10-16-91

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM: Adverse Data Flagged

Subject: EPA ID # 1624-117: Boric Acid. Review of Boric Acid Developmental Toxicity Studies in the Rat and Mouse (MRID # 417254-021)

EPA Shaughnessy No. 011001
EPA Record No. S338591
Tox. Chem No. 109
HED Project No. 1-0466

From: Myron S. Ottley, Ph. D. MSOttley 10/15/91
Section 4, Toxicology Branch I
Health Effects Division (H7509C)

To: Lois Rossie PM74
Registration Division (H7505C)

Thru: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 10/16/91
Section Head, Section 4, Toxicology Branch I
Health Effects Division (H7509C)

The Toxicology Branch I has reviewed the two studies submitted by Technology Services Group Inc. for the US Borax and Chemical Corp. regarding the Developmental Toxicity of Boric Acid in the Rat and the Mouse. The following conclusions have been drawn.

Rat (Study No. Rt88-BORT) (DER Attached)

Maternal
- NOEL 0.1% in diet (approx. 78 mg/kg/d)
- LOEL 0.2% in diet (approx. 163 mg/kg/d) Increased liver and kidney weights, increased food consumption. Decreased body wt. gain at 0.4% and above. Decreased food consumption at highest dose level (0.8%).

Developmental
- NOEL 0.1% (Minimal decrease in body weight noted. Considered to be the Threshold Dose)
- LOEL 0.2% in maternal diet. Decreased fetal b.w./litter. Increased fetuses with malformations/litter (short rib XIII) at 0.2% and above. Other gross, skeletal and visceral malformations at 0.4% and 0.8%.

1 of 25

CORE CLASSIFICATION

CORE SUPPLEMENTARY upgradeable. Data Submission is incomplete. Appendices V through XIII are missing. E.P.A. needs to have detailed data on the occurrence of specific developmental effects/litter (i.e. number of fetuses/litter and number of litter with the effect), the statistical significance of these occurrences, and historical control data which will help in assessing the biological significance of the observations and establishing a NOEL.

Several other deficiencies were noted, which of themselves did not render the study Supplementary (See Deficiencies Section of DER).

This study does not satisfy the guideline requirements for one rodent developmental toxicity study (83-3) because the exposure route was oral in diet, instead of oral gavage as stated in the guidelines. The Registrant may wish to justify use of this route of exposure, at which time the Agency will re-evaluate this data gap.

MOUSE (Study No. Mi88-BORT) DER Attached)

Maternal
- NOEL <0.1% in diet (< approx 248 mg/kg/d).
- LOEL 0.1% in diet. Renal Tubular dilatation/regeneration. Increased food consumption, decreased body wt. and body wt. gain observed 0.4%

Developmental
- NOEL 0.1%
- LOEL 0.2% (approx. 452 mg/kg/d) in maternal diet. Decreased average fetal body wt./litter, increased % resorptions/litter and malformations/litter observed at 0.4% (approx 1003 mg/kg/d).

CORE CLASSIFICATION

CORE MINIMUM in spite of the following deficiencies:

1. Dams were dosed throughout gestation days (gd) 0 to 17. Guidelines call for dosing on days 6 to 15 of gestation, inclusive.

2. Dams were exposed to boric acid in the diet. Guidelines call for oral dosing by gavage.

3. The occurrence and statistical significance of specific developmental endpoints was not reported according to the guidelines. For example, the occurrence of short rib XIII was not reported in terms of the number of fetuses per litter or the number of litters with this effect. Reviewers had to wade through raw data in appendices to properly evaluate this and similar endpoints.

This study does not satisfy the guideline requirements for one rodent

developmental toxicity study (83-3) because the exposure route was oral in diet, instead of oral gavage as stated in the guidelines. The Registrant may wish to justify use of this route of exposure, at which time the Agency will re-evaluate this data gap.

Both of these studies were noted by the registrant as meeting or exceeding the adverse effects flagging criteria number 5. However, since the developmental effects only occur at relatively high doses (rats and mice - 0.2% of diet), regulatory action is unnecessary at this time.

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GUIDELINE: 83-3

Primary Review by: Myron S. Ottley, Ph.D. 10/9/91
Review Section IV, Tox. Branch I/HED
Secondary Review by: Marion P. Copley, D.V.M., D.A.B.T. 10/10/91
Section Head, Review Section IV, Toxicology Branch I, HED (H7509C)

DATA EVALUATION RECORD

I. STUDY IDENTIFICATION

Study Type:	**DEVELOPMENTAL TOXICITY -- MOUSE**
EPA I.D.Numbers	MRID No. 417254-02 Shaughnessy No. 011001 Tox. Chem. No. 109
Test Material:	**BORIC ACID**
Synonyms:	AI3-02406, Boracid Acid, Borofax, Boron Trihidroxide, Hydrogen Orthoborate, Orthoboric Acid
Sponsor:	United States Borax and Chemical Corporation
Study Number:	Mi88-BORT
Testing Facility:	Research Triangle Institute, and National Toxicology Program
Title of Report:	Final Report on the Developmental Toxicity of Boric Acid (CAS No. 10043-35-3) in CD-1-Swiss Mice. EPA Reg. No. 1624-117.
Authors:	Elizabeth A. Field, Catherine J. Price, Melissa C. Marr, Christina B. Myers, Richard E. Morrissey, Bernard A. Schwetz.
Report Issued:	December 14, 1990

II. CONCLUSIONS

MATERNAL EFFECTS
NOEL: <0.1% in diet (< approx 248 mg/kg/d).
LOEL: 0.1% in diet. Renal Tubular dilatation/regeneration. Increased food consumption, decreased body wt. and body wt. gain observed (0.4%).
DEVELOPMENTAL EFFECTS
NOEL: 0.1%
LOEL: 0.2% (approx. 452 mg/kg/d) in maternal diet. Decreased average fetal body wt./litter, increased % resorptions/litter and malformations/litter observed at 0.4% (approx 1003 mg/kg/d).

