



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
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APP - 5

OFFICE OF  
PESTICIDES AND TOXIC  
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 011001, Two Generation Reproduction Study in  
Mice with Boric Acid

Tox. Chem. No.: 109  
Project No.: 0-1827  
Record No.: 268437

FROM: Melba S. Morrow, D.V.M. *M.S. Morrow 1/11/91*  
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THRU: Marion P. Copley, D.V.M. *Marion P. Copley 4/13/91*  
Section Head, Review Section II  
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CONCLUSIONS: The results of the two generation reproduction  
study in CD-1 Swiss mice show that the parental and reproductive  
NOEL for boric acid is 1000 ppm in both males and females.

The parental and reproductive LEL is 4500 ppm based on decreased  
organ weights in both sexes and based on decreased fertility and  
pup weights.

The study has been classified as core minimum. A copy of the DER  
is attached for your reference.

CC Gross

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Reviewed by: Melba S. Morrow, D.V.M. *MSMorrow 1/11/91*  
Section II, Tox. Branch I (H7509C)  
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DATA EVALUATION REPORT

STUDY TYPE: 2 - Generation Reproduction - mice

GUIDELINE #: 83-4

TOX. CHEM. #: 109

MRID #: 41589101

TEST MATERIAL: Boric acid

SYNONYMS: BORA

STUDY NUMBERS: 90-105

SPONSOR: U.S. Borax Research Corporation

TESTING FACILITY: NTP, NIEHS  
Research Triangle Park, N.C.

TITLE OF REPORT: Reproductive Toxicity of Boric Acid in CD-1 Swiss Mice

AUTHORS: Fail, George, Grizzle, Heindel and Chapin

REPORT ISSUED: April 13, 1990

CONCLUSIONS: The parental NOEL and the reproductive NOEL were both 1000 ppm (110 mg/kg and 182 mg/kg in males and females, respectively). The parental LEL was 4500 ppm (598 mg/kg in males and 846 mg/kg in females) based on decreased organ weights in both sexes. The reproductive LEL is also 4500 ppm, based on decreased fertility and decreased pup weights.

Also, at the 4500 ppm level, the average number of days between litters increased after the second litter and the number of dams producing litters decreased significantly when compared to controls.

At 9000 ppm, no litters were produced and males in this group had a decrease in sperm concentration and motility when compared to controls.

CLASSIFICATION: minimum  
TOX. CATEGORY: N/A

**MATERIALS:** The test compound was boric acid (BORA) which was 99% pure and stable at room temperature. The lot number of the test material was 872703 and the chemical was obtained from Fisher Scientific Company.

The test animals were CD-1 Swiss mice which weighed from 27 to 40 grams (males and from 26 to 32 grams (females). Animals were obtained from Charles River Laboratories and were 11 weeks of age at the start of the breeding phase of the study.

**METHODS:** The entire study was divided into specific tasks. The first was to be a dose finding trial which lasted for 14 days. This was not conducted because sufficient data existed that could be used in selecting appropriate doses. Task 2 was the continuous breeding portion of the study. This phase included a 1 week pre-mating period, a 14 week cohabitation period and a holding period. Animals were assigned to test groups as follows and fed boric acid throughout the study:

Test Group	Conc. in Diet (ppm)	# F <sub>0</sub>		# F <sub>1</sub>	
		male	female	male	female
Control	0	38	38	40	40
Low	1000	19	19	20	20
Mid	4500	20	20	--	--
High	9000	20	20	--	--

Task 3 was concerned with the determination of the affected sex and used a cross-over mating scheme. Animals were cross paired in the following manner:

	<u>control females</u>	<u>mid -dose females</u>
<u>control males</u>	20 pairs	20 pairs
<u>mid-dose males</u>	20 pairs	

For the offspring assessment (Task 4), the test animals were randomly selected from the last litters produced in Task 2. Males and females from different litters within the same treatment group were paired and prior to pairing, these animals received dosed feed at the same levels as their parents. In Task 4, no litters were available from the mid and high dose groups.

