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

009301

FEB 21 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: Review of Toxicology Studies with Borax (sodium tetraborate decahydrate) to support registration of test substance.

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 12/17/91
Tox. Branch II, Review Section IV
HED (H7509C)

TO: Susan Lewis, Product Manager (21)
Registration Division
(H7505C)

THRU: Elizabeth Doyle, Ph.D., Section Head E A Doyle 2/3/92
Tox. Branch II, Review Section IV
HED (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Tox Branch II
HED (H7509C)

ACTION REQUESTED: Review of toxicology studies

Overview of Studies:

The average LD_{50} in male rats was 5.40 gm/kg and 5.0 gm/kg in females. A LD_{50} was not obtained in dogs because the material irritated the stomach and was subsequently vomited.

Severe eye irritation would be expected to occur as evidenced by the eye irritation studies but may be prevented by immediately washing the material from the eye.

Dermal irritation and/or absorption of the test material through the skin would not be expected as evidenced by the primary dermal and acute dermal studies.

0001 of 143

Printed on Recycled Paper

Sodium tetraborate decahydrate, as noted in the following studies, produced testicular atrophy in rats and dogs. Effects on ovulation and possible other reproductive effects were seen in the rat. These effects, for the most part, appeared to be dose related.

The subchronic oral toxicity studies in the rat and the dog showed testicular atrophy and reduction in the weight of the testes at dose levels of 0.0463 and 1.54% in the rat and 1.54% in the dog.

In the dog chronic study and the rat combined chronic/oncogenic study a similar effect was noted at 1.03%. The test material was not considered to be carcinogenic in the rat.

The fertility and reproductive study again showed testicular atrophy and decrease in testes weight at 1.03%. Moreover, the ovaries and quite possibly other organs concerned with reproduction were also affected as evidenced by a decrease in ovulation and the lack of litters. These effects occurred in the first parental generation only.

Studies Summarized:

1. MRID 406923-01 Acute Oral Chronic Study - rat (81-1)
Core - supplementary

The LD_{50} in the male rat was calculated to be 6.08 gm/kg (Toxicity Category IV).

2. MRID 406923-02 Acute Oral Administration - rat (81-1)
Core - supplementary

The LD_{50} in male rats was 5.56 (5.15 - 6.0) gm/kg (Toxicity Category IV).

3. MRID 406923-03 Acute Oral Toxicity Study - rat (81-1)
Core - Guideline

The LD_{50} for male rats was 4.55 gm/kg. The LD_{50} for female rats was 4.98 gm/kg (Toxicity Category III).

4. MRID 406923-04 Acute Oral Toxicity Study - dog (81-1)
Core - supplementary

No deaths were observed in dogs at 0.246, 0.387, 0.615 and 0.9741 gm/kg of body weight (Toxicity Category III).

5. MRID 406923-05 Subchronic Oral Toxicity - rat (82-1)
Core - Supplementary

All animals died at the high dose of 4.63%. In both sexes at 1.54% (high middle dose), the testes weight and ratio, the ovary weight and ratio and the corresponding organ/brain weight ratios were markedly decreased. At 0.0463% (low dose), 4/10 male animals were similarly affected.

The NOEL in female rats was 0.463%. The LOEL was 1.54% based on reduced body weight gain. A NOEL in males was not obtained. The LOEL was 0.0463% based on testicular atrophy.

6. MRID 406923-06 Subchronic Oral Toxicity - rat (82-1)
Core - Supplementary

No compound related effects were noted at 0.0154, 0.0463, 0.154, and 0.463%. The systemic toxicity NOEL was 0.463%.

7. MRID 406923-07 Subchronic Oral Toxicity - dog (82-1)
Core - Supplementary

Atrophy of the testes occurred at 1.54% (high dose) together with decreased testes weight, testes/body weight and testes/brain weight ratios.

The systemic toxicity NOEL was 0.0154%, the LOEL was 0.154% based on pathology findings.

8. MRID 406923-08 Chronic Toxicity - dog (83-1)
Core - Supplementary

Testicular atrophy occurred at 1.03% (only dose used) together with decreased testes weight, testes/body weight and testes/brain weight ratios and lack of ejaculate.

Tissue storage of test material was not seen at any time period. The test substance was eliminated from the body in the urine and feces less than 4 days after dosing was stopped.

The NOEL and LOEL could not be determined for only 1 dose level was employed in this study.

9. MRID 406923-09 Combined Chronic Toxicity/Oncogenicity - rat (83-5) Core - Supplementary

The No Observed Effect Level (NOEL) = 0.130% (low dose).
The Lowest Observed Effect Level (LOEL) = 0.308% (middle dose) - decreased body weight gain.

The Maximum Tolerated Dose (MTD) = 1.030% (Highest Dose Tested, HDT) - decreased body weight gain and testicular tubular atrophy.

10. MRID 406923-10 Chronic Oral Toxicity - dog (83-1)
core - supplementary

No definitive test article effect were seen at any dose level.

No Observed Effect Level (NOEL) = 0.309% (Highest Dose Tested, HDT)
Lowest Observed Effect Level (LOEL) = none (>HDT)

11. MRID 406923-11 Fertility and Reproductive Effects - rat (83-4) Core - Supplementary

The testes at 1.03% (high level) in the P1 generation were grossly atrophied as evidenced by a severe decrease in the organ weight and organ/body weight ratio. A decrease in the number of corpora lutea was indicative of a decrease in ovulation in the P1 generation. No litters were produced at 1.03% when the test animals were mated with test females. P1 test females at 1.03% mated with control males resulted in a decreased number of litters and pup survival.

NOEL for systemic and reproductive toxicity - 0.308% (middle level)
LOEL for systemic and reproductive toxicity - 1.03%. (low level)

The following studies (Number 12, 13, 14 and 15) were not issued MRID numbers due to procedural problems that the sponsor must rectify.

12. MRID N/A Primary Eye Irritation - rabbit (81-4) [Labeled as study A] Core - guideline

Severe irritation was evidenced (no-washout) in albino rabbits (Toxicity Category I)

13. MRID N/A Primary Eye Irritation - rabbit (81-4) [Labeled as study B] Core - minimum

Sodium tetraborate decahydrate was considered to be an eye irritant in albino rats when no wash out procedure was used. Minimal corneal opacity was still evident at day 14 termination (Toxicity Category I).

Minimal irritation occurred in animals whose eyes were irrigated after administration of the test article. Eye irritation was judged unremarkable on day 14, the study termination.

14. MRID N/A Primary Dermal Irritation Study - rabbit (81-5) Core - guideline

A primary skin irritation score of zero (0) was obtained when 0.5 gm of the test substance was applied to the unabraded skin of albino rabbits (Toxicity Category IV).

15. MRID N/A Acute Dermal Toxicity Study - rabbit (81-2)
Core guideline

The LD_{50} for the test compound was greater than 2.0 gm/kg (Toxicity Category III).

Reviewed by Steven L. Malish, Ph.D. S.L. Malish 10/26/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 10/25/91
Tox. Branch II, Section IV (H7509C)

009301

DATA EVALUATION REPORT

STUDY TYPE:	Acute Oral Toxicity Study - Rat (81-1)
MRID NO.:	406923-01
TEST MATERIAL:	Sodium tetraborate decahydrate
SYNONYMS:	Borax
SPONSOR:	U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA 92801-6794
TESTING FACILITY:	Hazelton-Nuclear Science Corp. 4062 Fabian Way, Palo Alto, CA
TITLE OF THE REPORT:	Acute Oral Administration - Rats with Sodium tetraborate decahydrate
AUTHORS:	George D. Meyding
REPORT NO:	not available
REPORT ISSUED:	January 25, 1961
QUALITY ASSURANCE:	No quality assurance documentation was presented.
CONCLUSIONS:	Under the conditions of this 7 day study, the LD_{50} of borax in the male rat was calculated to be 6.08 gm/kg (3.54-10.4).
CLASSIFICATION:	Core supplementary - can not be upgraded. The study does not satisfy the guideline requirements (81-1) for an an "Acute Toxicity Study in Rats." The study was deficient in the fact that 1. only 5 male animals were used at each dose level, 2. observation period should be extended from 7 to 14 days.
TOXICITY CATEGORY:	-IV- (LD_{50} greater than 5000 mg/kg) as per the Federal Register, Vol. 49, No. 188, September 28, 1984, proposed rule.

0006

A. Materials:

1. Test Compound:

Chemical:	sodium tetraborate decahydrate
Label:	10-Mol Borax
Description:	white solid, no characteristic odor
Purity:	considered to be 100%
Stability:	data not available

2. Test Animals:

Species:	Rat
Strain:	Long-Evans
Groups:	five (5) groups of 5 males each
Age:	data not available
Weight:	87-118 gms
Source:	data not available

B. STUDY DESIGN: Groups of five male received orally by stomach tube a distilled water suspension of the test material at 1.0, 2.15, 4.64, 6.81 and 10.0 gm/km of body weight. Food and water were withheld for 3 to 4 hours before dosing but were given ad libitum after dosing.

All animals were observed for clinical signs and mortality several times throughout the day of dosing and daily, thereafter, until sacrifice and necropsy on day 7. Statistical analysis of the mortality data was calculated by the Moving Average Method (H.J. Horn, Biometrics 12, 311, 1956).

C. RESULTS: The oral LD_{50} for sodium tetraborate decahydrate (borax) was calculated to be 6.08 gm/kg with confidence limits of 3.54-10.4 gm/kg of body weight.

All animals at the 1.0, 2.15 and 4.64 gm/kg dose level showed normal appearance and behavior during the day of administration of the test compound and daily, thereafter, for the duration of the study.

At the 6.81 gm/kg dose level, 2/5 deaths occurred on day two which increased to 4/5 deaths by day 3. The animal at the 6.81 gm/kg dosage level appeared normal during the day of dosing. At the 24 and 48 hour observation periods, the surviving animals appeared depressed and showed slight diarrhea. On the third day and thereafter, the survivor appeared normal.

The rats at the 10.0 gm/kg level exhibited depression and shallow, rapid respiration within approximataly two hours following dosing. Moribund animals were noted to

have excessive urination and showed deep yellow urine stains. All animals (5/5) died on day one, after 4 hours but before the 24 hour observation periods.

The survivors at the 1.0, 2.15, and 4.64 gm/kg dose levels showed no gross pathology changes. Gross autopsies performed upon animals that died revealed markedly erythemic lungs, congested adrenals and pale livers. In the majority of the fatalities, the muscular region of the stomach appeared injected. The surviving animal at the 6.81 gm/kg level showed a slightly pale liver.

No body weights parameters were reported.

D. SUMMARY:

The oral LD_{50} in the male rat for sodium tetraborate decahydrate (borax) was calculated to be 6.08 gm/kg (3.54-10.4) of body weight.

Symptomatic animals evidenced shallow rapid respiration, depression, slight diarrhea and excessive urination.

The survivors at the 1.0, 2.15, and 4.64 gm/kg dose levels showed no gross pathology changes. Gross autopsies performed upon animals that died revealed markedly erythemic lungs, congested adrenals and pale livers. In the majority of the fatalities, the muscular region of the stomach appeared injected. The surviving animal at the 6.81 gm/kg level showed a slightly pale liver.

E. CONCLUSIONS:

Under the conditions of this 7 day study, the LD_{50} of borax in the male rat was calculated to be 6.08 gm/kg (3.54-10.4).

Reviewed by: Steven L. Malish, Ph.D. S.L. Malish 10/26/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 10/25/91
Section II, Tox. Branch IV (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Oral Toxicity Study - Rat (81-1)
MRID:	406923-02
TEST MATERIAL:	Sodium tetraborate decahydrate
SYNONYMS:	Borax
SPONSOR:	U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA 92801-6794
TESTING FACILITY:	Hazleton Laboratories, Inc., Vienna, VA
TITLE OF REPORT:	Acute Oral Administration - Rats with Sodium tetraborate decahydrate
AUTHORS:	George D. Meyding
REPORT ISSUED:	August 2, 1961
QUALITY ASSURANCE:	No quality assurance documentation was provided.
CONCLUSIONS:	Under conditions of this study, the LD_{50} for sodium tetraborate decahydrate in male rats was calculated to be 5.56 (5.15 - 6.0) gm/kg with a slope of 1.2.
CLASSIFICATION:	Core - supplementary. This study does not satisfy the guideline requirements (81-1) for an "Acute Oral Toxicity Study in Rats". The study can be upgraded by providing a LD_{50} for female animals and animal weight data for both sexes.
TOXICITY CATEGORY:	-IV- (LD_{50} greater than 5.0 gm/kg) as per Federal Register, Vol. 49, No. 188, September 28, 1984, proposed rule.

A. MATERIALS:

1. Test Compound:
Chemical: Sodium tetraborate decahydrate
Label: 10-Mol Borax
Description: white solid, no odor
Purity: considered to be 100%
Stability: data not available

2. Test Animals:
Species: Rats
Strain: Sprague-Dawley
Groups: 7 groups of 10 male animals
Age: data not available
Weight: 222 - 350 gms.
Source: data not available

Study Design: Food was withheld from the animals for a three to four hour period prior to dosing. Groups of 10 male rats received orally by stomach tube a 50% weight/volume suspension of the test material in distilled water. The dosage levels used were 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 gm/kg of body weight. Food and water were available ad libitum after dosing.

The animals were observed for gross effects several times during the day of administration and at least once daily, thereafter, for a period of 14 days.

Gross autopsies were performed on the animals that died. At the end of the observation period, the surviving animals were weighed and necropsied.

Statistical analysis of the mortality data was performed by the method of Litchfield and Wilcoxon (Litchfield, J., Wilcoxon, F. J. Pharmacol. Exptl. Therap. 96, 99, 1949.

B. RESULTS: Under the conditions of the study, the LD_{50} for sodium tetraborate decahydrate was calculated to be 5.56 gm/kg in male rats with confidence limits of 5.15 to 6.00 gm/kg. The slope function was calculated to be 1.2.

All animals succumbed by the 24 hour observation period except at the 6.0 gm/kg dose level where an additional animal was found dead by the third day.

009301

Table 1

Cumulative Mortality

Dose (gm/kg)	Mortality*
4.0	0/10
4.5	1/10
5.0	3/10
5.5	3/10
6.0	6/10
6.5	9/10
7.0	10/10

* Number of animals dead/total number of animals in the group. All animals died by the 24 hour observation period except for an additional animal that succumbed by day 3 at the 6.0 gm/kg dose level.

The author reported that surviving animals showed normal body weight gains for the 14 day observation period. [The reviewer notes, however, that weight parameters were not included in the report.]

Each of the animals at 4.0 gm/kg dosage level exhibited normal appearance and behavior during the day of administration of the test material and daily, thereafter, for the remainder of the 14 day observation period.

The animals at the 4.5, 5.0, 5.0 and 5.5 gm/kg dosage level appeared normal during the day of administration. At the 24 - hour observation period, the majority of the animals at these dosage levels exhibited depression, diarrhea, ataxia, lacrimation, ptosis and deep yellow urine stains. The surviving animals appeared normal at the next observation period (day 2) and, thereafter, during the 14 day study.

Within 3.5 to 4.5 hours following intubation, the animals at the 6.0, 6.5 and 7.0 gm/kg levels appeared depressed and exhibited ataxia, diarrhea, ptosis and deep yellow urine stains. These signs, in general, persisted up until the time of death. The survivors at the 6.0 and 6.5 gm/kg levels appeared normal within 3-6 days following dosage and daily, thereafter.

Gross autopsies performed on the animals that died revealed congested lungs, congested, adrenals, distension of the stomach and small intestines. The walls of the stomach and small intestine appeared pale.

Gross necropsies performed on the surviving animals revealed, in the majority, pale mottled kidneys, pale livers and slightly congested adrenals. Several animals showed slightly congested lungs.

C. SUMMARY:

The LD_{50} for sodium tetraborate decahydrate in male rats was calculated to be 5.56 gm/kg with confidence limits of 5.15 - 6.00 gm/kg. The slope function was 1.2.

Symptomatic animals exhibited depression, diarrhea, ataxia, lacrimation, and ptosis.

Gross autopsies performed on the animals that died revealed congested lungs, congested, adrenals, distension of the stomach and small intestines. The walls of the stomach and small intestine appeared pale.

Gross necropsies performed on the surviving animals revealed, in the majority, pale mottled kidneys, pale livers and slightly congested adrenals. Several animals showed slightly congested lungs.

Reviewed by: Steven L. Malish, Ph.D. S.L. Malish 10/26/91 [1]
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 10/25/91
Tox. Branch II, Section IV (H7509C)

009301

DATA EVALUATION REPORT

STUDY TYPE:	Acute Oral Toxicity Study - Rat (81-1)
MRID:	406923-03
TEST MATERIAL:	Sodium tetraborate decahydrate
SYNONYMS:	Borax
SPONSOR:	U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA 92801-6794
TESTING FACILITY:	Hazleton Laboratories, Inc., Vienna, VA
TITLE OF REPORT:	Acute Oral Administration - Rats with Borax (Sodium tetraborate decahydrate)
AUTHORS:	John G. Keller, Ph.D.
REPORT NO.	not available
REPORT ISSUED:	March 16, 1962
QUALITY ASSURANCE:	No quality assurance documentation was provided.
CONCLUSIONS:	The LD_{50} for sodium tetraborate decahydrate in male rats was calculated to be 4.55 (4.14 - 5.01) gm/kg with a slope of 1.12. The LD_{50} for female rats was calculated to be 4.98 (4.31 - 5.76) gm/kg with a slope of 1.16.
CLASSIFICATION:	Core - guideline
TOXICITY CATEGORY:	-III- (LD_{50} between 0.5 and 5.0 gm/kg) as per the Federal Register, Vol. 40, No. 188, September 28, 1984.

A. MATERIALS:

1. Test Compound:	Chemical:	Sodium tetraborate decahydrate
	Label:	not available
	Description:	soft, fine white powder with no noticeable odor
	Purity:	boron content was 104% of theoretical; considered to be free of impurities
	Stability:	not available
2. Test Animals:	Species:	Rats
	Strain:	Sprague-Dawley
	Groups:	6 groups of 5 animals per sex
	Age:	data not available

Weight: males: 272 - 318 gms
females: 206 - 243 gms
Source: data not available

B. STUDY DESIGN: Groups of 5 animals per sex received orally by stomach tube a 50% weight/volume suspension of the test material in 0.5% aqueous methyl cellulose. The dosage levels used were 3.09, 3.87, 4.88, 6.14, 7.73 and 9.74 gm/kg of body weight. The observation period was 14 days.

Food was withheld from the animals for a three to four hour period prior to dosing. Following oral administration, food and water were available ad libitum.

After dosing, the animals were observed for mortality and toxic effects immediately and at one, two, four and 24 hours, and once daily, thereafter, for 14 days. At the end of the observation period, the surviving animals were weighed, sacrificed and autopsied.

Statistical analysis of the mortality data was conducted in female rats by the method of Thompson, W.R., Bact. Rev. 11 115, 1947, utilizing the tables of Weil, C.S., Biometrics, 8, 249, 1952 and in male rats by the method of Litchfield, J.T. and Wilcoxon, F. J. Pharmacol. Exptl. Therap. 96, 99, 1949.

C. RESULTS: Under the conditions of the study, the LD_{50} for sodium tetraborate decahydrate was calculated to be 4.55 gm/kg in male rats with confidence limit of 4.14 to 5.01 gm/kg. The slope function was calculated to be 1.12.

Under conditions of the study, the LD_{50} in female rats was calculated to be 4.98 with a confidence limit of 4.31 to 5.76 gm/kg. The slope function was calculated to be 1.16.

Mortality:

The male and female animals that died between 5 and 24 hours are listed in Table 1 below. A single female animal at the 6.14 gm/kg dosa level was found dead on day 2.

D. CONCLUSIONS: The LD_{50} for sodium tetraborate decahydrate in male rats was calculated to be 4.55 (4.14 - 5.01) gm/kg with a slope of 1.12. The LD_{50} for female rats was calculated to be 4.98 (4.31 - 5.76) gm/kg with a slope of 1.16.

Table 1

Cumulative Mortality*

Dose gm/kg	Time of Death hours Male 5-24	Female 5-24
3.05	0/5	0/5
3.87	1/5	0/5
4.88	3/5	4/5
6.14	5/5	3/5
7.73	5/5	5/5
9.74	5/5	5/5

* Total number of animals dead/total number of animals in the group. All animals died between 5 and 24 hours except for a single animal that was found dead on day 2 at the 6.14 gm/kg dose level.

Symptomatology increased in severity as the dose was increased. Symptoms were generally noted within 2 to 4 hours after compound administration except at the 9.74 and 3.87 gm/kg dose levels. Symptoms were noted within 30 to 60 minutes at the higher dose level and within 24 hours in females at the lower dose level (Table 2).

Table 2

Signs	Symptomatology Dose (g/kg) 3.05	3.87	4.88	6.14	7.73	9.74
depression	-	mf+	mf	mf	mf	mf
dyspnea	-	mf	mf	mf	mf	mf
diarrhea	-		mf	mf	mf	mf
ataxia	-		mf	mf	mf	mf
sprawling of limbs	-		m	mf		mf
depressed righting/ placement reflexes	-			mf	m	mf

+ m=male, f=female

Pathological examination of the surviving animals was unremarkable.

D. SUMMARY: The calculated LD_{50} of borax in male rats was 4.55 gm/kg with a confidence limits of 4.14 to 5.01

gm/kg. In female rats the LD_{50} was 4.98 gm/kg of body weight with a confidence limit of 4.31 to 5.76 gm/kg.

The surviving animals at the 3.09 and 3.87 gm/kg dose levels showed an increase in body weight above the initial weights.

Symptomatology, except at the lowest dose levels included depression, dyspnea, diarrhea and ataxia.

Male and female animals in the 3.09 gm/kg dose group and females at the 3.87 gm/kg dose group showed no gross pathology.

Males at the 3.87 gm/kg dose were noted to have congestion of the lungs, kidneys and adrenals.

In the males at the 4.88 gm/kg dose level, congestion of the lungs, kidneys and adrenals and inflammation of the pyloric portion of the stomach and small intestine were noted. Females showed hemorrhage of the lungs rather than congestion together with the other abnormalities.

At the 6.14 and 7.73 gm/kg dose levels, males and females evidenced hemorrhage of the lungs together with the other abnormalities.

At the 9.74 gm/kg dose level males and females showed congestion or hemorrhage of the lungs, congestion of the kidneys and adrenals and inflammation of the small intestine. While males showed hemorrhage or inflammation of the pyloric portion of the stomach, females showed only hemorrhage.

Animals showed no pathology at necropsy following sacrifice.

E. CONCLUSIONS: The LD_{50} for sodium tetraborate decahydrate in male rats was calculated to be 4.55 (4.14 - 5.01) gm/kg with a slope of 1.12. The LD_{50} for female rats was calculated to be 4.98 (4.31 - 5.76) gm/kg with a slope of 1.16.