III. CORE CLASSIFICATION

CORE MINIMUM in spite of the following deficiencies:

1. Dams were dosed throughout gestation days (gd) 0 to 17. Guidelines call for dosing on days 6 to 15 of gestation, inclusive.

2. Dams were exposed to boric acid in the diet. Guidelines call for oral dosing by gavage.

3. The occurrence and statistical significance of specific developmental endpoints was not reported according to the guidelines. For example, the occurrence of short rib XIII was not reported in terms of the number of fetuses per litter or the number of litters with this effect. Reviewers had to wade through raw data in appendices to properly evaluate this and similar endpoints.

This study does not satisfy the guideline requirements for one rodent developmental toxicity study (83-3) because the exposure route was oral in diet, instead of oral gavage as stated in the guidelines. The Registrant may wish to justify use of this route of exposure, at which time the Agency will re-evaluate this data gap.

IV. MATERIALS

Test Compound:
Purity: 98-99%
Description: Colorless Crystals
Lot No.: 872703
Contaminant: Not Specified (other [redacted]

Vehicle(s): Purina Ground Certified Rodent Chow (5002)

Test Animal:
Species: Mouse
Strain: Crl:CD®-1(ICR) VAF/Plus™ outbred Swiss albino (CD-1 mice)
Source: Charles River Laboratories, Inc. Raleigh, N.C.
Age: Not Specified
Weight: 20.5 - 33.5 gm. on gestation day 0.

V. STUDY DESIGN

This study was designed to assess the developmental toxicity potential of boric acid when administered in diet to CD-1 mice on gestation days one through 17, inclusive.

Mating

Individual Breeding pairs were cohabited overnight.

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Group Arrangement

The complement and arrangement of treatment groups is displayed in Table 1.

TABLE 1 COMPLEMENT AND ARRANGEMENT OF GROUPS EXPOSED TO BORIC ACID ON GD 0 TO 17.

Test Group/ Dose Level	Dose Levels		No. Assigned Per Group
	Percent in Diet	mg/kg/d *	
Controls	0.0	0	29
Low Dose	0.1	248	28
Mid Dose	0.2	452	29
High Dose	0.4	1003	28

* Estimated average intake

Dosing

Fresh boric acid-treated food was obtained from refrigerated stores every three days (gd 0, 3, 6, 9, 12, 15, and 17) during the dosing period (gd 6,9 and 12 for high dose group). Dosing solutions were analyzed for concentration and stability. Dose level estimations were based on most recent body weights, which were obtained every three days beginning on gestation day 0.

Observations

The animals were checked daily for mortality or other abnormal condition. Food and water intake and body weight measurements were taken on the mornings of gd 0, 3, 6, 9, 12, and 17.

At sacrifice on gd 17, sperm-positive dam weights were taken, as were liver, kidney and intact uterine weights. Corpora lutea were counted. All uteri without visible implantation sites were stained with 10% ammonium sulfide to detect very early resorptions. Randomly selected kidneys from 10 dams of each dose group were prepared for microscopic examination.

The fetuses were weighed and examined for external morphological and visceral abnormalities. 50% of fetal heads were fixed in Bouin's solution and examined by free-hand sectioning technique. All fetal carcasses were stained and examined for skeletal malformations.

VI. STATISTICAL ANALYSES

The authors provided this summary of statistical procedures: General Linear Modes GLM) were applied for the analyses of variance (ANOVA) of maternal and fetal parameters. Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data and Bartlett's test for homogeneity of variance was performed on all data to be

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analyzed by ANOVA. 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, Williams' or Dunnett's Multiple Comparison Tests compared BORA-exposed to control groups. One-tailed tests were used for all pairwise comparisons except maternal body and organ weights 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 for related measures were presented separately for each replicate in the study, as well as for all replicates combined. Nominal scale measures were analyzed by a X^2 test for independence and by a test for linear trend on proportions. When a X^2 test showed significant group differences, a one-tailed Fisher's exact probability test was used for pairwise comparisons of BORA and control groups.

VII. COMPLIANCE

A signed Statement of Confidentiality Claim was provided.
A signed Statement of compliance with EPA GLPs was provided.
A signed Quality Assurance Statement has been provided.

VIII. RESULTS

A. Maternal Effects

Clinical Observations

Alopecia and rough coat were observed in some animals, neither of which appeared dose-related.

Mortality

All dams survived until scheduled sacrifice.

Body Weight

As seen in Table 2, maternal body weight was significantly reduced below control during late treatment (gd 12, 15 and 17) in the high-dose group. Uncorrected body weight gains were affected only at the 0.4% dose level (25% less than controls). Corrected body weight gain was not affected by treatment.

Food and Water Consumption

Food consumption was not affected by boric acid treatment, except for a transient increase (15%) at the high dose level during gd 15 to 17. Water consumption was similarly increased (15%) in the high dose level during gd 15 to 17.

Gross Pathology

At necropsy pale kidneys were observed, especially in the high-dose group (0.4%). One dam from the high dose group had fluid accumulation in the kidney. Two dams, one at 0.1% and the other at 0.4%, each had an ovarian cyst. At the high dose level, gravid uterine weight and absolute liver weight

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declined, while relative and absolute kidney weights increased.

