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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 01101: Mouse Oncogenicity Study for Boric Acid

Tox. Chem. No.: 109
Project No.: 2-1142
Record No. : S411260
Bar Code #: D174253

FROM: Melba S. Morrow, D.V.M. MSM 11/3/92
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CONCLUSIONS:

Based on the results, of this study, when boric acid was administered for two years to B6C3F1 mice at dietary concentrations of 0, 2500 and 5000 ppm (0, 400 - 500 and 1100 - 1200 mg/kg, respectively), the test material was not associated with an increase in tumor incidence. At the highest dose tested, boric acid was associated with an increase in the incidence of testicular atrophy as characterized by loss of spermatogonia, primary and secondary spermatocytes, spermatids and sperm from the seminiferous tubules. Interstitial cell hyperplasia was also present at the 5000 ppm dose level.

There was a dose related increase in the incidence of splenic lymphoid depletion in male mice that was reported to be associated with stress. In females, a dose related increase in the incidence of pulmonary hemorrhage was reported; however, the significance of this finding was unknown. Both of these findings were present at 2500 ppm. A NOEL for systemic effects was not obtained in this study.

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Based on the body weight decreases (10 - 20% lower than controls) reported for both sexes at 5000 ppm, it can be concluded that the study was conducted at adequate dose levels.

The study satisfies the minimum data requirements for an oncogenicity study. Deficiencies included failure to conduct appropriate clinical pathology tests; failure to provide information on the weights of liver, kidneys, brain and testes; and failure to provide information on whether the study was conducted under Good Laboratory Practices. It should be noted that this study was an NTP study and clinical pathology and organ weights are not routinely reported. In addition, the study was audited by Dynamac Corporation and the data were examined for completeness, accuracy and consistency and for procedures consistent with Good Laboratory Practices.

In spite of these deficiencies under the Subdivision F guidelines (83-2), the carcinogenic potential of boric acid, under the conditions that were present in this study, could be adequately assessed.

A copy of the DER is provided for your reference.

Reviewed by: Melba S. Morrow, D.V.M. 11/3/92
Section II, Tox. Branch I (H7509C)
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DATA EVALUATION REPORT 009834

STUDY TYPE: Mouse Oncogenicity

GUIDELINE #: 83-2

TOX. CHEM. #: 109

MRID #: 418613-01

TEST MATERIAL: Boric Acid 99.7%

SYNONYMS: Borax

STUDY NUMBERS: TR324

SPONSOR: U.S. Borax and Chemical Corporation
Los Angeles, California

TESTING FACILITY: E.G and G. Mason Research Institute

TITLE OF REPORT: Toxicology and Carcinogenesis of Boric Acid in B6C3F1 Mice, NTP Technical Report Series 324

AUTHORS: Dieter, Bishop, Eustis, Haseman, et. al.

REPORT ISSUED: April 1991

CONCLUSIONS:

The study was conducted at the E.G. and G. Mason Research Institute for the NTP. Administration of boric acid (99.7%) at dietary concentrations of 0, 2500 and 5000 ppm (0, 400 -500 and 1100 -1200 mg/kg, respectively), for two years to B6C3F1 mice, did not cause an increase in tumor incidence. At the highest dose tested, boric acid was associated with an increase in the incidence of testicular atrophy as characterized by loss of spermatogonia, primary and secondary spermatocytes, spermatids and sperm from the seminiferous tubules. Interstitial cell hyperplasia was also present at the 5000 ppm dose level.

There was a dose related increase in the incidence of splenic lymphoid depletion in male mice which was associated with stress. In females a dose related increase in the incidence of pulmonary hemorrhage was reported; however, the significance of this finding is not known. A NOEL for systemic effects was not obtained in this study because both of these effects were noted at 2500 ppm.

The study was conducted at adequate dose levels based on the observed decreases in body weights for both sexes at the highest dose tested (10 - 20% lower than controls).

Classification: Minimum. (No clinical pathology or organ weights).

MATERIALS:

Boric acid, a colorless, crystalline solid, with a purity of 97% was the test material. The test animals were male and female B6C3F1 mice (50/sex/group). The animals were approximately 6 to 8 weeks of age when placed on the study.