Reviewed by: Steven L. Malish, Ph.D. S.L. Malish 10/24/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 10/25/91
Tox. Branch II, Section IV (H7509C)

009301

DATA EVALUATION REPORT

STUDY TYPE:	Acute Oral Toxicity Study - Dog (81-1)
MRID:	406923-04
TEST MATERIAL:	Sodium tetraborate decahydrate
SYNONYMS:	Borax
SPONSOR:	U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA 92801-6794
TESTING FACILITY:	Hazleton Laboratories, Inc., Vienna, VA
TITLE OF REPORT:	Acute Oral Administration - Dogs with Borax (sodium tetraborate decahydrate)
AUTHORS:	John G. Keller, Ph.D.
REPORT NO.:	not available
REPORT ISSUED:	March 16, 1962
QUALITY ASSURANCE:	No quality assurance documentation was provided.
CONCLUSIONS:	Borax was administered to mongrel dogs at 0.246, 0.387, 0.615 and 0.9741 gm/kg of body weight. No deaths were observed. Slight seizures were noted at the two lowest dose levels; diarrhea ws seen at the three lower dose levels and vomiting occurred at all dose levels. Animals were considered to be unremarkable after the initial symptomatology had passed.
CLASSIFICATION:	Core - supplementary An LD_{50} was not determined.
TOXICITY CATEGORY:	-III- (LD_{50} greater than 0.5 but less than 5.0 gm/kg as per the Federal Register, Vol. 49, 188, September 28, 1984.

0017

A. MATERIALS:

1. Test Compound:
Chemical: Sodium tetraborate decahydrate
Label: not available
Description: soft, fine, white powder, no odor
Purity: boron content was 104% of theoretical
Stability: NA (not available)

2. Test Animals:
Species: dog
Strain: mongrel
Groups: four (4) groups of 3 dogs each
Age: NA
Weight: NA
Source: NA

3. Study Design: Borax was administered orally, by capsule, to 4 groups of 3 dogs at dosages of 1.54, 2.46, 3.87 and 6.15 gm/kg of body weight. Because of emesis occurring at these dose levels, the dogs were rested for 6 days and retested at lower dose levels of 0.246, 0.387, 0.615 and 0.974 gm/kg.

The dogs were fasted overnight prior to test compound administration; otherwise, dog chow and water were available ad libitum. All animals were observed continuously for gross signs of toxicity and pharmacological effects after administration of the compound and daily, thereafter, for the remainder of the seven-day observation period.

B. RESULTS: At the two lowest dose levels of 0.246 and 0.387 gm/kg, two (2) of the three (3) dogs showed slight emesis of a foamy, white-brown liquid which occurred within 13 to 19 minutes of dosing. These animals also evidenced slight seizures. [The reviewer notes that no other information was given about the seizures]. One (1) dog of each group had diarrhea 37 minutes to 2 hours after dosing. The dogs were unremarkable for the remainder of the study. Slight gains in body weight occurred from the initial values in these groups.

At dose levels of 0.615 and 0.974 gm/kg, all dogs experienced emesis within 18 to 31 minutes which continued for 10 to 15 minutes. Diarrhea was noted in 1 dog at 0.615 but not at the 0.974 gm/kg dose level. The dogs were unremarkable for the remainder of the study. Dogs maintained their initial body weight during the study.

No body weight data was provided and no necropsy was performed.

C. CONCLUSIONS: Borax was administered to mongrel dogs at 0.246, 0.387, 0.615 and 0.9741 gm/kg of body weight. No deaths were observed.

Slight seizures were noted at the two lowest dose levels; diarrhea ws seen at the three lower dose levels and vomiting occurred at all dose levels. Animals were considered to be unremarkable after the initial symptomology had passed.

009301

Reviewed by: Steven L. Malish, Ph.D. S.L. Malish 10/26/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 10/25/91
Tox. Branch II, Section IV (H7509C)

Data Evaluation Report

STUDY TYPE: Subchronic Oral Toxicity (Rodent): 90-day study (82-1)

MRID NO: 406923-05

TEST MATERIALS: Sodium tetraborate decahydrate

SYNONYMS: Borax

SPONSOR: U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA

TESTING FACILITY: Hazleton Laboratories America, Inc., Vienna, VA

TITLE OF REPORT: 90-Day Dietary Administration - Rats with 20 MULE TEAM^{R} Borax (Sodium tetraborate decahydrate)

STUDY NUMBER: Laboratory study number was not reported

AUTHORS: Orville Paynter, Ph.D.

REPORT ISSUED: December 13, 1962

CONCLUSIONS:

Sprague-Dawley rats were administered sodium tetraborate decahydrate at concentrations of 0.0 (Control), 0.0463%, 0.154%, 0.463%, 1.54% and 4.63% in the feed for 13 weeks.

All animals died in both sexes at the 4.63% level. In both sexes at 1.54%, decreased weight gain and food consumption occurred. The testes weight and ratio, the ovary weight and ratio and the corresponding organ/brain weight ratios were markedly decreased. At 0.0463%, 4/10 male animals were similarly affected.

The NOEL in females rats was 0.463%. The LOEL was 1.54% based on reduced body weight gain. A NOEL in males was not obtained. The LOEL was 0.0463% based on testicular atrophy.

CLASSIFICATION:

Core - Supplementary

The study does not satisfy the guidelines requirements (82-1) for a subchronic oral toxicity (rodent): 90 day study.

The study is deficient in that no NOEL in males was obtained. The hematology, clinical biochemistry and ophthalmological examinations were not performed; the tissues and organs required under microscopic examination in the guidelines were not evaluated.

A. MATERIALS:

1. Quality Assurance:

No quality assurance statement was issued.

2. Test Compound:

Chemical: Sodium Tetraborate Decahydrate
Label: not available
Description: fine white powder without noticeable odor
Storage: not available

Purity:

Two samples of the test material were submitted for analysis during the study, sample 1 had a boron content of 104.8% while sample 2 had a boron content of 104.7% of the theoretical value. For the first 8 weeks of the study, boron content calculations were adjusted from 104% to 100% from week 9 through 13 boron content calculations were adjusted from 105% to 100% of theoretical.

Stability, Dietary Concentration and Homogeniety in Feed:

No studies was done to assess the stability in feed, the analysis of the feed for the actual dietary concentration or the homogeniety during the 13 week study.

2. Test Animals:

Species: Rat
Strain: Sprague-Dawley, pathogen free
Age: not available
Weight: Males 89 to 114 gm
Females 81 to 114 gm
Source: Charles River Breeding Laboratories, Wilmington MA.

B. STUDY DESIGN:

1. Animals:

The animals were selected by stratified randomization and housed individually in wire mesh cages elevated above the droppings.

2. Animal Assignments:

Fifty animals per sex were assigned to six (6) test groups consisting of 10 animals per sex per dose level and administered either 0% (Control), 0.0463%, 0.154%,

0.463%, 1.54%, or 4.63% of the test compound in the diet.

Table 1

Animals Assignments and Dose Levels

Group No.	No. of Rats		Dietary Level	
	Male	Female	Test Cpd. Conc (%)	Boron^ Equiv (%)
1 (Control)	10	10	0	0
2	10	10	0.0463	0.00525
3	10	10	0.154	0.0175
4	10	10	0.463	0.0525
5	10	10	1.54	0.175
6	10	10	4.63	0.525

^ theoretical boron content assuming 100% sodium tetraborate decahydrate

3. Diet:

Animals received Purina Rodent Laboratory Chow and water ad libitum.

4. Diet Preparation:

Test diets was prepared weekly. The test material, as received, was incorporated into the basal diet on a weight/weight basis to provide the desired dietary levels. The feed was thoroughly mixed in a twin-shell blender. The dietary level was adjusted to 100% of the theoretical value based on the boron equivalent assay purity.

5. Compound Consumption:

Mean compound consumption is presented below and was calculated from the mean food consumption and mean body weight.

Table 2

Mean Compound Consumption Throughout The 13 Week Study

Conc. Level	males		females	
%	mg/kg/day		mg/kg/day	
0.0	0.0		0.0	
0.0463	31.4	(1.25)^	36.7	(4.34)^
0.154	112.9	(13.3)	113.5	(14.2)
0.463	311.3	(38.8)	367.5	(43.5)
1.54	1025.4	(120.5)	1087.7	(128.8)
4.63	4752.5	(560.8)	4466.7	(193.8)

adapted from p. 25 thru 30 of the original report
^ based on boron assay value of 104% for weeks 1 thru 8 and 105% for weeks 9 thru 13.

6. Statistics:

Statistical analyses were not performed at the 4.63% dose level but were performed at the lower levels. Survival was analyzed by the life-table technique; all other criteria were examined by the F-test or analysis of variance at the 5% probability level. Before completing each F-test, the variances were analyzed for heterogeneity by the method of Bartlett. If the variance was homogeneous, the F-test was completed in the normal fashion. Whenever a significant F-value was obtained, those groups significantly different from the control were determined by the method of Scheffe. Whenever heterogeneous variances were obtained, comparisons were made by the Fisher-Behrens modified "t" technique.

Significance levels (P values) were not reported.

C. METHODS and RESULTS

1. Observations:

Weekly records were kept of the individual body weights and food consumption, together with observations of the physical appearance and behavior of the animals.

a. General Appearance and Behavior: The physical appearance of the test animals fed borax at the 0.0463%, 0.154% and 0.463% levels were generally comparable to that noted for the controls.

From the first week of the study, male and female animals at the 1.54% level appeared in poor physical condition.

The rats at the 4.63% level were in very poor condition from the first week of the study. The following symptomatology was seen (Table 3).

Table 3

Observations throughout the 13 Week Study[a]

Observations	Concentration(%) 1.54	4.63
rapid respiration	x	x
swollen paws	x[1]	x
blue paws	-	x
coarse fur	x	x
desquamation of skin of paws and tail	x[2]	x
hunched position	x	x
bloody nasal discharge	x	x
sensitivity to touch	-	x
protruding penis	x[3]	x[a]
shrunken scrotum	x[4]	-
unsure gait	x	x[b]
inflamed eyes	x	-
emaciation	x	x

[a] adapted from p. 41 thru 44 of the original report
[1] several males, majority of females during second and third weeks
[2] paws and tail only
[3] sixth thru 13th week
[4] last week of study
[a] third week to time of death
[b] one male, several females

2. Mortality

No mortality was noted at the 0.0463%, 0.154%, and 0.463% level. At the 1.54% level, 1/10 males died during week 5 while at the 4.63% level 10/10 males died within 5 weeks and 10/10 females died within 4 weeks (Table 4).

Table 4

Number of Animals Dying During the 13 Week Study

Conc. Level (%)	weeks 1	2	3	4	5	6-12
0.0	-	-	-	-	-	-
0.0463	-	-	-	-	-	-
0.154	-	-	-	-	-	-
0.463	-	-	-	-	-	-
1.54	-	-	-	-	1M	-
4.63	1M	2M, 3F	6M 4F	- 3F	1M *	* *

from p. 41 thru 44 of the original report
M = males
F = females
* = all animals died

3. Body Weight

At the 13 week sacrifice, the terminal body weights for the males and females at the 1.54% level showed significant decreases in body weight gains compared to those of the controls. At week 13, the lower concentration levels were considered to be unremarkable. Animals of both sexes at the 4.63% concentration showed severe body weight losses starting at week 1. Male animals at the 0.0463% showed significantly decreased body weight gains during the first 4 weeks of the study when compared to the controls. Body weight gains were similar to the control values by week 8. (Table 5).

Table 5

Mean Body Weight Gains^

	Concentration(%)					
Weeks	0.00	0.0463	0.154	0.463	1.54	4.63
			Male			
1	57	50(-12%)[a]	55(-4%)	38(-33%)	6(-89%)	-25 L
2	107	69(-36%)	107(0%)	83(-22%)	16(-85%)	-30 L
4	191	152(-20%)	197(3%)	169(-12%)	25(-87%)	-21 L
8	306	284(-7%)	322(5%)	297(-4%)	69(-78%)	*
13	378	365(-3%)	397(5%)	385(2%)	125(-67%)	-
			Female			
1	38	35(8%)	36(5%)	28(-26%)	8(-79%)	-22 L
2	58	66(14%)	62(7%)	58(0%)	23(- 6%)	-29 L
4	92	99(8%)	93(1%)	96(4%)	40(-56%)	*
8	129	142(10%)	133(3%)	131(4%)	72(-44%)	*
13	153	165(8%)	154(1%)	152(0%)	104(-32%)	*

adopted from p. 21 thru 24 of the original report
^ = gms of weight gained from week 0
[a] = compared to control
* = all animals died
L = body weight loss

4. Food Consumption:

Food consumption in both the male and female animals at the 0.0463%, 0.154% and 0.463% were comparable to the respective control group. Male and female animals at the 1.54% and 4.63% concentration showed a severe decrease in food consumption when compared to the corresponding control animals (Table 6).

Table 6

Mean Food Consumption Throughout 13 Week Study^

Sex	Concentration in feed (%) 0.00	0.0463	0.154	0.463	1.54	4.63
M	141	141	162	146	77	57
F	103	111	109	107	76	80

from p. 25 thru 30 of the original report
^ gm/rat/week

5. Food Efficiency

The mean weekly food efficiency was calculated by dividing the grams of weight gained/rat/week by the grams of food consumed/rat/week as noted in Table 7.

The mean food efficiency averaged over the 13 week study in both male and female animals was comparable to the respective control animals at the 0.046%, 0.154%, and 0.463% levels (Table 7).

Over the 13 week study, a severe decrease (37%) in food efficiency was noted at the 1.54% level in male animals but only a slight decrease (10%) was seen in females when compared to their respective controls (Table 7).

Males and females at the 4.63% level, showed a negative food efficiency over the 13 week study (Tables 7).

Table 7

Mean Weekly Food Efficiency Throughout The 13 Week Study^

Sex	Concentration in feed (%) 0.00	0.0463	0.154	0.463	1.54	4.63
M	0.197	0.209	0.190	0.200	0.124‡	-0.246
F	0.117	0.114	0.111	0.109	0.105&	-0.300

^ food efficiency = weight gain (gm)/food consumption (gm) (adopted from p. 21 thru 31 of the original report).
‡ 37% decrease from the control value
& 10% decrease from the control value

6. Clinical Studies:

No hematology or clinical chemistry parameters were measured.

7. Sacrifice and Pathology:

All animals that died or were scheduled for sacrifice were subject to gross and pathological examination. The checked (X) tissue were collected for histological examination.

Organs and tissues marked with a (+) were also weighed while those organs and tissues marked with a (*) are required by the guidelines (Table 8).

Table 8

Tissues and Organs Subjected for Histological Examination and Organ and Tissue Weights.

Digestive	Cardiovas./ Hematol.	Neurologic
- Tongue	- Aorta*	X+Brain*
- Esophagus*	X Heart*	- Perip. Nerve*
- Stomach*	- Bone Marrow*	- Spinal Cord* (3 levels)
- Duodenum*	- Lymph Nodes*	- Sciatic nerve
- Jejunum*	cervical/	- Pituitary*
- Ileum*	mesenteric	- Eyes* & optic nerve*
- Cecum*	X+Spleen*	
- Colon*	- Thymus*	Glandular
- Rectum*	Urogenital	X+Adrenals
X+Liver*	X+Kidney*	- Lacrimal gland
X Pancreas*	- Urinary Blad*	- Mammary gland*
Respiratory	X+Testes(b)*	- Parathyroids*
Trachea*		X+Thyroids*
X Lung*	- Prostate*	Other
	- Seminal Ves.	- Bone (femur)*
	X+Ovaries(a)*	- Skeletal muscle*
	X Uterus*	- Skin*
		- gross lesions*
		- Harderian Gland
		- Salivary gland*
		- exorbital lachrymal glands*
		- target organs*

(a) with fallopian tubes
(b) with epididymis

a. Gross Pathology

Gross autopsies were performed on all animals that died during the study or at terminal sacrifice at week 13. Autolysis made it difficult to evaluate gross changes in animals that died during the study.

Gross autopsies performed on the animals sacrificed after 13 weeks revealed small, soft testes in 3 controls males, 4 males at the 0.0463%, 1 male at the 0.463% level and 10 males at the 1.54% level (Table 13).

No consistent changes were observed in the other test groups except for pale livers noted in 15 of 20 male and female animals at the 1.54% level and pale thyroids noted in 10 of 20 rats of both sexes.

Gross necrospy findings of all animals that died (10/10 at

the 4.63% level and 1/10 at the 1.54% level) were noted to have congestion of the liver and kidneys and bright red lungs in the majority of the rats; in several animals, a swollen appearance of the brain, small gonads and a thickened pancreas were noted.

b. Organ Tissue Weights and Ratios

The organs and tissues scheduled for weighing at the 13 week sacrifice were noted in Table 8. The actual weights and ratios were noted in Tables 9, 10, 11 and 12.

At the 0.0463% level, the organ weight and organ weight ratios of the brain, spleen, kidneys of the male and the organ weight of the ovaries for the females and the kidneys and kidney organ weight ratio for the males at the 0.154% level were significantly higher than the controls. The organ weights of the liver, spleen and gonads at the 1.54% level for both sexes, the organ weights of the brain and kidney for the males and the adrenals for the females at this level were significantly lower when compared to the controls (Table 9, 10).

The weights of the testes from 4/10 low level males (0.0463%) were lower than those from the remaining 6 animals. Statistical analysis revealed the average testes weight for the 4 animals to be significantly lower than for the controls. When analyzed for the whole group, there was no significant difference in the lower level testes weight as compared with the control group.

An analysis of the organ/brain weight ratio was performed. The liver/brain weight and spleen/brain weight ratios were significantly lower for both males and females at the 1.54% level. The males at this level showed significantly lower testes/brain weight and kidney/brain weight ratios. The females showed lower ovary/brain weight and adrenal/brain weight ratios (Table 11, 12).

The testes/brain weight ratio was significantly lower for 4 male rats in this group when compared to the control weights. No significant difference in testes/brain weight ratio was found when the whole group was compared to the values for the control rats (Table 11).

The significant alterations in organ/body weight ratios observed at the 1.54% dose levels in both males and females were of limited biological significance due to the marked weight loss in these animals.

Table 9

Body Weights, Organ Weights, Organ/Body Weight Ratios for Male Animals at the 13 Week Terminal Sacrifice

Conc. Level %	Body Wgt gm	Brain Wgt gm	Brain ratio %	Thyroid Wgt gm	Thyroid ratio %	Liver Wgt gm	Liver ratio %
Control	477	2.13	0.447	0.019	0.0039	16.85	3.54
0.0463	478	2.14	0.448	0.022	0.0046	16.63	3.48
0.154	508	2.21	0.435	0.023	0.0044	18.46	3.62
0.463	498	2.10	0.424	0.021	0.0042	15.86	3.15^{L}
1.54	215^{L}	1.86^{L}	0.814^{H}	0.015	0.0067^{H}	7.04^{L}	2.96^{L}
4.63	A	A	A	A	A	A	A

Table 9 (cont.)

Conc. Level %	Body wgt gm	Spleen wgt gm	Kidneys wgt. gm	Kidneys ratio %	Adrenals wgt gm	Adrenals ratio %	Testes wgt gm	Testes ratio %
Control	477	0.78	3.08	0.648	0.046	0.0095	3.50	0.734
0.0463	478	0.84	3.34	0.697	0.048	0.0098	2.79*	0.592*
0.154	508	0.90	3.73^{H}	0.734^{H}	0.050	0.0099	3.95	0.741
0.463	498	0.80	3.36	0.676	0.054	0.011	3.64	0.724
1.54	215^{L}	0.34^{L}	1.92^{L}	0.813^{L}	0.039	0.015^{H}	0.79^{L}	0.335^{L}
4.63	A	A	A	A	A	A	A	A

---

from p. 33 thru 36 of the original report

^{L} significantly lower than controls

^{H} significantly higher than controls

* The weights of the testes from 4/10 0.0463% level male animals were lower than those from the remaining 6 animals. Statistical analyses revealed the average testes weight for the 4 animals to be significantly lower than for the controls. [The reviewer notes that this data was not included in the report]. When analyzed for the whole group there was no significant difference as compared with the control group.

A autolysis

Table 10

Body Weights, Organ Weights, and Organ/Body Weight Ratios for Female Animals at the 13 Week Terminal Sacrifice

Conc. Level %	Body wgt gm	Brain wgt gm	Brain ratio %	Liver wgt gm	Liver ratio %	Spleen wgt gm	Spleen ratio %
Control	247	1.91	0.778	7.92	1.12	0.51	0.20
0.0463	265	2.15^{H}	0.814^{H}	8.56	0.57	0.69^{H}	0.26^{H}
0.154	256	1.92	0.769	8.14	1.01	0.52	0.20
0.463	256	2.02	0.800	8.04	0.70	0.53	0.21
1.54	222^{L}	1.91	0.888^{H}	6.40^{L}	1.46	0.39$_{L}$	0.18
4.63	A	A	A	A		A	A

from p. 33 thru 36 of the original report
L significantly lower than controls
H significantly higher than controls
A autolysis

Table 10 (cont.)

Conc. Level %	Body wgt gm	Kidneys wgt gm	Kidneys ratio %	Adrenals wgt gm	Adrenals ratio %	Ovaries wgt gm	Ovaries ratio %
Control	247	1.88	0.746	0.054	0.022	0.124	0.0503
0.0463	265	2.20^{H}	0.832^{H}	0.059	0.022	0.145^{H}	0.0552
0.154	256	1.99	0.783	0.058	0.023	0.127	0.498
0.463	256	1.96	0.770	0.058	0.023	0.123	0.474
1.54	222^{L}	1.95	0.791	0.040^{L}	0.019	0.071^{L}	0.034^{L}
4.63	A	A	A	A	A	A	A

from p. 33 thru 36 of the original report
L significantly lower than controls
H significantly higher than controls
A autolysis

Table 11

Mean Organ/Brain Weight Ratios For Male Animals at the 13 Week Terminal Sacrifice

Conc. Level %	Thyroid ratio %	Liver ratio %	Spleen ratio %	Kidneys ratio %	Testes ratio %
Control	0.88	795	37	146	165
0.0463	1.0	778	39	156	129*
0.154	1.0	835	41	169	170
0.463	0.99	755	38	160	172
1.54	0.81	378[L]	18[L]	103[L]	42[L]
4.63	A	A	A	A	A

from p. 37 of the original report

L significantly lower than controls

* the testes/brain weight ratio was significantly lower for 4 male rats in this group when compared to the control weights. [The reviewer notes that data was not in the original report]. No significant differences in the testes/brain weight ratio was found when the whole group was compared to the values for the control rats.

A autolysis

Table 12

Mean Organ/Brain Weight Ratios For Female Animals at the 13 Week Terminal Sacrifice

Conc. Level %	Liver ratio %	Spleen ratio %	Adrenals ratio %	Ovaries ratio %
Control	414	27	2.8	6.49
0.0463	400	32	2.7	6.72
0.154	425	27	2.9	6.57
0.463	398	26	2.9	6.07
1.54	334[L]	20[L]	2.1[L]	3.7[L]
4.63	A	A	A	A

from p. 37 of the original report

L significantly lower than controls

A autolysis

c. Microscopic Pathology:

The 0% and 1.54% animals were examined microscopically at 13 weeks. The majority of the animals at the 4.63% level evidenced advanced autolysis and were not microscopically examined.