Microscopic Pathology (Table 2)

A dose-related increase in the incidence of renal tubular dilatation (with or without regeneration) was observed. No other dose-related microscopic effects were observed.

B. Embryo/Fetal Toxicity (Tables 4 & 5)

Boric Acid exposure to the conceptus resulted in statistically significant embryofetal toxicity at 0.2% and 0.4%.

Fetal Body Weight

Boric Acid treatment caused a dose related decrease in fetal body weight (5.7%, 11.3%, and 34.0% for the low through high dose levels, respectively). These reductions were statistically significant at the two highest dose levels.

Resorptions

Boric acid treatment increased the percentage of resorptions/litter at the high dose level when compared statistically to the controls (19.3% vd 6.1%).

Malformations

Boric Acid treatment also caused an increase in the percentage (9% vd. 3% in controls) of all morphological anomalies/litter at the high dose level. Of special note, an increase in short rib XIII was observed (10 fetuses from 5 litters vd. none in controls).

Variations

Decreases in full or rudimentary Lumbar I rib(s) were observed at the low and mid-dose levels, but not at the high dose level. In the absence of a dose-response relationship, these latter effects are not considered to be of biological importance.

IX. DISCUSSION

These data, as summarized in Table 6, show that boric acid exerts deleterious effects on the developing mouse conceptus at maternal dietary levels of 0.2% and above. No significant effects were observed at 0.1%, the lowest dose level tested. Therefore, this study has identified a NOEL for the rat conceptus.

As seen in Table 5, a variety of abnormalities were observed in a generally non-dose related manner. When the incidence of these effects is pooled, the frequency of occurrences/litter appears dose-related (9% at high dose vd. 3% in controls).

Overt maternal toxicities were evident at dietary levels of 0.4%, while the more subtle effects on the maternal kidney (renal lesions) were observed at

0.2% and at 0.1%. Since the lesions observed at 0.1% were considered to be mild, and were not associated with compromised renal function, 0.1% may be the threshold dose for toxicity--very near the NOEL.

TABLE 2. MATERNAL TOXICITY IN CD-1 MICE EXPOSED TO BORIC ACID IN FEED ON GESTATIONAL DAYS 0 TO 17

DAMS	Boric Acid (% in Feed)			
	0.0	0.1	0.2	0.4
No. Treated	29	28	29	28
No (%) Pregnant	27(93)	27(96)	27(93)	26(93)
Maternal body wt. (g)[a]				
gd 0	26.4±0.5	25.5±0.5	26.6±0.5	25.8±0.4
gd 12	36.3±0.6	35.5±0.5	36.2±0.6	33.3±0.7*
gd 15	43.2±0.9	42.1±0.7	43.1±0.8	38.3±0.9*
gd 17	49.3±1.1	48.3±0.8	49.0±1.0	43.1±1.1*
Maternal body wt. gain				
Gestation/trtmnt 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
Maternal Liver Weight (g)				
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 kidney wts. (g)				
Right kidney				
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*
Left kidney				
Absolute (g)	0.19±0.01	0.19±0.01	0.19±0.01	0.22±0.01*
Relative (%body wt.)	0.39±0.02	0.40±0.02	0.41±0.02	0.55±0.04*
Renal Tubular Dilatation/Regeneration[c]	0/10	2/10	8/10	10/10

[a] Maternal Body weights taken on gestational days 3, 6, and 9 were not statistically different from controls.
[b] Weight gain during gestation minus gravid uterine weight.
[c] Expressed as number affected/number examined.

* $p<0.05$ Dunnett's or Williams' Test.

TABLE 3 MATERNAL FOOD AND WATER CONSUMPTION FOR CD-1 MICE EXPOSED TO BORIC ACID ON GESTATIONAL DAYS 0 TO 17.

DAMS	Boric Acid (%in Feed)			
	0.0	0.1	0.2	0.4
RELATIVE FOOD CONSUMPTION (g/kg/d)				
gd 0 to 3	356.4±36.9	398.6±36.1	353.6±29.7	407.6±31.7
gd 3 to 6	307.6±21.7	346.7±26.5	308.8±28.1	292.4±26.8
gd 6 to 9	308.9±38.1	291.3±22.7	265.9±17.4	252.3±14.3
gd 9 to 12	231.3±18.0	216.0±13.6	215.8±15.4	243.3±16.3
gd 12 to 15	181.9± 4.7	182.0± 6.2	180.7± 5.6	208.8±11.2*
gd 15 to 17	163.8± 4.0	168.6± 2.5	160.3± 3.7	182.9±22.3
gd 0 to 17	234.0±13.8	248.1±10.9	225.8± 9.5	250.8±11.7
RELATIVE WATER CONSUMPTION (g/kg/d)				
gd 0 to 3	295.3±10.3	330.9±25.5	299.5± 9.4	306.9±10.0
gd 3 to 6	382.8±16.5	363.6± 8.1	359.3±11.2	371.3±17.9
gd 6 to 9	338.1±15.1	349.6±15.6	348.1±11.4	369.8±15.8
gd 9 to 12	332.5±19.6	317.8±17.1	340.4±32.9	323.8±11.3
gd 12 to 15	271.7± 9.2	294.3±29.2	280.5± 6.8	312.0± 8.0
gd 15 to 17	248.2± 8.3	233.1± 5.8	251.0± 8.6	285.4±11.8*
gd 0 to 17	292.8± 7.0	301.3±10.5	300.3± 9.2	317.8± 8.0

* $p<0.05$ Dunnett's or Williams' Test

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TABLE 4. DEVELOPMENTAL TOXICITY IN CD-1 MICE FOLLOWING MATERNAL EXPOSURE TO BORIC ACID IN FEED ON GESTATIONAL DAYS 0 TO 17.