#### Mating Procedure

In Task 2, after the seven day pre-mating period in which dosed feed was administered at the designated levels, the mice were housed in pairs for 98 days. At the end of the 98 day period, the pairs were separated and housed individually. Any litters born after the 98 day cohabitation period were reared by the dam until weaning. Litters born during the cohabitation phase were

removed from the dam within 24 hours of birth.

In the cross-over mating trial, the pairs were kept together for 7 days or until a copulatory plug was evident. During the mating period, a non-dosed feed was administered. This was the only discontinuity in the dosing that was reported.

For the offspring assessment, the male and female animals were paired for 7 days or until a copulatory plug was evident. Prior to pairing, these animals were reared in same-sex groups until they were  $74 \pm 10$  days of age. After mating, the animals were housed individually until litters were delivered.

#### Termination of study

Sperm morphology and vaginal cytology were conducted on all animals prior to the termination of the study. Both  $F_0$  and  $F_1$  mice were necropsied at the end of the trial.

#### Parameters for Evaluation:

The following endpoints were used for evaluating both the  $F_0$  and the  $F_1$  mating trials:

1. clinical signs of toxicity
2. parental body weights
3. fertility (# pairs with litters/ # pairs bred)
4. # litters /pair
5. # live pups /litter
6. # pups born dead
7. sex of live pups
8. pup body weight at 24 hours
9. feed and water consumption

For all males the following parameters were evaluated: selected organ weights (liver, kidney, adrenal, testes, and prostate), body weights, epididymal sperm motility, morphology and concentration. For females in the control and mid-dose groups selected tissues (ovaries, uterus, liver, kidneys and adrenals) were prepared and examined microscopically.

The methods used to evaluate epididymal sperm for concentration, motility and morphology involved dissecting the cauda epididymus and placing it in a petri dish with 2.0 ml of sterile phosphate buffered saline. A small portion of the tubule was removed from the cauda tissue and was swirled in the solution to release sperm. The sample was viewed under a 40x magnification and the data was reported as percent motile sperm per sample.

For determining the concentration of sperm, the sample was milked out of the cauda and placed in a test tube with a suspension of 1.0 ml phosphate buffered saline. The tube was placed in boiling water, reagitated and two aliquots were obtained. Sperm were counted under a 10x or a 20x magnification and the concentration was recorded in terms of the number of sperm per mg of cauda

epididymal tissue.

Sperm morphology was evaluated using 500 sperm semen samples and by scoring the morphology as either normal or abnormal. Data were recorded as percent abnormal sperm per sample. (See Table VII for sperm data from both F<sub>0</sub> and F<sub>1</sub> animals).

Feed samples were collected to determine the stability of the test material. At nominal concentrations of 1, 4.5 and 9 mg/g, analysis revealed that 102, 99.6 and 98.4 percent of these levels were found, respectively.

QUALITY ASSURANCE: A statement of Quality Assurance, dated 11/20/89 has been provided.

RESULTS (F<sub>0</sub>):

In the portion of the study involving the F<sub>0</sub> parental animals, evidence of fighting occurred in all dose groups. There was also an increase in the number of clinical observations in the 9000 ppm males that involved the eyes. These observations included lacrimation, exophthalmos and cloudiness of the eyes.

There were no significant changes in the body weights of animals in the control 1000 and 4500 ppm groups. Body weights were measured on weeks 1, 2, 5, 9, 13 and 18 in the continuous breeding phase and on weeks 22 through 25 of the crossover phase. From weeks 2 through 18, there was a decrease in the body weight gain reported for males and females in the high group. At weeks 9 to 23, a decreased body weight gain was also present in females at the 4500 ppm level. The observed decrease in weight gain at the 9000 ppm level is suggestive of generalized toxicity, while changes in females at the 4500 ppm level may have been the result of diminished fertility and the absence of gravid uterine weights. (See Table I).