Microscopic examination revealed significant histologic changes in the testes. Atrophy of the spermatogenic

epithelium was complete in the 10 males at the 1.54% level and in 4 males at the 0.0463% level. The one male at the 0.463% level presented spermatogenic arrest involving practically all of the tubules of the section. The control and 0.154% levels were unremarkable (Table 13).

The testicular changes at the above levels were characterized by the complete atrophy of the spermatogenic epithelium, decrease in the size of the tubules without thickening of the basement membrane and a parallel increase in the amount interstitial tissue. The tunica albuginea was not altered.

With the exception of two animals, the uterus and ovaries of the females at the 1.54% level presented no suggestions of a compound related effect. These two animals presented unusual alterations. One animal was noted to have severe focal vacuolation of the interstitial cells. The other animal presented no corpora lutea and no Graafian follicles larger than those with small antra. None of the primary follicles present contained viable or even identified ova.

The adrenals of the males at 1.54% level revealed a consistent, slight to moderate increase in the lipid and in the size of the cells in the zona reticularis, that may have been related to the testicular atrophy found in these animals. In some instances this zone retained its differentiation from the middle zone only because of the trabecular arrangement of the cells. The adrenal glands of the male and females at the 0.0463, 0.154 and 0.463% levels revealed no consistent changes indicative of compound effect.

8. Discussion:

No effects on physical appearance or behavior were noted at 0.0463%, 0.154% and 0.463% level when compared to the control animals.

The test animals at the 1.54% and 4.63% level were observed to have a hunched appearance, rapid or labored respiration, inflamed eyes, desquamation of the skin and emaciation (Table 2). The males showed a protruding penis and a shrunken scrotum. At these dose levels a reduced body weight gain and reduced food consumption were noted in both sexes (Table 5, 6). Food efficiency was decreased more in the males than the females (Table 7).

In the females at the 0.0463% level, organ weights and ratios were increased for the brain, spleen, and kidneys while only the organ/body weight ratio was increased for the ovaries (Table 9, 10).

The organ weights of the liver, spleen and gonads at the 1.54% level for both sexes, the brain and kidneys for the male and the adrenals for the females at this level were significantly lower as compared to the controls. These effects were due to the rapid decrease in body weight (Table 9, 10).

Because of the marked reduction in growth, the organ/brain weight ratios were calculated. The brain weights were not as affected by a decrease in the body weight. It was noted that the liver and spleen/brain weight ratios were decreased for both male and females at the 1.54% level. The males at this level showed significantly lower gonad and kidney/brain weight ratios and the female showed lower adrenal/brain weight ratios (Table 11, 12).

At the 0.0463 level, weights of the testes from 4/10 males were lower than those from the remaining 6 animals. Statistical analysis revealed the average testes weight for the 4 animals to be significantly lower than for the controls. When analyzed for the whole group, however, there was no significant difference in the lower level testes weight as compared with the control group (Table 9). The testes/brain weight ratio was also significantly lower for these animals when compared to the control weights. No significant difference in testes/brain weight ratio was found when the whole group was compared to the values for the control rats (Table 11).

The decrease in the weight of the testes and the testes/brain weight ratio are correlated with the gross and microscopic pathology. Gross autopsies performed on the animals sacrificed at 13 weeks revealed small, soft testes in 3 control males (report p. 16), 4 males at the 0.0463% level, 1 male at the 0.463% level and all 10 males at the 1.54% level (Table 13).

Severe autolysis prevented the collection of organ weight data at the 4.63% level.

Microscopic examination revealed complete testicular atrophy at the 1.54% and 0.0463% levels. Spermatogenic arrest in the tubules of the testis were seen in 1 male at the 0.0463% level. The control animals proved microscopically unremarkable (Table 13).

Table 13

Comparison of Incidence of Testicular Gross and Microscopic Pathology Findings

Necropsy

Conc. %	Gross Obs./Total	Microscopic Obs./Total
0.0	3/10	0/10
0.0463	4/10	4/10
0.154	0/10	0/10
0.463	1/10	1/10
1.54	10/10^	10/10
4.63	A	A

adapted from p. 45 thru 47 of the original report
^ decreased testicular weight when compared to the controls.
A autolysis

9. CONCLUSIONS:

Sprague-Dawley rats were administered sodium tetraborate decahydrate at concentrations of 0.0 (Control), 0.0463%, 0.154%, 0.463%, 1.54% and 4.63% in the feed for 13 weeks.

All animals died in both sexes at the 4.63% level. In both sexes at 1.54%, decreased weight gain and food consumption occurred. The testes weight and ratio, the ovary weight and ratio and the corresponding organ/brain weight ratios were markedly decreased. At 0.0463%, 4/10 male animals were similarly affected.

The NOEL in females rats was 0.463%. The LOEL was 1.54% based on reduced body weight gain. A NOEL in males was not obtained. The LOEL was 0.0463% based on testicular atrophy.

Reviewed by: Steven L. Malish, Ph.D. Steven L. Malish 10/26/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 10/25/91
Tox. Branch II, Section IV (H7509C)

009301

Data Evaluation Report

STUDY TYPE: Subchronic Oral Toxicity (Rodent): 90-day study (82-1)

MRID NO: 406923-06

TEST MATERIALS: Borax

SYNONYMS: Sodium tetraborate decahydrate

SPONSOR: U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA

TESTING FACILITY: Hazleton Laboratories America, Inc., Vienna, VA

TITLE OF REPORT: 90-Day Dietary Administration - Rats with 20 MULE TEAM^{R} Borax (Sodium tetraborate decahydrate)

STUDY NUMBER: Laboratory study number was not reported

AUTHORS: Robert J. Weir, Ph.D.

REPORT ISSUED: February 15, 1963

CONCLUSIONS:

Sprague-Dawley male albino rats were administered sodium tetraborate decahydrate in the feed at levels of 0.0% (Control), 0.0154%, 0.0463%, 0.154%, and 0.463% for 13 weeks.

No compound related effects were noted. Based on the above findings, the systemic toxicity NOEL was 0.463%.

CLASSIFICATION:

Core Supplementary

The study does not satisfy the guidelines requirements (82-1) for a subchronic oral toxicity (rodent): 90 day study.

The 82-1 guidelines were not followed with regard to the following: only male animals were use; clinical pathology studies - hematology and clinical chemistry were not done. Ophtalmological examinations and complete necropsies were not done.

A. MATERIALS:

1. Test Compound:

Chemical: Sodium Tetraborate Decahydrate
Label: not available
Description: fine white powder without noticeable odor
Storage: not available

a. Purity:

Two samples of the test material were submitted for analysis during the study, sample 1 had a boron content of 105% while sample 2 had a boron content of 105.4% of the theoretical value.

2. Test Animals:

Species: Rat
Strain: Sprague-Dawley, pathogen free
Age: not available
Weight: Males 85 to 119 gm
Source: Charles River Breeding Laboratories, Wilmington MA.

B. STUDY DESIGN:

1. Quality Assurance:

No quality assurance statement was issued.

2. Animals

The animals were selected by stratified randomization and housed individually in wire mesh cages.

3. Animal Assignments:

Fifty male animals were assigned to 5 test groups consisting of 10 male animals per dose level and administered either 0%, 0.0154%, 0.0463%, 0.154% or 0.463% of the test compound in the diet.

Table 1

Animals Assignments and Dose Levels

Group No.	No. of Rats Male	Dietary Level (%)*
1 (Control)	10	0.0
2	10	0.0154
3	10	0.0463
4	10	0.154
5	10	0.463

* assuming 100% sodium tetraborate decahydrate.

4. Diet

Animals received Purina Rodent Laboratory Chow and water ad libitum.

5. Diet Preparation:

Test diets were prepared weekly. The test material, as received, was incorporated into the basal diet on a weight/weight basis to provide the desired dietary levels. The feed was thoroughly mixed in a twin-shell blender. The dietary level of the test material in the study was adjusted to 100% of theoretical.

a. Stability, Dietary Concentration and Homogeniety in Feed:

No studies were done to assess the stability of the test substance in the feed, the actual dietary concentration or the homogeniety during the 13 week study.

6. Statistics:

The criteria chosen for statistical evaluation were growth, total food consumption, food efficiency, organ weight and organ/body weight ratios. The degree of statistical significance (P value) was not stated.

C. METHODS AND RESULTS:

1. Observations:

Observations of the physical appearance and behavior of the animals was conducted daily.

The physical appearance of the test animals fed sodium tetraborate decahydrate at all dietary levels were generally comparable with those of the controls.

2. Mortality

One (1) animal at the 0.0463% level died during week 5 of the study. Death was not related to the administration of the test compound.

3. Body Weights:

Individual body weights were measured weekly.

No change was noted in the absolute weight or the change in body weight gain at any dose level over the 13 week study (Table 2).

Table 2

Mean Body Weight Change Over The 13 Week Study^

	Concentration (%)				
Week	0.0	0.0154	0.0463	0.154	0.463
0-13	419	422	414	418	433

^ gain in gms from week 0.
adopted from p. 13-14 of original report.

4. Food Consumption and Food Efficiency

Individual food consumption was measured weekly; food efficiency and compound consumption were calculated weekly.

Statistical analyses of food consumption for the test groups did not reveal any significant differences as compared with the control group. Food efficiency at all concentration levels was unremarkable.

5. Compound Consumption:

Compound consumption and the equivalent boron concentration were noted below.

Table 3

Mean Food and Compound Consumption Throughout the 13 Week Study*

Dietary Level (%)	Compound mg/kg/day	Boron Equiv.^ mg/kg/day
0.0	0.0	0.0
0.0154	11.7	1.32
0.0463	35.5	1.30
0.154	115	13.1
0.463	362	41.0

* adapted from p 15-16 original report
^ based on boron assay value adjusted to 100%.

6. Ophthalmological Examinations:

Examinations were not performed nor were the eyes examined microscopically at the terminal necropsy.

7. Clinical Studies:

Hematology or clinical chemistry parameters were not measured.

8. Sacrifice and Pathology:

After 13 weeks on test, all surviving animals were sacrificed by exsanguination, gross autopsies were performed, organs weighed and ratios calculated. Organ/brain weight ratios were not calculated. Microscopic examinations were performed only on the testes.

The one animals that died at the 0.0463% concentration during week 5 was evaluated only for gross pathology.

Organ Weights:

The following tissues were weighed and the organ/body weight ratios calculated:

brain, pituitary, thyroid, lung, heart, spleen, kidney, adrenal, stomach, pancreas, small and large intestines, urinary bladder, testis, sternum and femur.

Statistical analysis of the data revealed no significant differences between the control and test groups.

Gross Pathology:

Autopsies performed on the control and test rats sacrificed after 13 weeks revealed no consistent gross changes in the viscera or body cavities that could be attributed to the ingestion of the test compound.

The gross pathology examination of the animal that died during week 5 at the 0.0463% concentration revealed lesions unrelated to the administration of the test material.

c. Microscopic Pathology:

Microscopic examinations were performed only on the testes at the 13 week terminal sacrifice.

Sections of the testes from each animal in all groups were examined microscopically. No other tissue or organ was microscopically examined. No impairment of spermatogensis or other pathological alteration was found in the testes from the control or test rats at any of the dietary levels.

D. CONCLUSION:

Sprague-Dawley male albino rats were administered sodium tetraborate decahydrate in the feed at levels of 0.0% (Control), 0.0154%, 0.0463%, 0.154%, and 0.463% for 13 weeks.

No compound related effects were noted. Based on the above findings, the systemic toxicity NOEL was 0.463%.

Reviewed by: Steven L. Malish, Ph.D. S.L. Malish 10/26/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 10/25/91
Tox. Branch II, Section IV (H7509C)

009301

Data Evaluation Report

STUDY TYPE: Subchronic Oral Toxicity (non-rodent): 90-day study (82-1)

MRID NO: 406923-07

TEST MATERIALS: Sodium tetraborate decahydrate

SYNONYMS: Borax

SPONSOR: U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA

TESTING FACILITY: Hazleton Laboratories America, Inc., Vienna, VA

TITLE OF REPORT: 90-Day Dietary Feeding - Dogs with 20 MULE TEAM^{R} Borax (Sodium tetraborate decahydrate)

STUDY NUMBER: Laboratory study number was not reported

AUTHORS: Orville E. Paynter, Ph.D.

REPORT ISSUED: January 17, 1963

CONCLUSIONS:

Sodium tetraborate decahydrate was administered to 20 beagles per sex at dietary levels of 0 (Control), 0.0154%, 0.154% and 1.54% ad-mixed in feed.

Atrophy of the testes occurred at 1.54% together with decreased testes weight, testes/body weight and testes/brain weight ratios.

In both sexes, a decreased hematocrit and hemoglobin seen at 1.54% correlated with increase hemosiderin pigment in the spleen, liver and kidneys.

Thyroid glands of male animals had more epithelial nests than females at the 0.154% and 1.54% levels. Decreased thyroid/body weight and the thyroid/brain weight ratios occurred at 1.54%. A widened adrenal cortex at 0.154% occurred with widening of the zona reticularis and lipid accumulation at 1.54%. Brain weights of 1.54% females were increased.

The systemic toxicity NOEL was 0.0154%, the LOEL was 0.154% based on pathology findings.

CLASSIFICATION:

Core: Supplementary - upgradeable

This study does not satisfies guidelines 82-1 for a Subchronic Oral Toxicity (non-rodent): 90-day study.

The study may be upgraded by submitting pathology evaluation of the ovaries. Since the target organ in this study is the testes, a comparison with the female reproductive organs i.e. ovaries are essential.

Although the study was also deficient in the required hematology and clinical chemistry parameters and no ophtalmological examinations were performed, the reviewer is of the opinion that the evaluation of these parameters would not add to the validity of the study.

A. MATERIALS:

1. Test Compound:

Chemical:	sodium tetraborate decahydrate
Label:	not available
Description:	fine white powder without noticeable odor
Storage:	not available
Purity:	boron content 104% of theoretical value

2. Test Animals:

Species:	dog
Strain:	purebred beagles
Age:	not avalable
Weight:	males: 7.2 kg - 10.6 kg
	females 4.2 kg - 9.7 kg
Source:	not available

B. STUDY DESIGN:

1. Quality Assurance:

No quality assurance statement was issued.

2. Animals:

Twenty (20) male and 20 female purebred beagle dogs were selected at random and housed individually in metal cages. The dogs were maintained in the laboratory for at least 6 weeks prior to the initiation of the study.

a. Animal Assignments:

Five (5) animals per sex were administered either 0% (Control), 0.0154%, 0.154% or 1.54% of the test compound in the diet Table 1.

Table 1

Animals Assignments and Dose Levels[a]

Group No.	No. of Dogs Male	Female	Dietary Level (%)
1 (Control)	5	5	0
2	5	5	0.0154
3	5	5	0.154
4	5	5	1.54

[a] p. 6 of original report

3. Diet:

Animals received ad libitum water and either the basal laboratory diet (ground Wayne Dog Meal) or the test diet. The dry diet was supplemented with a 100 gm ration of canned meat (p/d ration, Hill Packing Company), 5 days per week.

4. Diet Preparation:

Test material was added to the basal diet of ground Wayne Dog Meal on a weight/weight basis to provide the desired dietary levels. The feed was thoroughly mixed in a large volume blender.

a. Stability, Dietary Concentration and Homogeniety in Feed:

No studies was done to assess the stability in feed, the analysis of the feed for the actual dietary concentration or the homogeniety during the 13 week study.

5. Statistics:

Statistical analyses was used to compare the organ weights, the organ/body weight ratios and the organ/brain ratios with the corresponding controls. Other parameters were not subjected to statistical evaluation. The degree of statistical significance (P values) were not indicated.

C. METHODS and RESULTS:

1. Observations:

Animals were observed daily for appearance, behavior, elimina-

tion and signs of systemic toxicity or pharmacologic effects.

The control and test animals were unremarkable with respect to appearance and behavior and elimination throughout the study.

Mortality - One animal died on day 68. Death was not considered to be related to the administration of the test compound.

2. Body Weights:

Once each week, individual body weights were recorded.

Individual body weights gains showed some fluctuations during the study but were within -0.5 to 1.6 kg of the starting weight except for one 1.54% male animal that died on day 68 of the study. Mean weight gains of the control and treated animals were unremarkable as noted in Table 2.

Table 2

Mean Body Weights (kg) Over the 13 Week Study[a]

Week	Control		0.0154%		0.154%		1.54%	
	M	F	M	F	M	F	M	F
1	8.2	6.7	8.9	7.9	8.8	7.8	8.8	7.5
13	9.0	7.0	9.4	8.3	9.4	8.1	9.1	7.6
Wgt. Gain[b]	+0.8	+0.3	+0.5	+0.4	+0.6	+0.3	+0.3	+0.1

[a] adopted from p. 16 thru 29 of the original report
M = males
F = females
[b] = increase in body weight from week 1 thru 13

3. Food and Compound Consumption:

Food and compound consumption were determined weekly.

Food consumption between controls and treated animals were comparable over the 13 week period.

4. Compound Consumption:

Mean compound consumption is presented below and was calculated from the mean food consumption and mean body weight.

Table 3

Mean Compound Consumption Throughout The 13 Week Study[a]

Conc. Level	Compound (mg/kg/day) males	Compound (mg/kg/day) females
0.0	0.0 (0.0)^	0.0 (0.0)^
0.0154	2.9 (0.34)	2.1 (0.25)
0.154	34.6 (4.1)	22.0 (2.6)
1.54	268.0 (32.0)	192.0 (22.6)

[a] adapted from p. 18 thru 29 original report
^ boron equivalent in mg/kg/day

6. Clinical Studies:

Hematological, biochemical studies and urine analyses were performed on all dogs initially (0 week) and at weeks 2, 4 and 13. (termination).

a. Hematology:

The following parameters (X) were determined. Parameters marked with an (*) are designated in the latest guidelines.

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
- Erythrocyte count (RBC)*
X Leukocyte count (WBC)*
X Leukocyte differential count*
X Sedimentation Rate (Sed. rate)
- Platelet count* or
- Prothrombin time*

The 0.0154% and 0.154% hematology values were unremarkable throughout the study.

Two (2) males and 3 females in the 1.54% group exhibited a moderate decrease as the study progressed in the individual hematocrit and hemoglobin values when compared to the respective initial values. When mean values were evaluated, both sexes showed a decrease over time in the hematocrit values when compared to the respective initial test group values. Hemoglobin values were decreased in both sexes only at 13 weeks (Table 4).

The respective hematocrit and hemoglobin parameters of both sexes also revealed a percentage decrease from 0 to 13 weeks in the test group when compared to the mean control values (Table 4).

Table 4

Mean Hematocrit and Hemoglobin Values in Rats at Selected Time Intervals[a]

Males

Time (weeks)	Hematocrit (%) Control	1.54%	Hemoglobin (gm/dl) Control	1.54%
0 (Initial)	47.4	46.5	17.5	17.0
2	47.0	44.5	17.4	16.7
4	45.7	42.1	17.7	17.9
13	49.9 (5%)^	39.5 (-15%)^	17.7 (1%)^	15.2 (-11%)^

Females

Time (weeks)	Hematocrit (%) Control	1.54%	Hemoglobin (gm/dl) Control	1.54%
0 (Initial)	47.3	44.5	17.7	16.8
2	47.4	45.0	17.5	16.0
4	49.4	42.8	18.9	18.0
13	51.3 (8%)^	41.8 (-6%)^	18.8 (6%)^	14.9 (-11%)^

[a] adapted from p. 30 thru 40 of the original report
^ percent difference between 0 (initial) week values

b. Clinical Chemistry:

Clinical Chemistry parameters determined in the study are designated by an (X) while those marked with a (-) were not evaluated. The parameters marked with an (*) are designated in the latest guidelines.

Blood glucose (non-fasting) and blood urea nitrogen were the only clinical chemistry parameters measured.

X Blood urea nitrogen*
- Cholesterol*
- Glucose (fasting)*
- Total bilirubin*
- Total serum protein*
- Triglycerides*
- Serum alanine aminotransferase (SGPT)*
- Serum aspartate aminotransferase (SGOT)*
- Albumin*
- Sodium*
- Chloride*
- Potassium*
- Calcium*

Clinical chemistry parameters at all levels were unremarkable throughout the study.

c. Urinalysis:

The following marked with an (X) were examined while those marked with a (-) were not. Parameters marked with an (*) are required by the guidelines.

X Appearance*	X Glucose*
- Volume*	X Ketones*
X Specific Gravity	X Bilirubin
X pH	X Blood (occult)*
X Sediment (microscopic)*	X Microscopic findings

Urine parameters at all levels were unremarkable throughout the study.

7. Sacrifice and Pathology:

Following completion of the 13th week of the study, the animals were sacrificed by exsanguination under thiamylal (Surital sodium) anesthesia and gross and microscopic pathological examinations performed.

The checked (X) tissue were collected for histological examination. Organs and tissues marked with a (+) were also weighed while those organs and tissues marked with a (*) are required by the 82-1 guidelines (Table 5).

Special Studies

Sections of the liver and spleen of 2 control and 3 animals at the 1.54% level that received sodium tetraborate decahydrate were stained for ferric iron by Perl's method. The specific animal and sex that these tissues were obtained from were not fully defined in the report.

Table 5

Tissues and Organs Subjected for Histological Examination and Organ and Tissue Weights.

Digestive	Cardiovas./ Hematol.	Neurologic
- Tongue +	- Aorta*	X+Brain*
- Esophagus*	X Heart*	- Perip. Nerve*
X Stomach*	X Bone Marrow*	- Spinal Cord* (3 levels)
X Duodenum*	- Lymph Nodes*	- Sciatic nerve
X Jejunum*	cervical/	- Pituitary*
X Ileum*	mesenteric	- Eyes* & optic nerve*
X Cecum*	X+Spleen*	
X Colon*	- Thymus*	Glandular
X Rectum*	Urogenital	X+Adrenals
X+Liver*	X+Kidney*	- Lacrimal gland
X Pancreas*	X Urinary Blad*	- Mammary gland*
Respiratory	X+Testes*	- Parathyroids*
- Trachea*		X+Thyroids*
X Lung*	- Prostate*	Other
	- Seminal Ves.	X Bone (femur)*
	- Ovaries*	- Skeletal muscle*
	- Uterus*	- Skin*
		- gross lesions*
		- Salivary gland*
		- exorbital lachrymal glands*
		X target organs*

a. Gross Pathology

Gross autopsies were performed on the 1 animal that died on day 68 and on the remainder of the animals at the week 13 terminal sacrifice.

At the terminal sacrifice, all organs of the surviving animals appeared within normal limits.

b. Organ Weights and Ratios

Organ weights, organ/body weight and organ/brain weight ratios were determined at week 13 of the study (Table 6, 7).