	Boric Acid (% in Feed)			
	0.0	0.1	0.2	0.4
All litters	27	27	27	26
No. Implant sites/litter	12.4±0.6	12.0±0.4	12.1±0.4	12.1±0.5
% Resorptions/litter	6.1±1.6	6.2±1.3	4.8±1.4	19.3±4.5*
% Litters w/ resorptions	44	56	37	73#
Average Fetal bod; wt/litter (g) (% of controls)	1.06±0.02 100	1.00±0.02 94.3	0.94±0.02* 88.7	0.70±0.02* 66.0
% malformed fetuses/litter	2.70±1.20	4.50±1.90	1.60±0.70	9.10±2.40*
% litters with malformed fetuses	22	22	19	44
% fetuses with variations/litter	29.3±3.5	18.8±4.0*	11.9±2.4*	26.3±5.9
%litters with variations	96	67	70	80

*p<0.05 Dunnett's or Williams' Test.

TABLE 5. Occurrence of Specific Morphological Abnormalities in CD-1 Mice Following Maternal Exposure to Boric Acid on Gestational Days 0 to 17.

Boric Acid (% in Feed)	0.0		0.1		0.2		0.4	
Litters with Malformations/Total Examined	6/27		6/27		5/27		11/25	
Malformed Fetuses/Total Examined	7/311		14/295		5/309		20/250	
MALFORMATIONS OBSERVED	Fetus	Litter	Fetus	Litter	Fetus	Litter	Fetus	Litter
Gross Malformations								
Cleft Palate	2	1			1	1	2	2
Exencephaly	1	1						
Meningoencephalocele			2	2				
Microphthalmia							1	1
Visceral Malformations								
Enlarged Lateral Ventricle			1	1				
Hydronephrosis	1	1						
Transposition of Aorta and Pulmonary Artery			1	1				
Skeletal Malformations								
Agenesis or Arch: thoracic							1	1
Agenesis of Rib							1	1
Agenesis of Vertebrae: Lumbar	1	1	11	4	2	2	3	1
Cleft Sternum	2	2						
Fused Arches-Thoracic							2	2
Fused Cartilage: Thoracic Centrum							1	1
Fused Ribs							2	2
Misalignment of Centrum-Thoracic							1	1
Rib on Lumbar I: Bilateral Full			1	1				
Short Rib XIII					2	2	10	5
Unilateral Cartilage, Unilateral ossification center, thoracic centrum							1	1

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TABLE 6 SUMMARY OF MATERNAL AND DEVELOPMENTAL RESPONSES TO BORIC ACID EXPOSURE ON GD 0 TO 17

EFFECTS OBSERVED	Boric Acid (% in feed)		
	0.1	0.2	0.4
Maternal			
Body Weight (gd 12 to 17)			-
Body Weight Gain (treatment and gestation)			-
Relative Food Consumption (gd 12 to 15)			+
Relative Water Consumption (gd 15 to 17)			+
Gravid Uterine Weight			-
Absolute liver Weight			-
Relative Kidney Weight			+
Renal Tubular dilatation/regeneration[a]	2/10	8/10	10/10
Embryo/Fetal			
Percent Resorptions/litter			+
Average fetal body wt./litter		-	-
Percent malformed fetuses/litter			+
Percent fetuses w/ variations/litter[b]	-	-	

+ indicates significantly greater ($p<0.05$) than control
- indicates significantly less ($p<0.05$) than control
[a] expressed as # affected/# examined; the incidence in controls was 0/10
[b] not dose-related, therefore not considered biologically important.

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GUIDELINE: 83-3

Primary Review by: Myron S. Ottley, Ph.D. 11/15/91
Review Section IV, Toxicology Branch I (H7509C)
Secondary Review by: Marion P. Copley, D.V.M., D.A.B.T. 11/16/91
Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION RECORD

I. STUDY IDENTIFICATION

Study Type: **DEVELOPMENTAL TOXICITY -- RAT**

EPA I.D. Numbers: MRID No. 417254-01
Shaughnessy No. 011001
Tox. Chem. No. 109

Test Substance: **BORIC ACID**

Synonyms: A13-02406, Boracid Acid, Borofax, Boron Trihydroxide, Hydrogen Orthoborate, Orthoboric Acid

Registrant: United States Borax and Chemical Corporation

Study Number: Rt88-BORT

Testing Facility: Research Triangle Institute, and National Toxicology Program

Title of Report: Final Report on the Developmental Toxicity of Boric Acid (CAS No. 10043-35-3) in Sprague- Dawley Rats. EPA Reg. No. 1624-117

Authors: Catherine J. Price, Elizabeth A. Field, Melissa C. Marr, Christina B. Myers, Richard E. Morrissey, Bernard A. Schwetz.

Report Issued: May 1, 1990

II. CONCLUSIONS

MATERNAL EFFECTS

NOEL 0.1% in diet (approx. 78 mg/kg/d)

LOEL 0.2% in diet (approx. 163 mg/kg/d) Increased liver and kidney weights, increased food consumption. Decreased body wt. gain at 0.4% and above. Decreased food consumption at highest dose level (0.8%).

DEVELOPMENTAL

NOEL 0.1% (Minimal decrease in body weight noted. Considered to be the Threshold Dose)

LOEL 0.2% in maternal diet. Decreased fetal b.w./litter. Increased fetuses with malformations/litter (short rib XIII) at 0.2% and above. Other gross, skeletal and visceral malformations at 0.4% and 0.8%.