Feed consumption (measured on weeks 1, 2, 5, 9, 13 and 18 in the continuous breeding phase and on weeks 22 through 25 in the crossover phase) was decreased in mid and high dose males and in high dose females. After five weeks, the feed consumption was higher in pairs receiving 9000 ppm boric acid. When compared to controls, feed consumption was higher at week 13 for animals in the 4500 ppm group; however following separation, a decrease in food consumption was reported in females in both the mid and high dose groups. At week 18, a decrease in body weight with no concurrent decrease in food consumption was recorded in females fed 4500 ppm. This may be indicative of decreased feed efficiency at this dose level. (See Table II for feed consumption data).

There was no significant difference between the male body and organ weights at 0 and 1000 ppm of boric acid. Significant

differences in organ weights were reported when males from the mid dose and high dose groups were compared to controls. (See Table III). These differences included decreased kidney/adrenal weights at 4500 ppm (10%), decreased testicular weights at the 4500 (40 - 50%) and 9000 ppm levels (68 - 86%), and decreased right caput and corpus epididymal weights at the two highest doses (20% at 4500 ppm and 32.5% at 9000 ppm).

In females, significant differences in liver and adrenal/kidney weights were reported at the 4500 ppm level when compared to controls. The mean absolute liver weight for females in the 4500 ppm group was 2.15 grams compared to 2.36 grams for the control animals. The mean absolute (combined) kidney/adrenal weight was 0.63 grams for the 4500 ppm group compared to 0.69 grams for the controls.

No other changes were reported in the parental animals that could be attributed to boric acid intake.

#### Reproductive parameters (F<sub>0</sub>)

In the testes, seminiferous tubular atrophy, characterized as severe was observed in the high dose males. Atrophy was also present in the mid-dose males, along with degeneration of the seminiferous tubules (reported in 17/20 males). Sperm was characterized as abnormal for mid and high dose males based on the lack of progressive motility. Additionally, a decrease in the number of sperm was reported at the 4500 ppm level, but this did not have an immediate adverse affect on fertility.

Differences in sperm concentration and motility were noted between controls and males fed 1000 ppm boric acid in both the F<sub>0</sub> and the F<sub>1</sub> animals. At 1000 ppm, the F<sub>0</sub> animals had a higher concentration of sperm, but a lower percent of the sperm were motile when compared to controls. Conversely, the F<sub>1</sub> males at this dose level had a lower concentration of sperm but increased motility. These findings appear to be incidental when other parameters such as fertility, the number of days between litters and pathological findings are considered.

At 9000 ppm, 12/15 males did not produce sperm. The average concentration for this group was only 2.8 x 1000%, with less than 50% of the sperm being motile. *sperm/mg caudal tissue*

At the 4500 ppm level, the number of pairs producing litters was significantly reduced after the first litter. (See Table IV.a.). This may be indicative of a cumulative effect of boric acid, the result of the compound's effect in causing reproductive senescence or the effect on sperm motility and/or concentration. The number of days between litters was also affected by boric acid at levels of 4500 ppm. The amount of time between the second and third litters was 48 days compared to 19 days for control and 21 days for the 1000 ppm group. This finding was

stated as being the result of biological variability; however, it is more likely to be treatment/dose related. (See Table IV).

At the 4500 ppm dose, there was a significant adverse effect on fertility, the average number of litters per pair, the average number of live pups and the percent pups born alive. Average pup weight was also affected at this dose level. At the 9000 ppm dose, no fertility occurred. (See Table V).

#### RESULTS CROSSOVER STUDY

In the crossover portion of the study which was designed to determine the sex that was affected by boric acid, the mating and fertility indices were as follows:

<u>Male</u>	x	<u>Female</u>	<u>mating index</u>	<u>fertility index</u>
control		* control	79%	74%
4500 ppm		control	30%	5%
control		4500 ppm	70%	64%

These results demonstrated that the males were more sensitive to the effects of boric acid on the reproductive system. The effects observed in females were on body weights, with females from the 4500 ppm group having lower body weights when compared to controls at weeks 22 and 23. The observed effect on female body weights was probably incidental because at weeks 24 and 25, the weights of the females fed 4500 ppm boric was slightly higher than controls.

