL is of limited value to evaluate the potential for barium to induce developmental effects because there was no exposure of the females during gestation.

3.10.1.14 [Study 14] Prenatal developmental toxicity of barium chloride in rats (OECD TG 414)

Reference Study Report (2014) Prenatal Developmental toxicity of barium chloride (as summarised in the publically disseminated REACH Registration dossier of barium chloride).

Guideline OECD TG 414
GLP guideline

Reliability Klimisch 1: reliable without restriction (reliability according to the publically disseminated REACH Registration dossier of barium chloride)

Species / strain Rat, Wistar (female)

Test material Barium chloride dihydrate
Purity: unknown
Form: crystalline powder

Study design **Materials and methods**
Route of administration: oral gavage
Exposure: GD 0 – 20

Doses / Concentrations: 0, 10, 30 and 100 mg/kg bw

Dose selection rationale:
The dose levels have been selected in consultation with the study monitor on the basis of a dose range finding study with the test item in pregnant rats. During the dose range finding study groups of 5 mated females, were administered different dose levels of the test substance by gavage from gestation day 0 up to gestation day 21. A dose volume of 10 mL/kg body weight was applied and demineralized water was used as vehicle and control item. Dose levels of 0, 50, 175 and 250 mg/kg were administered.
Based on the preterm death of 3/5 females in the high dose group and 2/5 females in the mid dose group after a single dose, dosing was discontinued in both groups.
All surviving animals in the mid and high dose group were re-allocated to a new mid dose group and received 100 mg/kg body weight barium chloride from gestation day 2 onwards.
On gestation day 21 the animals were sacrificed and caesarean section was performed.
In-life parameters included clinical signs, morbidity, mortality, body weight and food consumption. At sacrifice uterus weight, number of corpora lutea, number of implantation sites, early and late resorptions, number of live and dead foetuses and foetus weight were recorded. In addition, foetuses were examined for external abnormalities/malformations and dams were observed for gross anatomical changes.

Results:
Oral administration of 0, 50, 100, 175 and 250 mg/kg barium chloride to mated females resulted in:
- the preterm death of 3/5 animals in the 250 mg/kg group and 2/5 animals in the 175 mg/kg group after a single oral

dose.
- the spontaneous death of one animal in the 100 mg/kg group on gestation day 21. This animal was found dead before cesarean section and had 11 dead foetuses. This animal had received one dose of 250 mg/kg on gestation day 0 and daily doses of 100 mg/kg from gestation day 2 to 21.
- limited clinical observations in the 250 and 175 mg/kg group, including hunched posture an piloerection.
- no effect on body weight or body weight gain, food consumption, mean number of corpora lutea, implantation sites, early and late resorptions and the mean number of live foetuses.
- although based on a limited number of litters (four in the 50 mg/kg group and three in the 100 mg/kg group) an effect on foetus weight could not be ruled out.
- no foetuses showing external malformations

Details on analytical verification of doses or concentrations**:** From all three batches of the test items prepared in the study, samples were taken immediately after preparation and stored in a refrigerator until analysis. The following analyses were conducted by Inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis. The test item was quantified using barium as a marker component:
- Homogeneity: the homogeneity of the test substance in the experimental test items was demonstrated in the first batch prepared, by analysing three samples (taken at different locations in the gavage liquid container) of each level.
- Concentration: the concentration of test substance at each level was determined in all three batches of test items prepared in the study.
- Stability: samples of the low-dose, mid-dose and high-dose level were analysed in the first batch prepared in the study at t=0 and after storage in the refrigerator (2 – 10 °C) for twelve days.

Results:
- Homogeneity: the relative standard deviations between the mean content at three different locations was < 5% in the low, mid and high dose level. Therefore barium chloride dihydrate was considered to be homogeneously distributed in each test.
- Stability: upon storage at refrigerator temperature from 22 November 2013 till 4 December 2013, the relative difference in test substance concentration between t=0 and t=4 days was -3.6, +1.5 and +4.2% in the low, mid and high dose level, respectively. And all the dose levels met the criteria for stability (relative difference ≤10%). Therefore it was concluded that there was no loss of test substance from any tests items during storage for twelve days in the refrigerator.
- Content: the content of barium chloride dihydrate determined in the test items are compared with the intended content. The relative difference between the mean determined content and the intended content was between 1.5 and 2.5% at all nominal levels of 1, 3 and 10 mg/ml which was within the acceptance criteria (relative difference ≤10%). Therefore, the actual content was considered to meet the intended level in each test item.

No. of animals: 24 rats/dose group

Details on test animals and environmental conditions

TEST ANIMALS- RccHan: WIST strain
- Source: Harlan, Horst, the Netherlands
- Age at study initiation: approx. 12 weeks of age
- Weight at study initiation: control group: 197.9 - 230.9 g; low dose group: 198.1 - 230.2 g; mid dose group: 190.9 - 232.5 g; high dose group: 191.6 - 239.4 g
- Housing: animals were housed in Macrolon cages with a bedding of wood shavings (Lignocel) and strips of paper (Enviro-dri) and a wooden block as environmental enrichment. During the quarantine and acclimatization periods, the animals were housed in groups of 4 per sex. Mated females were housed individually in Macrolon cages.
- Diet (ad libitum): cereal-based (closed formula) rodent diet (Rat & Mouse No.3 Breeding Diet; RM3) (supplier: SDS Special Diets Services, Witham, England)
- Water (ad libitum): domestic mains tap-water
- Quarantine period: 9 days (upon arrival the rats were quarantined and checked for overt signs of ill health and abnormalities. During the quarantine period, serological examinations of the microbiological status of the rats were conducted in a random sample.)
- Acclimation period: 2 days

ENVIRONMENTAL CONDITIONS
- Temperature: 20 - 24°C
- Relative humidity: exceeded 65% for short times only during cleaning activities
- Air changes: about 10 air changes per hour
- Photoperiod (hrs dark / hrs light): 12/12

Details on mating procedure:

Impregnation procedure: cohoused
- M/F ratio per cage: 2 females : one male
- Length of cohabitation: until a sperm positive smear was detected
- Proof of pregnancy: sperm in vaginal smear referred to as gestation day 0 of pregnancy

Maternal examinations:

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: daily
- Cage side observations: clinical signs and mortality

DETAILED CLINICAL OBSERVATIONS: No

BODY WEIGHT: Yes
- Time schedule for examinations: gestation days (GD) 0, 3, 6, 10, 14, 17 and 21

FOOD CONSUMPTION AND COMPOUND INTAKE: Yes (gestation days 0-3, 3-6, 6-10, 10-14, 14-17 and 17-21)
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: No

WATER CONSUMPTION AND COMPOUND INTAKE: No

POST-MORTEM EXAMINATIONS: Yes
- Sacrifice on gestation day #21
The females were killed by decapitation after CO2/O2 anaesthesia on gestation day 21 and examined for gross abnormalities.

Ovaries and uterine content:

The ovaries and uterine content was examined after termination: Yes
Examinations included:
- Gravid uterus weight: Yes
- Weight of empty uterus: Yes
- Weight of ovaries: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes
- Number of early resorptions: Yes
- Number of late resorptions: Yes
- Gross evaluation of placentas: Yes
For seemingly non pregnant females (part of) the uterus was stained with Na2SO3 in order to visualize possible implantation sites (Salewski E, 1964). Upon staining non pregnancy was confirmed for these females.

Foetal examinations:

- External examinations: Yes
- Soft tissue examinations: Yes: half per litter
- Skeletal examinations: Yes: half per litter
- Head examinations: No

Further examinations:
- number of live and dead foetuses
- sex of the foetuses
- live foetuses (individually) and corresponding placentas
- foetal weight

Statistics: Tests were generally performed as two-sided tests with results taken as significant where the probability of the results was $p<0.05$ or $p<0.01$.
Continuous data were subjected to the ‘Decision tree for continuous data’ and dichotomous data to the ‘Decision tree for dichotomous data’.

Findings **Details on maternal toxic effects:**

CLINICAL SIGNS AND MORTALITIES
- two animals in the high dose group were found dead on day 21 of gestation. Both animals were pregnant and all foetuses were dead. Although death was not preceded by clinical signs, growth retardation or gross anatomical observations at necropsy that could clarify the death of these animals, the death of these rats is ascribed to treatment.
- one animal in the high dose group felt cold and was weakened and showed piloerection on gestation day 21. Upon necropsy this animal showed hydrothorax, haemorrhages in the liver and haemorrhagic discharge in the vagina. Also the death of this high-dose rat is ascribed to treatment.
- the spontaneous death of two rats, and the conditional decline of one rat on day 21 of gestation were considered to be treatment-related and to represent severe maternal toxicity in the high dose group.
- all foetuses were dead in the above three rats. The foetal deaths observed in these animals are considered to be related to the severe maternal toxicity in the high-dose group.

BODY WEIGHT AND BODY WEIGHT CHANGE
- no effects were observed on body weight. A slightly, but statistically significantly reduced body weight gain was observed in the high dose group as compared to the control group during the first three days of dosing. This was considered to be related to treatment and recovered thereafter.
- no effects on body weight or body weight gain were observed in the low dose group and the mid dose group as compared to the control group.

FOOD CONSUMPTION
- no effects were observed on food consumption.

REPRODUCTIVE PERFORMANCE
- 23, 22, 23 and 22 pregnant females in the control group, low dose, mid dose and high dose group, respectively.
- reproduction indices were comparable for the control, low dose, mid dose and high dose group
- no effects were noted in mean number of corpora lutea, mean number of implantation sites, preimplantation loss, mean number of early resorptions, late resorptions and mean number of live foetuses.

MACROSCOPY
- no treatment-related effects were observed

FEMALE REPRODUCTIVE ORGANS
- mean ovary weight, mean full and empty uterus weight were comparable in all groups
- mean carcass weight and net body weight change were comparable in all groups
- mean placenta weight was comparable in all groups

Details on embryotoxic / teratogenic effects

FOETUS WEIGHT AND SEX
- mean foetus weight was comparable in all groups
- mean percentages male littermates was comparable in all groups

FOETAL EXAMINATION
- foetal external, visceral, and skeletal examinations did not reveal any treatment-related effects.

Conclusion **Applicant's summary and conclusion**

Daily administration of barium chloride dihydrate at dose levels of 0, 10, 30 or 100 mg/kg body weight to pregnant rats from gestation day 1 up to and including gestation day 20, resulted in maternal toxicity as evidenced by the spontaneous deaths of two animals on gestation day 21 and the conditional decline of another animal on gestation day 21 in the high dose group. No developmental toxicity was observed. The NOAEL for maternal toxicity was therefore 30 mg/kg body weight (recalculated for barium chloride: 25.6 mg/kg bw/day) . In absence of developmental effects the NOAEL for prenatal developmental toxicity in the rat was ≥ 100 mg/kg body weight (recalculated for barium chloride: ≥ 85.3 mg/kg bw/day).

3.10.2 Human data

3.10.2.1 [Study 1] Retrospective study (occupational exposure)

Reference Duydu, Y., Başaran, N., Aydın, S., Üstündağ, A., Yalçın, C. Ö., Anlar, H. G., and Ickstadt, K. (2018a). Evaluation of FSH, LH, testosterone levels and semen parameters in male boron workers under extreme exposure conditions. Archives of toxicology, 92(10), 3051-3059.

Exposure The study investigated boron-occupational exposure of workers from a borate-processing plant (Bandirma) and a boron-mining plant (Bigadic Boron Works), both located in Turkey.

Study design

HYPOTHESIS TESTED:

The global hypothesis was that the means of the five groups are equal (Kruskal-Wallis test).

METHOD OF DATA COLLECTION

Details: A questionnaire survey was carried out to gather information on demographic data and possible confounding variables (age, duration of employment, pesticide application, smoking and alcohol consumption). As lunch was regularly provided for all employees in the central cafeteria, which was located within the boric acid production zone, drinking water and meal samples were taken also from there.

- Air sampling:

Bandirma: static air sampling was performed at 5 different stations (central cafeteria/garage, mechanical workshop, steam power plant, infirmary and acid production plant), representing the whole sampling area. Static air sampling was also performed at one air sampling station in downtown Bandirma.

Bigadic: personal air sampling was performed in workers (n = 65) working in the high exposure (packaging unit) areas. Static air sampling was performed for the rest of the workers (n = 45). Static air sampling was also performed in the village centres of Osmanca and Iskele at two locations, representative of both villages. Both, personal air sampling and static air sampling, were performed using IOM samplers and personal air sampling pumps (SKC, AirCheck 2000). The flow rate was 2 L/min, and the sampling time was 8 h. SKC (GLA-5000), 5 µm, 25 mm filters were used to sample boron compounds within inhalable dust.

- Biological sampling: performed at the day at which the workers completed their work shift periods (the working programme of the enterprise consisted of three work shifts, of 8h each). Peripheral blood samples were drawn from veins of the volunteers into appropriate vacutainer tubes. The blood samples in heparin tubes were stored at 4 °C for subsequent determination of boron. The tubes containing clot activator (BD vacutainer) were used to determine follicle-stimulating hormone (FSH), luteinizing hormone (LH) and total testosterone levels, using Immulite 2000 Immunoassay. The semen samplings and analysis were in accordance with the recommendations of World Health Organization (WHO 2010). Sperm concentration, motility and morphology parameters were determined in fresh semen samples using SQA-V Gold Sperm Quality Analyzer. Spot urine samples (post-shift) were collected in polypropylene containers and stored at – 20 °C for subsequent determination of boron and creatinine (Cayman chemical). Analysis of dust collected in cassettes by gravimetric and instrumental methods, boron determination in body fluids was performed with inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass spectrometry.

STUDY PERIOD:

2014 – 2017

STUDY POPULATION

- Total population: 212 workers from both Bandirma and Bigadic, classified as follows:

Low exposure group: blood boron concentrations < 100 ng B/g blood were (n = 12);

Medium exposure group: with blood boron concentrations between 100 – 150 ng B/g blood (n = 17);

High exposure group: with blood boron concentrations between 150 – 400 ng B/g blood (n = 85);

Extreme exposure group: with blood boron concentrations ≥ 400 ng B/g blood (n = 98).