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III. CORE CLASSIFICATION

CORE SUPPLEMENTARY upgradeable. Data Submission is incomplete. Appendices V through XIII are missing. E.P.A. needs to have detailed data on the occurrence of specific developmental effects/litter (i.e. number of fetuses/litter and number of litter with the effect), the statistical significance of these occurrences, and historical control data which will help in assessing the biological significance of the observations and establishing a NOEL.

Other deficiencies were noted, which of themselves did not render the study Supplementary (See Deficiencies Section).

This study does not satisfy the guideline requirements for one rodent developmental toxicity study (83-3) because the exposure route was oral in diet, instead of oral gavage as stated in the guidelines. The Registrant may wish to justify use of this route of exposure, at which time the Agency will re-evaluate this data gap.

IV. MATERIALS

Test Compound: Purity: 98-99%
Description: Colorless Crystals
Lot No.: 872703
Contaminant: Not Specified (other [redacted]

Vehicle(s): Purina Ground Certified Rodent Chow (5002)

Test Animal:
Species: Rat
Strain: Crl CD® BR VAF/Plus™ Outbred Sprague-Dawley (CD)
Source: Charles River Laboratories, Inc. Raleigh, N.C.
Age: Not Specified
Weight: 213 - 275 gm. on gestation day 0.

V. STUDY DESIGN

This study was designed to assess the developmental toxicity potential of boric acid when administered in diet to rats on gestation days one through 20 (gd 6-15 in high dose group), inclusive.

Mating
Individual Breeding pairs were cohabited overnight.

Group Arrangement TABLE 1.

Test Group/ Dose Level	Dose Levels		No. Assigned Per Group
	Percent in Diet	mg/kg/d	
Controls	0.0	0	29
Low Dose	0.1	78	29
Low-Mid Dose	0.2	163	29
High-Mid Dose	0.4	330	29
High Dose	0.8	539	14

Dose Selection

In a preliminary study pregnant rats (4 - 7 per group) were exposed to boric acid in dose levels of 0%, 0.2%, 0.4%, 0.8%, 1.2% and 2.4% in diet (approx. 0, 162, 311, 617, 928, and 1704 mg/kg/d, respectively) throughout gd 0 - 20.

Maternal toxicity was observed at 0.8% and above. In the conceptus, decreased average fetal body weight/litter was observed at all dose levels, while resorptions and external malformations were observed at 0.8% and above. Based on these findings, dose levels of 0, 0.1%, 0.2%, and 0.4% were selected for the definitive study. The highest dose level, 0.8% was administered in a replicate study to evaluate the nature and extent of malformations caused by exposure at this high level.

The authors began dosing on gd 0 for the three lower dose levels, and on gd 6 for the highest dose level. Their rationale was that it would take some days for each dam to reach steady state concentrations of boric acid, so that it was necessary to begin dosing before gd 6 to achieve best results. At 0.8%, however, malformations were the primary endpoint of interest. So exposure was begun at gd 6, in order to minimize the high levels of preimplantation loss and early embryolethality observed at this level in the preliminary study, where dosing was begun on gd 0.

Dosing

Fresh boric acid-treated food was obtained from refrigerated stores every three days (gd 0, 3, 6, 9, 12, 15, and 18) during the dosing period (gd 6,9 and 12 for high dose group). Dosing solutions were analyzed for concentration and stability. Dose level estimations were based on most recent body weights, which were obtained every three days beginning on gestation day 0.

Observations

The animals were checked daily for mortality or abnormal condition. Food and water intake and body weight measurements were taken on the mornings of gd 0, 3, 6, 9, 12, 18, and 20.

At sacrifice on gd 20, sperm-positive dam weights were taken, as were liver, kidney and intact uterine weights. Corpora lutea were counted. Uteri devoid of visible implantation sites were stained with 10% ammonium sulfide to detect very

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early resorptions. Randomly selected kidneys from each dose group 10 dams/group) were prepared for microscopic examination.

The fetuses were weighed and examined for external morphological and visceral abnormalities. 50% of fetal heads were fixed in Bouin's solution and examined by free-hand sectioning technique. All fetal carcasses were stained and examined for skeletal malformations.

Historical control data were not provided to allow comparison with concurrent controls.

VI. STATISTICAL ANALYSES

See attached.

VII. COMPLIANCE

A signed Statement of Confidentiality Claim was provided.
A signed Statement of compliance with EPA GLPs was provided.
A signed Quality Assurance Statement has been provided.

VIII. RESULTS

A. Maternal Effects

Clinical Observations

The most common findings were alopecia and discoloration of the fur, neither of which appeared dose-related.

Mortality

All dams survived until scheduled sacrifice.

Body Weight

As seen in Table 2, uncorrected and corrected body weight gains were affected (>10%) only at and above the 0.4% dose level. At 0.4% uncorrected body weight gains was 10.6% below controls, while corrected body weight gain was 14.5% above controls. At 0.8%, uncorrected body weight gains were substantially below controls (35% for gd 0 to 20, 57.6% for gd 6 to 15), while corrected body weight gain was not different from controls.

Food and Water Consumption

Food consumption was increased at dose levels of 0.2% and 0.4%, for each time period measured (gd 12 to 15, 15 to 18, 18 to 20, 0 to 20), as depicted in Table 3. By contrast, food consumption was decreased at the 0.8% dose level during the periods of dosing (gd 6-9 = 21.3%, gd 6-15 = 13.1%). A rebound occurred after dosing ceased. Water consumption was also decreased during gd 6-9. It was not affected significantly at other times.