#### RESULTS (F<sub>1</sub>):

In the second generation mating study, animals receiving doses of 0 and 1000 ppm boric acid were evaluated. The average pup weight appeared to be the only parameter that was affected. Adjusted live pup weights were slightly lower for females at the 1000 ppm level, with no concurrent effect on male offspring. This is more than likely an incidental finding.

Food consumption was reported as being unaffected by the test material; however, body weights for these animals were not provided. It is also noted that feed consumption for the F<sub>1</sub> pairs was higher than feed consumption for the F<sub>0</sub> pairs at 0 and 1000 ppm. This is more than likely an incidental finding.

A difference was noted in the length of the estrous cycles of treated and control females. The average length of the cycle in control females was 4.7 days, whereas the average length in females receiving 1000 ppm boric acid was 4.19 days. Diestrus was prolonged by 8% in the 1000 ppm group. This finding was not reported in females in the F<sub>0</sub> generation and it is probably an incidental finding. (See Table VI).

DISCUSSION: Based on the results of this study, the parental NOEL and the reproductive NOEL were both 1000 ppm. The parental

LEL was 4500 ppm based on the finding of decreased organ weights in both sexes. The reproductive LEL was 4500 ppm based on decreased fertility and decreased pup weights. In addition, at the 4500 ppm level, the average number of days between litters increased after the second litter and the number of dams producing litters decreased.

At 9000 ppm, males had a decrease in sperm concentration and motility. At this dose level no litters were produced.

The study is classified as core minimum.

TABLE I - BODY WEIGHTS

<u>Females</u>	0	Dose group (ppm)		9000
		1000	4500	
<u>Week</u>	<u>Average Body Weight (g)</u>			
1	28.9	29.2	29.0	28.6
5	40.2	41.1	41.3	32.6
9	48.0	46.8	40.3	34.9
13	51.1	51.0	42.8	36.8
18	49.4	50.0	38.2	34.1
25*	43.2	--	45.1	--
<u>Males</u>				
<u>Week</u>	<u>Average Body Weight (g)</u>			
1	36.1	36.2	35.8	35.0
5	37.3	36.9	37.8	32.8
9	38.8	38.5	39.0	34.5
13	39.8	39.2	39.9	35.7
18	40.8	40.3	40.2	37.0
25*	41.4	--	40.5	--

\* = Weights recorded at week 25 represent weights of those animals participating in the cross-over phase of the study. Animals from the low and high dose groups were not used.

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TABLE II - FEED CONSUMPTION (g/kg)

<u>Females</u> <u>Week</u>	<u>Dose (ppm)</u>			
	0	1000	4500	9000
1	190	182	193	163
5*	155	146	146	171
9*	130	136	130.6	163.8
13*	120.7	128	139	159.5
18**	426	513	151.6	183.5
<u>Males</u> <u>Week</u>				
1	151.7	152.2	141.3	140.3
18	133.6	145.7	145.4	152.3

\* - represents feed consumption of pairs

\*\* - represents feed consumption of dams and pups at 0 and 1000 ppm.