- Age and sex of the study population (mean ± SD (range)):

Low exposure group (*n* = 12): 33.75 ± 7.85 (24–46), males;

Medium exposure group (*n* =17): 35.71 ± 6.75 (27–48), males;

High exposure group (*n* = 85): 34.24 ± 6.20 (22–49), males;

Extreme exposure group (*n* =98): 36.69 ± 6.52 (23–50), males.

-Duration of employment (years, mean ± SD (range)):

Low exposure group: 4.79 ± 2.37 (2.5–11,0);

Medium exposure group: 9.06 ± 7.31 (1–22);

High exposure group: 6.33 ± 2.98 (1–15);

Extreme exposure group: 6.28 ± 4.76 (1–27).

- Selection criteria:

Bandirma: Part of the workers employed at the Bandirma boric acid production zone had been enrolled in the previous "Boron Project I" (Duydu et al. 2011), thus, for the current "Boron Project II" only workers who were not involved in the previous project, were selected. In the current (second) project, 102 workers participated from acid production facilities, steam power plant, mechanical workshop, garage, steelyard, demineralized water production unit, construction units and central cafeteria (cooks), but not from the boric acid production facilities.

Bigadic: In total, 110 workers participated in the study, employed at the Bigadic Boron Works and residing in Iskele or Osmanca (these villages are located near the boron deposits).

MEASURED PARAMETERS:

-DBE (daily boron exposure), boron concentrations in biological fluids (i.e. blood, urine, semen), sperm parameters (i.e. concentration, motile sperm concentration, progressively motile sperm concentration, functional sperm concentration, total sperm number, total motile sperm number, total progressive motile sperm number, total functional sperm, total morphologically normal sperm, morphologically normal forms), sperm motility parameters (i.e. total motility, progressive motility, non-progressive motility, immotility, velocity, sperm motility index), and FSH, LH and total testosterone levels.

Detailed study summary and results

Bandirma: Boron concentrations in the drinking water samples taken from the central cafeteria ranged between 16.60 and 45.02 mg B/L.

Bigadic: The workers who participated in the study were employed at the Bigadic Boron Works and residing in Iskele or Osmanca. Boron concentrations in the drinking water (environmentally) of Iskele were very high, i.e. around 18 mg B/L. Boron concentrations in environmental air samples from the residential areas of Osmanca and Iskele were < LOQ (i.e. 0.9 µg/filter of air samples).

DBE levels (mg B/day, Mean ± SD (range)):

Low exposure group: 15.07 ± 10.50 (3.61–35.61);

Medium exposure group: 19.85 ± 15.06 (4.10–47.18);

High exposure group: 26.84 ± 15.03 (3.84–55.10);

Extreme exposure group: 47.17 ± 17.47 (7.95–106.8).

Blood boron levels (ng B/g blood, Mean ± SD (range)):

Low exposure group: 74.03 ± 28.16 (23.80–99.37);

Medium exposure group: 126.6 ± 14.41 (102–149.8);

High exposure group: 269.2 ± 73.81 (151–391.9);

Extreme exposure group: 570.6 ± 160.1 (402.5–1100).

Semen boron levels (ng B/g semen, Mean ± SD (range)):

Low exposure group: 475.9 ± 639.4 (110.6–2455);
Medium exposure group: 1019 ± 1082 (346.7–3863);
High exposure group: 1158 ± 1449 (179.4–10543);
Extreme exposure group: 1772 ± 1791 (188.7-18072).

Table: Boron concentration in biological fluids

Parameters	Exposure groups				*p* value
	Low exposure, < 100 ng B/g blood ($n = 12$)	Medium exposure, 100–150 ng B/g blood ($n = 17$)	High exposure, 150–400 ng B/g blood ($n = 85$)	Extreme exposure, > 400 ng B/g blood ($n = 98$)	
Age	33.75 ± 7.85 (24–46)	35.71 ± 6.75 (27–48)	34.24 ± 6.20 (22–49)	36.69 ± 6.52 (23–50)	> 0.05
Duration of employment, year	4.79 ± 2.37 (2.5–11,0)	9.06 ± 7.31 (1–22)	6.33 ± 2.98 (1–15)	6.28 ± 4.76 (1–27)	> 0.05
Blood boron, ng B/g blood	74.03 ± 28.16 (23.80–99.37)	126.6 ± 14.41 (102–149.8)	269.2 ± 73.81 (151–391.9)	570.6 ± 160.1 (402.5–1100)	< 0.05*
Urine boron, mg B/g creat	7.54 ± 17.68 (0.79–63.46)	7.01 ± 6.13 (1.79–27.12)	5.63 ± 3.09 (1.13–19.09)	14.20 ± 7.91 (1.06–43.09)	< 0.05**
Semen boron, ng B/g semen	475.9 ± 639.4 (110.6–2455)	1019 ± 1082 (346.7–3863)	1158 ± 1449 (179.4–10543)	1772 ± 1791 (188.7–18072)	< 0.05**
DBE, mg B/day	15.07 ± 10.50 (3.61–35.61)	19.85 ± 15.06 (4.10–47.18)	26.84 ± 15.03 (3.84–55.10)	47.17 ± 17.47 (7.95–106.8)	< 0.05***

Mean ± SD (range)

DBE daily boron exposure, Kruskal–Wallis for global hypothesis, Wilcoxon–Mann–Whitney as post hoc test with Bonferroni–Holm correction

*All pairwise

**L-M, L-H, L-E, M-E, H-E

***L-H, L-E, M-E, H-E

In general, the boron concentrations in the biological fluids were very much paralleled by the levels of calculated daily boron exposure (DBE). The correlations between blood boron-DBE, blood boron-urine boron and blood boron-semen boron levels were all statistically significant ($p < 0.01$). The mean semen boron concentrations of the workers were 6.4, 8.0, 4.3 and 3.1 times higher than the mean blood boron concentrations of workers classified in low, medium, high and extreme exposure groups.

Sperm parameters:

Sperm quality parameters (and reproductive hormone levels) were compared between the differently exposed groups of workers to identify possible reproductive effects attributable to boron exposure. No statistically significant ($p > 0.05$) differences were observed in pairwise comparisons of the exposure groups for the following parameters: sperm concentration and sperm morphology parameters (sperm counts, motile sperm, progressively motile sperm concentrations, functional sperm, total sperm number, total motile sperm, total progressive motile sperm, total functional sperm, total number of morphologically normal sperm, percentage of morphologically normal sperm forms).

Table: Sperm parameters as determined by SQA-V gold sperm quality analyzer

Parameters	Low exposure ($n=12$)	Medium exposure ($n=17$)	High exposure ($n=85$)	Extreme exposure ($n=98$)	p value
Sperm concentration, M/mL. RV ≥ 15	81.51 ± 67.23 (14.8–234.5)	79.36 ± 58.6 (4.6–195.3)	89.69 ± 59.74 (8.0–276.3)	82.44 ± 62.85 (4.9–260.3)	> 0.05
Motile sperm conc., M/mL	39.43 ± 29.53 (7.8–108.1)	38.15 ± 28.42 (2.1–101.1)	43.45 ± 31.13 (0.4–143)	38.77 ± 30.33 (0.4–140.7)	> 0.05
Progressively motile sperm conc., M/mL	32.05 ± 26.22 (5.7–93.2)	31.35 ± 25.05 (0–86)	35.83 ± 27.94 (0–129.3)	31.87 ± 27.37 (0–127.1)	> 0.05
Functional sperm conc., M/mL	8.35 ± 8.11 (1–24.4)	8.79 ± 8.44 (0–27.6)	10.37 ± 9.83 (0–46)	9.1 ± 9.68 (0–44.3)	> 0.05
Total sperm number, M/ejac., RV: ≥ 39	215.4 ± 193.8 (24.5–583.7)	254.7 ± 244.4 (11.5–825.6)	263.4 ± 241.9 (16–1253.4)	276.8 ± 323.8 (8.5–1973.7)	> 0.05
Total motile sperm, M/ejac	100.2 ± 79.46 (14.50–258.7)	120.9 ± 110.2 (5.3–404.4)	129.7 ± 132.4 (1.8–679)	127.8 ± 134.8 (1.2–566)	> 0.05
Total progressive motile sperm, M/ejac.	79.88 ± 67.09 (10.3–213.9)	98.41 ± 93.64 (0–344)	108 ± 115.9 (0–592.3)	104.8 ± 114.9 (0–486.5)	> 0.05
Total functional sperm, M/ejac	19.25 ± 18.45 (2–56.8)	26.46 ± 27.21 (0–110.4)	32.45 ± 41.41 (0–207)	27.94 ± 33.15 (0–147)	> 0.05
Total morphologically normal sperm, M/ejac	21.82 ± 20.28 (2.3–57.5)	29.24 ± 30.23 (0.3–120)	33.95 ± 41.29 (0.5–207.8)	32.19 ± 37.26 (0.3–160.3)	> 0.05
Morph. normal forms, RV ≥ 4%	11.33 ± 5.26 (6–23)	10.31 ± 4.85 (3–21)	11.13 ± 5.05 (3–30)	10.53 ± 5.42 (3–24)	> 0.05

Mean ± SD (range)

M million, *RV* reference value, *ejac*. ejaculate, Kruskal–Wallis for global hypothesis, Wilcoxon–Mann–Whitney as post hoc test with Bonferroni–Holm correction

Sperm motility parameters:

The mean values of total motility, progressive motility, non-progressive motility, immotility, velocity, and sperm motility index were compared between the low, medium, high and extreme exposure groups, and no statistically significant difference was observed ($p > 0.05$) in pairwise comparisons of the exposure groups. The mean values of these parameters were again well above their reference values (i.e. according to WHO, the reference values for "total motility" and "progressive motility" are ≥ 40% and ≥ 32%, respectively).

Table: Sperm motility parameters determined by SQA-V gold sperm quality analyser

Parameters	Low exposure ($n=12$)	Medium exposure ($n=17$)	High exposure ($n=85$)	Extreme exposure ($n=98$)	p value
Total motility (PR + NP), RV ≥ 40%	52.08 ± 10.32 (38–75)	48.53 ± 11.17 (28–74)	48.25 ± 15.07 (0–97)	46.85 ± 15.68 (2–83)	> 0.05
Progressive motility (PR), RV ≥ 32%	40.92 ± 9.89 (28–57)	35.71 ± 16.36 (0–60)	37.93 ± 15.01 (0–88)	35.96 ± 15.86 (0–67)	> 0.05
Non-progressive motility (NP), %	11.33 ± 4.72 (5–21)	12.82 ± 10.22 (6–44)	10.32 ± 4.49 (0–28)	10.90 ± 5.37 (2–32)	> 0.05
Immotility, %	47.25 ± 10.27 (25–62)	51.47 ± 11.17 (26–72)	51.75 ± 15.07 (3–100)	53.15 ± 15.68 (17–98)	> 0.05
Velocity, mic./s	11.75 ± 3.19 (8–18)	11.12 ± 4.44 (1–18)	11.31 ± 3.80 (1–18)	10.83 ± 4.08 (1–18)	> 0.05
Sperm motility index	176.5 ± 166.3 (32–580)	163.2 ± 139.5 (0–540)	178.8 ± 137.4 (0–556)	167.7 ± 147.2 (0–593)	> 0.05

Mean ± SD (range)

mic./sec. micron/second, Kruskal–Wallis for global hypothesis, Wilcoxon–Mann–Whitney as post hoc test with Bonferroni–Holm correction

Hormone levels:

FSH, LH and total (free and protein-bound) testosterone concentrations were determined in the blood samples: no statistically significant differences (p>0.05) of mean FSH, LH and total testosterone concentrations between the low, medium, high and extreme exposure groups, were found. Statistically significant correlations between blood boron-FSH, blood boron-LH and blood boron-total testosterone concentrations were not apparent ($p > 0.05$).

Table: FSH, LH and total testosterone levels in low, medium, high and extreme exposure groups

Parameters	Low exposure ($n=12$)	Medium exposure ($n=17$)	High exposure ($n=85$)	Extreme exposure ($n=98$)	p value
FSH, mIU/mL	4.15 ± 1.95 (1.6–7.69)	3.91 ± 2.88 (1.17–9.95)	4.04 ± 3.40 (1.02–26.56)	4.21 ± 2.63 (0.42–16.65)	>0.05
LH, mIU/mL	3.71 ± 1.1 (1.80–5.13)	4.01 ± 1.63 (1.33–7.09)	3.82 ± 1.68 (0.83–9.18)	3.72 ± 1.70 (1.18–8.66)	>0.05
Total testosterone, ng/dL	323.8 ± 135.8 (162.3–615.6)	373 ± 183.7 (172.8–922.4)	354 ± 152.9 (44.3–899.7)	332.5 ± 129 (120.9–731.2)	>0.05

Mean ± SD (range), Kruskal–Wallis for global hypothesis, Wilcoxon–Mann–Whitney as post hoc test with Bonferroni–Holm correction

Conclusion Boron-mediated adverse effects on semen parameters and reproductive hormone levels in males have not been observed under extreme exposure conditions.

3.10.2.2 [Study 2] Retrospective study (occupational and environmental exposure)

Reference Duydu, Y., Başaran, N., Üstündağ, A., Aydın, S., Ündeğer, Ü., Ataman, O. Y. and Golka, K. (2011). Reproductive toxicity parameters and biological monitoring in occupationally and environmentally boron-exposed persons in Bandırma, Turkey. Archives of toxicology, 85(6), 589-600.

Exposure The study investigated boron-environmental and occupational exposure (i.e. boric acid and borax) of workers from a borate-processing plant (Bandirma), located in Turkey.

Study design

HYPOTHESIS TESTED:

The null hypothesis for each biologic fluid was that the means of the respective four groups are equal.

METHOD OF DATA COLLECTION

Details:

- Personal sampling:

exposed group only, personal air sampler (SKC, AirCheck 2000), flow rate 2 L/min, sampling time 8 hours; low-ash PVC filters (SKC, 5 37 mm, preweighed) and SureSeal cassettes (SKC, 37 mm).Analysis of dust collected in cassettes by gravimetric and instrumental methods (Selin B (2010) Boron Determination in Body Fluids by Inductively Coupled Plasma Optical Emission Spectrometry and Inductively Coupled Plasma Mass Spectrometry.

- Area air sampling: control group only: same devices and parameters were used as for the personal sampling but the devices were not carried by individuals, but used statically, to determine an average value for the control workers.