At the 1.54% level, male animals showed a non-statistically significant decrease of 44% in the mean testes weight (9.6 gm) when compared to the weight (17.2 gm) of the corresponding control. Significantly lower mean testes/body weight and mean testes/brain weight ratios were also observed (Table 6, 7).

In the 1.54% and 0.0154% males, the thyroid/body weight and spleen/body weight ratios, respectively, were decreased when compared to the corresponding controls. Variable spleen weights without an apparent dose response were observed (Table 6).

Table 6

Body Weights, Organ Weights, Organ/Body Weight Ratios for Male Animals at the 13 Week Terminal Sacrifice[a]

Conc. Level %	Body Wgt kg	Testes Wgt gm	Testes Ratio %	Thyroid Wgt gm	Thyroid Ratio %	Spleen Wgt gm	Spleen Ratio %
Control	8.5	17.2	0.20	0.77	0.0091	24.2	0.28
0.0154	9.1	14.4	0.16	--	--	17.1	0.19[L]
0.154	9.2	15.8	0.17	--	--	19.2	0.23
1.54	9.1	9.6	0.10[L]	0.59	0.0064[L]	28.9	0.32

[a] from p. 87 thru 91 of the original report
[L] Significantly lower than controls. Significance levels (P values) were not included in the original report.

Table 7

Mean Organ/Brain Weight Ratios for Male Animals at the 13 Week Terminal Sacrifice[a]

Conc. Level %	Brain wgt gm	Testes wgt gm	Testes ratio %	Thyroid wgt gm	Thyroid ratio %
Control	79.3	17.2	22	0.77	0.95
0.0154	77.8	14.4	18	--	--
0.154	80.0	15.8	20	--	--
1.54	80.3	9.6	12[L]	0.59	0.73[L]

[a] from p. 88 thru 91 of the original report
[L] significantly lower than controls. Significance levels (P values) were not included in the original report.

c. Microscopic Pathology:

Data at the 0.0154% level were unremarkable between the control and treated groups.

Listed below are the summary of the important pathological findings at the 0.154% and 1.54% levels.

Table 8

Microscopic Pathology at the Various Concentration Levels at the 13 Week Necropsy

Conc.	Sex	Organ	Comparison to Controls
0.0154%	M	---	unremarkable
	F	---	"
0.154%	F	adrenal	Cortex distinctly widened;
	M	thyroid	greater proportion of small and solid epithelial nests compared to females but did not exceed that occasionally found in the controls.
	M	testes	No significant alterations·*
1.54%	M	testes	Complete atrophy in 4 males partial atrophy in the 5th male. The interstitial cells were increased in amount and the Leydig-like cells in the capsule were greatly increased in some cases.
	M/F	liver } spleen} kidney}	Hemosiderin deposition, indicative of red cell destruction occurred in reticular cells of liver and spleen and proximal tubule of the kidney, greater in males than females.
	M/F	spleen	Myelopoiesis present; white pulp decreased in some animals.
	M/F	thyroid	Male animals presented a slightly greater proportion of solid epithelial nests and minute follicles; females more affected.
	M/F	adrenal	Zona reticularis showed a consistent increase in width, with accumulation of lipid.

M = males
F = females
* 5/5 males presented artifactual distortions of the tubules in the outer third of the testes.

Special Studies:

The sections of the liver and spleen stained for ferric iron by the method of Perl confirmed the impression gained from the H&E section of an increased amount of hemosiderin in the liver and spleen of the 1.54% animals of both sexes.

8. DISCUSSION:

Mean body weights gains in all the test groups were generally similar to the control group. Food consumption of the test feed was comparable to the controls over the 13 week study.

No effect was seen for any of the parameters at 0.0154% except for a decrease in the spleen/body weight ratio of male animals when compared to the corresponding control. This effect is considered to be a spurious event for the effect was not seen at either the 0.154% or 1.54% levels; pathology was considered to be unremarkable at the 0.0154% and 0.154% levels.

The testes of all males at 0.154% showed artifactual distortion of the tubules in the outer third of the gland. No other significant alteration was found.

The 0.154% level of both sexes showed a widened zona reticularis of the adrenal cortex. Males presented a somewhat greater proportion of small and solid epithelial nests in the thyroid gland than females.

Animals of both sexes at the 1.54% level showed decreases in hematocrit and hemoglobin values at week 13. Microscopic pathology examination revealed hemosiderin pigment accumulation from the breakdown of red blood cells in the liver, spleen and kidney. This effect was more severe in males than females.

Male dogs at 1.54% exhibited lower mean testes/body weight and testes/brain weight ratios. Although a 44% decrease in the weight of the testes occurred compared to the weight of the controls, this effect was not statistically significant. Microscopic pathology of the testes revealed complete atrophy in 4 males and partial atrophy in the fifth male. The interstitial cells were increased in amount and the Leydig-like cells in the capsule were greatly increased in some cases.

At 1.54% other effects were noted in both sexes, the zona reticularis of the adrenal gland showed a consistent increase in width and lipid accumulation and the thyroid gland showed a greater proportion of solid epithelial nests and minute follicles. Male animals showed a decreased thyroid/body weight and thyroid/brain weight ratios and female animals showed an increase in the mean brain weight when compared to the controls. The spleen of both sexes showed myelopoiesis with a decrease in the white pulp in some animals.

9. CONCLUSIONS:

Sodium tetraborate decahydrate was administered to 20 beagles per sex at dietary levels of 0 (Control), 0.0154%, 0.154% and 1.54% ad-mixed in feed.

Atrophy of the testes occurred at 1.54% together with decreased testes weight, testes/body weight and testes/brain weight ratios.

In both sexes, a decreased hematocrit and hemoglobin seen at 1.54% correlated with increase hemosiderin pigment in the spleen, liver and kidneys.

Thyroids of male animals had more epithelial nests than females at the 0.154% and 1.54% levels. Decreased thyroid/body weight and the thyroid/brain weight ratios occurred at 1.54%. A widened adrenal cortex at 0.0154% occurred with widening of the zona reticularis and lipid accumulation at 1.54%. Brain weights of 1.54% females were increased.

The systemic toxicity NOEL was 0.0154%, the LOEL was 0.154% based on pathology findings.

009301

Reviewed by: Steven L. Malish, Ph.D. Steven L. Malish 9/26/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 9/26/91
Tox. Branch II, Section IV (H7509C)

Data Evaluation Report

STUDY TYPE: 83-1, Chronic Toxicity Study

MRID NO: 406923-08

TEST MATERIALS: Sodium tetraborate decahydrate

SYNONYMS: Borax

SPONSOR: U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA

TESTING FACILITY: Hazleton Laboratories America, Inc., Vienna, VA

TITLE OF REPORT: 38-Week Dietary Feeding - Dogs with 20 MULE TEAM^{R} Borax (Sodium tetraborate decahydrate)

STUDY NUMBER: Laboratory study number was not reported

AUTHORS: Robert J. Weir, Ph.D.

REPORT ISSUED: February 28, 1967

CONCLUSIONS:

Sodium tetraborate decahydrate was fed for 38 weeks to 4 dogs per sex at 0% (Control) and 1.03% ad-mixed in the feed.

A decrease of about 11% in the rate of weight gain occurred at 1.03%.

Microscopic findings of testicular atrophy occurred at 1.03% together with decreased testes weight, testes/body weight and testes/brain weight ratios. No ejaculate specimens could be obtained from the 1.03% male animals.

No tissue storage of the test material was seen at any time period. The test substance was eliminated from the body in the urine and feces less than 4 days after dosing was stopped.

The NOEL and LOEL could not be determined for only 1 dose level was employed in this study.

CLASSIFICATION:

Core: supplementary - not upgradeable

This study does not satisfy the guideline requirements 81-3 for a chronic toxicity study.

The study is deficient with regard to the fact that because only a single dose level was employed, a NOEL and LOEL could not be determined. The required clinical chemistry parameters were not determined and ophthalmologic examinations were not done.

A. MATERIALS:

1. Test Compound:

Chemical:	sodium tetraborate decahydrate
Label:	not available
Description:	fine white powder without noticeable odor
Storage:	not available
Purity:	Purity of the sample ranged between 103.29% and 105.10% of the theoretical boron content. (Appendix A, p. 18.)

2. Test Animals:

Species:	dog
Strain:	purebred beagles
Age:	not available
Weight:	males 5.5 kg - 7.6 kg females 3.8 kg - 5.7 kg
Source:	not available

B. STUDY DESIGN:

1. Quality Assurance:

No quality assurance statement was issued.

2. Animals:

Sixteen young, purebred beagles, 8 males and 8 females were selected and individually housed in metal cages during the study. The dogs were maintained in the laboratory for at least 1 month prior to the initiation of the study.

a. Animal Assignments:

Sodium tetraborate decahydrate was fed for 38 weeks to 4 dogs per sex at 0% (Control) and 1.03% ad-mixed in the feed (Table 1).

Toxicological parameters and the boron content of various tissues and excreta were measured at various time periods as listed under the Methods and Results section.

Table 1

Animals Assignments and Dose Levels[a]

Dietary Level (%)	26 Week Sac. No. of Dogs M	F	38 Week Sac. No. of Dogs M	F	41 Week Sac.[b] No. of Dogs M	F
0	2	2	2	2	0	0
1.03	2	2	1	1	1	1

[a] Adapted from p. 8 of original report.
M = males
F = females
[b] Recovery sacrifice (week 41) occurred at end of the recovery phase - week 38 + 3 weeks. The recovery phase was also noted as 38 weeks + 25 days in various sections of the original report.

3. Diet:

Animals received a control (basal) laboratory diet of ground Wayne Dog Meal o. the test diet and water ad libitum.

4. Diet Preparation:

The test compound was passed through a wire strainer prior to incorporation. Fresh diets were prepared weekly by incorporating the test material into the control diet of ground Wayne Dog Meal on a weight/weight basis and thoroughly mixed in a Kelly-Patterson twin-shell blender. The test compound was adjusted at monthly intervals according to the assay results (Appendix A, p. 18). The test diet was feed ad libitum in self-feeders 7 days a week.

a. Stability, Dietary Concentration and Homogeniety in Feed:

No studies was done to assess the stability of the test compound in feed, the analysis of the feed for the actual dietary concentration or the homogeniety during the 38 week study.

5. Statistics:

Statistical evaluation was not performed on any parameter.

6. Boron Analyses:

Whole blood, tissues, urine and feces were analyzed for boron content according to the method of Konikowski, T. and Farr, L. E. (Clin. Chem., 11 378-385, 1965).

C. METHODS and RESULTS

1. Observations:

Animals were observed daily for appearance, behavior, and gross signs of systemic toxicity or pharmacologic effects. The testes were manually palpated at the beginning and end of the recovery phase.

The control and test animals appeared normal with respect to appearance, behavior and appetite throughout the study.

Three (3) instances of diarrhea were seen in the control dogs and 1 to 6 scattered instances of diarrhea in 6 of the test dogs. Scattered instances of soft stools were seen in the test group during the first 26 weeks.

Manual testicle palpation at the beginning of the recovery phase in the single dog revealed the testes of the test animal were smaller and firmer than those of the control animals examined at previous time periods. Moreover, no apparent change in the testes were noted at the end of the recovery period when compared to the beginning of the period.

2. Body Weights:

Once each week, individual body weights were recorded.

All animals either maintained or gained body weight during the study. A decrease in the mean body weight gain of about 11% occurred at the 1.03% level when compared to the control level (Table 2).

Table 2

Mean Body Weight(kg) and Mean Body Weight Gains (%) During the 38 Week Study[a]

	Male		Female	
	Dietary Level (%)		Dietary Level (%)	
Week	0.0	1.03	0.0	1.03
0	6.4[b] (---)	6.3[b] (---)	4.7[b] (---)	4.7[b] (---)
26	8.1[b] (27%)	7.3[b] (16%)	6.4[b] (36%)	5.9b (26%)
38	8.0[c] (25%)	7.2[c] (14%)	6.5[c] (38%)	6.0[c] (28%)
41	--- ---	7.1[d] (13%)	--- --	6.1[d] (30%)

[a] Adapted from p. 19 thru 24 of the original report.
[b] 4 dogs/sex, treated and control
[c] 2 dogs/sex, treated and control
[d] 1 dog/sex, treated and control

3. Food Consumption:

Food consumption was determined weekly. Mean food consumption was equivalent to the control values throughout the study.

4. Compound Consumption:

Mean compound consumption was presented below and was calculated from the mean food consumption and mean body weight at the various time periods (Table 3).

At 38 weeks, 1 dog per sex was place on the control diet for an additional 3 weeks (25 days) for evaluation of boron storage in the tissues and elimination of boron in the urine and feces.

Table 3

Compound Consumption Over The 38 Week Study[a]

	Males		Females	
	compound	boron equiv.	compound	boron equiv.
Week	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
0	-	-	-	-
26	337	39	407	47
38	359	41	393	45
41	b	b	b	b

[a] Adapted from p. 19 thru 24 of the original report.
b Only control diet administered.

5. Clinical Studies:

Hematological, biochemical and urine parameters were evaluated on 4 dogs per sex in both the control and test groups during week 0 (initial), 4, 12, and 26 and 2 dogs/sex in the control on week 38. The 26 and 38 week time periods were referred to as the 6 or 9 months time periods, respectively, in the hematology, clinical chemistry and urinalysis tables (p. 25, 29, 32 of the original report).

a. Hematology:

The parameters marked with an (X) were determined while those marked with a (-) were not evaluated. Parameters marked with an (*) are designated in the latest guidelines (Table 4).

Table 4

Hematology Parameters

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Erythrocyte count (RBC)*
X Leukocyte count (WBC)*
- Prothrombin time*
X Leukocyte differential count*
X Sedimentation Rate (Sed. rate)
- Platelet count*
X Reticulocyte counts (week 38 only)

Hematology values at the 1.03% concentration level were comparable to the control values and generally within the accepted normal limits throughout the study.

b. Clinical Chemistry:

Clinical chemistry parameters determined in the study were designated by an (X) while those marked with a (-) were not evaluated. The parameters marked with an (*) are designated in the latest guidelines (Table 5).

Non-fasting blood glucose values were determined.

Table 5

Clinical Chemistry Parameters

X Blood urea nitrogen*
- Cholesterol*
- Glucose (fasting)*
- Total bilirubin*
- Total serum protein*
- Triglycerides*
X Serum alanine aminotransferase (SGOT)*
X Serum aspartate aminotransferase (SGPT)*
- Albumin*
- Sodium*
- Chloride*
- Potassium*
- Calcium*

Clinical chemistry values at the 1.03% level were comparable to the control values and generally within accepted normal limits throughout the study.

c. Urinalysis:

The following parameters parameters marked with an (X) were examined on a 24 hour urine sample while those marked with a (-) were not evaluated. Parameters marked with an (*) are required by the guidelines (Table 6).

Table 6

Urinalysis Parameters

X Appearance*
- Volume*
X Specific Gravity
X pH
X Sediment (microscopic)*
X Glucose*
X Ketones*
X Bilirubin
X Blood (occult)*
X protein*

Urinalysis values at the 1.03% level were comparable to the control values and within normal limits throughout the study.

d. Ejaculation Study:

Male dogs were evaluated at 26 weeks for ejaculation volume, sperm count and motility. Ejaculation specimens were obtained from 3/4 control dogs, no specimens, however, were obtained from 4/4 dogs from the 1.03% level.

6. Sacrifice and Pathology:

After 26 weeks, two male and 2 female animals in the control and treated groups were sacrificed by exsanguination under thiamylal (Surital sodium) anesthesia and gross and microscopic pathological examinations performed.

After 38 weeks on study, 2 animals per sex in the control group and 1 test animal per sex at 1.03% were sacrificed for gross and microscopic examination in the same manner as those at the 26 week interval.

The remaining 2 test dogs, one from each sex, that received the test diet for 38 weeks were given the control diet for an additional 3 weeks (25 days) and were sacrificed for gross and microscopic examination during week 41.

The checked (X) tissue were collected for histological examination while those tissues marked with an (-) were not evaluated. Organs and tissues marked with a (+) were also weighed. Those organs and tissues marked with a (*) are required by the guidelines (Table 7).

Table 7

Tissues and Organs Subjected for Histological Examination and Organ and Tissue Weights

Digestive	Cardiovas./ Hematol.	Neurologic
- Tongue	- Aorta*	X+Brain*
- Esophagus*	X Heart*	- Perip. Nerve*
X Stomach*	X Bone Marrow*	- Spinal Cord* (3 levels)
X Duodenum*	- Lymph Nodes*	- Sciatic nerve
X Jejunum*	cervical/	X Pituitary*
X Ileum*	mesenteric	- Eyes* & optic nerve*
X Cecum*	X+Spleen*	
X Colon*	- Thymus*	Glandular
X Rectum*	Urogenital	X+Adrenals
X+Liver*	X+Kidney*	- Lacrimal gland
X Pancreas*	X Urinary Blad*	- Mammary gland*
Respiratory	X+Testes*	- Parathyroids*
- Trachea*		X+Thyroids*
X Lung*	- Prostate*	Other
	- Seminal Ves.	X Bone (femur)*
	X+Ovaries*	- Skeletal muscle*
	- Uterus*	- Skin*
		- gross lesions*
		- Salivary gland*
		- exorbital lachrymal glands*
		X target organs*
		- gallbladder*

a. Gross Pathology

Gross pathology were performed on all animals scheduled for sacrifice.

In all 4 male test animals (all sacrifices), the testes at the 1.03% level appeared smaller than in the control levels.

b. Organ Weights, Organ/Weight Ratios, Organ/Brain Weight Ratios

The number of animals evaluated at each time period can be found in Table 1.

At the 26 week sacrifice, organ weights were determined and organ/body weight ratios were calculated. At 38 weeks, in addition to the above, organ/brain weight ratios were calculated. At the recovery sacrifice on week 41, organ weights were recorded and the organ/body weights and organ/brain weight ratios were calculated on the single remaining animal at each sex. Week 41 parameters were compared to the week 26/38 controls.

Inspection of the mean and individual parameters - testes weights, testes/body weight and testes/brain weight ratios at the 26, 38 and 41 week sacrifices revealed a decrease in the mean and individual testicular values at the 1.03% level compared to the respective control level (Table 8,9).

Table 8

Mean Body Weight, Mean Testes Weight and Mean Testes/Body Weight Ratios

Conc. Level (%)	Body Weight (kg)	Testes Wgt (gm)	Testes Ratio (%)
26 Weeks[a]			
Control	7.3	14.4	0.20
1.03	7.1	8.7	0.13
38 Weeks[b]			
Control	7.9	13.5	0.18
1.03	6.9	7.3	0.11
41 Weeks[c]			
Control	NA	NA	NA
1.03	7.0	9.4	0.13

---

[a] Mean values adapted from p. 41 thru 42 of the original report.
[b] Mean values adapted from p. 43 thru 44 of the original report.
[c] Values adapted from p. 45 of the original report.
NA = not applicable.

Table 9

Mean Brain Weight, Mean Testes Weight and Mean Testes/Brain Ratios

26 Weeks[a]

Conc. Level (%)	Brain Weight (gm)	Testes Wgt (gm)	Testes Ratio (%)
Control	77.0	14.4	19
1.03	69.2	8.7	13

38 Weeks[b]

Control	76.0	13.5	18
1.03	70.8	7.3	10

41 Weeks[c]

Control	NA	NA	NA
1.03	80.3	9.4	12

---

[a] Mean testes/brain weight ratio calculated by the reviewer.
[b] Mean values adapted from p. 46 of the original report.
[c] Values adapted from p. 47 of the original report.

Sporadic variations in weights and ratios were noted in various test animal organs when compared with the same organ or ratio in the control group. These changes were considered to be no toxicological significance became of the absence of any pathological alterations and the small sample size. (Table 10, 11).

Table 10

Mean Organ Weights(gm) and Ratios(%) at the 26, 38 and 41 Week Sacrifices[a]

Liver

Conc. (%)	26 Weeks wgt M	26 Weeks wgt F	26 Weeks ratio M	26 Weeks ratio F	38 Weeks wgt M	38 Weeks wgt F	38 Weeks ratio M	38 Weeks ratio F	41 Weeks wgt M	41 Weeks wgt F	41 Weeks ratio M	41 Weeks ratio F
Control	203	174	-	-	278	181	3.6	3.0	*	*	*	*
1.03	183	147	-	-	214	141	3.1	2.6	-	200	-	-

Thyroid

Conc. (%)	26 Weeks wgt M	26 Weeks wgt F	26 Weeks ratio M	26 Weeks ratio F	38 Weeks wgt M	38 Weeks wgt F	38 Weeks ratio M	38 Weeks ratio F	41 Weeks wgt M	41 Weeks wgt F	41 Weeks ratio M	41 Weeks ratio F
Control	0.61	-	0.0084	-	-	-	0.0070	0.0085	*	*	*	*
1.03	0.47	-	0.0064	-	-	-	0.0085	0.0010	0.75	-	0.011	0.009

Spleen

Conc. (%)	26 Weeks wgt M	26 Weeks wgt F	26 Weeks ratio M	26 Weeks ratio F	38 Weeks wgt M	38 Weeks wgt F	38 Weeks ratio M	38 Weeks ratio F	41 Weeks wgt M	41 Weeks wgt F	41 Weeks ratio M	41 Weeks ratio F
Control	-	22	-	0.37	-	-	-	-	*	*	*	*
1.03	-	16	-	0.30	-	-	-	-	-	-	-	-

Ovaries

Conc. (%)	26 Weeks wgt M	26 Weeks wgt F	26 Weeks ratio M	26 Weeks ratio F	38 Weeks wgt M	38 Weeks wgt F	38 Weeks ratio M	38 Weeks ratio F	41 Weeks wgt M	41 Weeks wgt F	41 Weeks ratio M	41 Weeks ratio F
Control	-	0.69	-	-	-	1.31	-	0.21	*	*	*	*
1.03	-	-	-	-	-	0.61	-	0.11	-	1.0	-	-

Brain

Conc. (%)	26 Weeks wgt M	26 Weeks wgt F	26 Weeks ratio M	26 Weeks ratio F	38 Weeks wgt M	38 Weeks wgt F	38 Weeks ratio M	38 Weeks ratio F	41 Weeks wgt M	41 Weeks wgt F	41 Weeks ratio M	41 Weeks ratio F
Control	77	65	-	1.1	76	76	-	-	*	*	*	*
1.03	69	71	-	1.4	71	65	-	-	80	69	-	-

[a] Mean values adapted from p. 41-42, 43-44 and 45 of the original report.
* No controls values at this period.
M= males, F= females

Table 11

Mean Organ/Brain Weight Ratios(%) at the 26, 38 and 41 Week Sacrifce[a]

Liver

Conc.(%)	26 Weeks M	26 Weeks F	38 Weeks M	38 Weeks F	41 Weeks M	41 Weeks F
Control	-	268	365	238	*	*
1.03	-	207	302	219	-	292

Thyroids

Conc.(%)	26 Weeks M	26 Weeks F	38 Weeks M	38 Weeks F	41 Weeks M	41 Weeks F
Control	0.79	-	0.71	-	*	*
1.03	0.68	-	0.83	-	0.93	-

Spleen

Conc.(%)	26 Weeks M	26 Weeks F	38 Weeks M	38 Weeks F	41 Weeks M	41 Weeks F
Control	25	33	-	-	*	*
1.03	30	23	-	-	-	-

Kidneys

Conc.(%)	26 Weeks M	26 Weeks F	38 Weeks M	38 Weeks F	41 Weeks M	41 Weeks F
Control	57	51	56	-	*	*
1.03	63	42	80	-	45	-

Ovaries

Conc.(%)	26 Weeks M	26 Weeks F	38 Weeks M	38 Weeks F	41 Weeks M	41 Weeks F
Control	-	1.1	-	1.7	*	*
1.03	-	-	-	0.95	-	1.5

Adrenals

Conc.(%)	26 Weeks M	26 Weeks F	38 Weeks M	38 Weeks F	41 Weeks M	41 Weeks F
Control	-	-	-	1.4	*	*
1.03	-	-	-	1.7	-	-

---

[a] Mean values adapted from p. 41-44, 46 and 47 of the original report.
b At 26 weeks, organ/body weight ratios calculated by reviewer.
c * no controls at this time period. M=males, F=females

c. Microscopic Pathology:

A summary of important pathological findings at the 0% and 1.03% levels noted that at 26 weeks, although testicular atrophy was seen in both the control and test animals, atrophy was more severe in the test group (Table 12).