TABLE III - ABSOLUTE ORGAN WEIGHTS

Males	0	Dose (ppm)		
		1000	4500	9000
Body (g)	42.24	42.11	40.70	35.70
Liver (g)	2.38	--	2.24	--
Kidney/adrenal(g)	0.91	--	0.82	--
sem. vesicles(mg)	422	--	385	--
l. testis/epid(mg)	201	205	126	66
rt testis	140	140	69	20
rt.cauda epid	18.4	20.1	16.7	15
rt. caput/corpus epididymus	40.6	42.9	32.1	27
prostate	48.32	--	38.7	--
<u>Females</u>				
Body	38.98	--	37.78	--
Liver	2.36	--	2.15	--
kidneys/adrenal	0.69	--	0.63	--
uterus	340	--	345	--

TABLE IV(a) - NUMBER of DAYS to LITTER (F<sub>0</sub>)

Litter	Dose (ppm)			
	0(n*)	1000	4500	9000
1st	24.1(38)	23.0(19)	23.9(19)	--
2nd	45.5(38)	44.6(19)	44.65(17)	--
3rd	64.2(36)	65.8(19)	92.5(6)	--
4th	85.5(36)	87.3(19)	98(1)	--
5th	103.0(31)	103.6(19)	119(1)	

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 \* = number of dams producing litters.

TABLE IV(b) - AVERAGE NUMBER of LIVE PUPS per LITTER

Litter	Dose (ppm)			
	0	1000	4500	9000
1st	12.68	12.05	9.7	--
2nd	13.76	13.85	8.24	--
3rd	14.22	14.32	4.17	--
4th	13.47	13.53	--*	--
5th	13.66	13.53	3.00	--

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 \* = One dam had five litters. All pups in the fourth litter were born dead.

TABLE V - REPRODUCTIVE PARAMETERS (F<sub>0</sub>)

DOSE (ppm)	0	1000	4500	9000
<b>Parameters:</b>				
Avg. fertility (%)	94	97	44	0
Avg. # litters/pair	4.7	4.8	2.3	--
Avg. # live pups/litter	13.52	13.31	8.67	--
% alive	99	97	88	
M:F ratio	52:48	53:47	52:48	
Avg. pup weight @ 24 hrs. (g)	1.62	1.64	1.39	

TABLE VI - Stages and Length of Estrous Cycle in F<sub>0</sub> and F<sub>1</sub> Females

Dose	STAGE					
	P	E	M (percent)	D	N	L (days)
F <sub>0</sub>						
0 ppm	18.4	23.7	18	38.2	1.8	4.5
0 ppm	20.6	14.6	15	46.7	3.1	5.2
1000 ppm	25.8	20.0	15	38.5	0.6	4.7
F <sub>1</sub>						
0 ppm	25.6	22.2	16.0	23.3	12.8	4.7
1000 ppm	23.5	22.2	19.7	31.2	3.4	4.1

P = proestrus, E = estrus, M = metestrus, D = diestrus, N = stage not clear. L = Length of cycle in days.

NOTE: Control females were divided into two groups in evaluating the parental animals.

TABLE VII - Sperm Data from F<sub>0</sub> and F<sub>1</sub> Males

Parameter	Dose Group (ppm)			
	0	1000	4500	9000
(F <sub>0</sub> ) sperm/mg Caudal Tissue Conc. (x1000)	518.64	532	146.9	2.80 <sup>a</sup>
% motile	78.13	68.96	53.26 <sup>b</sup>	42.86
% abnormal	11.34	6.43	61.17	6.83
(F <sub>1</sub> ) Conc.	585.6	442.6	--	--
% motile	66.58	75.13		
% abnormal	2.66	3.05		

a = 12/15 animals had no sperm

b = no motility observed in one animal, 2 animals had fewer than 20 sperm/field

TABLE VIII - REPRODUCTIVE PARAMETERS (F<sub>1</sub>)

Parameter	Dose (ppm)	
	0	1000
Fertility (%)	90	90
Avg. # pups/litter	12.64	12.94
Sex of F <sub>2</sub> pups (% male)	47	51
Avg. pup weight (g)	1.55	1.50

TABLE IX - FEED CONSUMPTION of F<sub>1</sub> MICE (g/kg)

Day	Dose (ppm)			
	males		females	
	0	1000	0	1000
77*	195	210	195	210
84	171	170	209	224
91	160	166	188	206
98	161	158	164	186

\* = Feed intake at day 77 represents the average for pairs.