Gross Pathology

The investigators supplied the following data: Changes in body and organ weights were observed: liver and kidney weights increased (see Table 2) at dose levels of 0.2% and above, while gravid uterine weights and body weight gains decreased during the treatment period and gestation at dose levels of 0.4% and above. Corrected body weight gains were not affected.

Microscopic Pathology

Microscopic kidney evaluation revealed a low, non-dose-related incidence of pathology.

B. Embryo/Fetal Toxicity (Tables 4 & 5)

Boric acid exposure to the conceptus resulted in statistically significant embryofetal toxicity at all dose levels tested. The incidence of percent litters with adversely affected implants was 50% higher than controls at the lowest dose level, rising to 100% above controls in the two highest dose levels. Essesntial parameters, such as number of litters and number of fetuses/litter with a specific effect, were not provided by the Submitter. In addition, the raw data were not submitted, making proper evaluation impossible at this time.

Body Weight

All dose levels of boric acid caused a statistically significant decrease in average fetal body weight per litter. Decreases of 6%, 13%, 37% and 54% were observed for the low through the high dose levels, respectively.

Mortality

Prenatal mortality [resorptions, fetal deaths] was significantly increased at the high dose level only (0.8%).

Malformations (Table 5)

The incidence of malformations, [% malformed fetuses/litter, and % litters with malformations], increased significantly at dose levels of 0.2% and above. The most commonly observed were: **Gross** -- Short and/or curly tail, anophthalmia, microphthalmia. **Visceral** -- enlarged lateral ventricles of the brain, displaced eyes, cardiovascular anomalies. **Skeletal** -- agenesis and/or shortening of rib XIII. The authors did not provide statistical data on either the individual types of malformations, or on the subgroupings just mentioned.

Variations (Table 5)

The incidence of skeletal and other variations appeared to be increased at all dose levels. Most common were wavy ribs, delayed ossicifation of thoracic centrum. Statistical data on individual variations were not provided.

IX. STUDY DEFICIENCIES

Several deficiences were noted in this report:

1. Data Submission is incomplete. Appendices V through XIII are missing.

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E.P.A. needs to have detailed data on the occurrence of specific developmental effects/litter (i.e. number of fetuses/litter and number of litter with the effect), the statistical significance of these occurrences, and historical control data which will help in assessing the biological significance of the observations and establishing a NOEL.

2. A NOEL for developmental toxicity was not established. However, a threshold dose appears to have been reached. Retesting using lower dose levels would not yield more useful information.

3. Pregnant animals were dosed on gestation days (gd) 0-20. Guidelines specify gd 6-15. Inadequate justification was given for variance. However, while some of the developmental endpoints affected may be expected to change, i.e., increased preimplantation loss and embryolethality vs. malformations, there is no reason to expect the NOEL and/or LOEL to change. Thus, repeating the study is not expected to cause the level of concern to change.

4. It is sometimes an arduous task to locate pertinent data in this study. The alternate pagination listed in the Table Of Contents, e.g., III-10, V-23, which would have facilitated the locating of certain appendices, etc., was not implemented throughout the document. This deficiency should be corrected when these data are resubmitted.

X. DISCUSSION

As summarized in Table 6, these data show that boric acid appears to exert statistically significant deleterious effects on the developing rat conceptus at maternal dietary levels of 0.1% and above. The most sensitive endpoint was average fetal body weight, which was decreased at all treatment levels.

Since 0.1% is the lowest dose level tested, this study has not identified a NOEL for the rat conceptus. As such, the Core Classification would automatically be Supplementary. However, the actual decreases in average fetal body weights were 6.3%, 13.1%, 36.8% and 53.7% for the low through the high dose levels, respectively. Since the percent decrease at the lowest dose level (0.1%) was within the normal 10% body weight fluctuation, the biological significance of this observed effect is diminished, and the lowest dose level can be regarded as a threshold dose level. Testing at a dose level lower than 0.1% seems unjustified. Therefore, 0.1% can be considered to be a NOEL for developmental toxicity, and this deficiency will not be used to determine its CORE classification.

Overt maternal effects, such as changes in body weight gain, were evident at dietary levels of 0.4% and higher, while the more subtle effects on the maternal liver and kidney were observed at 0.2%.

Changes in uterine weight and live fetuses/litter paralleled changes in body weight gain. Statistically significant decreases in these three parameters were observed at 0.4% and 0.8%, suggesting that the sensitivities of these endpoints to toxicological insult by boric acid are quite similar in the rat.

While food consumption increased at 0.2% and 0.4% throughout the treatment period (Table 3), body weight gain remained the same at 0.2%, and decreased at 0.4%. This observation suggests a treatment-related decrease in food efficiency in the dams. Food consumption and body weight gain were both depressed at 0.8%.

As stated earlier, the authors departed from guideline procedure by beginning dosing on gd 0 instead of gd 6, and concluded dosing on gd 20, five days after the usual day 15 cutoff. Their rationale was that since it takes time for boric acid to reach steady state concentrations in the dam, dosing must begin early. This reasoning is somewhat weakened by the observation in their preliminary study that at the high dose level (0.8%), the incidences of preimplantation loss and early embryolethality were both significantly above controls, indicating that boric acid was reaching target sites rapidly. And since preimplantation loss and early embryolethality as toxicological endpoints are of as much concern as malformations, the deviation from guidelines seems unjustified. No rationale was given for dosing the five extra days to gd 20.