The ovary pathology findings at all time periods were considered to be unremarkable when compared to the corresponding control groups (Table 12).

Table 12

Microscopic Pathology Findings in the Testes and Ovaries at the 26, 38 and 41 Week Necropsies

Conc.(%)	Sex	Animals on test	Organ	Pathological Findings
26 Weeks Sacrifice				
0	M	2	testes	In 1/2 of the control males, the seminiferous epithelium in a few scattered tubules was atrophied to various degrees and in a few other tubules the nuclei were hyperchromatic and spermatogenesis ceased at the spermatocyte stage. The second animal was considered to be unremarkable.
0	F	2	ovaries	unremarkable
1.03	M	2	testes	In 2/2 animals generalized spermatogenic arrest at the spermatocyte stage progressing to complete atrophy of the seminiferous epithelium in various number of tubules.
1.03	F	2	ovaries	unremarkable
38 Weeks Sacrifice				
0	M	2	testes	In 1/2 control dogs, the seminiferous tubules were rather severely degenerated. In the second control dog 10-15% of the tubules were undergoing atrophy.
0	F	2	ovaries	unremarkable
1.03	M	1	testes	In 1/1 test animals only 5-10% of the tubules presented degenerative changes.
1.03	F	1	ovaries	unremarkable
41 Week Sacrifice[a]				
1.03	M	1	testes	Moderate degree of degeneration and evidence of complete cessation of spermatogenesis.
1.03	F	1	ovaries	unremarkable

[a] no controls for either sex

d. Boron Analyses:

Blood for boron analyses were collected from 1 animal per sex in the control and treated groups at week 0 (initial), 4 and 12 and from 2 animals per sex per dose level prior to the week 26 and 38 sacrifices. The test and control animals were considered to be unremarkable with regard to the content of boron in the blood throughout the study.

Brain, liver, fat, kidney and muscle were collected at the 26 and 38 week sacrifices for analysis of boron. Boron levels were not elevated in these tissues when compared to the corresponding control animals.

Twenty-four (24) hour urine samples were collected from 1 animal per sex in the control and treated groups at week 0 (initial), 1, 4, 12 and from 2 animals per sex per dose level prior to the week 26 and 38 sacrifices for determination of the amount of boron excreted.

Boron was excreted into the urine as noted in Tables 13, and 14 below.

Table 13

Boron Equivalent(ug/ml) in Urine of Male Animals at Various Intervals During the Study After Receiving the Test Substance in the Diet^

Conc. (%)	Weeks 0	4	12	26	38	41
0.0	14.0[a]	12.8[a]	11.2[a]	51.0[b]	14.8[b]	--
1.03	27.0[a]	573.0[a]	668.0[a]	456.0[b]	653.0[b]	NA[c]

^ adopted from p. 50 of the original report.
[a] Urine samples obtained from 1 control and 1 test animals.
[b] Urine samples obtained from 2 controls and 2 test animals.
[c] Urine samples obtained from 1 test animal after stopping of the test diet; data not available (NA).

Table 14

Boron Equivalent(ug/ml) in Urine of Female Animals At Various Intervals During the Study After Receiving the Test Substance in the Diet^

Conc. (%)	Weeks 0	4	12	26	38	41
0.0	16.0[a]	21.8[a]	14.8[a]	50.0[b]	22.7[b]	--
1.03	27.0[a]	500.0[a]	265.0[a]	373.0[b]	660.0[b]	NA[c]

^ adopted from p. 50 in the original report.
[a] Urine samples obtained from 1 control and 1 test animals.
[b] Urine samples obtained from 2 controls and 2 test animals.
[c] Urine samples obtained from 1 test animal after stopping of the test diet; data not available (NA).

Twenty-four (24) hour fecal samples were collected prior to the 38 week sacrifice from 2 control and 2 test animals per sex. The results indicated that boron was eliminated in the feces at the 38 week time period. [Data was not included in the original report, only a summation of the results].

Recovery Phase:

At the end of the 38 week feeding study, 1 animal per sex in the test group was placed on control feed for an additional 3 weeks (25 days). Blood, urine and fecal samples were collected 3 times a week for the determination of boron excretion.

Urine and fecal levels of boron were within normal limits within 4 days after the dogs were removed from the test diet when compared to the control values. Data was not available for these parameters (Table 13, 14). Blood levels continued to be unremarkable.

D. DISCUSSION:

Sodium tetraborate decahydrate was fed to 4 dogs per sex at 0% (Control) and 1.03% dose levels ad-mixed in feed for 38 weeks. Toxicological parameters were measured together with tissue boron levels at various time periods. Boron was also analyzed in the blood, urine and feces during the administration of the test compound and after removal of the compound from the feed.

Test animals showed scattered incidences of soft stools when compared to the control group in the first 26 weeks of the study.

The test animals at all time period were noted to have an about a 11% decrease in the rate of weight gain when compared to the control animals (Table 2).

Palpation of the testes revealed no difference at the beginning versus the end of the recovery phase study with regard to firmness and size of the testes. The testis appeared to be smaller and firmer than the control animals.

No ejaculatory specimens could be obtained from 4/4 animals at the 1.03% level at the week 26 sampling period. Specimens, however, were obtained from 3/4 control dogs.

The individual and mean testes weight, testes/body weight and testes/brain weight ratios of the test animals at week 26 and 38 showed a decrease when compared to the corresponding individual or mean control value. The single animal at 41 weeks was noted to have a decreased testes weight, testes/body weight and testes/brain weight ratios when compared to the corresponding 26 and 38 week control values (Table 8, 9).

Severe testicular atrophy with spermatogenic arrest was noted in 2/2 test animals sacrificed at 26 weeks compared to only 1/2 control animals showing a minimal degree of idiopathic testicular atrophy seen in the control group (Table 12).

At the 38 week sacrifice, testicular atrophy in 1/1 animals at 1.03% was considered to be minimal. Control animals showed either a slight (1/2) or severe (1/2) testicular degeneration (Table 12).

During the 41 week recovery sacrifice, 1/1 males showed a moderate degree of testicular degeneration and evidence of complete cessation of spermatogenesis. A control was not run at this time period (Table 12).

Although idiopathic testicular atrophy occurred in the control groups at both the 26 and 38 week sacrifice periods, it is concluded that the severe testicular atrophy seen at the 26 week necropsy in 2/2 male animals at the 1.03% level was, in fact, related to the administration of the test compound.

The ovary pathology findings at all time periods were considered to be unremarkable when compared to the corresponding control groups (Table 12).

Variation in various organ weights and organ weight ratios were seen in the test animals when compared with the same organ or ratio in the control group. Except for the testes, these changes were considered to be of little toxicological significance, particularly in the absence of pathological alterations and the small sample size (Tables 8, 9, 10, 11 and 12).

No appreciable storage of boron was demonstrated in the brain, liver, fat, kidneys or muscle at the 26 and 38 week periods. At all time periods (4, 12, 26, 38 weeks and the recovery phase samples), blood levels of boron were not

increased when compared to the 0 (initial) week values or the corresponding control values. Boron levels, however, were increased in the urine at the 4, 12, 26, and 38 week time periods when compared to the 0 (initial) week values or to the corresponding control group values (Table 13, 14).

In the recovery phase, the urine and fecal boron levels were comparable to the control values at the first sampling time after the test feed was stopped, indicating that boron elimination occurred before this time period.

E. CONCLUSIONS:

Sodium tetraborate decahydrate was fed for 38 weeks to 4 dogs per sex at 0% (Control) and 1.03% ad-mixed in the feed.

A decrease of about 11% in the rate of weight gain occurred at 1.03%.

Microscopic findings of testicular atrophy occurred at 1.03% together with decreased testes weight, testes/body weight and testes/brain weight ratios. No ejaculate specimens could be obtained from the 1.03% animals.

No tissue storage of the test material was seen at any time period. The test substance was eliminated from the body in the urine and feces less than 4 days after dosing was stopped.

The NOEL and LOEL could not be determined for only 1 dose level was employed in this study.

Appendix A

Monthly Assay Purity of Various Test Samples of Sodium Tetraborate Decahydrate

Time Interval (month)[a]	Boron Content of Test Sample[b] (%)
March	105.05
April	105.10
May	104.78
June	not sampled
July	not sampled
August	not sampled
September	104.39
October	103.70
November	104.39
December	103.29

---

[a] year = 1965
[b] percent of theoretical value

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy Dec. 4, 1991
Section IV, Tox. Branch II (H7509C)

Secondary reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 12/4/91
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Combined Chronic Toxicity/Oncogenicity (rats) (§83-5)

TEST MATERIAL: BORAX - Sodium tetraborate decahydrate

SYNONYMS: none TOX. CHEM. NO.: 406B

STUDY NUMBER: 182-104 MRID NO.: 406923-09

SPONSOR: U.S. Borax Research Corporation

TESTING FACILITY: Hazleton Laboratories, Incorporated
Falls Church, VA

TITLE OF REPORT: Two Year Dietary Administration - Albino Rats - Borax (Sodium Tetraborate Decahydrate) and Addendum

AUTHORS: Robert J. Weir and Louis M. Crews

REPORT ISSUED: July 8, 1966; addendum, April 10, 1967

CONCLUSIONS:

BORAX, administered to rats as a dietary admix for 2 years at concentrations of 0, 0.130, 0.308 and 1.030% (elemental boron - 0, 117, 350 and 1170 ppm) appeared to cause the following:

0.130% - no effects
0.308% - decrease in body weight gain
1.030% - decrease in body weight, possible anemia, clinical observation of being "unkempt" and testicular tubular atrophy

The No Observed Effect Level (NOEL) = 0.130% (1,300 ppm, mg/kg/day: males = 140-32, females = 140-37

The Lowest Observed Effect Level (LOEL) = 0.308% (3,080 ppm, mg/kg/day: males = 410-100, females = 422-118 - decreased body weight gain

The Maximum Tolerated Dose (MTD) = 1.030% (Highest Dose Tested, HDT) - decreased body weight gain (19% in males and 41% in females) and testicular tubular atrophy

The test article did not appear to increase the number of any tumors over control values.

This study is classified Core Supplementary.

This study does not satisfy the Guideline requirements (§-83-5) for a combined chronic toxicity/oncogenicity rat study.

NOTE: This study was conducted before Good Laboratory Practices and the 1982 EPA Guidelines. Some clinical chemistry parameters were not examined. Ophthalmic examinations were not performed. Histopathology on the eyes was not done. In spite of the lack of examination of the above mentioned parameters, it is felt that sufficient toxicological and oncogenic information was obtained.

The study may be upgraded from Core Supplementary to Core Minimum provided the Registrant's response regarding percent survival is acceptable.

I. MATERIALS, METHODS AND RESULTS

NOTE: This report was completed July 8, 1966. The in-life portion of the study was conducted 7/1/63-6/28/65. This was prior to the implementation of Good Laboratory Practices and Quality Assurance Units. It was also before the EPA 1982 Guidelines.

A. Test Article

Name: BORAX, Sodium tetraborate decahydrate

Formula: $Na_2B_4O_7.10H_2O$

Lot Number: none given; received from U.S. Borax Research Corporation on 10/21/61

Physical Properties: fine, white powder; no noticeable odor

Purity: Boron content was 103.2-105.4% of theoretical value

B. Compound Purity and Diet Analysis

Samples of test article were sent to the Registrant approximately monthly during the two-year study for the analysis of boron content. The results (detailed on Report page 2) indicated 103.2-105.4% of the theoretical boron content of the material. The report indicated that dietary levels were adjusted based on the results of the analyses.

[No dietary admix or homogeneity data were included in the report. It is stated (Report page 3) that beginning at 15 months, freshly mixed samples of the diets for the control and each test group were shipped at 3-month intervals to the Registrant for boron analyses (4 shipments).]

C. Animals

Male and female (175 of each) young albino rats of the Sprague-Dawley Charles River Cesarean-derived strain were used. At study initiation, the males weighed 93-130 g and the females, 86-128 g. The rats were placed into the following groups (Table 1) by stratified randomization:

Table 1

NUMBERS OF ANIMALS AND DIETARY LEVELS IN A TWO-YEAR RAT STUDY WITH BORAX

Group	No. of Rats		Dietary Level (%)			
			Total Material†	Total Material†† (corrected)		
	Male	Female		weeks: 0-20	21-30	31-104
1 (con.)	70	70	0	0	0	0
2	35	35	0.103	0.1	0.099	0.098
3	35	35	0.308	0.3	0.297	0.293
4	35	35	1.03	1.0	0.99	0.98

† = Assuming 100% sodium tetraborate decahydrate.
†† = Calculations based on change in boron content.
REVIEWER'S CALCULATIONS: 0.103% = 1,030 ppm
0.308% = 3,080 ppm
1.030% = 10,300 ppm
Data extracted from table on Report page 3.

The animals were housed individually in suspended wire mesh cages with food and water available ad libitum. [No mention of: room temperature, humidity or light/dark cycle.] The test article was incorporated into the basal diet (Purina Laboratory Chow) on a weight/weight basis. Mixing was accomplished by use of a twin shell blender. Fresh diets were prepared weekly.

D. General Observations

1. Mortality and Moribundity - Daily observations were made. Table 2.

As presented in Table 2, survival of treated groups was at least as great as in the respective control groups with the possible exception of the 0.308% female group (58% survival versus 67% for control). As the 1.030% group had 80% survival, it is not considered that the lower value at 0.308% was due to BORAX administration

2. Clinical Observations - Daily cage-site observations were made. In addition, physical appearance and behavior were specifically noted when body weights and food consumption were recorded on a weekly basis for the first 52 weeks and every four weeks thereafter.

According to the report, there did not appear to be any clinical observations which distinguished any treated groups from the control.

Table 2

SURVIVAL OF ANIMALS IN A TWO-YEAR RAT STUDY WITH BORAX

Week \ % =	Males				Females			
	0	0.103	0.308	1.030	0	0.103	0.308	1.030
0	70/70	35/35	35/35	35/35	70/70	35/35	35/35	35/35
8	70/70	35/35	35/35	35/35	70/70	35/35	35/35	35/35
28a	65/65	29/30	30/30	28/30	65/65	30/30	29/29	30/30
56a	59/60	24/25	25/25	23/25	60/60	25/25	24/24	25/25
80	52/60	22/25	24/25	23/25	57/60	23/25	23/24	22/25
104	35/60	19/25	16/25	18/25	40/60	17/25	14/24	20/25
104-% surv.†	58	76	64	72	67	68	58	80
Report Data	58.4	76.9	76.9	73.8	66.7	68.0	58.3	80.0
Mean surv. time - days	671b	679	695	673	697	694	696	694

a = Interval sacrifice
b = Adjusted to compensate for interval sacrifice.
† = Values by - males: 58=35/60, 76=19/25, 64=16/25 and 72=13/25;
report states 58.4, 76.9, 76.9 and 73.8
females: 67=40/60, 68=17/25, 58=14/24 and 80=20/25;
report states 66.7, 68.0, 58.3 and 80.0
surv. = Survival

Data extracted from Report Tables 1 and 2, pages 32-35.

NOTE: There appears to be a difference in the % survival as calculated from the data versus what the report states; particularly, 0.308% males - 64% versus 76.9%. How were the % survival ± S.D. (?) determined, especially for males? Also, for male groups 0.103 and 0.308%, Report data indicate 76.9% but values are 19/25 and 16/25. The Registrant is requested to clarify these.

Males and females administered 1.030% were reported to gradually (beginning at weeks 4-6 with the majority showing the observation by 26 weeks) show the following (severity increased with time):

tails - dry and scaly
fur - rough and coarse, discolored
appearance - hunched
respiration - wheezing
bloody discharge - nose or eyes
eyelids - drooping, inflamed
paws - dark pink, swollen pads
toenails - abnormally long, curving inward
scrotum - appeared shrunken

3. Body Weights - Individual body weights were recorded weekly for the first 52 weeks and every 4 weeks thereafter. Table 3.

Table 3

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS FOR RATS ADMINISTERED BORAX FOR TWO YEARS

	Males				Females			
Week % =	0	0.103	0.308	1.030	0	0.103	0.308	1.030
Body Wt. g								
0	110	111	110	111	101	101	101	101
1	164	157	158	138	134	134	134	123
2	222	214	212	174	165	163	166	147
4	313	309	304	246	207	207	209	183
8	430	424	421	351	259	255	258	228
12	495	488	486	418	281	280	290	253
24	593	592	576	499	333	326	336	283
36	641	646	623	535	369	364	372	299
48	677	679	657	564	404	394	404	316
60	696	696	658	575	429	403	428	332
72	721	710	689	582	470	442	447	340
84	738	765	695	602	502	461	459	353
96	683	704	679	578	504	447	455	348
104	667	657	609	560	506	419	460	339
Body Wt. gain g								
0 - 4	203	198	194	135	106	106	108	82
0 - 48	567	568	547	453	303	293	303	215
0 - 84	628	654	585	491	401	360	358	252
0 - 104	557	546	499	449	405	318	359	238

NOTE: Body weight ranges were reported; S.D. were not included. Statistical significances were not noted on these Report pages.

Data extracted from Report Tables 1 and 2, pages 32-35.

In males, the 0.103% group body weights and gains were similar to control values. At 0.308% there were decreases (from control) in both of these parameters (at week 104 = 9%; for 104 weeks = 10%). The 1.030% group

body weights were 16% below controls and the weight gains were 19% less. The 1.030% group mean terminal body weight was reported to be statistically significantly (p=0.05) lower than control.

For females, the lowest concentration of test article (0.103%) appeared to cause lower body weights over 104 weeks (17%) with the difference from control values becoming more evident after the first year. The difference in body weight gain in this 0.103% group versus control was 21%. The middle concentration group (0.309%) had mean body weights and weight gains somewhat greater than the 0.103% group and closer to control values (% from control for body weight = 9% and for weight gain = 11%). At 1.030%, the difference (decrease) from control values was 33% for body weight and 41% for weight gains. It therefore appears that the lower values for the 0.103% group were probably not due to test article administration but were a result of normal biological variation as a dose response would have been expected. Both the 0.103 and 1.030% group mean terminal body weights were reported to be statistically significantly (p=0.05) lower than control.

4. Food consumption and Test Article Intake - Food consumption was recorded weekly for the first 52 weeks and every four weeks thereafter.

At the 0.103 and 0.308% male and female groups, food consumption, expressed as g/rat/week or g/kg/day was similar to control values (Report Tables 3 and 4, pages 36-39). During the first 13 weeks, food consumptions of the 1.030% males and females were less than controls. Also, from weeks 27-52, female (only) consumption was below controls. During the second year, female food consumption was less at all intervals than was the control value. In all instances, the food consumption was lower when expressed as g/rat/week; whereas, values were higher when expressed as g/kg/day. This is a reflection of lower body weights.

Table 4 presents compound consumption values.

Table 4

GROUP MEAN RANGES FOR COMPOUND AND BORON EQUIVALENT CONSUMPTION IN A TWO-YEAR RAT STUDY WITH BORAX

Concentration %	Males		Females	
	Compound mg/kg/day	Boron Equivalent mg/kg/day	Compound mg/kg/day	Boron Equivalent mg/kg/day
0.103	140-31.8	15.9-3.61	140-37.4	15.9-4.24
0.308	410-99.8	46.5-11.3	422-114	47.9-12.9
1.030	1160-358	132-40.6	1190-448	135-50.8

Data extracted from Report Tables 3 and 4, pages 36-39.

5. Ophthalmoscopic Examinations - None performed [Histopathology not performed]

E. Clinical Pathology - [No mention was made of fasting prior to sampling.]

1. Hematology - These parameters were examined from tail vein blood of 5/sex/group at 1, 2, 3, 6, 12 and 18 months as well as at termination. The CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	-	Total plasma protein (TP)
x	Hemoglobin (HGB)*	x	Leukocyte differential count*
x	Leukocyte count (WBC)*	-	Mean corpuscular HGB (MCH)
x	Erythrocyte count (RBC)*	-	Mean corpusc. HGB conc. (MCHC)
-	Platelet count *	-	Mean corpusc. volume (MCV)

* = EPA Guideline Requirement, 1982 "-" = Not examined
Erythrocyte count - 2, 3, 18 and 24 months

Table 5 presents selected hematology results.

Hemoglobin and hematocrit values in the 1.030% male and female groups were below controls (statistically significant or not significant) at all intervals. Male 1.030% erythrocyte counts were below control values (significant only at the second month), but were not considered to be below an expected normal range (no apparent differences in females). Neither the 0.103 nor 0.308% groups differed from controls. Treated mean leukocyte counts were sometimes different from control values, but it was not considered that these differences were due to test article administration.