Biological sampling: taken at the end of a work shift; no samples taken on the first working day of the week or shift period; workers were informed of the importance to avoid a possible contamination (sampling after showering and changing of clothes).

STUDY PERIOD: not described in detail.

Exposure periods (years employed, boron blood level based groups):

Control 15.30 + 8.63

Low exposure 16.85 + 7.06

Medium 17.21 + 6.77

High 13.96 + 8.04

STUDY POPULATION

- Total population (Total no. of persons in cohort from which the subjects were drawn):

exposed: 428 workers, 102 participated: boric acid production workers (n=57), borax (disodium tetraborate decahydrate) production workers (n=31), sodium perborate production unit workers (n=5), boric acid plus borax (disodium tetraborate decahydrate) production workers (n=5), laboratory workers (n=2), a storage worker (n=1), a mechanic technician (n=1) controls: 432 workers, acid production plant workers (n=28), steam power plant workers (n=17), demineralized water production (DWP) unit workers (n=2), energy suppliers (n=11), mechanical workshop workers (n=19), garage workers (n=14), steelyard workers (n=2), construction service workers (n=3), laboratory technicians (n=3), and office workers (n=3).

- Selection criteria: original groups: exposed: all married workers of the plants described above, wishing to participate, were enrolled. Controls: probably matched for age and years of employment (and possibly additional

parameters), not described in detail boron blood level based groups:

Exposure groups n (204) Re-classification (ng boron/g blood)

New control group 49 <LOQ (48.5)

Low exposure group 72 >LOQ–100

Medium exposure group 44 >100–150

High-exposure group 39 >150

Significant background exposure to boron via the diet prepared in the same cafeteria for both groups made a regrouping necessary which was based on the blood boron levels. All participating workers were re-classified both according to their calculated daily boron exposure levels and to the blood boron levels. For the re-classification of dose groups blood boron levels published in recent epidemiological studies were taken into account. Workers with a blood boron concentration below the LOQ were combined to form the new control group.

- Total number of subjects participating in study: 204

- Sex/age/race: males original groups:

Exposed: 42.62 ± 4.76 (range: 28-50) years, Caucasian;

Controls: 41.75 ± 6.29 (range: 23-53) years, Caucasian.

- Smoker/non-smoker: not reported

- Total number of subjects at end of study: 204

- Matching criteria: not reported, probably age and years of employment (and possibly additional parameters)

COMPARISON POPULATION

- Type: Control group

- Details: The control group was defined as the group which had blood boron levels below the LOQ (level of quantification).

HEALTH EFFECTS STUDIED

-DBE and blood boron concentrations effects on: Sperm concentration parameters, motility parameters of sperm cells, sperm morphology parameters, DNA integrity with COMET assay, hormone levels (FSH, LH, total testosterone) and total PSA.

Detailed study summary and results

The high boron contamination (9.47± 0.18 mg B/L) of water sources for cafeteria and infirmary was not anticipated in the planning phase of the study. This “background” exposure lead to relatively high exposure of the control group.

Total average exposure of occupationally exposure exposed workers: 12.08 ± 6.18 mg boron/day).

Total average exposure of control workers: 5.83 ±1.71 mg boron/day.

The average daily boron exposure (DBE, in mg B/d) calculated for the reclassified groups are:

Control 4.68 ± 1.63

Low exposure 7.39 ± 3.97 Medium 11.02 ± 4.61 High 14.45 ± 6.57

• Mean calculated daily boron exposure levels (DBE): significantly higher in exposure groups than in the new control group.

Exposure to boron:

• Restricted to the tap water in the infirmary and the cafeteria of the company (oral) and to the atmosphere in the boron production sites (inhalation).

• The mean levels of inhaled boron (mg/8 h) 0.23 ± 0.79, 1.15 ± 3.14, 1.47 ± 2.69, and 2.58 ± 4.96 in control, low, medium and high exposure groups respectively. Medium and high exposure group significantly higher than in the control group

Boron levels in biological fluids:

• Mean urine boron levels: 2.59 ± 1.32, 5.01 ± 2.07, 7.03 ± 2.37, and 9.83 ± 5.13 mg/g creat. In control, low, median and high exposure groups. Significantly higher in exposure groups than in the new control group.

• Mean blood boron (ng/g) levels: < 48.5, 72.94 ± 15.43, 121.68 ± 15.62, and 223.89 ± 69.49 in control, low, med

and high exposure groups, respectively.

• Calculated DBE levels: positively correlated with the blood boron concentrations of the workers (Pearson corr. Coeff.: 0.635).

• Urine boron concentrations: positively correlated with the blood boron concentrations of the workers (Pearson corr. Coeff: 0.633).

• Semen boron concentrations (ng/g): 807.92 ± 1625.58, 1422.07 ± 1939.03, 1482.19 ± 1410.71 and 1875.68.2255.07 ± 2255.07 in control, low, med and high exposure groups.

• Semen boron concentrations in exposure groups vs. new control group significantly different; the dose response trend was not significant, variations within groups were great.

• Correlation between semen boron concentration and blood boron concentration: very low (Pearson corr. Coeff.: 0.222).

Table: Boron concentrations in biological fluids and calculated DBEs

Parameters	Control (C)	Low exposure (L)	Medium exposure (M)	High exposure (H)	*P* value
Age	41.69 ± 6.52 (26–52)	42.32 ± 5.66 (23–50)	42.19 ± 4.65 (32–50)	42.67 ± 5.33 (28–53)	>0.05
Years employed	15.30 ± 8.63 (0.17–26)	16.85 ± 7.06 (0.17–25)	17.21 ± 6.77 (0.17–25)	13.96 ± 8.04 (0.17–23)	>0.05
Urine boron, mg/g creat.	2.59 ± 1.32 (0.78–5.62)	5.01 ± 2.07 (2.38–13.54)	7.03 ± 2.37 (2.57–13.43)	9.83 ± 5.13 (3.34–32.68)	<0.05 (all pairwise comparisons)
Blood boron, ng/g	<48.5 (LOQ)	72.94 ± 15.43 (48.46–99.91)	121.68 ± 15.62 (100.51–146.07)	223.89 ± 69.49 (152.82–454.02)	no test performed
Semen boron, ng/g	807.92 ± 1,625.58 (<LOQ–8,597)	1,422.07 ± 1,939.03 (<LOQ–8,615)	1,482.19 ± 1,410.71 (<LOQ–4,897)	1,875.68 ± 2,255.07 (<LOQ–9,522)	<0.05 (C-L; C-M; C-H)
Inhaled boron, mg/8 h	0.23 ± 0.79 (<LOQ–4.09)	1.15 ± 3.14 (<LOQ–22.16)	1.47 ± 2.69 (<LOQ–11.34)	2.58 ± 4.96 (<LOQ–26.9)	<0.05 (C-M; C-H; L-H)
DBE, mg B/day	4.68 ± 1.63 (0.2–7.54)	7.39 ± 3.97 (2.56–24.72)	11.02 ± 4.61 (2.56–20.88)	14.45 ± 6.57 (3.32–35.62)	<0.05 (all pairwise comparisons)

Mean ± SD, range in brackets. *LOQ* Limit of quantification *DBE* calculated daily boron exposure levels of the workers (please refer to the electronic annex for the calculation method of DBE and the related LOQ values)

Hormone levels:

• no significant differences between dmini except for LH, mid dose vs. high dose.

• Very weak correlation between blood boron concentration and hormone levels (FSH: Pearson corr. Coeff: 0.143; LH: Pearson corr. Coeff: 0.164; total testosterone level: -0.053).

• No statistical significant difference in testosterone levels between new control group and exposure groups.

Table: Hormone levels and total PSA levels in workers.

Exposure groups	Control (C)	Low exposure (L)	Medium exposure (M)	High exposure (H)	*P* value
Total PSA ng/mL	1.18 ± 0.62 (0.28–3.04)	1.22 ± 0.70 (0.34–4.34)	1.30 ± 0.94 (0.40–4.07)	1.25 ± 0.65 (0.31–2.76)	>0.05
FSH mIU/mL	5.97 ± 2.71 (2.06–17.40)	5.26 ± 2.39 (1.63–17.40)	4.97 ± 2.29 (1.81–14.10)	7.47 ± 6.36 (1.81–40.20)	<0.05(*)
LH mIU/mL	4.15 ± 1.77 (1.49–8.91)	4.30 ± 2.00 (1.41–11.80)	3.67 ± 1.34 (1.29–7.15)	5.38 ± 3.20 (1.95–20.00)	<0.05 (M-H)
Total testosterone ng/dL	351.78 ± 133.84 (159–907)	337.40 ± 118.64 (127–773)	347.30 ± 110.91 (157–668)	329.56 ± 106.04 (95.9–581)	>0.05

Mean ± SD, range in *brackets*. * The global null hypothesis that all group means are equal is rejected, but the pairwise Mann–Whitney U tests provide no *P* values below 0.05 after adjustment for multiple testing (Bonferroni-Holm). M-H: The global null hypothesis is rejected and the only significant pairwise difference is between medium and high-exposure group

Table: Mean total testosterone levels of workers in different work shifts

	Shift time, *n* = 204		
	00:00–08:00	16:00–24:00	08:00–16:00
n	41	30	133
Total testosterone, ng/dl	355 ± 118.25 (108–668)	325.53 ± 97.14 (95.9–512)	340.93 ± 122.49 (127–907)

Mean ± SD, range given in *brackets*. The difference in mean total testosterone levels was statistically not significant between the shifts ($P > 0.05$)

Semen and sperm parameters (including morphology and DNA integrity testes):

• No significant difference in parameter tested between the exposure groups and the new control group.

• Correspondingly only a weak correlation between the percentages of the normal morphology and blood boron levels.

• Only weak correlation between inhaled boron (mg/8 h) and blood boron (0.279), inhaled boron– semen boron (0.185), and inhaled boron–urine boron (0.106) levels.

• Boron effects on semen parameters, reproductive hormone levels, or DNA integrity in sperm cells is absent. No significant dose-dependent relationship between reproductive toxicity biomarkers and blood boron concentration. The relatively extreme boron exposure conditions did not result in blood boron concentrations above considered safe.The PSA level was not statistically significantly different when groups are compared.

Table: Characteristics of the semen samples and sperm concentrations determined by SQA-V Gold Sperm Quality Analyzer

Semen parameters	Control *n* = 49/An. = 48	Low exposure *n* = 72/An. = 68	Medium exposure *n* = 44/An. = 43	High exposure *n* = 39/An. = 39	*P* value
Abstinence, day	3.98 ± 1.59 (1–7)	3.78 ± 1.17 (2–7)	3.95 ± 1.33 (2–7)	4 ± 1.32 (2–7)	>0.05
Volume of ejaculate, mL, RV: ≥2	3.24 ± 1.24 (1.5–7)	3.13 ± 1.19 (1–7)	3.65 ± 1.93 (1.5–10)	3.13 ± 1.34 (1–6)	>0.05
pH	7.9 ± 0.29 (7–9)	8.04 ± 0.30 (7.5–8.5)	7.94 ± 0.35 (7.5–8.5)	7.92 ± 0.34 (7.5–8.5)	>0.05
Sperm conc. M/mL, RV: ≥20	69.84 ± 40.76 (5.2–166.3)	64.94 ± 48.97 (5.2–231)	70.49 ± 61.26 (5.9–277.2)	65.34 ± 55.74 (5.9–292.3)	>0.05
Motile sperm conc., M/mL	35.86 ± 28.33 (1.4–161.2)	35.15 ± 27.62 (1.40–133.5)	33.48 ± 26.14 (0.3–135.3)	35.92 ± 36.06 (0.3–207)	>0.05
PMSC (grade a), M/mL	15.69 ± 14.3 (0.1–55.9)	16.28 ± 15.64 (0–52.8)	16.95 ± 14.68 (0.3–51.1)	17.48 ± 23.72 (0.3–136.4)	>0.05
PMSC (grade b), M/mL	14.03 ± 13.95 (0–89.8)	12.51 ± 11.94 (0–67.6)	11.18 ± 11.03 (0–66.4)	12.6 ± 11.45 (0–53.9)	>0.05
Functional sperm conc., M/mL	19.02 ± 20.75 (0–120.8)	18.7 ± 18.02 (0.1–82.8)	17.41 ± 15.33 (0.1–69.37)	19.9 ± 26.44 (0.1–152.9)	>0.05
All sperm, M/ejac., RV: ≥40	223.59 ± 157.68 (15.60–795.5)	187.65 ± 140.49 (13–752.5)	233.55 ± 224.26 (11.8–1,297)	185.69 ± 139.01 (8.9–584.6)	>0.05
Motile sperm, (a + b+c), M/ejac.	114.53 ± 93.33 (4.2–503.5)	102.1 ± 81.07 (3.5–409.5)	111.49 ± 72.24 (3.2–298)	104.85 ± 81.4 (2.6–414)	>0.05
PMS (grades a + b), M/ejac, RV: ≥20	92.84 ± 84.77 (0.3–460)	83 ± 72.91 (0–355.5)	89.86 ± 60.5 (0.6–229)	84.37 ± 73.14 (0.5–380.6)	>0.05
Funtional sperm, M/ejac.	58.45 ± 63.92 (0–342.5)	53.86 ± 52.6 (0.4–249.6)	54.57 ± 37.32 (0.2–122.4)	55.19 ± 56.18 (0.2–305.8)	>0.05

Mean ± SD, range in *brackets*. *An*. Analyzed samples, *Conc*. concentration, *PMSC* progressive motile sperm concentration, *PMS* progressive motile sperm, *M/ejac* million/ejaculate, *RV* reference value

Table: The motility parameters of the sperm cells determined by SQA-V Gold Sperm Quality Analyzer