METHODS:

Prior to conducting the 2 year oncogenicity study, preliminary studies were conducted to determine the the toxicity of boric acid. Studies are summarized as follows:

Fourteen Day Dietary Studies:

Two studies were conducted with Boric acid being administered at dietary levels ranging from 600 to 100,000 ppm. (In the first study, the highest dose tested was 9800 ppm).

In the first 14 day study, all 5 males and 1/5 females in the highest dose group experienced either weight loss or no weight gain. At 4900 ppm, there was no effect on body weight.

Higher levels of boric acid were administered in the second 14 day study and deaths were reported in 5/5 males and 4/5 females in the 100.000 ppm group. Deaths were also reported in 3/5 males receiving 50,000 ppm and in 1/5 males receiving 25,000 ppm.At 25,00 ppm final body weights in males were 10% lower than the final body weights reported for controls. In both sexes, hyperplasia and dysplasia of the stomach were also reported at this dose level.

Based on the body weight loss and mortality at doses of 25,000 ppm and above, a 13 week study was conducted using a high dose of 20,000 ppm.

Thirteen Week Dietary Study

Boric acid was administered in the feed at doses of 1200 to 20,000 ppm to B6 mice (10/sex/group). Animals in the control and high dose groups received the test material for 5 days/week for 13 weeks; animals in the low and mid dose groups received the test material for 5 days a week for up to 16 weeks.

Deaths were reported in 8/10 males and 6/10 females in the high dose group. At 10,000 ppm, 1/10 males died before the end of the study. In males, decreases in final body weights of > 10% of

controls were reported in animals receiving 5000, 10,000 and 20,000 ppm. In females, body weight decrements were 8 to 18% below control body weights.

At necropsy, extramedullary hematopoiesis was observed in the spleen of high dose animals. Additional findings at necropsy in the high dose group included hyperkeratosis and /or acanthosis of the stomach. Testicular degeneration or atrophy of the seminiferous tubules were present in males receiving doses of boric acid greater than and equal to 5,000 ppm.

Based on the results of the thirteen week feeding study, doses selected for the 2 year study were 2,500 ppm and 5000 ppm.

Two Year Study:

After a two week acclimation period, boric acid was administered to 50 mice/sex/group at dietary levels of 0, 2500 and 5000 ppm (0, 400-500 mg/kg and 1100 -1200 mg/kg, respectively) for a duration of two years. (See Table I, below).

TABLE I
DOSE GROUPS

Group	Conc. (ppm)	Dose (mg/kg)	# Animals M	# Animals F
Control	0	0	50	50
Low	2500	400 - 500	50	50
High	5000	1100 - 1200	50	50

Animals were housed 5/cage and food and water were provided ad libitum. Observations for clinical signs of toxicity were conducted twice daily and body weights were recorded weekly for the first 12 weeks. After the first twelve weeks, body weights were recorded every 4 weeks. Necropsies were performed on all animals and tissues and organs were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin and stained with hematoxylin and eosin. Tissues from all control and high dose animals and from any low dose animals dying before the end of the study were subjected to a microscopic examination. (See Table II for the tissues that were examined microscopically). In the low dose males and females, the liver, lungs, testes/ovaries and brain were examined microscopically. For low dose males, stomach, kidneys, salivary glands and pancreas were also examined histologically.

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STATISTICAL ANALYSIS:

The following statistical methodologies were employed:
- Kaplan- Meier for survival probability
- Cox's method used to determine dose related effects on survival
- Tarone's life table test for trend.
- Mantel-Hanszel for life table analysis
- Fisher's Exact test for pair wise comparison and Cochran Armitage for linear trend used for tumor analysis.

Levels of significance were not provided in the report.

DIET PREPARATION/ANALYSIS:

Boric acid was added to NIH 07 rat and mouse ration. Formulated diets were analyzed for boric acid content by the Azomethine H Method for determination of boron. Stability was also determined for boric acid blends that were stored sealed and protected from light. Analysis for verification of concentration was conducted periodically.

RESULTS:

Two Year Study:

Homogeneity of the dietary samples was confirmed. The concentration of boric acid in the feed was also determined to be within acceptable limits. At each sampling interval, the concentration was within 10% of the target concentration. Boric acid was stable in the feed for periods up to two weeks.