The authors departed from guidelines again by dosing animals in diet instead of by gavage. This departure was not explained. However, in this case the data are accepted because clear dose-response relationships were shown in both the dam and conceptus, and the profile of toxicity demonstrated is not expected to be altered significantly if the study were repeated using gavage.

In conclusion, these data provide the basis for concern for the developmental toxicity potential of boric acid, since the conceptus is being affected at doses where there are no maternal warning signs.

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TABLE 2. MATERNAL TOXICITY IN CD RATS EXPOSED TO BORIC ACID ON GESTATIONAL DAYS 0 TO 20 OR 6 TO 15

DAMS	Boric Acid (% in feed) on gd 0 to 20				Boric Acid (% in feed) on gd 6 to 15	
	0.0	0.1	0.2	0.4	0.0	0.8
Total Treated[a]	29	29	29	29	14	14
No (%) Pregnant at Sacrifice	28(97)	28(97)	26(90)	26(90)	14(100)	14(100)
Body Weight (gd 0) (g)[b]	248.2 ± 2.8	247.6 ± 3.1	247.8 ± 3.0	249.4 ± 2.7	258.5 ± 3.1	261.1 ± 2.8
Weight Gain (gd 0 to 20) (g)[b] (% compared with controls)	160.6[d] ± 3.8	157.5 ± 3.0 (98.1)	156.6 ± 3.6 (97.5)	143.6 * ± 3.9 (89.4)	157.8 ± 6.1	102.5 * ± 5.3 (65.0)
Weight Gain (treatment) (g)[b] (% compared with controls)					54.0 ±2.9	22.9 * ±3.1 (42.4)
Weight Gain (corrected) (g)[b,c] (% compared with controls)	71.1[d] ±2.9	72.1 ±2.1 (101.4)	74.6 ±3.1 (105.0)	81.4 * ±2.5 (114.5)	66.6 ±4.8	66.2 ±6.2 (99.4)
Gravid Uterine Weight(g)[b]	88.35[d] ±2.57	85.32 ±2.05	82.04 ±2.03	62.14 * ±3.11	88.87 ±3.58	36.23 * ±4.39
Liver Weight (g)[b]	17.15 ±0.15	17.59 ±0.27	17.86 ±0.30	17.54 ±0.35	17.27 ±0.40	17.12 ±0.59
Relative Liver Wt. (% body wt.)[b]	4.20[d] ±0.05	4.35 ±0.06	4.42 * ±0.07	4.46 * ±0.07	4.15 ±0.07	4.70 * ±0.13
Right Kidney Weight (g)[b]	1.23[d] ±0.02	1.25 ±0.02	1.35 ±0.06	1.32 ±0.03	1.21 ±0.03	1.37 * ±0.04
Relative Right Kidney Weight (% body weight)[b]	0.302[d] ±0.006	0.309 ±0.006	0.335 * ±0.016	0.338 * ±0.007	0.289 ±0.009	0.376 * ±0.009
Left Kidney Weight (g)[b]	1.19[d] ±0.02	1.21 ±0.03	1.29 ±0.05	1.28 * ±0.03	1.19 ±0.03	1.31 * ±0.04
Relative Left Kidney Weight (% body weight)[b]	0.292[d] ±0.006	0.300 ±0.007	0.320 * ±0.015	0.326 * ±0.007	0.286 ±0.010	0.360 * ±0.009

[a] All females survived until scheduled sacrifice; no females were removed from the study.
[b] Includes all dams pregnant at sacrifice; mean ± S.E.
[c] Weight gain during gestation minus gravid uterine weight.
[d] $p<0.05$ Test for Linear Trend.
* $p<0.05$ pairwise comparison to controls.

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TABLE 3. MATERNAL FOOD AND WATER CONSUMPTION FOR CD RATS EXPOSED TO BORIC ACID ON GESTATIONAL DAYS 0 TO 20 OR 6 TO 15[a]

	Boric Acid (% in feed) on gd 0 - 20				Boric Acid (% in feed) on gd 6 - 15		
	0	0.1	0.2	0.4		0	0.8
Number of Dams	28	28	26	26		14	14
FOOD CONSUMPTION (g/kg/day)					FOOD CONSUMPTION (g/kg/day)		
gd 12 to 15	78.1[b] ±1.8	78.6 ±1.4	83.2* ±1.9	83.8* ±1.6	gd 6 to 9	78.1 ±1.6	61.5* ±1.4
gd 15 to 18	76.5[b] ±1.4	76.8 ±1.7	81.5* ±1.6	85.4* ±1.9	gd 6 to 15	77.3 ±2.3	67.4* ±2.8
gd 18 to 20	69.8[b] ±1.5	71.9 ±2.1	82.6* ±4.9	79.4* ±3.9	gd 15 to 18	71.4 ±1.0	87.4* ±2.5
gd 0 to 20	77.5[b] ±1.2	78.0 ±1.1	81.4* ±1.4	82.6* ±1.5	gd 0 to 20	74.4 ±1.5	73.4 ±1.8
WATER CONSUMPTION (g/kg/day)					WATER CONSUMPTION (g/kg/day)		
gd 12 to 15	129.3[b] ± 5.5	130.4 ± 3.1	135.6 ± 3.7	140.7 ± 2.9	gd 6 to 9	129.3 ± 6.4	107.7* ± 5.3
gd 15 to 18	136.8[b] ± 6.5	137.6 ± 3.6	143.0 ± 4.0	150.5 ± 3.3	gd 6 to 15	130.5 ± 7.5	126.2 ± 5.8
gd 18 to 20	130.4[b] ± 5.7	133.2 ± 3.6	137.6 ± 4.1	160.0* ±11.5	gd 15 to 18	141.7 ±11.9	166.5 ± 5.8
gd 0 to 20	130.4 ± 4.6	131.1 ± 3.3	135.4 ± 3.1	139.4 ± 3.2	gd 0 to 20	131.3 ± 8.0	133.8 ± 5.5