2. Blood Chemistry - Determinations were made as follows:

urea nitrogen - months, 1, 2, 3, 6, 12, 18 and 24 - 5/sex/group - urea nitrogen from tail vein blood at months 1, 2, and 3; 6 month determinations from abdominal aorta; 12 and 24 month determinations from intestinal vein

pH, sodium, potassium, carbon dioxide combining power - 6 months - 2/sex/group

serum glutamic pyruvic transaminase (SGPT, ALT), serum glutamic oxaloacetic transaminase (SGOT, AST) - 6, 12 and 24 months - 5/sex from control and high concentration (1.030%)

Table 5

GROUP MEAN HEMOGLOBIN, HEMATOCRIT AND ERYTHROCYTE VALUES FOR RATS ADMINISTERED BORAX FOR TWO-YEARS

Parameter (month) | % =	Males 0	Males 0.103	Males 0.308	Males 1.030	Females 0	Females 0.103	Females 0.308	Females 1.030
HEMATOCRIT (%)								
1	42.6	45.3	43.1	40.1	42.1	44.5	46.2	38.2
2	44.1	40.8	40.9	38.8a	41.7	44.0	40.9	38.4a
3	45.9	47.6	43.3	42.8	44.2	45.6	44.2	41.2a
6	45.4	44.3	45.8	42.8	43.3	43.8	44.9	41.1
12	47.3	45.7	46.8	42.1a	42.8	44.9	41.0	38.2a
18	47.8	44.9	44.8	41.8a	43.0	46.3	44.1	41.3a
24	46.4	42.0a	40.8a	43.9	46.2	44.4	41.6	41.4
HEMOGLOBIN (g/100 ml)								
1	14.5	14.4	14.2	13.7	14.6	15.4	14.7	13.5
2	14.7	14.1	14.1	13.0a	14.9	15.0	15.1	13.0a
3	15.7	15.5	14.8	14.2	14.0	14.7	13.7	12.6
6	15.4	14.2a	14.7	13.2a	14.5	13.9	14.1	14.0
12	14.1	13.9	13.7	12.4a	12.9	13.7	12.9	12.4
18	15.6	14.4	15.0	13.6a	14.8	15.9	14.8	14.0
24	14.7	13.6	13.3a	13.5	14.4	13.7	13.6	12.2a
ERYTHROCYTE ($x10^6$/cmm)								
1	-	-	-	-	-	-	-	-
2	8.2	7.9	7.7a	7.2a	7.4	7.7	7.3	7.1
3	7.1	7.4	6.3	6.4	6.6	6.1	6.1	6.7
6	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
18	5.2	5.1	5.2	5.2	6.6	6.7	6.5	6.5
24	7.1	6.8	6.8	8.3	6.2	5.1	5.7	5.7

a = Statistical Significance (p=0.05)

- = Not examined

Data extracted from Report Tables 7 and 8, pages 42-69.

The CHECKED (X) parameters were examined.

X	Electrolytes
-	Calcium*
-	Chloride*
-	Magnesium
-	Phosphorous*
x	Potassium*
x	Sodium*

X	Other
-	Albumin*
-	Blood creatinine
x	Blood urea nitrogen*
-	Total Cholesterol*
-	Globulins
-	Glucose*
-	Total bilirubin*
-	Total protein*
-	Triglycerides

	Enzymes
-	Alkaline phosphatase
-	Cholinesterase
-	Creatinine phosphokinase*
-	Lactic acid dehydrogenase
x	Serum alanine aminotransferase (also SGPT)*
x	Serum aspartate aminotransferase (also SGOT)*
x	Carbon dioxide combining power

* = EPA Guideline requiremnt - 1982 "-" = Not examined

There did not appear to be test article effects on any of the parameters examined.

3. Urinalysis - Urine was collected by housing rats overnight in metabolism cages. At 6 months, pH was determined for 5 consecutive days on individual daily samples from 2/sex/group. At 18 and 24 months, analyses were performed on pooled samples from 5/sex/group.

The CHECKED (X) parameters were examined.

X	
x	Appearance*
-	Volume*
x	Specific gravity*
x	pH
x	Sediment (microscopic)*
x	Protein*

X	
x	Glucose*
x	Ketones*
x	Bilirubin
x	Blood*
-	Nitrate
-	Urobilinogen

* = EPA Guideline Requirement - 1882 "-" = Not examined

There were no apparent effects of BORAX administration on any of the parameters examined.

F. Sacrifice and Pathology -

Necropsies were performed on all rats which died, were sacrificed moribund or were sacrificed at a scheduled interval. Five/sex/group were sacrificed at 6 and 12 months with all survivors being sacrificed at study termination (24 months). Sacrifice was by exsanguination. Animals designated for biochemical or boron analyses were anesthetized with pentobarbital sodium. Tissues were preserved in 10% formalin (did not mention "buffered").

Histopathology was performed at 6 and 12 months from all sacrificed rats. At terminal sacrifice, there was microscopic examination of tissues from 10/sex from control and 1.030% groups plus target tissues from 10/sex at the 0.103 and 0.309% groups.

Individual blood samples plus samples of brain, liver and kidney, pooled by sex and by group from all control and test rats sacrificed at 6 and 12 months as well as from 10/sex from the control, 0.103% and 0.308% groups sacrificed at 24 months, were frozen for future analyses of boron content.

The CHECKED (X) parameters were examined. Organs with (XX) were also weighed.

X	Digestive System
-	Tongue
-	Salivary glands*
-	Esophagus*
x	Stomach*
a	Duodenum*
a	Jejunum*
a	Ileum*
a	Cecum*
a	Colon*
a	Rectum*
xx	Liver*
x	Pancreas*

X	Respiratory
-	Trachea*
x	Lung*

X	Cardiovascular/Hemat.
-	Aorta*
x	Heart*
-	Bone marrow*
-	Lymph nodes*
xx	Spleen*
-	Thymus*

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

X	Neurologic
xx	Brain*
-	Peripheral nerve*
-	Spinal cord (3 levels)*
x	Pituitary*
-	Eyes (optic nerve)*

X	Glandular
xx	Adrenals*
-	Lacrimal gland
-	Mammary gland*
-	Parathyroids*
xx	Thyroids*

X	Other
x	Bone*
-	Skeletal muscle*
-	Skin*
x	All gross lesions and masses*

x Rib junction

* = EPA Guideline Requirement - 1982 "-" = Not examined
a = Report mentions examination of small and large intestine.

1. Macroscopic -

Interim Sacrifices (6 and 12 months) and Rats that Died: The only reported apparent test article related finding was small, underdeveloped and soft testes in all males in only the 1.030% group.

Terminal Sacrifice: Small testes in the 1.030% group was the only reported test article related finding.

2. Organ Weights -

Only isolated statistically significant ($p=0.05$) absolute or relative-to-body weight ratios (no relative-to-brain weight ratios calculated) were seen in males or females at either of the scheduled interim sacrifices at 6 and 12 months in the two lowest concentration levels (0.103 or 0.308%). Table 6 presents absolute and relative-to-body weight organ weights for the 1.030% rats at the three scheduled sacrifices.

Table 6

GROUP MEAN ABSOLUTE AND RELATIVE ORGAN WEIGHTS AT SCHEDULED SACRIFICES IN A TWO-YEAR RAT STUDY WITH BORAX ADMINISTERED AT A CONCENTRATION OF 1.030%

Sacrifice (months) =	6		12		24	
Concentration (%) =	0	1.030	0	1.030	0	1.030
MALES						
TERMINAL BODY WEIGHT - g .	610	547	647	554	671	566a
THYROID - absolute - g ...	.03	.04	.04	.04	.05	.04a
relative - % ...	.005	.007a	.006	.008a	.007	.006
BRAIN - absolute - g	2.13	2.24	2.27	2.28	2.28	2.31
relative - %	0.35	0.41a	0.35	0.41	0.35	0.41a
LIVER - absolute - g	19.2	15.9a	17.1	13.8	20.7	15.9a
relative - %	3.2	2.9	2.9	2.5	3.1	2.8
SPLEEN - absolute - g	0.88	0.73	1.13	0.75	1.20	0.83a
relative - %	0.15	0.13	0.17	0.13	0.18	0.15a
KIDNEY - absolute - g	3.4	3.2	4.1	3.4	5.5	3.8a
relative - %	0.56	0.59	0.63	0.61	0.79	0.67
ADRENAL - absolute - g ...	0.06	0.06	0.08	0.07	0.08	0.06a
relative - % ...	0.01	0.01	0.01	0.01	0.01	0.01a
TESTES - absolute - g	3.8	0.6	3.7	0.9a	3.7	1.0a
relative - %	1.0	0.2a	0.6	0.2a	0.6	0.2a

009301

Table 6 continued

Sacrifice (months) =	6		12		24	
Concentration (%) =	0	1.030	0	1.030	0	1.030
FEMALES						
TERMINAL BODY WEIGHT - g	338	287a	394	294a	512	328a
THYROID - absolute - g	.02	.03	.04	.04	.04	.03
relative - %	.007	.007a	.010	.012	.007	.009a
BRAIN - absolute - g	1.96	2.13	2.06	2.08	2.06	2.09
relative - %	0.58	0.75a	0.53	0.74	0.41	0.65a
LIVER - absolute - g	10.3	9.1	10.1	8.6	15.3	11.0a
relative - %	3.1	3.2	2.6	3.0a	3.0	3.4
SPLEEN - absolute - g	0.59	0.47	0.59	0.51a	0.82	0.53a
relative - %	0.18	0.16	0.15	0.18	0.16	0.16
KIDNEY - absolute - g	2.1	2.0	2.2	2.3	3.0	2.3
relative - %	0.63	0.71	0.56	0.80a	0.60	0.86a
ADRENAL - absolute - g	0.08	0.07	0.09	0.08	0.11	0.10
relative - %	0.02	0.02	0.02	0.03	0.02	0.03a

a = Statistically Significant (p=0.05)
Data extracted from Report Tables 15-17, pages 93-104.

Only the effects on testes weights (absolute and relative) are considered to be a result of test article administration (1.030%, Highest Dose Tested, HDT). The statistically significant differences regarding other organs appear to be the result of lower terminal body weights.

3. Microscopic -

The following tisses were examined microscopically from all rats at scheduled 6 and 12 month interim sacrifices, 10/sex from 0 and 1.030% groups at the terminal sacrifice and target organs (including testes and ovaries) from 10/sex from the lower concentratrion levels (0.103 and 0.308%): brain, thyroid, heart, lung, liver, kidney, adrenal, pancreas, stomach, small intestine, large intestine, gonads and all unusual lesions. In addition, selected tissues from a number of control and treated animals which died during the study and which exhibited unusual lesions were examined histopathologically.

The only apparent test article related microscopic findings concerned the testes. Table 7 presents these data.

Table 7

HISTOPATHOLOGICAL TESTICULAR FINDINGS IN RATS ADMINISTERED BORAX FOR TWO YEARS

Observation % =	0	0.103	0.308	1.030
6 MONTH SACRIFICE				
Tubular atrophy	0/5	0/5	0/5	5/5, 4a
12 MONTH SACRIFICE				
Tubular atrophy	0/5	0/5	0/5	5/5, 3-4
24 MONTH SACRIFICE				
Focal tubular atrophy	3/10 10-60%	1/10 15%	4/10 5-100%	10/10 90-100%
Panarteritis	0/10	1/10, 4	0/10	1/10, 2
Calcific arteriosclerosis	0/10	1/10, 4	2/10, 1-3	6/10, 2-4

a = Severity: 0 = present but insignificant
1 = slight
2 = moderate or average
3 = moderate to severe
4 = severe

Data extracted from Report Appendices A, B and C; pages 133-244.

6-Month Sacrifice - Five/five 1.030% males showed severe (grade 4) testicular tubular atrophy compared with none in any of the other groups.

12-Month Sacrifice - Five/five 1.030% males showed moderate to severe (grade 3) or severe (grade 4) testicular tubular atrophy (none in other groups).

24-Month Terminal Sacrifice - As noted in Table 7, focal tubular atrophy was noted in 3/10 controls, 1/10 low dose (0.103%), 4/10 mid dose (0.308%) and 10/10 high dose (1.030%). Panarteritis was present in no more than one rat/group. Calcific arteriosclerosis was observed (none in the control group) in one rat at low dose, in 2 at mid dose and in 6 at the high dose. The highest dose tested (HDT), 1.030%, had an effect on microscopic findings in the testes as early as 6 months.

There did not appear to be any increase in tumor incidence related to test article administration.

ADDENDUM TO FINAL REPORT

The purpose of this Addendum (April 10, 1967) was to combine all data from boron analyses which had been presented in progress reports as well as terminal analyses which had not been previously reported. The analyses were conducted to determine the levels of boron and borax equivalent in samples of whole blood, brain, liver and kidney from male and female rats which received borax at dietary levels of 0 (control), 0.103, 0.308 and 1.030%.

Analyses were made on individual blood samples and on pooled (by group and sex) brain, liver and kidney samples from 5 rats/sex/group that were sacrificed at 26 and 52 weeks, and from 10/sex from 0.103 and 0.308% groups sacrificed at 104 weeks. At the 26-week interval, analyses were performed on two aliquots of each sample. Results are presented in Table 8.

REVIEWER'S COMMENT: The 52 week male brain boron and equivalent values in the 1.030% group (19.0 and 167.4 ug/g) are the same as the male liver values in the 0.308% group. This duplication of values also occurs for male liver and kidney at 52 weeks for the 1.030% group as well as at 104 weeks (male liver and kidney - see all three groups).

Amounts of boron in examined tissues at 104 weeks were less than at the 26 and 52 week intervals with the exception of brain at 1.030%, which had higher values at 52 weeks than at 26 weeks (both sexes). At the lowest concentration tested (0.103%), blood and kidney levels appeared to increase with sampling intervals.

The Reviewer has no comments regarding the materials and methods section.

A description of the statistical analyses employed was included in the report.

Good Laboratory Practice (GLP) statements are not applicable as the study was conducted (1963-65) prior to GLP being in effect.

II. DISCUSSION

NOTE: As this study was conducted during the period 1963-65 and the report was not completed until July 8, 1966, the work described was completed prior to the presence of Good Laboratory Practrices. The report was signed by the Registrant's agent on May 6, 1988 for submission to the Agency.

Table 8

GROUP MEAN BORON AND BORAX EQUIVALENTS IN TISSUES FROM RATS ADMINISTERED BORAX BY DIETARY ADMIX FOR TWO YEARS

% =	0		0.103		0.308		1.030	
	boron	equiv	boron	equiv	boron	equiv	boron	equiv
BLOOD (ug/ml)								
Males - wk 26a	2.1	18.5	2.8*	24.7*	5.4*	47.6*	10.9*	96.0*
52	1.4	12.3	2.9*	25.5*	4.0*	35.2*	5.5*	48.5
104	4.7	41.3	6.1	54.2	6.9	60.8	-	-
Females - wk 26a	1.9	16.7	3.2*	28.2*	5.1*	44.9*	10.3*	90.7*
52	0.8	7.0	2.3*	20.3*	3.1*	27.3*	4.4*	38.8*
104	4.9	42.8	6.2	54.5	7.2	63.2	-	-
BRAIN (ug/g)								
Males - wk 26a	ND	ND	ND	ND	ND	ND	9.7	85.9
52	ND	ND	3.0	26.4	7.0	61.7	19.0	167.4
104	ND	ND	ND	ND	11.0	96.9	-	-
Females - wk 26a	ND	ND	ND	ND	ND	ND	10.8	95.6
52	ND	ND	3.0	26.4	11.0	96.9	15.0	132.2
104	ND	ND	ND	ND	5.5	48.4	-	-
LIVER (ug/g)								
Males - wk 26a	ND	ND	ND	ND	17.0	150.2	34.0	299.5
52	3.0	26.4	15.0	132.2	19.0	167.4	23.0	202.6
104	1.5	13.2	7.0	61.6	7.0	61.6	-	-
Females - wk 26a	ND	ND	ND	ND	ND	ND	19.6	172.6
52	3.0	26.4	7.0	61.7	11.0	96.9	19.0	167.4
104	7.0	61.6	11.0	96.9	13.0	114.5	-	-
KIDNEY (ug/g)								
Males - wk 26a	ND	ND	ND	ND	6.9	60.8	33.9	299.0
52	3.0	26.4	7.0	61.7	11.0	96.9	23.0	202.6
104	7.0	61.6	19.0	167.3	7.0	61.6	-	-
Females - wk 26a	5.3b	46.7b	ND	ND	9.1	80.6	20.7	182.8
52	ND	ND	3.0	26.4	11.0	96.9	23.0	202.6
104	3.5	30.8	15.0	132.1	7.0	61.6	-	-

wk = week
ND = not detectable
a = analyses performed on two aliquot samples
b = possible contamination of sample
* = Significantly higher than control
Data extracted from Report Addendum Tables 1-4, pages 250-255.

Although there are some Good Laboratory Practice and 1982 Agency Guideline deficiencies, this Reviewer feels that the available data are sufficient in order to conduct a comprehenisve review and make the determination as to whether the study is acceptable as Core Minimum.

Analyses of boron concentration in the test diets appeared to be within acceptable ranges (103-105% of theoretical boron content).

Survival of treated groups did not appear to be affected by test article administration. The 58% survival for the 0.308% group females was less than controls (67%). However, this is considered to be a normal biological variation as the 0.103% showed 68% and 1.030% group had 80% survival.

Only the 1.030% animals showed clinical signs of test article administration as evidenced by increased severity of the following observations: dry scaly tails, rough coarse discolored fur, hunched appearance, wheezing, blood discharge of nose or eyes, drooping or inflamed eyelids, dark pink/swollen paws, abnormally long curving toenails and shrunken scrotums.

In males, body weight gain was decreased in the 0.308 and 1.030% groups with the lowest concentration (0.103%) group mean being similar to control values. Body weight gain was less in the 0.103% females than in the 0.308% group. Because the percent decrease from the female control value was 21, 11 and 41 (0.103, 0.308 and 1.030%), it is considered that, primarily because there was no dose response plus the fact that this was not seen in males, the 0.103% difference is most likely within normal biological variation.

Food consumption values for the 0.103 and 0.308% males and females were similar to respective controls. At 1.030%, there was a decrease during the first 13 weeks in both sexes as well as a decrease from weeks 27-52 in females. In the second year of the study, only females showed a decreased food consumption when expressed as g/rat/day, but an increase when calculated as g/kg/day (a reflection of lower body weights).

There was the suggestion of possible anemia because of lower hematocrit and hemoglobin values throughout the study in males and females at 1.030%. However, RBC values were either not reduced or only slightly lower than controls.

Borax did not appear to have an effect on any blood chemistry or urinalysis parameters examined.

Macroscopic pathology and organ weight data indicated only underdeveloped/small testes in the 1.030% rats at the 6 and 12 month as well as at the terminal sacrifice. This finding was corroborated by histopathological findings which indicated both an increase in the number of males with testicular tubular atrophy as well as an increase in the severity of this observation. The 0.308% males were not reported to have this change at the 6 or 12 month sacrifices, and there was only the possible suggestion that an effect occurred at the terminal sacrifice.

No increase in tumor incidence in any of the treated groups was reported.

In an addendum to the final report, boron analyses were made at 26, 52 and 104 weeks from blood as well as brain, liver and kidney tissues. There appeared to be less boron in tissues at 104 weeks than at earlier samplings (26 and 52 weeks), except for brain which had more boron at 52 than at 26 weeks. Blood and kidney levels were greater with each sampling interval at the 0.103% concentration.

III. CONCLUSIONS

BORAX, administered to rats as a dietary admix for 2 years at concentrations of 0, 0.130, 0.308 and 1.030% (elemental boron = 0, 117, 350 and 1170 ppm) appeared to cause the following:

0.130% - no effects
0.308% - decrease in body weight gain
1.030% - decrease in body weight gain, possible anemia, clinical observation of being "unkempt" and testicular tubular atrophy

The No Observed Effect Level (NOEL) = 0.130% (1,300 ppm, mg/kg/day: males = 140-32, females = 140-37)

The Lowest Observed Effect Level (LOEL) = 0.308% (3,080 ppm, mg/kg/day: males = 410-100, females = 422-118) - decreased body weight gain

The Maximum Tolerated Dose (MTD) = 1.030% (Highest Dose Tested, HDT) - decreased body weight gain (19% in males and 41% in females) and testicular tubular atrophy

The test article did not appear to increase the number of any tumors over control values.

This study is classified Core Supplementary

This study does not satisfy the Guideline requirements (§83-5) for a combined chronic toxicity/oncogenicity rat study.

NOTE: This study was conducted before Good Laboratory Practices and the 1982 EPA Guidelines. Some clinical chemistry parameters were not examined. Ophthalmic examinations were not performed. Histopathology on the eyes was not done. In spite of the lack of examination of the above mentioned parameters, it is felt that sufficient toxicological and oncogenic information was obtained. The study may be upgraded from Core Supplementary to Core Minimum provided the Registrant's response regarding percent survival is acceptable.

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy
Section IV, Tox. Branch II (H7509C) Dec. 4, 1991

Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E A Doyle
Section IV, Tox. Branch II (H7509C) 12/4/91

DATA EVALUATION REPORT

STUDY TYPE: Chronic Oral Toxicity - Dog (§83-1)

TEST MATERIAL: BORAX - Sodium Tetraborate Decahydrate

SYNONYMS: none **Tox. Chem. No.:** 406B **MRID No.:** 406923-10

STUDY NUMBER: 182-106 **HED Project No.:** 1-1119

SPONSOR: U.S. Borax Research Corporation

TESTING FACILITY: Hazleton Laboratories, Incorporated
Falls Church, VA

TITLE OF REPORT: Two-Year Dietary Feeding - Dogs - Borax (Sodium Tetraborate Decahydrate) and Addendum

AUTHORS: Robert J. Weir and Louis M. Crews

REPORT ISSUED: July 8, 1966; Addendum, April 10, 1967

CONCLUSIONS:

Male and female beagles were given BORAX (boron) by dietary admix at concentrations of 0, 0.051, 0.103 and 0.309% for 104 weeks. There was a 52 week interim sacrifice and a 13 week "recovery" period after 104 weeks on test article for some dogs. There did not appear to be any definitive test article effect on any parameter examined. A questionable sperm/testes effect is raised by this Reviewer concerning the 0.309% dogs. However, the study pathologist considered the histopathological findings as being, "not compound-induced."

CONCENTRATIONS/DOSES

Percent	ppm elemental boron	ppm BORAX	mg/kg estim.
0.051	117	510	13 (510÷40)
0.103	350	1,030	26
0.309	1,170	3,090	77

No Observed Effect Level (NOEL) = 0.309% (Highest Dose Tested, HDT)

Lowest Observed Effect Level (LOEL) = none (>HDT)

Classification: **Core Supplementary** - No definitive test article effect was reported. This study is not considered to be upgradable. A new chronic dog study is required.

This study does not satisfy the Guideline Requirements (§83-1) for a chronic oral toxicity study in dogs.

I. MATERIALS, METHODS AND RESULTS

NOTE: This report was completed July 8, 1966. The in-life portion of the study was conducted 6/26/63 - 104 weeks + 3 months recovery. This was prior to the implementation of Good Laboratory Practices and Quality Assurance Units. It was also before the EPA 1982 Guidelines.

A. Test Article

Name: BORAX, Sodium Tetraborate Decahydrate

Formula: $Na_2B_4O_7.10H_2O$

Lot Number: none given; received from U.S. Borax Research Corporation on 10/12/61.

Physical Properties: fine white powder; no noticeable odor

Purity: Boron content was 103.2-105.4% of theoretical value

B. Compound Purity and Diet Analysis

Samples of the test article were sent to the Registrant approximately monthly during the two-year study for the analysis of the boron content. The results (detailed on Report page 7) indicated 103.2-105.4% of the theoretical boron content of the material. The report stated that dietary levels were adjusted based on the results of the analyses.