Semen parameters	Control *n* = 49/An. = 48	Low exposure *n* = 72/An. = 68	Medium exposure *n* = 44/An. = 43	High exposure *n* = 39/An. = 39	*P* value
Motility %, (grades a + b+c)	50.6 ± 15.1 (6.1–96.9)	54.1 ± 14.1 (20.7–96.6)	52.41 ± 14.08 (21.4–74.1)	56.13 ± 15.57 (15.3–88.9)	>0.05
Rapid prog. mot. %, (grade a), RV: ≥25	20 ± 11.64 (0.4–42.3)	22.37 ± 14.74 (0–63.3)	22.65 ± 12.49 (3–48.4)	23.91 ± 13.86 (1.4–49.7)	>0.05
Slow prog. mot. %, (grade b)	18.8 ± 8.7 (0–54)	18.7 ± 7.92 (0–33.7)	17.67 ± 8.85 (0–37.5)	18.87 ± 8.85 (0–35.5)	>0.05
Non prog. mot. %, (grade c)	11.82 ± 5.1 (5.40–28.90)	12.95 ± 5.66 (5.3–27.3)	12.1 ± 5.7 (5.3–27.7)	13.35 ± 5.44 (5.6–3.1)	>0.05
Immotility %, (grade d)	49.38 ± 15.09 (3.1–93.1)	45.9 ± 14.1 (3.4–79.3)	47.59 ± 14.08 (25.9–78.6)	43.88 ± 15.57 (11.1–84.7)	>0.05
Sperm motility index, RV: ≥80	163.06 ± 141.67 (1–663)	174.06 ± 141.61 (0–659)	173.53 ± 130.08 (0–475)	157.9 ± 146.68 (0–776)	>0.05
Velocity (APV), mic/sec, RV: ≥5	11 ± 3.3 (1–19)	11.01 ± 3.6 (1–19)	11.20 ± 3.56 (1–17)	10.86 ± 3.89 (2–22)	>0.05
Morphology (N.F.%), WHO criteria, RV: ≥30	35.48 ± 11.51 (14.6–63.7)	37 ± 10.68 (15.60–60.30)	36.57 ± 10.57 (16.5–57.8)	37.96 ± 11.46 (16.4–60.5)	>0.05

Mean ± SD, range in *brackets*. *An.* Analyzed samples, *Prog.* progressive, *Mot.* motility, *N.F.* normal forms, *APV* average path velocity, *mic.* micron, *sec*. second, *RV* reference value

Table: Sperm morphology parameters as per the Kruger's criteria

Parameters	Control *n* = 49/An = 47	Low exposure *n* = 72/An = 65	Medium exposure *n* = 44/An = 42	High exposure *n* = 39/An = 37	*P* value
Normal morph. (%) RV: >14%	13.87 ± 8.05 (0–30)	16.74 ± 9.98 (1–36)	15.49 ± 7.94 (2–34)	17.95 ± 9.60 (2–40)	>0.05
Head defects (%)	56.55 ± 11.49 (32–90)	56.11 ± 10.17 (34–76)	57.10 ± 10.16 (31–80)	56.43 ± 9.26 (33–80)	>0.05
Neck/mid-piece defects (%)	12.23 ± 7.07 (2–36)	12.52 ± 6.87 (0–34)	14.48 ± 6.43 (2–29)	13.57 ± 6.65 (4–31)	>0.05
Tail defects (%)	9.09 ± 8.30 (0–34)	7.18 ± 5.96 (0–26)	7.79 ± 6.07 (0–24)	7.68 ± 5.95 (0–26)	>0.05
Cytoplasmic droplets (%)	0.45 ± 0.93 (0–4)	1.09 ± 1.97 (0–10)	0.79 ± 1.41 (0–6)	0.86 ± 1.51 (0–4)	>0.05

Mean ± SD, range in *brackets*. *RV* Reference value as per the Kruger's strict criteria, *An.* Analyzed samples

Conclusion Due to the background exposure via drinking water no clear relation could be found between inhalation exposure and boron levels in biological fluids. Blood and urine boron levels increased steadily with rising DBE, while semen boron levels failed to follow a steady trend. Variation in semen boron levels was high. Boron is accumulated in semen and the concentration factor is the highest at the lowest exposure. Adverse effects in hormone levels were absent when exposure groups are compared to the new control group. For any of the semen parameters a statistically significant difference was not seen between the new control.

3.10.2.3 [Study 3] Retrospective study (environmental exposure)

Reference Sayli, B. S., Tüccar, E., and Elhan, A. H. (1998). An assessment of fertility in boron-exposed Turkish subpopulations. Reproductive Toxicology, 12(3), 297-304.

Exposure The study investigated boron-environmental exposure of residents from 5 villages located near the borate-processing plant Bigadic, Balikesir county, Turkey.

Study design

HYPOTHESIS TESTED:

Relationships between elevated boron intake and fertility were sought by comparing reproduction in the residents of two Turkish villages with high levels of boron in their drinking water (one with 8.5 to 29 mg B/L and the other with 2.05 to 2.5 mg B/L), with three nearby villages with more typical lower boron levels (0.03 to 0.45 mg B/L). The two high boron villages were designated as Region I, and the three villages with lower boron in the drinking water were designated Region II. In addition to exposure to elevated boron in drinking water, 28.3 % of the probands in Region I were employed in borate mining or processing, whereas in Region II, 11.7 % were so employed. The data on fertility from these two populations was also compared with that from an area with a very low boron concentration in drinking water and no occupational exposure, and also from data for the whole Turkish population.

STUDY POPULATION

- Total population: The group with the high boron exposures in Regions I and II comprised 927 probands and by the use of a pedigree technique covering three generations, fertility data on 5934 marriages were investigated.

- Selection criteria: Relationships between elevated boron intake and fertility were sought by comparing reproduction in the residents of two Turkish villages with high levels of boron in their drinking water (one with 8.5 to 29 mg B/L and the other with 2.05 to 2.5 mg B/L), with three nearby villages with more typical lower boron levels (0.03 to 0.45 mg B/L). The two high boron villages were designated as Region I, and the three villages with lower boron in the drinking water were designated Region II. In addition to exposure to elevated boron in drinking water, 28.3 % of the probands in Region I were employed in borate mining or processing, whereas in Region II, 11.7 % were so employed.

-Sex/age/race: Males and females; 40 % of the probands were 30-39 y; 35 % 40-60 y; and 15 % < 30 y

-Smoker/non-smoker: Smokers and non-smokers

COMPARISON POPULATION

- Type: Other comparison group: The data on fertility from the study populations was also compared with that from an area with a very low boron concentration in drinking water and no occupational exposure, and also from data for the whole Turkish population. National population of Turkey 49,856 randomly chosen families. The regional population of Camlidere (relatively boron free soils) was 625 families, covering three generations.

Detailed study summary and results

In high boron areas, the average concentrations ranged from 0.7-29.0 mg B/L.

In other lower boron areas 0.05- 0.45 mg B/L. Drinking water in 5 supplies from the very low control area of Camlidere had levels <0.1 mg B/L.

Table: Distribution of children of probands from Region I by gender and vital status

	Alive		Deceased		Total	
Gender	Number	Percent	Number	Percent	Number	Percent
Male	181	48.39	8	28.57	189	47.01
Female	193	51.61	19	67.85	212	52.73
Unspecified	0	0	1	3.57	1	0.24
Total	374	100.00	28	100.00	402	100.00
Sex ratio	0.93		0.42		0.89	

Table: Fertility of kindreds over three generations, Region I

Generation	Family status	Number of families	Percent infertile	Number of families	Percent infertile
I	With children	290	97.97		
	Without children	6	2.03		
	Cause:				
	Recent marriage			0	0.00
	Primary infertility			6	2.03
	Secondary infertility			0	0.00
	Subtotal	296			
II	With children	589[a–c]	95.46		
	Without children	28	4.53		
	Cause:				
	Recent marriage			13	2.11
	Primary infertility			15[d]	2.43
	Secondary infertility			0	0.00
	Subtotal	617			
III	With children	143	92.26		
	Without children	12	7.74		
	Cause:				
	Recent marriage			8[e]	5.16
	Primary infertility			4[f]	2.58
	Secondary infertility			0	0.00
	Subtotal	155			
Subtotal	With children	1022	95.69		
	Without children	46	4.31		
	Cause:				
	Recent marriage			21	1.97
	Primary infertility			25	2.34
	Secondary infertility			0	0.00
Total		1068		46	4.31

[a]Divorced, with children: 1.
[b]Offspring from two wives: 1.
[c]Married twice, first wife had habitual abortion: 1.
[d]No children with three wives: 1.
[e]Married twice, no child with first marriage, first husband had two wives without children.
[f]No pregnancy with first wife, 1 abortion with second, over a 17-year period.

Table: Children of proband families by number and sex, Region II

Children per family[a]	Sex	Number of families	Percent
1	Male	5	3.40
	Female	8	5.44
2	2 Males	12	8.16
	2 Females	7	4.76
	1 Male, 1 Female	29	19.73
3	3 Males	4	2.72
	3 Females	5	3.40
	1 Male, 2 Females	18	12.25
	2 Males, 1 Female	30	20.41
4	1 Male, 3 Females	5	3.40
	2 Males, 2 Females	10	6.80
	3 Males, 1 Female	4	2.72
5	1 Male, 4 Females	3	2.04
	2 Males, 3 Females	1	0.68
	3 Males, 2 Females	1	0.68
6	2 Males, 4 Females	2	1.36
	3 Males, 3 Females	1	0.68
	5 Males, 1 Female	1	0.68
7	3 Males, 4 Females	1	0.68
Totals		147	100.00

[a]Couples with no children in a category omitted.

Table: Fertility of kindreds over three generations, Region II

Generation	Family status	Number of families	Percent infertile	Number of families	Percent infertile
I	With children	46	93.88		
	Without children	3	6.72		
	Cause:				
	Recent marriage			0	0.00
	Primary infertility			3	6.12
	Secondary infertility			0	0.00
	Subtotal	49			
II	With children	439	96.27		
	Without children	17	3.73		
	Cause:				
	Recent marriage			4	0.80
	Primary infertility			12	2.63
	Secondary infertility			1	0.02
	Subtotal	456			
III	With children	98	93.33		
	Without children	7	6.67		
	Cause:				
	Recent marriage			6	5.71
	Primary infertility			1	0.96
	Secondary infertility			0	0.00
	Subtotal	105			
Subtotal	With children	583	95.57		
	Without children	27	4.43		
	Cause:				
	Recent marriage			10	1.64
	Primary infertility			16	2.62
	Secondary infertility			1	0.16
Total		610		27	4.43

In the high boron exposure region the infertility rate was 3.17 % in the probands and 3.0 % averaged over 3 generations. In the very low exposure control area infertility was 4.48 %, and in the general Turkish population was 3.84 %.

Conclusion No difference in fertility was observed between 399 men with occupational exposure to boron, and 222 men with similar occupations but not exposed to boron. It was concluded that within the limits of the study, there was no evidence that boron interfered with human fertility and reproduction.

3.10.2.4 [Study 4] Retrospective study (occupational exposure)

Reference Sayli, B. S. (2003). Low frequency of infertility among workers in a borate processing facility. Biological trace element research, 93(1-3), 19-29.

Exposure The study investigated boron-occupational exposure (i.e. boric acid and borax) of workers from a borate-processing plant (Bandirma), located in Turkey.

Study design

METHOD OF DATA COLLECTION

- Details : First phase:

The questionnaire covered marital status and childbearing properties of the proband, and included the age at marriage, its duration, the period of first conception, the number of pregnancies, births, foetal losses and congenital malformations, and the number and sex of children both alive and deceased. No physical examination was conducted but medical records if available were recorded.

Second phase:

Computerised individual files of all workers as well as all general management people were checked without interview.

SETTING: Borates plant, prior to or immediately after an 8 h shift.

STUDY POPULATION

- Total number of subjects participating in study:

Phase 1: 191

Phase 2: 712

Detailed study summary and results

At the first phase of the investigation, 191 workers were interviewed. Among these there were six infertiles of the primary type with a rate of 3.1 %. Boron-unrelated infertile couples among sibs were found to be 2.6 – 3.6 % and 3.2 % for three-generation marriages – none being higher than those revealed in different sets of controls.

Table: Dust Concentration of Different Units in the Facility*

Sampling site	Air, mg/m^3	Dust, mg/m^3
Drying unit of boric acid plant	0.252	5.952
Worker at drying unit of boric acid plant	0.189	1.058
Crushing unit of boric acid plant	0.204	4.166
Worker at crushing unit of boric acid plant	0.153	2.614
Drying unit of sodium perborate plant	0.240	3.333
Worker at drying unit of sodium perborate plant	0.180	2.080
Filling unit of borax penthydrate plant	0.264	3.787
Worker at filling unit of pentahydrate plant	0.198	3.224

* Representative figures as measured by Mürteza Hartamaci, Engineer for Dust Control Unit (kindly provided by Eti Bor, Inc.)

Table: Birth Places of Probands

Birth place	No.	%
Balıkesir (itself)	5	2.6
Vicinity	8	4.2
Bandırma (itself)	53	27.7
Vicinity	21	11.0
Other parts of the province	51	26.7
Close provinces	10	5.2
Distant provinces	38	19.9
Foreign country	5	2.6
TOTAL	191	100.0

Table: Age Distribution of Probands

Age group	Male		Female		Total	
	No.	%	No.	%	No.	%
20 – 29	6	3.3	1	12.5	7	3.6
30 – 39	121	66.1	5	62.5	126	65.9
40 – 49	47	25.7	-	-	47	24.6
50 – 60	3	1.6	2	25.0	5	2.6
60 – 69	3	1.6	-	-	3	1.5
Unspecified	3	1.6	-	-	3	1.5
TOTALS	183	100.0	8	100.0	191	100.0

In the second stage of work, computerised files of all workers of the facility and all employees of the general management sharing the same location were checked without an interview.

Twenty-four subjects (3.4 %) out of 712 workers were childless versus 2.7 % among 108 employees and 2.2 % among 91 workers of a distantly located acid plant of the same complex. The differences were not significant.

94.2 % of probands had at least 1 living child at the time of inquiry, including one widow and one separated. 307 children were born to proband families of which 50.1 % were males and 49.9 % females, all alive at the time of the investigation, with a sex ratio of 1.0.