No clinical signs of toxicity were observed during the course of the study. Sixty percent of male mice in the low dose group and 44% of males in the high dose group survived the two year study and were sacrificed at the termination of the study. Survivability in control males was 82%. For females, the survival rates were 66% in the control and low dose groups and 74% in the high dose group. Drowning was listed as the cause of death in 2/50 females and 5/50 males in the high dose group.

Lower body weights were reported for high dose males after week 32 (10 - 17% lower than controls) and for high dose females after 52 weeks (10 - 20%). In low dose males, at week 96 a 12% body weight difference was reported in comparison to controls; however, at week 100, the mean body weight was only 5% lower than controls, indicating that the observed decrease in body weight at week 96 was not related to treatment. Low dose females had non-significantly lower body weights when compared to controls throughout most of the study. (See Table III, below).

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TABLE III
MEAN BODY WEIGHT (g)

	Dose Level (ppm) 0	5000
Males		
Week		
0	21.7	21.8 (100)*
8	29.7	30.1 (101)
16	33.1	32.5 (98)
24	35.6	30.6 (86)
32**	38.8	35.0 (89)
40	41.1	35.7 (87)
52	43.4	38.3 (88)
72	43.4	36.3 (84)
92	42.6	37.3 (89)
96	42.3	35.2 (83)
104	42.0	36.5 (87)
Females		
Week		
0	17.6	17.1 (97)
8	23.7	23.7 (100)
16	27.0	25.9 (96)
24	29.7	26.1 (88)
32	33.2	29.6 (89)
52**	38.9	33.7 (87)
72	40.6	33.8 (83)
92	41.6	35.4 (85)
96	41.9	35.3 (84)
104	44.8	35.9 (80)

* Numbers in parenthesis indicate body weight as percent of controls

** Indicates interval at which body weight remained at least 10% less than that reported for controls.

- In both sexes, body weights at 2500 ppm were more than 90% of the body weights reported for controls, with the exception of weights recorded for males at week 96.

Average daily food consumption was not adversely affected by the administration of boric acid in the diet. Higher food consumption values were reported for dosed animals and the report stated that the higher values may have reflected food spillage due to decreased palatability.

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Microscopically, testicular atrophy and interstitial cell hyperplasia were present at an increased incidence in high dose males. Atrophy was characterized by loss of differentiating sperm cells. Additional microscopic findings included seminiferous tubules containing Sertoli cells and interstitial cell accumulation. Testicular pathology has been reported in other studies with boric acid.

A dose related increase in the incidence of splenic lymphoid depletion was also reported in males. The incidence was 5/48, 11/49 and 25/48 for control, low and high dose males. Lymphoid depletion was present in females but there was no relationship between the dose of boric acid and the frequency of this lesion. In males splenic lymphoid depletion was believed to be associated with stress. In females, there was a reported dose related increase in the incidence of pulmonary hemorrhage, but the significance of this finding is unknown.

There was a statistically significant increase in the incidence of hepatocellular carcinomas in males receiving 2500 ppm of boric acid (10% for controls, 24% for LD). In these males, there was also an increase in the incidence of hepatocellular adenomas or carcinomas (combined) when compared to controls (28% for controls; 38% LD). The observed increases were significant by life table test analysis only and were not significant by the incidental tumor test. It was further stated that the observed increases were within the historical control ranges (NTP Carcinomas: 20 $\pm$ 7%; Combined: 30$\pm$ 8%) and were not believed to be associated with the administration of boric acid. No similar findings were observed in female mice at corresponding treatment levels.

In low dose males there was also a significant increase in the combined incidence of tumors of the integumentary system when compared to controls. These included fibromas, sarcomas, fibrosarcomas and neurofibromas. Similar findings were not present in high dose males or in any dosed females. A copy of the incidence data table for neoplastic lesions (taken from the report) is attached.

QUALITY ASSURANCE:

Not provided. However, data were audited for the NTP under a contract with Dynamac Corporation. The audit summary states that the data were examined for completeness, consistency and accuracy and for procedures consistent with Good Laboratory Practices.