Fresh diets were prepared weekly by mixing the test material with ground Wayne Dog Meal (twin-shell blender) on a weight/weight basis. The dietary admixes were available via self-feeders seven days/week. Food and water were available ad libitum. The concentrations of Borax in this study were 0 (control), 0.051, 0.103 and 0.309%. [Reviewer's calculations: 0.051% = 510 ppm, 0.103% = 1,030 ppm and 0.309% = 3,090 ppm; Report (one-liner) as elemental boron = 117, 350 and 1,170 ppm.]

There was no mention of homogeneity analyses.

C. Animals

Young purebred beagles [no source mentioned] were housed individually in metal cages. There was a one-month acclimation period before study initiation. Four dogs/sex were randomly placed into each of the four groups. No pre-test body weights were reported nor was the age range of the dogs given.

D. Disposition of Animals

1. Interim Sacrifice (52 weeks) - One male and one female per group were sacrificed (exsanguination, thiamylal sodium anesthesia).

2. Terminal Sacrifice (104 weeks) - At this sacrifice there were 2/sex from the control and 0.309% groups and 3/sex from the 0.051 and 0.103% groups.

3. Recovery Sacrifice (117 weeks). At this sacrifice there was 1/sex from the control and 0.309% groups.

4. Metabolism Studies - One control male and one male from the 0.309% group were placed on a metabolism study for: one month at the beginning of the test period, two weeks at the one-year interval and three months at the two-year interval. [Three month dogs were on study a total of 117 weeks - 104 + 13.] The purpose was to evaluate the rate of storage and elimination of boron. One female from the control and one from the 0.309% group were added for the three month terminal metabolism study.

   REPORT NOTE: As the initial metabolism study was not started until 6 days after study initiation, a special study consisting of 2 stock colony dogs was conducted in order to obtain initial metabolism results. "The periods of observation were overlapped to confirm the general agreement of the analytical data."

E. General Observations

1. Clinical Observations - The dogs were observed daily for appearance, behavior and signs of toxicity or pharmacological effects.

   All animals appeared "normal" during the time they were on study with the following exceptions:

0.103% group -

dog No. 5652 - At the start of the 64th week this female delivered 4 puppies - 2 were stillborn and 2 were " very weak". [No comment in the report as to how this female got pregnant.] The 2nd day after birth the 2 surviving pups were found dead. Report pages 99-104 contain details regarding the 4 pups (radiologic examination for maturity as well as gross and histopathology).

dog No. 5994 - During week 68, this female was examined because of weight loss and a decrease in food consumption. Observations included a purulent vaginal discharge, a temperature of 102° F and a slightly elevated WBC count with an increase in immature forms. A diagnosis of metritis was made. [Details of antibiotic therapy were described on Report pages 14 and 15.] Various treatments were continued for about 5 weeks after which the animal appeared to be in in good physical condition (weight gain and increased appetite).

2. Body Weights - All dogs were weighed weekly. Selected group mean weights and weight gains are presented in Table 1.

The individual animal body weights at week one (no pre-test weights reported) ranged from 4.2-11.5 kg for males and 4.1-8.1 kg for females. Group mean body weights (4/sex/group) at week 1 ranged from 7.9-8.9 kg for males and from 5.6-7.1 kg for females. At week 52 (interim sacrifice of 1/sex/group), group mean body weight gains were similar for both males (1.0-1.2 kg) and females (0.3-0.7 kg). At the 104 week weighing (3/sex/group), the high concentration males (0.309%) appeared to have gained less than controls (1.8 kg versus 2.3 kg). From week 52-104, controls gained 1.1 kg and the 0.309% group gained 0.8 kg. Taking into consideration the number of dogs/sex/group at any weighing interval, the range of weights at study week one and the overall weight gains one would expect to see, it is doubtful that the dietary administration of BORAX had an effect on body weights or weight gains at any concentration.

Table 1

BODY WEIGHTS OF DOGS ADMINISTERED BORAX BY DIETARY ADMIX FOR TWO-YEARS (kg)

	Males				Females			
Week % =	0	.051	.103	.309	0	.051	.103	.309
1a	7.9	8.1	8.3	8.9	5.6	6.3	6.6	7.1
4	7.9	8.0	8.4	8.9	5.2	6.4	6.6	7.1
12	8.7	8.1	8.6	9.2	5.6	6.8	6.9	7.1
20	8.7	8.6	9.1	9.8	5.8	6.8	7.0	7.4
28	8.9	8.8	9.1	9.9	5.8	6.7	7.0	7.5
36	8.7	8.9	9.1	9.9	5.8	6.6	6.8	7.7
44	8.8	9.3	9.2	10.0	5.8	6.8	7.0	8.0
52b	9.1	9.1	9.4	9.9	5.9	6.9	6.9	7.8
60	9.8	9.0	10.4	10.4	5.8	7.4	7.1	7.7
68	9.8	9.1	10.6	10.4	5.8	7.2	6.8	7.9
76	9.9	9.1	10.6	10.8	5.6	7.5	6.8	8.1
84	10.1	9.0	10.7	11.2	5.9	7.2	7.4	8.1
92	10.3	9.4	10.7	11.0	5.8	7.5	7.6	7.9
104c	10.2	9.2	10.6	10.7	5.9	7.3	7.2	7.8
117d	12.3	-	-	12.2	3.9	-	-	8.1
BODY WEIGHT GAINS (kg)								
0-52	1.2	1.0	1.1	1.0	0.3	0.6	0.3	0.7
0-76	2.0	1.0	2.3	1.9	0.0	1.2	0.2	1.3
0-104	2.3	1.1	2.3	1.8	0.3	1.0	0.6	0.7

a = 4/sex/group at start of study
b = 1/sex/group sacrificed
c = 2/sex/group sacrificed
d = values are for one dog
Data extracted from Report Table 1, pages 22-34.

3. Food consumption - The amount of food consumed was expressed as kg/week.

There did not appear to be any test article effect on the amount of food consumed.

4. Ophthalmoscopic Examination - none performed [Histopathology was not performed on the eyes.]

E. Clinical Pathology - [No mention was made of fasting prior to sampling.] Hematology, blood chemistry and urinalysis parameters were examined on all available dogs initially as well as at 1, 3, 6, 12, 18, 24 and 27 months.

1. Hematology - The CHECKED (X) parameters were examined.

x	Hematocrit (HCT)*	-	Total Plasma Protein
x	Hemoglobin (HGB)*	x	Leukocyte differential*
x	Leukocyte count (WBC)*	-	Mean Corpuscular HGB (MCH)
x	Erythrocyte count (RBC)*	-	Mean Corpusc. HGB Conc. (MCHC)
-	Platelet*	-	Mean Corpusc. Volume (MCV)

x Sedimentation rate * = EPA Guideline Requirement
"-" = Not examined

Note: Erythrocyte counts on all survivors at months 18, 24 and 27.

There did not appear to be any differences in hematological values caused by test article administration.

2. Blood Chemistry - The CHECKED (X) parameters were examined.

Electrolyte

-	Calcium*	x	Potassium‡*
-	Chloride‡*	x	Sodium‡**
-	Magnesium	x	Carbon dioxide‡
-	Phosphorous*	x	pH‡

Other

-	Albumin*	-	Globulin
-	Blood Creatinine*	x	Glucose*
x	Blood urea nitrogen*	-	Total Bilirubin*
-	Cholesterol*	-	Total Protein*
		-	Triglycerides

Enzymes

-	Alkaline Phosphatase
-	Cholinesterase
-	Creatinine Phosphokinase*
-	Lactic Acid Dehydrogenase
x	Serum Alanine Aminotransferase (SGPT)*
x	Serum Aspartate Aminotransferase (SGOT)*

‡ = Determinations made only during week 28 and only on one male from each group on day 2 (blood sample 4 hours after feeder removal), day 2 (blood sample 4 hours following initiation of feeding) and day 4

(blood sample 4 hours after feeder removal). pH at 22°C and 37°C.

* = EPA Guideline Requirement "-" = Not examined

The only blood chemistry differences between treated and control animals regarded the SGOT and SGPT values (and ratio) in males at 0.051 and 0.309 % at the 12 month sacrifice. (See Table 2)

Table 2

SELECTED ENZYME LEVELS AND RATIOS OF DOGS ADMINISTERED BORAX FOR TWO-YEARS

Dog No.	Months	Conc. Gp.	SGOT	SGPT	SGOT-SGPT Ratio
5624-M	12	0	38	25	1.52
5982-F	12	0	55	29	1.90
5921-M	12	.051	22	34	0.65
5656-F	12	.051	22	20	1.10
6033-M	12	.103	38	32	1.19
5996-F	12	.103	34	18	1.89
5925-M	12	.309	20	25	0.80
5651-F	12	.309	26	20	1.30
5932-M	24	0	36	32	1.12
5936-M	24	0	42	35	1.20
5635-F	24	0	30	29	1.03
5660-F	24	0	38	27	1.40
5946-M	24	.309	25	24	1.04
6036-M	24	.309	22	21	1.05
5659-F	24	.309	32	31	1.03
5997-F	24	.309	25	23	1.09
5924-M	27	0	25	24	1.04
5628-F	27	0	32	27	1.18
5955-F	27	.309	22	22	1.00
5664-F	27	.309	28	24	1.17

Data extracted from Report Table 4, pages 55 and 56.

3. Urinalysis - The CHECKED (X) parameters were examined.

x	Appearance*	x	Glucose*
-	Volume*	x	Ketones*
x	Specific Gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment*	-	Nitrate
x	Protein	-	Urobilinogen

* = EPA Guideline Requirement "-" = Not examined

There were no apparent test article induced changes in any of the parameters examined.

4. Sperm Counts and Viability - No description of any procedures was provided. Table 3 presents the results.

Table 3

SPERM COUNTS AND MOTILITY IN DOGS ADMINISTERED BORAX IN THE DIET FOR TWO-YEARS

Borax Conc. %	Dog No.	Motility %	No. Sperm/ cu mm	Ejaculate Vol. ml	Total No. sperm
0	5932	100	120,000	4	480 mil.
0	5936	50	90,000	3	360 mil.
0.309	5946	0	0	QNS	0
0.309	5946a	0	0	QNS	0
0.309	6036	50	10,000	1	10 mil.
0.309	6036a	≥50	10,000	2	20 mil.

a = Repeat sample
QNS = Quantity not sufficient
mil. = million
Data extracted from Report Table on page 16.

Of the two 0.309 % males at 24 months, the ejaculate (prior to sacrifice) either had no observable or relatively few (10,000/cu mm) sperm. Also, in the dog which had sperm, motility was 50% or ">50%". One control had 100% motility and the other was reported to have 50%.

At the time of the 24 month necropsy, the vas

deferens was stripped with the following results:

dog No. 5946 (0.309%) - less than one-half drop of material was obtained which microscopically contained only cellular debris and amorphous material (no sperm present).

dog No. 6036 (0.309%) - one drop of thick creamy material was expressed from each vas deferens which microscopically was very dense with sperm that had almost no motility.

G. Sacrifice and Pathology - One male and one female from each group were sacrificed (exsanguination under thiamylal anesthesia) after one year on study. After 24 months, 2/sex from the control and 0.309% groups were sacrificed in addition to the remaining 3/sex in the 0.051 and 0.103% groups. The other 1/sex from control and 0.309% groups were removed from the BORAX diet and given untreated food for 13 weeks at which time they were sacrificed (117 weeks).

One male and one female from each group at 12, 24 and 27 (only control and 0.309% groups remaining) months had samples of the following tissues placed in plastic bags and frozen for future analyses of boron content: brain, liver, kidney, body fat and muscle.

The CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

	Digestive System
-	Tongue
-	Salivary Gland*
-	Esophagus*
x	Stomach*
x	Duodenum*‡
x	Jejunum*‡
x	Ileum*‡
x	Cecum*‡
x	Colon*‡
x	Rectum*‡
xx	Liver*
x	Gallbladder*
x	Pancreas*

	Respiratory
-	Trachea*
x	Lung*

	Cardiovascular/ Hematology
-	Aorta*
x	Heart*
x	Bone Marrow*
-	Lymph Nodes*
x	Spleen*
x	Thymus*

	Urogenital
xx	Kidneys*
x	Urinary Bladder*
xx	Testes*
-	Epididymides*
-	Prostate*
-	Seminal Vesicle*
-	Ovaries
-	Uterus*

009301

	Neurologic		Glandular		Other
xx	Brain*	xx	Adrenals*	x	Bone*
-	Periph. Nerve*	-	Lacrimal Gland	-	Skeletal Muscle*
-	Spinal Cord (x3)*	-	Mammary Gland*	-	Skin*
x	Pituitary*	-	Parathyroids*	-	All Gross Lesions & Masses*
-	Eyes (Optic N.)*	xx	Thyroids*		

‡ = Report states, "large and small intestine."
x3 = Three levels of spinal cord
* = EPA Guideline Requirement "-" = Not examined

1. Macroscopic

There were no findings which were considered related to test article administration.

2. Organ Weights

No absolute or relative (to body weight) organ weights appeared to be affected by test article administration.

3. Microscopic

The study pathologist concluded that, "The microscopic examination revealed no histological alterations in any of the tissues examined." The pathologist indicated that at the 2-year sacrifice, a possible test article related effect was noted in the testes of 1 of 2 0.309% dogs (No. 5946) as demonstrated by the following: spermatogenic epithelium atrophy of various degrees as well as small and empty epididymal loops. The pathologist stated, "These degenerative changes were of recent origin and probably unrelated to compound ingestion." See Table 4.

Table 4

HISTOPATHOLOGICAL FINDINGS IN TESTES OF DOGS ADMINISTERED BORAX FOR TWO-YEARS

Sac. Week	Finding	% =	0	.051	.103	.309
52	no reported findings		1	1	1	1
104	focal atrophy		0/2	2/3,0-1	0/3	0/2
	depression of spermatogenesis		0/2	0/3	1/3,2	1/2,3
117	tubular atrophy		1/1,1	-	-	0/1

NOTE: 2/3,0-1 = 2 of 3 dogs, 0-1 grading
Grading: 1 = slight, 2 = moderate or average, 3 = moderate to severe, 4 = severe
Data extracted from Report Appendix, pages 105-142.

H. Metabolism Studies

One male from the control and one male from the 0.309% groups were placed on a metabolism study for the following durations:

1. for one month at the beginning of the test period
2. for two weeks at the one-year interval
3. for three months at the two-year interval

The purpose of these studies was to evaluate the rate of storage and elimination of boron. One female from the control and one female from the 0.309% groups were added for the three month terminal interval.

Urine and feces were collected at 24-hour intervals, blood was collected three times weekly and diets were sampled weekly at the beginning and at one-year intervals. At two years, urine and feces were collected 24 hours prior to test article removal and daily thereafter for five weeks. Also, at two years, blood was collected 24 hours prior to test article removal, three times/week for the next five weeks and weekly thereafter until termination (13 weeks). During the terminal study interval, all dogs received control feed (sample obtained on the 12th week).

The initial metabolism study was begun six days after the study was started; therefore, two additional stock dogs were put on a special study in order to obtain initial metabolism results. The periods of observation were overlapped to confirm the general agreement of the analytical data.

One male from each group was selected during week 28 for a six-day study to determine pH and various electrolytes in the blood and urine in order to evaluate the acid-base balance. The animals were individually housed in metabolism cages and fed once daily [no mention of duration or amount of food]. Prior to feeding, the bladder was emptied by catheterization. After feeding, the first voided urine specimen was collected and analyzed by flame photometry. The remainder of the 24-hour specimen was frozen and stored for possible future analysis. On the 2nd and 4th days of the study, blood samples were drawn and frozen. The following parameters were examined on blood and urine samples: sodium, potassium, chloride and pH. Carbon dioxide combining power determinations were performed in the presence of known quantities of boron, which was initially added to these samples.

It should be noted in the tables that there were measureable amounts of boron/Borax in the control dogs. Table 5 indicates a slight dose response in both sexes during approximately the first year regarding boron/Borox blood levels. By the end of two years there appeared to be little or no differences between treated and control dogs. Urine content of boron/Borax showed a dose response which was noted during the 104 week study. However, at the highest percent tested (0.309), the urine values in both sexes were essentially half (or less) at the 104 week determination compared with the 13, 52 or 78 week intervals. [The blood or urine values were from one or two dogs/concentration level.]

Table 6 (Blood, Feces and Urine) which compares 0.309% dosed dogs with controls, shows that during the first year (no values given during the second year) at all intervals there were about 2-4 fold larger amounts of boron/Borax in the blood of treated dogs. In feces, the 0.309% dogs had 1.5-2 times the amount of control boron/Borax. Urine samples indicated a many fold (10 or more) increase in boron/Borax at all intervals. By day 14 of the depletion period (off test article) the control and 0.309% dog values were comparable.

Table 5

BORON AND BORAX EQUIVALENT IN BLOOD AND URINE OF DOGS ADMINISTERED BORAX FOR TWO-YEARS

Week	% =	Males 0	Males 0.051	Males 0.103	Males 0.309	Females 0	Females 0.051	Females 0.103	Females 0.309
WHOLE BLOOD									
Boron	4 µg/l	1.2b	1.8b	2.5b	3.2b	1.7b	1.8b	2.2b	3.7b
	13	1.1b	1.7b	1.7b	2.5b	1.8b	1.8b	2.1b	2.5b
	52	1.0a	1.5a	2.1a	3.2a	1.0a	1.5a	2.6a	3.2a
	78	2.0a	2.6a	2.6a	3.1a	3.7a	3.7a	4.8a	4.8a
	104	1.7b	1.5a	1.2a	1.2b	1.2b	2.0a	1.7a	1.8b
Borax	4 µg/l	11b	16b	22b	28b	15b	16b	19b	33b
	13	10b	15b	15b	22b	16b	16b	19b	22b
	52	9a	13a	19a	28a	9a	13a	23a	28a
	78	18a	23a	23a	27a	33a	33a	42a	42a
	104	11b	13a	11a	11b	11b	18a	15a	15a
URINE									
Boron	13 µg/l	14a	41a	53a	139a	11a	33a	67a	163a
	52	9a	29a	69a	145a	11a	33a	75a	188a
	78	21a	105a	48a	208a	17a	27a	44a	280a
	104	20b	64a	77a	109b	22b	45a	25a	84b
Borax	13 µg/l	120a	364a	470a	1221a	99a	293a	587a	1433a
	52	76a	258a	611a	1280a	99a	293a	657a	1656a
	78	182a	928a	423a	1832a	153a	235a	388a	2467a
	104	159b	564a	678a	1014b	194b	385a	220a	751b

a = value for one dog
b = value for two dogs
Data extracted from Report Tables 1-4, pages 150-153.

Table 7 (Report Table 6, page 160) shows boron/Borax equivalents in brain, kidney, liver, muscle and fat of males and females at weeks 52, 104 and 117 (13 weeks after cessation of test article administration).

Table 6

BORON AND BORAX EQUIVALENTS IN BLOOD, FECES AND URINE OF DOGS ADMINISTERED BORAX FOR TWO-YEARS

Days	Borax % Diet	Blood µg/ml Boron	Blood µg/ml Borax Equi	Feces µg/g Boron	Feces µg/g Borax Equi	Urine µg/ml Boron	Urine µg/ml Borax Equi
0m	0	1.5	9	40	228	6	34
0m	0.309	1.3	12	38	335	4	35
3m	0	1.3	7	38	217	6	34
3m	0.309	3.7	33	56	493	235	2067
5m	0	1.8	10	38	217	8	43
5m	0.309	4.0	35	70	617	211	1855
7m	0	2.1	12	38	217	6	31
7m	0.309	4.6	41	68	599	187	1644
10m	0	2.1	12	42	240	7	38
10m	0.309	4.3	38	62	546	167	1468
35m	0	1.8	16	a	a	a	a
35m	0.309	5.1	45	a	a	a	a
360m	0	1.5	13	46	405	11	97
360m	0.309	3.7	33	a	a	106	934
370m	0	2.1	19	42	370	6	50
370m	0.309	4.3	38	a	a	133	1168
DEPLETION PERIOD FOLLOWING 104 WEEKS OF DOSING							
0m	0	7.8	69	47	415	7	61
0m	0.309	7.8	69	103	907	91	803
0f	0	7.0	62	27	238	8	74
0f	0.309	10.0	88	35	308	60	529
1m	0	5.3	47	35	309	23	203
1m	0.309	7.5	66	63	555	114	1005
1f	0	8.6	76	27	238	20	176
1f	0.309	8.1	71	27	238	36	318
14m	0	7.2	64	2	18	6	55
14m	0.309	7.2	64	4	35	5	44
14f	0	6.3	56	ND	ND	7	61
14f	0.309	7.5	66	ND	ND	16	137

m = male
f = female
a = no determination
ND = No detectable amount
day 0 = 24 hours prior to test article withdrawal
Data extracted from Report Table 5, poages 154-159.

Table No. 7 - Boron and borax equivalent content in various tissues obtained from purebred male and female beagles which served as controls or received the indicated dietary levels of borax for 52 or 104 weeks; 117-week sacrifice followed 13 weeks on control food. Key: ND = no detectable amount.

DOG NO. AND SEX	LEVEL	TIME	BRAIN		KIDNEY		LIVER		MUSCLE		FAT	
			BORON	EQUIVALENT	BORON	EQUIVALENT	BORON	EQUIVALENT	BORON	EQUIVALENT	BORON	EQUIVALENT
	%	weeks	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
5624 (M)	0	52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5921 (M)	0.051	52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6033 (M)	0.103	52	ND	ND	3.0	26.0	3.0	26.0	ND	ND	ND	ND
5925 (M)	0.309	52	3.0	26.0	7.0	62.0	7.0	62.0	3.0	26.0	ND	ND
5982 (F)	0	52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5656 (F)	0.051	52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5996 (F)	0.103	52	ND	ND	3.0	26.0	ND	ND	ND	ND	ND	ND
5651 (F)	0.309	52	3.0	26.0	7.0	62.0	7.0	62.0	3.0	26.0	ND	ND
5932 (M)	0	104	19.0	167.5	13.0	114.6	21.0	185.2	23.0	202.8	ND	ND
6038 (M)	0.051	104	15.0	132.1	7.0	61.6	23.0	202.6	ND	ND	3.0	26.4
5938 (M)	0.103	104	29.0	255.7	ND	ND	3.0	26.4	ND	ND	ND	ND
5946 (M)	0.309	104	10.0	88.2	10.0	88.2	23.0	202.8	15.0	132.3	15.0	132.3
5660 (F)	0	104	14.0	123.4	ND	ND	35.0	308.7	7.0	61.7	ND	ND
5658 (F)	0.051	104	23.0	202.6	3.0	26.4	23.0	202.6	ND	ND	27.0	237.8
5994 (F)	0.103	104	ND	ND	7.0	61.6	23.0	202.6	3.0	26.4	3.0	26.4
5659 (F)	0.309	104	19.0	167.5	15.0	132.3	25.0	220.5	15.0	132.3	19.0	167.5
5924 (M)	0	117	ND	ND	3.0	26.4	33.0	291.0	25.0	220.2	ND	ND
5955 (M)	0.309	117	ND	ND	ND	ND	25.0	220.5	27.0	237.8	ND	ND
5628 (F)	0	117	ND	ND	7.0	61.6	25.0	220.5	25.0	220.2	ND	ND
5664 (F)	0.309	117	ND	ND	ND	ND	27.0	238.1	21.0	185.0	ND	ND

The data in Table 7 indicate the following:

52 Week - There were no detectable amounts in control or 0.051% dogs in any tissue. In the 0.309% dogs (1/sex), levels were noted in all but fat.