Table: Occupational/Professional Distribution of Probands

Service/unit at facility	No.	%
Administration	7	3.6
Boric acid plant	58	30.3
Borax plant	26	13.6
Perborate plant	25	13.0
Product depot	11	5.7
Warehouse	7	3.6
Operator and drivers	3	1.5
Loading/unloading at harbor	30	15.7
Laboratory assistance	5	2.6
Miscellaneous	14	7.3
Retired miner	5	2.6
TOTAL	191	100.0

Table: Fertile and Infertile Subjects in First and Second Phases

Marital state	Studied at firt stage.... at second stage			
	No.	%	No.	%
With children	180	94.2	674	94.6
Marriage continuing	178	93.2	669	93.9
Widowed	1	0.5	3	0.4
Separated	1	0.5	2	0.3
Without children	11	5.8	38	5 .4
Recent marriage	4	2.1	8	1.1
Primary	6	3.1	24	3.4
Secondary	1	0.5	6	0.8
TOTALS	191	100.0	712	100.0

Table: Fertile and Infertile Couples Among Sibs of Proband and His (Her) Spouse

Marital state	Proband's				Proband's spouse's			
	Male sib[a]		Female sib[b]		Male sib[c]		Female sib[d]	
	No.	%	No.	%	No.	%	No.	%
With children	166	87.3	177	92.6	150	89.3	165	92.2
Without children								
Recent marriage	17	8.9	4	2.1	10	5.9	7	3.9
Primary	5	2.6	7	3.6	6	3.5	6	3.3
Secondary	2	1.0	3	1.6	2	1.2	1	0.5
TOTALS	190	100.0	191	100.0	168	100.0	179	100.0

[a] χ^2=0.09 and p>0.05.
[b] Fisher's exact test, p>0.05.
[c] χ^2=0.05 and p>0.05.
[d] χ^2=0.01 and p>0.05.

Conclusion Nine males and 6 female infants were described as deceased early in life. There were 1.7 alive and 0.1 deceased offspring per family. Of 119 interviewed, 32.5 % had 1 child, 56.6 % had 2 children and 8.8 % had 3 children. The remaining 2.3 % had 4 – 7 children. No discussion of foetal losses or congenital malformations were included.

3.10.2.5 [Study 5] Cross-sectional descriptive study (exposure and environmental)

Reference Chang, B. L., Robbins, W. A., Wei, F., Wu, G. and Elashoff, D. A. (2006). Boron workers in China: exploring work and lifestyle factors related to boron exposure. Aaohn Journal, 54(10), 435-443.

Exposure The study investigated occupational boron-exposure of workers from boron mines and processing plants located in the city of Kuandian, China.

Study design The study was based on interviews with participants who had occupational exposure to boron and a comparison group selected from an environment without significant exposure to boron.

This article described the lifestyle patterns of boron mining and processing workers (N = 936) and a comparison group (N = 251) in Northeast China, and explores relationships between boron exposure and reproductive health. An English version of an interview guide addressing areas of work and lifestyle relevant to boron exposure and metabolism was developed by an occupational health research team, translated to Chinese, and translated back, for clarity.

Modifications incorporated suggestions from local community advisory board and boron industry workers; the translation-back translation process was reapplied and cultural settings and semantic equivalence was attained.

The environmental boron exposure for the boron works (mean) and the comparison group (mean) were 2.6 – 3.8 mg/L for boron workers and 0.005 – 0.67 mg/L for the comparison group in surface water; 1.2 – 25.1 mg/L in boron workers well water and 0.002 – 0.67 mg/L for the comparison group's well water.

Detailed study summary and results

34 % of boron workers reported eating in the contaminated work areas.

Nearly all boron workers (99 %) showered or bathed after work although approximately 10 % redressed in their contaminated clothes.

Reproductive health outcomes were explored, including delayed pregnancy, multiple births, spontaneous miscarriages, induced abortions, stillbirths and unusual male:female offspring.

On average, boron workers fathered nearly 2.0 pregnancies compared with 2.1 pregnancies in the control group (P = 0.6). Of the self-reported pregnancies fathered by boron workers, an average of 1.3 resulted in livebirths, compared to an average of 1.4 for the comparison group (P = 0.3).

Table: **Environmental Boron Exposure for the Two Groups**

Substance Tested	*Boron Workers Mean*	*Comparison Group Mean*
Surface water		
Area 1	2.6 mg/L	0.005 mg/L
Area 2	3.8 mg/L	0.67 mg/L
Well water		
Area 1	1.2 mg/L	0.002 mg/L
Area 2	25.1 mg/L	0.67 mg/L
Soil		
Area 1	133 mg/kg	38.8 mg/kg
Area 2	1,195 mg/kg	82.4 mg/kg
Legumes	40.7 mg/kg	24.9 mg/kg
Potatoes	12.4 mg/kg	6.0 mg/kg
Soybeans	34.0 mg/kg	43.0 mg/kg

Table: Reproductive Health of the Two Groups* *(N* =1,087)

Variable	*Boron Workers (n = 843)*	*Comparison Group (n = 244)*	p
Delay in pregnancy†	78 (9.42%; total = 828)	11 (4.62%; total = 238)	.018†
Multiple births	6 (0.71%)	3 (1.23%)	.428
Spontaneous miscarriage	65 (7.71%)	12 (4.92%)	.134
Induced abortion	332 (39.38%)	115 (47.13%)	.030
Stillbirth	9 (1.07%)	5 (2.05%)	.329
Tubal or ectopic pregnancy	3 (0.36%)	0 (0%)	> .999
More boys than girls‡	387 (55.52%; total = 697)	117 (60.31%; total = 194)	.234
Mean no. of pregnancies fathered altogether (*SD*)	1.98 (1.08)	2.11 (1.10)	.064
Mean total no. of live births (*SD*)	1.26 (0.61)	1.35 (0.65)	.028

**Males currently married, widowed, or divorced.*
†Based on 828 of the 843 boron workers and 238 of the 244 comparison group members who had been married for at least 1 year at the time of interview and answered "yes" to "trying for a child without success for more than a year." p = .11 after adjusting for age, educational level, race, smoking, alcohol use, and soybean intake.
‡Participants with equal numbers of boys and girls were excluded.

A significant difference existed between groups in delay in pregnancy, defined as the inability to conceive within 1 year of desiring a child, with boron workers experiencing greater delays. However in logistic regression models adjusting for age, education, race, tobacco, alcohol and soybean consumption the difference was no longer statistically significant (P = 0.11) with an odds ratio of 1.7 for boron workers compared to the control group (95 % confidence interval, 0.09 to 3.5).

Conclusion No statistically significant differences in reproductive health outcomes (i.e. delayed pregnancies, multiple births, spontaneous miscarriages, stillbirths and tubal or ectopic pregnancies) for the exposed workers were observed.

3.10.2.6 [Study 6] Retrospective study (occupational exposure)

Reference Whorton D, Haas J and Trent L. (1994a). Reproductive effects of inorganic borates on male employees: Birth rate assessment. Environ. Health Perspect. 102 (Suppl. 7) 129 - 131.

Whorton MD, Haas JL, Trent L and Wong O. (1994b). Reproductive effects of sodium borates on male employees: Birth rate assessment. Occupational and Environmental Medicine 51, 761 - 767

Exposure The study investigated occupational boron-exposure of workers from a sodium borates mining and production facility located in the Mojave Desert, California.

Study design

METHOD OF DATA COLLECTION

The fertility data were obtained primarily by self administered questionnaire, and a section of the group by telephone interview. A 10 % sample of questionnaires was checked against the relevant medical insurance records. The work and exposure data were provided from company records.

STUDY POPULATION

- Selection criteria: All male employees at the U.S. Borax mine and production facility in Southern California with more than 6 months service were invited to participate in the study.

- Total number of subjects participating in study: Of the 753 eligible male employees with more than 6 months service, 542 (72 %) participated. The demographic data, length of employment, age and year at hire and medical insurance records of the non-participants and the participants were compared and no significant differences were found.

- Sex/age/race: Males; wide range with average duration of employment in the facility of 16 years; race not specified

- Smoker/non-smoker: Smokers and non-smokers.

EXPOSURE

The range of exposure in one year was 2 to 35.7 mg/m^3 (sodium borates). Based on an average of 23.2 mg/m^3, the authors calculated the average exposure to borate dusts to be 203 mg/day assuming a 7 hour day and a respiratory volume of 8.75 m^3 (based on 10 m^3 for 8 hours). They assumed an average or usual boron content of 14% of the dust which, for the high exposure group, is equivalent to a mean of 28.4 mg B/d or 0.4 mg B/kg/d for a 70kg worker. The average exposure for the highest exposure group was 28.4 mg B/day (approximately 0.4 mg B/kg bw/day) for two or more years. The average duration of exposure was 16 years.

COMPARISON POPULATION

- Type: No specific local control group was studied, but the results expressed as the Standardised Birth Ratio (SBR) were compared with the SBR for the general US population adjusted for maternal age, parity, race and calendar year.

Detailed study summary and results There was a highly significant excess of offspring fathered by the male employees at the mine and production facility (529 observed births compared with 466.7 expected).

A statistically significant excess in the standardised birth ratio (SBR) of 113, significant at $p < 0.01$. The SBR for the workers with 'low' (< 3 mg/m3) exposures was not different from the SBR of those with 'medium' (3 – 8 mg/m3) and 'high' (> 8 mg/m3) exposures, and both exceeded 100. There was no evidence of a relation between exposure and this excess of offspring, nor were there any temporal differences during the more than 30 year period of observation. The SBR was also evaluated in 5 year periods from 1950-1990 and in every period the SBR was greater than 100.

9% of workers tried unsuccessfully to conceive for more than one year, which compares with the national average of 15 % of the adult population.

Table: Characteristics of study population forgive mean exposure categories: male employees

Characteristics	Exposure categories (mg/m³)				
	Mean (range) 0·37 (<0·82) (n = 108)	Mean (range) 1·34 (0·82–1·77) (n = 108)	Mean (range) 2·23 (1·78–2·97) (n = 108)	Mean (range) 3·98 (2·98–5·04) (n = 109)	Mean (range) 8·58 (⩾5·05) (n = 109)
Race (%):					
White	88	95	93	92	96
Non-white	12	5	7	8	4
Marital status (%):					
Single	5	2	5	6	7
Married	85	83	82	86	80
Divorced, separated, or widowed	10	15	13	8	13
Had vasectomy (%)	29	43	34	41	35
Tried to conceive >1 y (%)	12	7	13	4	9
Sought help for fertility (%)	8	3	7	5	4
Length of employment (y)	14·1	17·0	16·2	17·3	14·6
Age at hire	30·4	26·8	27·2	26·0	25·2
Year of birth	1945	1946	1946	1946	1947
Year of hire	1976	1973	1974	1973	1975
Average age	45·0	44·3	43·9	43·8	40·2

Table: Observed (Obs) and expected (Exp) births and standardised birth ratios (SBR) for five mean categories of exposure: male respondents

Category	n	Obs	Exp	SBR (95% CI)
<0·82	108	94	62·1	151 (121·8–185·5)**
0·82–1·77	108	108	104·2	104 (84·6–126·5)
1·78–2·97	108	93	94·1	99 (79·8–121·6)
2·98–5·04	109	114	110·2	103 (85·4–123·2)
⩾5·05	109	120	95·9	125 (103·6–149·5)*

*P < 0·05; **P < 0·01.

Table: Observed (Obs) and expected (Exp) births and standardized birth ratios (SBR) of male participants with > 2 consecutive years of high exposure (only exposed time)

Time after beginning/end of high exposure	Obs	Exp	SBR (95% CI)
12 months/12 months	26	21·5	121 (79·1–177·4)
15 months/12 months	24	20·0	120 (76·4–178·6)
18 months/12 months	22	19·2	115 (71·9–174·2)
Total work history	59	57·6	102 (77·9–131·3)

Table: Observed (Obs) standardised birth ratios (SBR) by 5y groups: male participants

Years	Obs	SBR (95% CI)
1950–1954	5	226 (73·1–528·0)
1955–1959	25	135 (87·1–199·1)
1960–1964	44	101 (73·7–135·2)
1965–1969	43	108 (78·8–144·6)
1970–1974	58	112 (85·5–144·1)
1975–1979	78	113 (89·7–140·7)
1980–1984	152	128 (108·9–149·4)**
1985–1990	124	101 (83·7–120·8)
All participants	529	113 (103·3–123·4)**

**P < 0·01

Table: Female offspring (%) for all participants and the five exposure categories

Category	*Female offspring (%) (95% CI)*
<0·82	55·3 (45·2–65·4)
0·82–1·77	56·5 (47·1–65·9)
1·78–2·97	51·6 (41·4–61·8)
2·98–5·04	50·9 (41·7–60·1)
⩾5·05	49·2 (40·2–58·2)
Total	52·7 (48·4–57·0)

Table: Female offspring (%) by 5 y groups: male employees

Years	*Female offspring (%) (95% CI)*
1950–1954	60·0 (17·1–100·0)
1955–1959	52·0 (32·4–71·6)
1960–1964	45·5 (30·8–60·2)
1965–1969	37·2 (22·8–51·7)
1970–1974	58·6 (45·9–71·3)
1975–1979	57·7 (46·7–68·7)
1980–1984	55·9 (48·0–63·8)
1985–1990	50·8 (42·0–59·6)
All participants	52·7 (48·4–57·0)

Conclusion An excess in the percentage of female offspring (52.7 % compared with 48.8 % expected) were fathered by these male employees, this increase was not statistically significant, and was not due to a deficit of boys since 249 were observed compared with 238 expected. Thus, there was an excess of 11 boys and 51 girls. There was no evidence of an exposure relationship to sodium borate exposures of the fathers and the excess of female offspring, nor were there any temporal differences. There was an inverse relationship between the increase percentage of female offspring and the sodium borate exposures of their fathers.

3.10.2.7 [Study 7] Retrospective study (environmental exposure)

Reference Duydu, Y., Başaran, N., Üstündağ, A., Aydın, S., Yalçın, C. Ö., Anlar, H. G. and Ickstadt, K. (2018b). Birth weights of newborns and pregnancy outcomes of environmentally boron-exposed females in Turkey. Archives of toxicology, 92(8), 2475-2485.

Exposure The study investigated boron-environmental exposure of women residing near a borate-processing plant (Bandirma) and a boron-mining plant (Bigadic Boron Works), both located in Turkey.

Study design HYPOTHESIS TESTED:

The global hypothesis was that the means of the three groups were equal (Kruskal-Wallis test).

METHOD OF DATA COLLECTION

Details: Demographic information and information on pregnancy outcomes were obtained by a questionnaire survey.