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DISCUSSION:

Based on the results from this study, there does not appear to be an association with the administration of boric acid to B6C3F1 mice and the development of tumors. Although there was an increase in the combined incidence of tumors of the integument in low dose males, this observation was not believed to be related to the administration of the test material because similar effects were not present in males receiving 5000 ppm of boric acid.

The observed increases in tumors of the liver were not believed to be associated with the administration of the test material because the incidence was in the historical range and because there was no increase in incidence at higher dose levels in males.

Testicular pathology at the highest level tested was associated with the administration of boric acid and has been demonstrated in other studies. Testicular changes included testicular atrophy and interstitial cell hyperplasia. Other pathological findings included a dose related increase in the incidence of splenic lymphoid depletion in male mice that was associated with stress and a dose related increase in pulmonary hemorrhage in female mice which was of unknown biological significance.

Based on the observed decreases in body weight in both sexes at the high dose (10 -17% M; 10 - 20% F), it is believed that the study was conducted at adequate dose levels.

This study satisfies the minimum requirement for an oncogenicity study in mice as set forth in the Subdivision F Guidelines (83-2). Deficiencies in the study included failure to conduct clinical pathology tests and failure to provide information on organ weights. (It should be kept in mind that this is an NTP study and clinical pathology and organ weights are not routinely reported). In spite of these deficiencies, the carcinogenic potential could be adequately assessed.

TABLE III

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TISSUES COLLECTED FOR MICROSCOPIC EXAMINATION

The following CHECKED (x) tissues were collected for histological examination

Digestive system
- X Tongue
- X Salivary glands
- X Esophagus
- X Stomach
- X Duodenum
- X Jejunum
- X Ileum
- Cecum
- X Colon
- Rectum
- X Liver
- X Gall bladder
- X Pancreas

Respiratory
- X Trachea
- X Lung
- Nose
- Pharynx
- X Larynx
- X Bronchi

Cardiovasc./Hemat.
- Aorta
- X Heart
- X Bone marrow
- X Lymph nodes
- x Spleen
- X Thymus

Urogenital
- x Kidneys
- X Urinary bladder
- x Testes
- Epididymides
- X Prostate
- X Seminal vesicle
- X Ovaries
- X Uterus
- Vagina

Neurologic
- X Brain
- Periph. nerves
- Spinal cord

Glandular
- X Parathyroids
- X Adrenals
- X Thyroid
- X Pituitary

Other
- X Bone & marrow
- X Skin
- X Skel. muscle
- x All tissue masses

TABLE IV
INCIDENCE SUMMARY OF PRIMARY NEOPLASMS IN MALE MICE

	DOSE GROUPS		
	CONTROL 0 ppm	LOW 2500 ppm	HIGH 5000 ppm
SYSTEM/TUMOR TYPE (# examined)			
INTEGUMENTARY			
Fibroma	1/50 (2)	3/50 (6)	0/50 (0)
Sarcoma	0/50 (0)	2/50 (4)	0/50 (0)
Fibrosarcoma	1/50 (2)	4/50 (8)	2/50 (4)
Neurofibroma	0/50 (0)	1/50 (2)	0/50 (0)
Combined *	2/50 (4)	10/50 (20)	2/50 (4)
* p (life table test)	0.239	0.005	0.493
HEPATIC			
Hepat. adenoma	11/50 (22)	9/50 (18)	8/49 (16)
p (life table test)	0.271	0.495	0.320
Hepat. carcinoma	5/50 (10)	12/50 (24)	8/49 (16)
p (life table test)	0.035	0.019	0.056
Combined	14/50 (28)	19/50 (38)	15/49 (31)
p (life table test)	0.023 *	0.043	0.038

Numbers in parenthesis are percents.

Historical Incidence of Subcutaneous Tumors
Mason Research Facility - 39/697 (6 ± 4%)
NTP studies - 156/2091 (7 ± 8%)

Historical Incidence of Hepatic Tumors
Mason Research Facility-
Adenomas 96/697 (14% ± 10%)
Carcinomas 131/697 (19% ± 6%)
Both 216/697 (31% ± 9%)

NTP Historical Incidence
Adenomas 228/2084 (11% ± 8%)
Carcinomas 424/2084 (20% ± 7%)
Both 627/2084 (30% ± 8%)