[a] Reported as mean ± S.E.M.
[b] $p<0.05$, Trend test
* $p<0.05$, pairwise comparison to controls

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TABLE 4. 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 (% in feed) on gd 0 to 20				Boric Acid (% in feed) on gd 6 to 15	
	0.0	0.1	0.2	0.4	0.0	0.8
ALL LITTERS [a]	28	28	26	26	14	14
% Adversely Affected Implants per Litter [b,d]	5.46 ± 1.35	8.66 ± 1.90	11.17 * ± 2.06	53.58 * ± 5.63	7.06 ± 2.41	77.71 * ± 6.77
% Litters with Adversely Affected Implants [d]	50	75 *	85 *	100 *	50	100 *
LIVE LITTERS [e]	28	28	26	25	14	14
Live Fetuses/ Litter [b]	15.39 ± 0.41	15.43 ± 0.46	15.69 ± 0.38	15.44 ± 0.48	15.36 ± 0.66	9.71 * ± 1.56
Average Fetal Body Wt. (g)/Litter [b]	3.695 ± .060	3.463 * ± .048	3.210 * ± .051	2.327 * ± .050	3.740 ± .068	1.730 * ± .146
% Fetuses Malformed per Litter [b,f,g]	2.05 ± 0.84	2.63 ± 1.41	7.83 * ± 2.41	50.20 * ± 5.43	2.78 ± 1.40	72.64 * ± 8.05
% Litters with Malformations [h]	21	21	50 *	100 *	29	100 *
% Fetuses/ Litter with Defects [b,f,k]	22.59 ± 3.40	10.12 * ± 2.05	15.64 ± 2.85	59.81 * ± 5.19	26.08 ± 5.54	82.15 * ± 6.28
% Litters with Defects [l]	96	71 *	73 *	100	93	100

a Includes all dams pregnant at sacrifice; litter size = no. implantation sites per dam. One dam in the 0.4% group had 100% resorptions.
b Reported as mean ± S.E.M.
d Adversely affected implants = nonlive implants plus malformed
e Includes only dams with live fetuses; litter size = no. live fetuses per dam.
f Only live fetuses were examined for malformations and variations.
g Fetuses with one or more malformations.
h Litter with one or more malformed fetuses.
k Fetuses with one or more defects (i.e. either a malformation or variation.
l Litter with one or more fetuses with defects (i.e. either a malformation or variation.
# $p<0.05$ Trend Test.
* $p<0.05$ Pairwise comparison to controls.

TABLE 5. INCIDENCES OF SPECIFIC EMBRYO/FETAL EFFECTS*

EFFECT OBSERVED	INCIDENCE					
	Percent in Feed, gd 0 to 20				Percent in Feed gd 6 to 15	
	0.0	0.1	0.2	0.4	0.0	0.8
GROSS MALFORMATIONS						
Short & Curly Tail						11
Short Tail						4
Anophthalmia	1				1	6
Microphthalmia				1		7
VISCERAL MALFORMATIONS						
Enlarged Lateral Ventricles of the Brain				21		24
Convoluted Retina						7
Cardiovascular						18
SKELETAL MALFORMATIONS						
Agenesis of Rib XIII	1	1		24		17
Short Rib XIII	1	11	28	152	1	50
Cleft Sternum			4	8		13

* Essesntial parameters, such as number of litters and number of fetuses/litter with a specific effect, were not provided by the Submitter. In addition, the raw data were not submitted, making proper evaluation in this table impossible at this time.

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TABLE 6. SUMMARY OF MATERNAL AND EMBRYO/FETAL EFFECTS

MATERNAL		Boric Acid (% in feed) *			
		0.1%	0.2%	0.4%	0.8%
Body Weight (gd 9 to 20)					-
Body Weight Gain	(treatment)			-	-
	(gestation)			-	-
	(corrected)			+	
Gravid Uterine Weight				-	-
Liver Weight:	Absolute (g)				
	Relative (% bw)		+	+	+
Kidney Weight:	Absolute (g)				+
	Relative (% bw)		+	+	+
Food Consumption (g/kg/day)	gd 6 - 9				-
	gd 6 - 15				-
	gd 12 - 15		+	+	
	gd 15 - 18		+	+	+
	gd 18 - 20		+	+	
	gd 0 - 20		+	+	
Water Consumption (g/kg/day)	gd 6 - 9				-
	gd 6 - 15				
	gd 18 - 20			+	
	gd 0 - 20				
EMBRYO / FETAL					
% Nonlive implants/litter					+
% Litters with nonlive implants					+
% Adversely affected implants/litter			+	+	+
% Litters with adversely affected implants		+	+	+	+
No. live fetuses/litter					-
Average Fetal Body Wt./litter		-	-	-	-
% Fetuses malformed/litter			+	+	+
% Litters with malformed fetuses			+	+	+
% Fetuses with variations/litter		-	-	+	+
% Litters with variations			-	-	
% Fetuses with defects/litter		-		+	+
% Litters with defects		-	-		

* Boric acid was administered on gd 0 - 20, except for the 0.8% concentration group which was administered on gd 6 - 15.
+ Significantly ($p<0.05$) greater than control.
- Significantly ($p<0.05$) less than control.

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