Information on possible confounders (alcohol consumption, smoking, pesticide application) was also obtained. This study did not include pregnant women, as pregnancy monitoring was not within the scope of the project. All participating women, both in Badirma and in Bigadic, accepted to provide biological samples (blood and urine) and specimens of food from their meals (breakfast, lunch, dinner), as well as drinking water samples.

- Air sampling:

Air sampling was performed at two and five different sites of

Bandirma and Bigadic, respectively. The sampling sites were representative of the appropriate study area. Static air sampling was performed using IOM samplers and personal air sampling pumps (SKC, AirCheck 2000). The flow rate was 2 L/min, and the sampling time was 8 h. SKC (GLA-5000), 5-µm and 25-mm filters, were used to sample inhalable dust.

- Biological sampling:

Bandirma: sampling was performed on pre-scheduled dates in the guesthouse of Eti Mine Works General Management that is located distant from both the local boric acid production plant and the commercial port. The participants were asked to bring samples of their actual meals (breakfast, lunch, dinner) and of their drinking water. Containers for food and water were provided. After completing the questionnaire, blood and urine samples were taken and stored.

Bigadic: sampling was performed by visiting the participants at home. Again, after completing the questionnaire, blood, urine, drinking water and meal samples (breakfast, lunch and dinner) were stored.

Vein blood samples were drawn into Vacutainer collection tubes containing heparin and stored at 4 °C for subsequent boron determination. Spot urine samples of all volunteers were collected in polypropylene containers and kept at − 20 °C until analysis of boron and creatinine. Creatinine analysis was performed using the creatinine assay kit of Cayman Chem. Corp.

Drinking water and food samples were stored in polypropylene containers at -20 °C until boron analysis.

STUDY PERIOD:

2014 – 2017

STUDY POPULATION

- Total population: 199 women residing near Bandirma and Bigadic, divided into 3 different groups based on the measured blood boron levels, as follows:

-Low exposure group: blood boron concentrations < 100 ng B/g blood were (*n* = 143);

-Medium exposure group: with blood boron concentrations between 100 – 150 ng B/g blood (n = 29);

-High exposure group: with blood boron concentrations between >150 ng B/g blood (n = 27).

- Age of the study population (mean ± SD (range)):

Low exposure group (*n* = 143): 32.31 ± 6.77 (17–49);

Medium exposure group (*n* =29): 36.28 ± 6.95 (23–49);

High exposure group (*n* = 27): 34.56 ± 6.10 (24–46).

MEASURED PARAMETERS:

-DBE (daily boron exposure), boron concentrations in biological fluids (i.e. blood, urine), preterm births, numbers of children, birth weights of newborns, congenital anomalies, abortions, miscarriage, stillbirth, early neonatal death, neonatal death and infant death.

Detailed study summary and results

Bandirma: Although significant boron exposure occurs in employees of the local boric acid production plant and the commercial port of Bandirma, environmental boron exposure is negligible for the general population living in Bandirma.

Bigadic: Boron concentrations in the drinking water (environmentally) of Iskele were very high, i.e. around 12.2 mg B/L.

Boron concentrations in air samples taken from Bandirma and Bigadic were lower than the limit of quantitation (LOQ). Therefore, environmental boron exposure by inhalation was not taken into account when estimating DBE levels. The major and relevant sources of boron exposure, in both Bandirma and Bigadic, were drinking water and food. The daily drinking water consumption of all participating females was assumed to be 2 L/day.

The daily boron exposure via food was estimated using the "double plate method" for both lunch and dinner (i.e. the provided food samples from lunch and dinner menus were equal to the amounts actually consumed). Local bread, cheese, eggs and olives were mostly consumed for breakfast. Boron concentration in these food samples was negligible. Therefore, boron exposure via breakfast was neglected.

DBE levels (mg B/day, Mean ± SD (range)):

Low exposure group: 9.73 ± 5.29 (2.26–38.27);

Medium exposure group: 21.62 ± 7.87 (8.08–39.71);

High exposure group: 24.67 ± 11.39 (10.47–57.86).

Blood boron levels (ng B/g
blood, Mean ± SD (range)):
Low exposure group: 39.74 ± 27.60 (3.28–99.28);
Medium exposure group: 124.19 ± 13.10 (100.35–1496.74);
High exposure group: 274.58 ± 213.00 (151.81–975.66).

The study covered a number of 199 women who altogether gave birth to 326 children (i.e. 162 girls and 164 boys), with the following measured parameters:

Number of childless women:
Low exposure group: 14;
Medium exposure group: 1;
High exposure group: 0.

Number of low body weight children (<2500 g):
Low exposure group: 21;
Medium exposure group: 6;
High exposure group: 7.

Number of very low body weight children(<1500g):
Low exposure group: 2;
Medium exposure group: 1;
High exposure group: 1.

Number of preterm births:
Low exposure group: 12;
Medium exposure group: 1;
High exposure group: 4.

Number of children with congenital anomalies:
Low exposure group: 6;
Medium exposure group: 1;
High exposure group: 1.

Number of spontaneous abortions (miscarriages):
Low exposure group: 21;
Medium exposure group: 6;
High exposure group: 6.

Number of stillbirths:
Low exposure group: 0;
Medium exposure group: 1;
High exposure group: 1.

Number of infant deaths:
Low exposure group: 2;
Medium exposure group: 2;

High exposure group: 0.

Birth weight of newborns (g, Mean ± SD (range)):
Low exposure group: 3213 ± 561 (1140 - 5000);
Medium exposure group: 3083 ± 563 (1400 - 4200);
High exposure group: 3112 ± 709 (1200 - 4750).

Birth weight of newborns - girls (g, Mean ± SD (range)):
Low exposure group: 3154 ± 536 (1140 - 4250);
Medium exposure group: 2991 ± 615 (1400 - 4000);
High exposure group: 3057 ± 674 (2000 - 4000).

Birth weight of newborns - boys(g, Mean ± SD (range)):
Low exposure group: 3269 ± 580 (1400 - 5000);
Medium exposure group: 3209 ± 464 (2000 - 4200);
High exposure group: 3142 ± 745 (1200 - 4750).

Table: Reproductive outcomes

Parameters	(T) Total population ($n=199$) Sum	(L) Low exposure ($n=143$) Sum	(M) Medium exposure ($n=29$) Sum	(H) High exposure ($n=27$) Sum	p value*
Number of children	326	215	57	54	>0.05
Number of girls	162	105	33	24	>0.05
Number of boys	164	110	24	30	>0.05
Sex ratio, male/female	1.01	1.05	0.72	1.25	>0.05
Low body weight (LBW), < 2500 g	34	21	6	7	>0.05
Very low body weight (VLBW), < 1500	4	2	1	1	>0.05
Number of childless women	15	14	1	0	>0.05
Number of pregnant women	6	6	0	0	>0.05
Preterm birth	17	12	1	4	>0.05
Congenital anomalies	8	6	1	1	>0.05
Induced abortion	23	18	4	1	>0.05
Spontaneous abortion (miscarriage)	33	21	6	6	>0.05
Stillbirth	2	0	1	1	>0.05
Early neonatal death	0	0	0	0	–
Neonatal death	0	0	0	0	–
Infant death	4	2	2	0	>0.05

*Statistical comparisons were performed between the low, medium and high exposure groups; χ^2 tests were used to determine the statistical significance between the groups

Table: Birth weights and reproductive characteristics of females

Parameters	(L) Low exposure ($n=143$)		(M) Medium exposure ($n=29$)		(H) High exposure ($n=27$)		p value
	Mean ± SD (min–max)	5th–95th percentile	Mean ± SD (min–max)	5th–95th percentile	Mean ± SD (min–max)	5th–95th percentile	
Age of mothers	32.31 ± 6.77 (17–49)	23.00–44.90	36.28 ± 6.95 (23–49)	24.40–46.60	34.56 ± 6.10 (24–46)	26.00–44.70	< 0.05 (L–M)
Duration of marriage, year	11.17 ± 7.02 (0.66–29)	2.00–25.00	16.61 ± 7.73 (0.58–29)	3.20–28.60	14.81 ± 8.64 (3–42)	5.30–28.10	< 0.05 (L–M)
Birth weight of newborns, g	3213 ± 561 (1140–5000)	2200–4000	3083 ± 563 (1400–4200)	1950–3820	3112 ± 709 (1200–4750)	2000–4000	> 0.05
Birth weight of newborns, girls, g	3154 ± 536 (1140–4250)	2600–3919	2991 ± 615 (1400–4000)	1690–3840	3057 ± 674 (2000–4000)	2015–4000	> 0.05
Birth weight of newborns, boys, g	3269 ± 580 (1400–5000)	2250–4000	3209 ± 464 (2000–4200)	2703–3800	3142 ± 745 (1200–4750)	1725–4055	> 0.05

*Kruskal–Wallis test for global comparison. Wilcoxon–Mann–Whitney test with Bonferroni–Holm correction as post hoc test

Birth weights of newborns (girls, boys, girls + boys) were statistically not different between low, medium and high exposure groups ($p < 0.05$). The boron-mediated effects on the birth weights analysed using linear spline regression models with two knots at 100 and 150 ng B/g blood, did not show any statistically significant associations. The numbers of newborns with LBW and VLBW were also compared between the low, medium and high exposure groups, and nostatistically significant differences were reported.

Conclusion Based upon the presented results, the authors concluded that environmental exposure to boron does not have an adverse effect on the development of the offspring.

3.10.2.8 [Study 8] Prospective mother-child cohort study (environmental exposure)

Reference Igra, A. M., Harari, F., Lu, Y., Casimiro, E. and Vahter, M. (2016). Boron exposure through drinking water during pregnancy and birth size. Environment international, 95, 54-60.

Exposure Boron environmental exposure of pregnant women residing in northern Argentina.

Study design METHOD OF DATA COLLECTION

Details: interviews conducted by the authors. At enrolment, the women were interviewed regarding family characteristics, including known diseases, preferred diet, last menstrual period (LMP), and pre-pregnancy weight. Data on maternal age, parity (number of born children), parental monthly income, years of maternal education, smoking, alcohol consumption, chewing of coca leaves, and prenatal vitamin supplementation were collected at the follow-up visits.

Biological samples:

-Serum samples were fractionated from whole blood samples collected in Trace Elements Serum Clot Activator tubes (Vacuette®, Greiner bio-one, Kremsmunster, Austria).

-Spot urine samples were collected in disposable trace element-free plastic cups and transferred to 20 mL polyethylene bottles (Zinsser Analytic GMBH, Frankfurt, Germany). Blood and spot-urine samples were collected at each visit, at which time the women were also interviewed about encountered health problems.

Waters samples: were repeatedly collected during the study period using 20-mL polyethylene bottles.

Boron concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700×, Agilent Technologies, Tokyo, Japan).

Because arsenic, cesium and lithium were also present at elevated concentrations in the drinking water and these elements may also impair foetal growth, the exposure to these was considered. We additionally measures lead, cadmium and selenium to test for confounding. Cesium and lithium were measured in whole blood and urine, lead and cadmium in whole blood and selenium in serum, all by ICP-MS. Exposure to arsenic was assessed by the sum concentrations of inorganic arsenic (iAs) and its mono- and dimethylated metabolites (MMA and DMA) in urine,

measured using HPLC-HG-ICPMS.

STUDY PERIOD:
2012 – 2013

STUDY POPULATION
- Total population: 180 women (out of the initial 194 women enrolled in the study, 182 were interviewed and provided samples and 2/182 had miscarriages).

- Age of the study population: average 25 years old (13 – 41 years)

MEASURED PARAMETERS:
-water boron levels, boron concentrations in biological fluids (i.e. blood, serum and urine);
-pregnancy outcomes: birthweight (g), length (cm) and head circumference (cm), (measured by the health care personnel immediately after birth (for most women) or after a few hours for seven women (3.9%) who delivered at home).

Detailed study summary and results

Parameters measured in the mothers:
-Average pre-pregnancy weight: 55 kg (range 38–86 kg);
-Average height: 153 cm (range 134–169 cm);
-BMI: 24% had a BMI above 25;
-Time of residing in the area: 96% had lived in the study area for several years (mean time 18 years, range 0.1–40 years);
-Median boron levels in drinking water (µg/L)
-tertile 1 (n=60): 5246;
-tertile 2 (n=60): 5965;
-tertile 3 (n=60): 6072;

-Median serum boron levels (µg/L) (range):
-first trimester (n=31): 118 (32-232);
-second trimester (n=99): 131 (20-273);
-third trimester (n=152): 135 (26-315);

-Whole blood boron levels (µg/L) (range):
-first trimester (n=31): 131 (55-245);
-second trimester (n=99): 119 (38-210);
-third trimester (n=152): 139 (47-280);

-Urinary boron levels (µg/L) (range):
-first trimester (n=31):
10 076 (3107-19681);
-second trimester (n=99): 9881 (2803-23058);
-third trimester (n=152):
10 307 (2972-21144);

-Whole blood lithium levels (µg/L) (range):
-first trimester (n=31): 21 (6.6-54);
-second trimester (n=99): 23 (4.1-52);
-third trimester (n=152): 26 (5.7-63);

-Urinary lithium levels (µg/L) (range):
-first trimester (n=31): 1117 (209-3768);
-second trimester (n=99): 1398 (262-3509);
-third trimester (n=152): 1465 (273-3732);

-Whole blood cesium levels (µg/L) (range):
-first trimester (n=31): 132 (12-288);
-second trimester (n=99): 107 (8.3-220);
-third trimester (n=152): 111 (8.9-253);

-Urinary arsenic levels (µg/L) (range):
-first trimester (n=31): 98 (31-458);
-second trimester (n=99): 104(26-282);
-third trimester (n=152): 129 (33-414);

Table: Maternal and infant characteristics by tertiles of serum boron concentrations during pregnancy

	Tertile 1 ($n = 60$)		Tertile 2 ($n = 60$)		Tertile 3 ($n = 60$)		
Variable	Median	5th–95th	Median	5th–95th	Median	5th–95th	p-Value[a]
Mothers							
Age (years)	23	16–37	24	16–37	25	15–37	0.84
Parity (n)	1	0–7.5	1	0–6	1	0–8	0.73
Pre-pregnancy weight (kg)	54	45–80	53	42–73	50	42–70	0.095
Height (cm)	153	145–166	153	146–161	152	144–158	0.079
Pre-pregnancy Body Mass Index (kg/m^2)	22	19–31	23	18–31	22	18–33	0.45
Education years (years)	9.0	3.0–14	9.0	2.0–14	9.0	0.5–14	0.96
Monthly income family (Argentinean pesos)	2050	0–6000	1700	0–5800	1000	0–4250	0.52
Boron in drinking water (µg/L)	5246	377–6525	5965	402–6525	6072	846–10,929	0.0049
Biomarker concentrations during pregnancy[b]							
Serum boron (µg/L)	75	11–101	133	111–161	205	168–381	0.0001
Whole blood boron (µg/L)	80	24–127	135	95–179	189	139–437	0.0001
Urinary boron (µg/L)	6592	1808–12,466	10,742	6132–15,832	13,684	7187–29,314	0.0001
Whole blood lithium (µg/L)	13	3.0–26	27	10–53	37	12–85	0.0001
Urinary lithium (µg/L)	916	169–1653	1631	451–3390	2106	1070–3846	0.0001
Whole blood cesium (µg/L)	75	4.3–198	108	9.8–223	166	11–362	0.0001
Urinary arsenic (µg/L)	74	24–273	145	32–274	129	43–469	0.0001
Infants							
Sex (boys, %)	51%		61%		51%		0.48
Gestational age at birth (weeks)	39	34–42	39	35–42	38	32–42	0.037
Birth weight (g)	3100	2320–3950	3040	2380–3650	3000	2110–3680	0.39
Birth length (cm)	49	42–51	48	42–51	47	43–50	0.037
Birth head circumference (cm)	34	31–37	34	31–36	34	32–36	0.99

[a] Kruskall Wallis H-test (for continuous variables) and chi-squared test (for categorical variables) across tertiles.
[b] Average during pregnancy for those with 2 or more measurements.

Table: Biomarker concentrations of boron, lithium, cesium and arsenic in the pregnant women by trimenter

	1st trimester ($n = 31$)		2nd trimester ($n = 99$)		3rd trimester ($n = 152$)	
	Median	5th–95th	Median	5th–95th	Median	5th–95th
Gestational week	12	7.6–14	22	16–27	33	28–38
Serum boron (µg/L)	118	32–232	131	20–273	135	26–315
Whole blood boron (µg/L)	131	55–245	119	38–210	139	47–280
Urinary boron (µg/L)	10,076	3107–19,681	9881	2803–23,058	10,307	2972–21,144
Whole blood lithium (µg/L)	21	6.6–54	23	4.1–52	26	5.7–63
Urinary lithium (µg/L)	1117	209–3768	1398	262–3509	1465	273–3732
Whole blood cesium (µg/L)	132	12–288	107	8.3–220	111	8.9–253
Urinary arsenic (µg/L)	98	31–458	104	26–282	129	33–414

Pregnancy outcomes:
-Mean birth weight: 3022 ± 459 g (range 1250–4500 g), with 9.4% weighing < 2500 g.
-Average birth length: 48 ± 2.3 cm (range 39–53 cm)
-Average head circumference: 34±1.7 cm (range 26–40cm)
-Average gestational age at birth: 39 weeks (range 29–42 weeks), and 18% of the infants were born pre-term (before 37 gestational weeks).

The adjusted mixed effect linear models showed that the serum boron concentration increased by 3.1 µg/L per gestational week on average (95% CI 1.9; 4.4, p-value < 0.001). The effect estimate for the inverse association between serum boron concentrations (above 80 µg/L) and birth length increased by 28% when considering only the third trimester instead of the whole pregnancy (B −0.088 for each 10 µg/L increase in serum boron concentration, 95% CI −0.14; −0.036, p-value = 0.001). The inverse association between serum boron concentrations (above 80 µg/L) and birth weight was statistically significant, and the fully adjusted effect estimate increased >2.5 times (from −4.5 to −12 g per 10 µg/L increase in serum boron) when considering only exposure in the third trimester. No statistically significant associations between serum boron concentrations > 80 µg/L and birth head circumference was found in any model.

Table: Linear spline regression analyses of birth outcomes in relation to maternal boron concentrations in serum (women's average concentrations during pregnancy)

	Basic adjustment[a,b]		Further adjustment[b,c]	
Outcomes	B (95% CI)	*p*-Value	B (95% CI)	*p*-Value
Birth length (*n* = 161)				
≤80 µg/L (n = 31)	0.27 (0.060, 0.47)	0.012	0.26 (0.061, 0.47)	0.011
>80 µg/L (*n* = 130)	−0.061 (−0.12, −0.0022)	0.042	−0.069 (−0.14, −0.0024)	0.043
Birth weight (*n* = 167)				
≤80 µg/L (n = 31)	38 (−2.6, 78)	0.067	36 (−3.0, 76)	0.070
>80 µg/L (*n* = 136)	−7.1 (−18, 4.3)	0.22	−4.5 (−17, 8.1)	0.48
Birth head circumference (*n* = 150)				
≤80 µg/L (*n* = 28)	0.077 (−0.11, 0.26)	0.41	0.13 (−0.055, 0.31)	0.17
>80 µg/L (*n* = 122)	−0.0050 (−0.043, 0.054)	0.84	0.021 (−0.033, 0.075)	0.45
Children born at term (≥37 gestational weeks)				
Birth length (*n* = 134)				
≤80 µg/L (*n* = 25)	0.24 (0.035, 0.45)	0.022	0.19 (−0.010, 0.39	0.063
>80 µg/L (*n* = 109)	−0.081 (−0.14, −0.024)	0.006	−0.11 (−0.17, −0.042)	0.001
Birth weight (*n* = 139)				
≤80 µg/L (n = 25)	34 (−7.3, 75)	0.11	28 (−12, 68)	0.17
>80 µg/L (*n* = 114)	−9.7 (−21, 1.6)	0.093	−8.7 (−21, 3.9)	0.18
Birth head circumference (*n* = 123)				
≤80 µg/L (*n* = 22)	0.093 (−0.11, 0.30)	0.38	0.12 (−0.093, 0.33)	0.27
>80 µg/L (*n* = 101)	−0.00027 (−0.052, 0.052)	0.99	0.019 (−0.041, 0.079)	0.53

[a] Basic models adjusted for gestational age at birth (weeks) and gestational age at birth squared (weeks^2).
[b] Coefficient per 10 µg/L change of boron concentration in serum.
[c] Further adjusted models include gestational age at birth (weeks), gestational age at birth squared (weeks^2), parity (number of children), height of the mother (cm), monthly family income (above or below 1700 Argentinean pesos), infant sex, cesium in whole blood (µg/L) and lithium in urine (µg/L).

Table: Linear spline regression analyses of birth outcomes in relation to maternal boron concentrations in serum during the third trimester

	Basic adjustment[a,b]		Further adjustment[b,c]	
Outcome	B (95% CI)	*p*-Value	B (95% CI)	*p*-Value
Birth length (*n* = 138)				
≤80 µg/L (n = 31)	0.23 (0.053, 0.41)	0.012	0.22 (0.049, 0.39)	0.012
>80 µg/L (*n* = 107)	−0.072 (−0.12, −0.026)	0.003	−0.088 (−0.14, −0.036)	0.001
Birth weight (*n* = 144)				
≤80 µg/L (n = 31)	47 (11, 82)	0.010	38 (4.9, 71)	0.025
>80 µg/L (n = 114)	−14 (−23, −4.9)	0.003	−12 (−22, −1.8)	0.021
Birth head circumference (n = 130)				
≤80 µg/L (*n* = 29)	0.16 (−0.011, 0.32)	0.067	0.19 (0.020, 0.35)	0.029
>80 µg/L (n = 101)	−0.026 (−0.066, 0.014)	0.21	−0.0065 (−0.053, 0.040)	0.78
Children born at term (≥37 gestational weeks)				
Birth length (*n* = 117)				
≤80 µg/L (*n* = 24)	0.16 (−0.0035, 0.33)	0.055	0.13 (−0.024, 0.29)	0.095
>80 µg/L (*n* = 93)	−0.071 (−0.11, −0.029)	0.001	−0.095 (−0.14, −0.048)	<0.001
Birth weight (n = 122)				
≤80 µg/L (n = 24)	31 (−3.5, 65)	0.078	24 (−8.5, 56)	0.15
>80 µg/L (*n* = 98)	−13 (−21, −3.9)	0.005	−11 (−21, −1.5)	0.025
Birth head circumference (n = 109)				
≤80 µg/L (n = 22)	0.13 (−0.057, 0.31)	0.17	0.15 (−0.039, 0.34)	0.12
>80 µg/L (*n* = 87)	−0.023 (−0.065, 0.020)	0.30	−0.0044 (−0.056, 0.047)	0.87

[a] Basic models adjusted for gestational age at birth (weeks) and gestational age at birth squared (weeks^2).
[b] Coefficient per 10 µg/L change of boron concentration in serum.
[c] Further adjusted models include gestational age at birth (weeks), gestational age at birth squared (weeks^2), parity (number of children), height of the mother (cm), monthly family income (above or below 1700 Argentinean pesos), infant sex, cesium in whole blood (µg/L) and lithium in urine (µg/L).

Conclusion The results of this study show that elevated environmental boron levels have a statistically significant effect on the birth size of newborn.

3.10.2.9 [Study 9] Retrospective study (environmental exposure)

Reference

Tuccar, E., Elhan, A. H., Yavuz, Y. and Şayh, B. S. (1998). Comparison of infertility rates in communities from boron-rich and boron-poor territories. Biological trace element research, 66(1-3), 401-407.

Exposure

The study investigated boron-environmental exposure of residents from villages located near the borate-processing plant Bigadic, Balikesir county, Turkey.

Study design

The study population was divided into three sub-groups. The individuals that were interviewed in each subgroup served as probands for the study. The first subgroup of probands was identified in Region 1 which covers an area near boron-rich territories. Dwellings of Region 1 were located close to borate pits and a processing plant. Region 2 probands were from villages far from boron deposits, but were within the same zone. Region 3 probands were born and lived in areas with a mixed group, some near to and some far from deposits and pits. In Region 1, drinking water forming from (natural) springs and wells contained 29 ppm boron, but in Region 2 the concentration was between 0.3 and 0.50 ppm. In the third region, no measurements were regularly made but boron content was not known to be too high. In all three areas there were active and former borate workers.
From Region 1, 226 families over three generations with respect to probands (that of the proband being the second) and from Region 2, 164 families were included. There were 177 families from Region 3 and 80 from Kirka.

Criteria for selection were the presence of legal marriage regardless of whether one member was dead or whether there had been a divorce. The study was carried out by home visits. Workers and other related individuals were contacted at borate plants and pits. Questionnaires were arranged in order to obtain the number of pregnancies, early infant deaths, congenital malformations, stillbirths and spontaneous abortions.

Detailed study summary and results

The infant death rate in Region 2 (low boron area) was higher than those of other regions (significantly different). Although difficult to recognise spontaneous abortions and stillbirths in a retrospective study based solely on the description of the probands (mostly females), these were considered separately, but no differences were found. The observed number of congenital malformation was not sufficient within the study groups to perform statistical tests.

Table: Infant deaths (conception basis)

	+		-		
	n	**%**	**n**	**%**	**Total**
Region I	41	8 86	422	91 14	**463**
Region II	57	15 75	305	84 25	**362**
Region III	18	6.10	277	93 90	**295**
Kırka	33	10 15	292	89 85	**325**
Total	**149**	**10.31**	**1296**	**89.69**	**1445**

$\chi^2 = 18.28$, $p < 0.001$.

Table: Infant deaths (live births)

	+		-		
	n	**%**	**n**	**%**	**Total**
Region I	37	8 47	400	91 53	**437**
Region II	57	18 10	258	81 90	**315**
Region III	18	6 45	261	93 55	**279**
Kırka	32	10 74	266	89 26	**298**
Total	**144**	**10.84**	**1185**	**89.16**	**1329**

$\chi^2 = 25.27$, $p < 0.001$.

Table: Still births (family basis)

	+		-		
	n	%	n	%	Total
Region I	5	2.79	174	97 21	179
Region II	4	3 92	98	96 08	102
Region III	5	4.90	97	95 10	102
Kırka	3	2.70	108	97 30	111
Total	17	3.44	477	96.56	494

$\chi^2 = 1.13$, $p > 0.05$.

Table: Stillbirths (conception basis)

	+		-		
	n	%	n	%	Total
Region I	6	1.30	457	98 70	463
Region II	4	1.10	359	98 90	363
Region III	5	1 69	290	98 31	295
Kırka	3	0 92	322	99 08	325
Total	18	1.24	1428	98.76	1446

$\chi^2 = 0.83$, $p > 0.05$.

Table: Spontaneous abortions (family basis)

	+		-		
	n	%	n	%	Total
Region I	13	8 13	147	91 88	160
Region II	8	7 84	94	92 16	102
Region III	11	10 78	91	89 22	102
Kırka	15	13 51	96	86 49	111
Total	47	9.89	428	90.11	475

$\chi^2 = 2.76$, $p > 0.05$.

Table: Congenital malformations (family basis)

	+		-		
	n	%	n	%	Total
Region I	4	2 30	170	97 70	174
Region II	0	0 00	101	100 00	101
Region III	0	0 00	103	100 00	103
Kırka	1	0.90	110	99 10	111
Total	5	1.02	484	98.98	489

Table: Congenital malformations (conception basis)

	+		-		
	n	%	n	%	Total
Region I	4	0 86	459	99 14	463
Region II	0	0 00	266	100 00	266
Region III	0	0.00	295	100 00	295
Kırka	1	0.31	324	99.69	325
Total	5	0.37	1344	99.63	1349

Conclusion Based on the gathered results (through questionnaires), the authors concluded that environmental, and both environmental and occupational exposure to boron do not induce developmental effects in humans.

3.10.2.10 [Study 10] Estimation of daily boron exposure limits (environmental exposure)

Reference Korkmaz, M., Şaylı, U., Şaylı, B. S., Bakırdere, S., Titretir, S., Ataman, O. Y. and Keskin, S. (2007). Estimation of human daily boron exposure in a boron-rich area. British journal of nutrition, 98(3), 571-575.

Exposure The study investigated environmental exposure to boron of residents of Balikesir area

Study design The aim of the study was to estimate daily boron exposure in 66 males in Turkey living in a B-rich area using water containing at least 2 mg/L boron with an average age of 38 - 55 (SE 1.66) years and an average number of years of residence in the boron rich area of 35 - 89 (SE 1.73). Another group of 57 males living in the city centres of Balikesir and Ankara were taken as controls; the average age and number of years of residence for this group were 29.44 (SE 1.43) and 10.26 (SE 1.83) years respectively. As it is assumed that boron levels in urine reflect daily boron exposure, the amount of urinary boron of both the study and control groups was analysed using an inductively coupled plasma optical emission spectrometry technique (ICP-OES).

Detailed study summary and results The average daily boron exposure was calculated as 6.77 (SE 0.47) mg in the study group and 1.26 (SE 0.1) mg in the controls. None of the subjects reported any health problems that may be linked to high boron exposure.

Table: Results of descriptive statistics for all characteristics in group I and group II

Variable	Group	*n*	Mean	SEM	Minimum	Maximum
Age	I	66	38·55[a]	1·66	20	79
	II	57	29·44[b]	1·43	20	52
Residency period (years)	I	66	35·89[a]	1·73	10	79
	II	57	10·26[b]	1·83	1	46
Height (m)	I	66	1·72[a]	0·009	1·58	1·91
	II	57	1·76[a]	0·009	1·59	1·92
Weight (kg)	I	66	73·98[a]	1·64	55·00	110
	II	57	74·60[a]	1·56	53·00	123
BMI (kg/m^2)	I	66	24·68[a]	0·66	23·4	39·44
	II	57	21·64[a]	1·08	21·0	35·17
Creatinine clearance (ml/min)	I	66	106·99[a]	2·36	66·20	147·7
	II	57	106·40[a]	2·86	73·91	180·2
Urine volume (24 h, ml)	I	66	1363[a]	63·8	650	2750
	II	57	1350[a]	63·1	500	2500
B (mg/d)	I	66	6·768[a]	0·473	1·766	22·81
	II	57	1·256[b]	0·104	0·212	2·901
B (mg/kg)	I	66	0·093[a]	0·006	0·017	0·285
	II	57	0·017[b]	0·001	0·003	0·045

Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).

Table: Pearson correlation coefficients among some traits for each group

	Group	Age	Residency period	B (mg/d)
Residency period (years)	I	0·852**		
	II	0·625**		
B (mg/d)	I	0·042	0·107	
	II	−0·380**	−0·170	
B (mg/kg)	I	0·036	0·088	0·949**
	II	−0·396**	−0·181	0·964**

**$P<0.01$.

Conclusion The estimated daily boron exposure was 6.77 mg B/kg bw/day for the high-exposure area (Region I).

3.10.2.11 [Study 11] Case control study (boric acid)

Reference Acs, N., Bánhidy, F., Puhó, E. and Czeizel, A. E. (2006). Teratogenic effects of vaginal boric acid treatment during pregnancy. International Journal of Gynecology and Obstetrics, 93(1), 55-56.

Exposure The study investigated the effect of vaginal tablets containing boric acid.

Study design The effects of the use of boric acid vaginal tablets for treatment of infectious diseases of the genital organs were evaluated in a Hungarian Case Control Surveillance of Congenital Abnormalities (HCCSCA) study.

In most cases, treatment consisted of two vaginal tablets of 30 mg each daily for 7 days.

Detailed study summary and results For the 22843 infants born with congenital abnormalities in the study group, 43 mothers (0.19 %) had received boric acid treatment and for the 38151 controls, 52 mothers (0.14 %) had received boric acid treatment.
There were no significant differences between the groups in maternal sociodemographic characteristics, occurrence of acute and chronic diseases and frequently used drugs. The extremely high prevalence of acute infectious diseases of the genital organs (85.8 % in the study group and 91.9 % among controls) explains the use of the boric acid.
Cases of congenital abnormalities affecting the skeletal system only occurred in the offspring of others who were treated with boric acid during their entire pregnancy. In this study there was a higher risk of neural tube defects when boric acid was used during the second and third months of pregnancy, but this finding was based on only two cases.

Table: Prevalence of vaginal boric acid treatment during pregnancy in different CA groups and adjusted prevalence odds ratio (POR) with 95% CI for confounders

Isolated CA	Total no. of cases	Entire pregnancy		Second and third months	
		No. (%)	POR (95% CI)	No. (%)	POR (95% CI)
Neural-tube defects*	1202	3 (0.3)	1.9 (0.6–6.1)	2 (0.2)	8.0 (1.7–37.8)
Posterior cleft palate	582	3 (0.5)	5.1 (0.5–50.5)	1 (0.2)	–
Cardiovascular CAs	4479	7 (0.2)	1.6 (0.5–4.9)	1 (0.0)	0.7 (0.1–8.0)
Hypospadias	3038	4 (0.1)	1.3 (0.4–4.7)	2 (0.1)	4.7 (0.4–53.3)
Undescended testis	2051	4 (0.2)	0.7 (0.2–2.4)	1 (0.1)	1.0 (0.1–15.8)
Poly/syndactyly	1744	4 (0.2)	4.0 (0.7–22.7)	1 (0.1)	2.1 (0.1–34.1)
Clubfoot	2424	8 (0.3)	3.1 (0.9–10.2)	1 (0.0)	1.8 (0.1–43.4)
CAs of skeletal system*	211	2 (1.0)	7.1 (1.7–29.5)	0 (0.0)	–
Other isolated CAs	5763	5** (0.1)	0.5 (0.2–1.3)	2 (0.0)	3.0 (0.3–33.0)
Cases with multiple CAs	1349	3 (0.2)	8.6 (0.9–83.5)	1 (0.1)	3.1 (0.2–49.5)
Total CAs	22,843	43 (0.2)	1.6 (1.0–2.4)	12 (0.1)	2.8 (1.1–7.1)
Total controls	38,151	52 (0.1)	Referent	8 (0.0)	Referent

POR adjusted for birth order, maternal age, employment status.
* It was not possible to evaluate the POR and 95% CI in a conditional logistic regression model, therefore we used all population controls (not only the matched control pairs) as a reference group in an unconditional logistic regression model.
** Esophageal stenosis, diaphragmatic CA, horseshoe kidney, and 2 cases of torticollis.

Conclusion The authors conclude that boric acid might have a weak teratogenic potential, but taking into account the reduced absorption through topical exposure, this would most likely appear in the case of a damaged vaginal epithelium.

3.10.3 Other data

3.10.3.1 [Study 1] Benchmark dose analysis for developmental toxicity

Reference

Allen BC, Strong PL, Price CJ, Hubbard SA & Daston GP (1996). Benchmark dose analysis of developmental toxicity in rats exposed to boric acid. Fundamental and Applied Toxicology 32: 194 - 204.

Study design

In this analysis of the developmental toxicity observed in rats exposed to boric acid in their diet, benchmark dose (BMD) analyses have been conducted using two existing studies. By considering various endpoints and modelling approaches for those endpoints, the best approach for incorporating all of the information available from the studies could be determined. In this case, the approach involved combining data from two studies which were similarly designed and were conducted in the same laboratory to calculate BMDs that were more accurate and more precise than from either study alone.

Detailed study summary and results

The benchmark dose is defined as the 95 % lower bound on the dose corresponding to a 5 % decrease in the mean fetal weight (BMDL05). Results are based on the studies of Heindel et al. 1992 and Price et al. 1996a,b.

Table: Dose-Response Modeling Results for Boric Acid

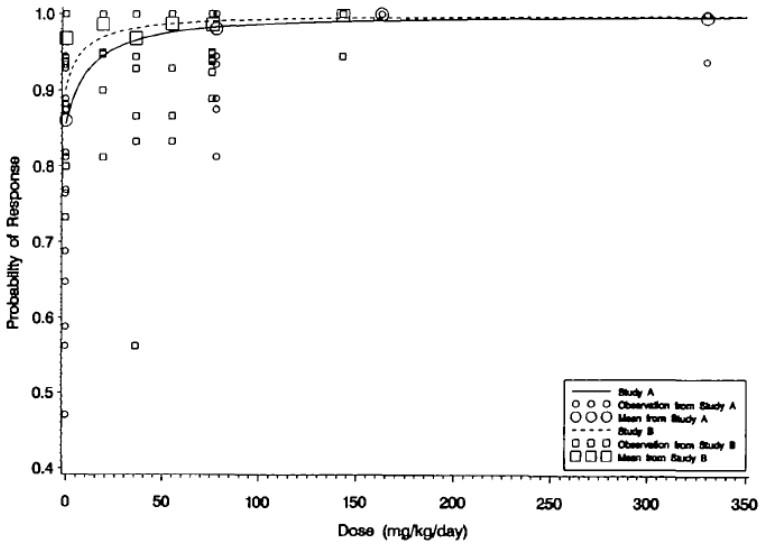
Endpoint	Study	Significant trend?[a]	Max LL[b]	Goodness-of-fit p value[c]	Doses corresponding to BME[d]	
					MLE	BMD
Fetal weight analysis	A	Yes	141.74	0.24	80	56
1a: BME	B	Yes	215.87	0.89	68	47
= 5% decrease[d]	Combined	—	353.43 (✓)	0.58	78	59
Fetal weight analysis	A	Yes	141.74	0.24	73	48
1b: BME	B	Yes	215 87	0.89	49	31
= Decrease to s(0)/2[d]	Combined	—	353.43 (✓)	0.58	65	48
Fetal weight	A	Yes	−401.69	0.44	129	115
analysis 2	B	Yes	−767.46	0.01	47	31
	Combined	—	−1177.83 (X)	—	—	—
Shortening or agenesis	A	Yes	−374.84	0.07	142	106
of rib XIII	B	Yes	−125.62	0.64	171	123
	Combined	—	−502.73 (✓)	0.42	140	120
Missing lumbar ribs	A	Yes	−216.70	0.99	6.7	1.7
	B	Yes	−196.94	0.78	8.3	3.4
	Combined	—	418.51 (✓)	0.99	12	5.2
Rib effects analysis 1a:	A	Yes	279.79	0.27	121	94
1/6 weighting	B	Yes	653.17	0.78	188	147
	Combined	—	920.41 (X)	—	—	—
Rib effects analysis 1b:	A	Yes	287.66	0.02	77	58
1/2 weighting	B	Yes	595.40	0.64	259	173
	Combined	—	865.90 (X)	—	—	—
Rib effects analysis 1c:	A	Yes	328.41	<0.001	142	117
5/6 weighting	B	Yes	568.19	0.53	307	178
	Combined	—	880.82 (X)	—	—	—
Rib effects analysis 2:	A	Yes	327.79	0.002	94	73
rib count	B	Yes	650.81	0.08	146	94
	Combined	—	964.33 (X)	—	—	—

[a] Mantel–Haenszel trend test results; significance of the trend corresponds to a p value less than 0.05. Combined study results were not tested for trend. Model predictions are presented for individual studies only when the trend was significant.

[b] The maximum values of the log-likelihoods of the models fit to the data, ignoring constant terms unrelated to parameter estimates. If the maximum log-likelihood for the combined data is not significantly different from the log-likelihood maximized for each study individually (the sum of the log-likelihoods shown for each study) at the $p = 0.01$ level (indicated by "✓"), model results for the combined data are presented. Otherwise (indicated by "X"), the two studies are not consistent with a single dose–response curve and combined data results are not shown.

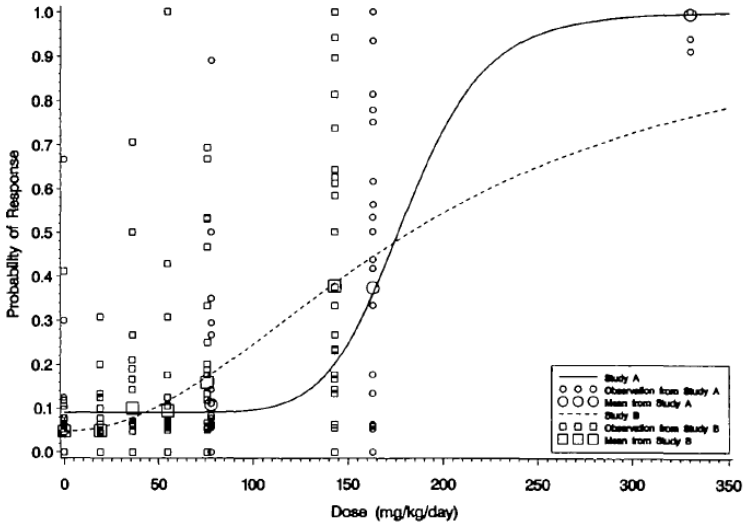
[c] p values assessing adequacy of the models for describing the observed data.

[d] The benchmark effect (BME) level is the value of the dependent variable for which maximum-likelihood estimates (MLEs) and their 95 % lower confidence limits (defining the BMD) are calculated (see methods).



Observed and predicted probabilities of missing lumbar ribs. The steep dose–response curves (solid and dotted curves) for both studies entail relatively low MLE and BMD estimates. The predicted background rates (for the average control litter size) differed across the two studies: for Study A the predicted background rate was 0.86, whereas for Study B that rate was 0.90, accounting for the differences in the curves at low doses.

Fig. Observed and predicted probabilities of missing lumbar ribs



Observed and predicted probabilities of low-weight fetuses. Mean probabilities of low-weight fetuses as a function of dose (large circles, with individual observations for each litter designated by smaller circles) were well-predicted by log-logistic model for Study A (solid curve; lack-of-fit p value was 0.44). Observed and predicted means for Study B (large squares and dotted curve, respectively) showed less agreement (lack-of-fit p value = 0.01). Study-specific dose–response curves were significantly better than a single common dose–response curve.

Fig. Observed and predicted probabilities of low-weight fetuses

Conclusion **BMD (developmental toxicity):** 59 mg/kg bw/day, equivalent to 10.3 mg B/kg bw/day, based on decreased foetal body weight provided the best basis for BMD calculations.

3.11 Specific target organ toxicity – single exposure

Not assessed in the CLH dossier.

3.12 Specific target organ toxicity – repeated exposure

Not assessed in the CLH dossier.

3.13 Aspiration hazard

Not assessed in the CLH dossier.

4 ENVIRONMENTAL HAZARDS

Not assessed in the CLH dossier.