104 Week - At this interval, controls had measurable amounts of boron/Borax in all tissues but fat. In the 0.309% dogs, levels of test article were found in all tissues; however, in some instances, these were in the range of control values. No dose-response pattern was noted.

117 Week - There did not appear to be any differences between control and 0.309% dog values (only concentration at this interval) regarding any tissue. [In some instances, the presence of boron/Borax was reported in the controls, but not in the treated animals.]

- -

Taking into consideration that the study was conducted in 1963-1965 (prior to Good Laboratory Practices and the 1982 EPA Guidelines), the Reviewer does not have any comments regarding the Materials and Methods.

Details of statistical analyses were not included in the report.

Compliance and Good Laboratory Practice statements were not applicable.

II. DISCUSSION

NOTE: The study was reported in 1966 and 1967. This was prior to Good Laboratory Practices as well as the 1982 EPA Guidelines.

Results of test article purity analyses were included in the report. There was no mention of homogeneity analyses.

There was no mortality and no test article related clinical signs reported. One female delivered 4 puppies at week 64 (2 still born and 2 that died the next day). A second female was diagnosed at week 68 as having metritis, was treated and after 5 weeks appeared to be in good condition.

Although there was the appearance of a decrease in group mean

body weight gains in the 0.309% males over the 104 week period, it is not felt that test article administration was a factor when the following are considered: only one sex with finding, overall weight gains expected, the number of dogs/sex/group at a given weighing interval and the group means as well as individual body weights at the start of the study.

Food consumption, hematology and urinalysis values were similar between treated and control groups for all parameters and intervals.

SGOT (AST) and SGPT (ALT) and/or their ratios in the 0.051 and 0.309% dogs appeared to be below control values at 12 months. These differences were not apparent at the 24 month interval. As toxicity is usually represented by an elevation of one or both of these enzymes and considering the number of animals involved, it is considered doubtful that the test article had a deleterious effect on these parameters.

Sperm counts and motility at 24 months indicated that the 0.309% dogs (lower concentration groups not examined) had lower sperm counts and sperm motility than did the controls. The pathologist indicated that one of the two 0.309% dogs showed spermatogenic epithelium atrophy as well as small and empty epididymal loops, but that, "These degenerative changes were of recent origin and probably unrelated to compound ingestion." This Reviewer feels that based upon the relatively limited data available (sperm count/motility and histopathology), there is at least the suggestion that a sperm/testicular effect may have been caused by the test article. Further investigation appears to be appropriate.

The metabolism aspects of this study indicated that there was little or no storage of boron. Blood, urine and fecal boron levels peaked during the first two weeks of the study and remained relatively constant during the rest of the study. Small amounts were present in some tissues at 52 weeks (0.103 and 0.309%). The 104-week results showed boron present in control samples and, therefore, evaluation was difficult. [No explanation in the report regarding control boron levels.]

III. CONCLUSIONS

Male and female beagles were given Borax (boron) by dietary admix at concentrations of 0, 0.051, 0.103 and 0.309% for 104 weeks. There was a 52 week interim sacrifice and a 13 week "recovery" period after 104 weeks on test article for some dogs. There did not appear to be any definitive test article affect on any parameter examined. A questionable sperm/testes effect is raised by this Reviewer concerning the 0.309% dogs. However, the

study pathologist considered the histopathological findings as being, "not compound-induced."

CONCENTRATIONS/DOSES

%	ppm elemental boron	ppm Borax	mg/kg(estimated)
0.051	117	510	13 (510÷40)
0.103	350	1,030	26
0.309	1,170	3,090	77

No Observed Effect Level (NOEL) = 0.309% (Highest Dose Tested, HDT)

Lowest Observed Effect Level (LOEL) = none (>HDT)

Classification: **Core Supplementary** - No definitive test article effect was reported. This study is not considered to be upgradable. A new chronic dog study is required.

This study does not satisfy the Guideline Requirements (§83-1) for a chronic oral toxicity study in dogs.

Reviewed by Steven L. Malish, Ph.D. Steven L. Malish 10/29/91
Review Section IV, Tox Branch II (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E. A. Doyle 10/30/91
Section IV, Tox Branch II (H7509C)

DATA EVALUATION RECORD

STUDY TYPE:	Fertility and Reproductive Effects Rat (83-4)
MRID NUMBER:	406923-11
TEST MATERIAL:	Sodium decaborate tetrahydrate
SYNONYMS:	Borax
SPONSOR:	U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Company 412 Crescent Way Anaheim, CA 92801-6794
TESTING FACILITY:	Hazleton Laboratories, Inc., Vienna, VA
LAB STUDY NO:	Project No. 182-105
TITLE OF REPORT:	Three-Generation Reproductive Study - Rats Borax (Sodium Tetraborate Decahydrate) and Addendum
AUTHORS:	Robert J. Weir, Ph.D., Louis M. Crews, M.D.
DATE ISSUED:	July 8, 1966

CONCLUSIONS:

Male and female albino rats were administered the test compound at levels of 0% (Control), 0.103%, 0.308% and 1.03% ad-mixed in the feed and evaluated for reproductive performance during 3 generations in each of 2 litters.

In the pre-mating period of the 1.03% P1 generation, a decrease in weight gain occurred in both sexes and food efficiency was decreased in the females.

The testes at 1.03% in the P1 generation were grossly atrophied as evidenced by a severe decrease in the organ weight and organ/body weight ratio. A decrease in the number of corpora lutea was indicative of a decrease in ovulation. No litters were produced at 1.03% when the test males were mated with test females.

P1 test females at 1.03% mated with control males resulted in a decreased number of litters and pup survival.

The ingestion of the test compound showed no compound related effects on the reproductive performance at 0.103% and 0.308% through 3 parental and 3 filial generations of 2 litters each.

NOEL for systemic and reproductive toxicity - 0.308%.
LOEL for systemic and reproductive toxicity - 1.03%

CLASSIFICATION:

CORE: Supplementary - not upgradeable

This study does not satisfy the guideline requirements for 83-4 "Reproductive and Fertility Effects". The study is deficient in the following:

1. Testes, epididymis, seminal vesicles and prostate were not microscopically examined at any of the dose level in the P1 or F1 generations.

2. In the 1.03% P1 generation, the ovaries and uteri but not the vagina were microscopically examined. These organs were not microscopically examined from the lower dose levels (0.103%, 0.308%) or from any dose level in the F1 generation.

3. The test and the control groups did not contain at least 20 males and a sufficient number of females to yield at least 20 pregnant females at or near term.

4. Sperm or vaginal plug determinations were not performed.

5. No data was available during the gestation period on body weights, food consumption, clinicaL observations etc.

6. Matings between a single male and a single female are required.

7. Lactation indices variation in the 0% (Control) values of the F1A, F1B and F2A litters and the F3A test animals compromised the validity of these values.

A. MATERIALS:

1. Test Compound

Chemical:	sodium tetraborate decahydrate
Description:	fine white powder without noticeable odor
Purity:	(see Two Year Dietary Administration - Rats, Sodium Tetraborate Decahydrate, July 8, 1966; MRID 406923-09)
Stability:	not available

2. Test Animals (P1 Generation)

Species:	Rat
Strain:	Charles River CD® strain
Sex:	64 females, 32 males for the first parental generation
Weight:	males - 130 to 150 gms females - 110 to 149 gms

B. STUDY DESIGN:

1. Quality Assurance

No quality assurance statement was issued.

2. Diet

Animals received a control (basal) laboratory diet of Purina Laboratory Chow or the test diet and water ad libitum.

3. Diet Preparation

The test material was incorporated into the basal diet on a weight/weight basis and thoroughly mixed in a twin-shell blender to provide the desired dietary levels.

The dietary levels for the test groups were calculated on the basis of the test material as received.

a. Stability, Dietary Concentration and Homogeneity In Feed

No studies were done to assess the stability of the test compound in the feed, the analysis of the feed for the actual dietary concentration or the homogeneity during the study.

4. Statistics

Terminal body weights, organ weights, and organ/body weight ratios for the P1 animals were examined by the analysis of variance, or F-test, at the 5% probability level. Before completing each F-test the variances were tested for heterogeneity by the method of Bartlett. If the variances were homogeneous, the F-test could be completed in the normal fashion and if a significant F-value was obtained, those groups significantly different from control could be determined by the method of Scheffe.

In instances of heterogeneous variances, the samples were examined or extreme values by Sach's test for rejection of measurements. If no legitimate, unbiased, adjustment to the variance could be made by rejection of "outliers", comparison of test to controls were effected by the Fisher-Behrens modified t-test. Breeding indices were analyzed by the chi-square test of significance.

C. METHODS:

1. Pre-mating Period

a. Animal Assignments

The study was initiated with 96 young albino rats; 64 females and 32 males were selected by stratified randomization and placed into 4 groups (Table 1). Prior to the initiation of the first breeding phase, the animals were maintained in individual cages and fed their respective diets for 14 weeks until they reached maturity.

Similar animal assignments and dietary levels were used for the P2 and P3 generations except that only the 0%, 0.103% and 0.308% dietary levels were employed.

Table 1

Animal Assignments and Levels[a]

Group No.	No. of Animals Males	Females	Dietary Level (%)
1 (Control[b])	8	16	0.0
2	8	16	0.103
3	8	16	0.308
4+	8	16	1.030

[a] Adapted from p. 7 of the original report.
[b] Received basal diet of laboratory chow.
\+ Group 4 (1.03%) was not included in the P2 and P3 generations.

b. Appearance, Behavior, Body weights, Food consumption, Food Efficiency

In the pre-mating period for all parental generations (P1, P2, P3), weekly records were kept of appearance, behavior, individual body weights, food consumption and test compound consumption; food efficiency was measured in the pre-mating period only in the P1 and P2 generations.

In the P1 generation animals, terminal body weights and organ weights were recorded and ratios were calculated on week 34 at the 0% (Control), 0.103% and 0.308% levels and on week 27 and 46 at the 1.03% level in the males and females respectively. Terminal body weights for the P2 generation were recorded during week 13 and during week 10 for the P3 generation animals.

2. Reproductive Phase

a. Mating Phase:

In the P1, P2 and P3 generations, one male and 2 females were placed in each breeding cage. The males remained with the females for 21 days and then were returned to their individual cages.

The experimental design for the 1.03% level was altered due to failure of the P1 parents to produce litters. The male animals in this group were not rebred.

In order to determine whether the female reproductive system was affected, the P1 females in the 1.03% level were mated with males of the same strain and approximately the same age which had received only the control diet. The males remained in the breeding cage for eight hours each day. To prevent the males from feeding on the test diet, no food was available to the animals during the daily mating period. The females were not rebred after the first litter.

b. Lactation Phase

Twenty-four hours after birth the litters were reduced to a maximum of 8 pups to be nursed.

Records were maintained for all litters on the number and size of litters, deaths and the weight of the pups at 24 hours and at weaning. The pups were observed for gross signs of abnormalities.

The first filial (F1A) litters were discarded when they reached 21 days of age. The P1 parents in the 0% (Control), 0.103% and 0.308% levels were remated to produce their second (F1B) litters. At the time of weaning, 16 females and 8 males each from the control and the 2 lower dose test groups were selected at random and designated as the second parental generation (P2) for continuation of the reproductive study. All excess weanlings were discarded.

The second (F2) and third (F3) filial generations litters for each group were obtained by the procedure described for the first generation.

c. Indices

The female Fertility Index (the number of pregnancies/number of matings) and the Gestation Index (the number litters with at least 1 live pup/number of pregnancies) were calculated for all parental (P1, P2 and P3) generations at all but the 1.03%. dose level.

The following indices were calculated for all litters (F1A, F1B, F2A, F2B, F3A, F3B) at all but the 1.03% dose level.

Live Birth Index - number of pups born alive/number of pups born,

Lactation Index - number of pups weaned/number of pups left to nurse.

3. Terminal Sacrifice and Pathology

Gross necropsies were performed at the terminal sacrifice period for the P1 generation animals (week 34 for the 0% (Control), 0.103% and 0.308% levels after the second breeding cycle, week 27 for the 1.03% test males after the first breeding cycle and week 46 for the 1.03% females after the first breeding cycle). Organs were weighed and organ/body weight ratios were calculated; the ovaries and uteri were microscopically examined (Table 2).

After completion of the second breeding cycle, all P2 and P3 animals were sacrificed and gross necropsies were performed. Organs were not weighed.

Gross necropsies were also performed on 5 animals per sex of the F3B weanlings at the 0% (Control), 0.103% and 0.308% levels.

Table 2

Organs Selected for Organ Weights and Organ/Body Weight Calculations In the P1 Generation Animals

	Dietary Levels in Feed			
Organ	0%[a]	0.103%[a]	0.308%[a]	1.03%[b]
brain	X	X	X	X
thyroid	X	X	X	X
liver	X	X	X	X
spleen	X	X	X	X
kidneys	X	X	X	X
adrenals	X	X	X	X
testes	X	X	X	X
ovaries	-	-	-	X+
uterus	-	-	-	X+

[a] terminal sacrifice at 34 weeks
[b] terminal sacrifice at 27 weeks for males and 46 weeks for females
+ microscopic pathology evaluated

D. RESULTS:

1. Pre-mating Phase

a. Appearance and Behavior

The appearance and behavior of the P1, P2 and P3 rats at the 0.103% and 0.308% levels during the pre-mating period were generally comparable with those of the controls. In the P1 group during the fifth to the seventh week, the males and females in the 1.03% level developed a rough haircoat, scaly tails and a hunched appearance. Inflamed eyelids were noted in a few females in this group towards the end of the 14th week period. No mortality occurred in the parental generations.

b. Body Weights

In the pre-mating P1 generation, body weight gains for the males and females at 1.03% level were lower than the corresponding controls (Table 3).

In the pre-mating P2 and P3 generations, absolute body weights and body weight gains were considered to be unremarkable when compared to the respective control groups.

Table 3

Mean Weekly Body Weights (gm) and Mean Body Weight Gains (%) for the P1 Generation*

Males

Week	0.0%	0.103%	0.308%	1.03%
Initial	138	138	142	142
14	531(--)	519(3%)	561(7%)	481(-14%)

Females

Initial	128	128	125	128
14	292(--)	299(4.0%)	292(2.0%)	268(-15%)

---

* Adapted from p. 24 thru 27 in the original report.
() Mean body weight gains (%) compared to 0% (control) weight gain.

c. Food Consumption

Food consumption was considered to be unremarkable in the P1, P2 and P3 parental generations.

d. Food Efficiency

Food efficiency in the P1 generation was unremarkable in the males at the 0.103%, 0.308% and 1.03% levels. The female animals showed a 13% decrease in food efficiency throughout the 14 week pre-mating phase.

Food efficiency in the P2 animals at all dietary levels were unremarkable when compared to the corresponding controls.

e. Compound Consumption

Compound consumption was calculated from the mean food consumption and mean body weight data throughout the pre-mating periods (Table 4).

Table 4

Mean Compound Consumption for the Parental Generations[a]

Level (%)	Compound (mg/kg/day) Males	Females
P1 Gen.		
0.0	0	0
0.103	72	81
0.308	218	241
1.030	729	814
P2 Gen.		
0.0	0	0
0.103	89	92
0.308	260	281
1.030	--	--
P3 Gen.		
0.0	0	0
0.103	90	94
0.308	270	301
1.030	--	--

[a] Adapted from p. 31 thru 37 of the original report.

2. Mating Phase

In the P1 generation, there were no conceptions at the 1.03% level during the F1A breeding cycle. The male animals in this group were not remated.

In the 1.03% P1 generation, the mating of female rats fed the 1.03% level with untreated male rats fed the control (basal) diet resulted in 2 deliveries and 1 abortion out of 16 matings. One female delivered 3 living and 2 stillborn pups. All pups died within 3 days. Birth of another litter was observed, but 24 hours later there were no traces of any pups in the breeding cage. The female animals were not remated.

The P2 and P3 animals at the 0.103% and 0.308% levels were comparable to the respective controls.

The appearance and behavior and the measured parameters of the P3 animals at the 0.103% and 0.308% levels during the breeding phase were also comparable with those of the controls except for the fertility index which showed a statistically significant increase of 45% at the 0.308% level in both the

F3A and F3B breeding cycle. This effect was not considered to be of any toxicological significance.

3. Lactation Phase

The lactation indices for the 0% (Control) of the F1A and F1B litters and the F2A were considered to be below the accepted historical values (90%) observed in the laboratory rat. Without acceptable control values, no conclusion can be made on the validity of the lactation indices at the 0.103% and 0.308% levels (Table 5).

In the same regard, while the F3A 0% (Control) lactation index was similar to the accepted historical values, the test values were depressed. Because of the variation seen in this cycle and the variation noted in the previous generations, no conclusion can be drawn on the validity of the lactation values (Table 5).

For the F2B and F3B litters, the test group lactation indices were similar to the 0% (Control) groups values (Table 5).

The other parameters measured in all three filial generations were considered to be unremarkable. The surviving first, second and third generation pups in the control and test groups developed normally.

Table 5

Lactation Indices for the Three Filial Generation Study[a]

Litter	Test Substance in Feed 0.0%	0.103%	0.308%	1.03%
F1A	56.3^	63.6	82.3	[b]
F1B	58.8^	60.0	74.2	--
F2A	48.3^	79.8	82.7	--
F2B	92.1	93.2	95.5	--
F3A	91.5	81.1	79.1	--
F3B	89.7	91.8	95.9	--

[a] Adapted from p. 42 thru 44 of the original report.
[b] Female test animals mated with male test animals did not conceive.
^ Values considered to below the accepted historical value of about 90% for control rats.

4. Terminal Sacrifice and Pathology

a. Gross Pathology

Gross necropsies performed on the P1 rats at the 1.03% level revealed atrophied, soft, blue testes in 8/8 male animals. The ovaries of the females presented a somewhat increased incidence of cyst formation, congestion or infection when compared to the controls. The other organs did not reveal any consistent gross changes.

Gross necropsies performed on the 0% (Control), 0.103% and 0.308% levels of the 3 parental generations sacrificed after completion of the second breeding cycle did not reveal any consistent gross changes in the test rats which were not also seen in the control rats.

The organs and viscera of representative third generation weanlings (F3B) sacrificed from the 0% (Control), 0.103% and 0.308% levels showed no gross abnormalities.

b. Organ Weights

Statistical analyses revealed significantly higher thyroid weight and thyroid/body weight ratios for the 0.103% and 0.308% males as compared to the male controls in the animals sacrificed during week 34 of the study. This finding were not considered to be of any toxicological significance for thyroid weights at 1.03% were comparable to the controls (Table 6).

The testes weight and ratio showed decreases of 76% and 73%, respectively, at the 1.03% dose level when compared to the corresponding controls (Table 6).

Table 6

Mean Terminal Body Weight, Organ Weights, Organ/Body Weight Ratios For P1 Male Rats Sacrificed on Week 34 of the Study[a]

Level (%)	Body Weight (gm)	Thyroid wgt (gm)	Thyroid ratio (%)	Testes wgt (gm)	Testes ratio (%)
Control	643	0.030	0.0047	4.08	0.634
0.103	606	0.036^	0.0059^	3.78	0.633
0.308	642	0.036^	0.0056^	3.70	0.576
1.030+	567	0.033	0.0059	0.98[b,c]	0.17#

[a] Adapted from p. 45, 48 and 49 of the original report.
+ terminal sacrifice at 27 weeks
^ significantly higher than the control; degree of statistical significance was not given
[b] 76% decrease from the control
[c] The original report reported the testes weight as 0.098 gm. The reviewer notes that the weight should be 0.98 gm. A 1 gm testes weight was denoted in the report - Two Year Dietary Administration - Rats, Sodium tetraborate Decahydrate, July 8, 1966; MRID 406923-09.
73% decrease from control.

The other organ weights and organ/body weight ratios determined for the P1 animals at the 0.103%, 0.308% and 1.03% levels were comparable with the control values.

c. Microscopic Pathology

Histopathology was evaluated from the ovary and uterus from the 1.03% level sacrificed during week 46. The incidence of animals showing decreased or non-functioning ovaries was higher than would normally be encountered in rats of this age. Only 4/15 ovaries contained corpora lutea.

Inconsistent alterations encountered in the uterus were not regarded as test compound related.

E. DISCUSSION:

The toxicity of the test substance upon reproduction in male and female rats was evaluated through 3 parental generations (P1, P2 and P3) and 3 filial generations (F1, F2 and F3) each consisting of 2 litterings.

The reproduction study was initiated at dietary levels of 0% (Control), 0.103%, 0.308% and 1.03%.

The appearance and behavior of the P1, P2 and P3 rats at the 0.103% and 0.308% during the pre-mating period were generally comparable with those of the controls. In the P1 group during the fifth to the seventh week of the study, animals of both sexes developed a rough haircoat, scaly tails and a hunched appearance.

In the P1 1.03% group, male and female animals showed decreases in the rate of weight gain of about 15% during the pre-mating period. Females, moreover, showed a decrease in food efficiency of 13%. No effect was seen in the males.

Mating of the P1 animals in the 1.03% test group was discontinued due to failure of the parents to produce litters. An attempt to obtain litters by mating the 1.03% level females with control males resulted in 2 litters and 1 abortion out of 16 matings. All pups were either cannibalized or died within 3 days.

Gross necropsies at the P1 1.03% level revealed atrophied testes. Severe decreases in the testes weight and the testes/body weight ratio were also noted (Table 6). No consistent gross changes were seen in other organs of the P1 animals or in any of the organs examined from the P2 and P3 generation parents.

Microscopic examination of the testes was not done in this study. Albeit, the report entitled Two Year Dietary Administration - Rats, Sodium Tetraborate Decahydrate, July 8, 1966; MRID 406923-09, revealed that viable sperm were not found when the testes were examined microscopically.

Gross pathology of the ovaries from the P1 generation females at the 1.03% level presented a somewhat increased incidence of cyst formation, congestion or infection when compared to the controls. Microscopic pathology indicated decreased functioning of the ovaries in the majority of the animals. Corpora lutea were noted in only 4 of 15 ovaries. The number of corpora lutea was regarded as a major indication of cyclic function, especially ovulation; a correlation usually exists between the degree of development of the corpora lutea and the maturation of the Graafian follicles.

The decrease in corpora lutea indicated that the incidence of non-functional ovaries or ovaries with decreased function was higher than normally encountered in rats of this age. The authors noted that this finding was not considered to be a sufficient explanation for the decreased fertility and speculated that a deleterious effect on the ovum, implantation or gestation after implantation, might have been the possible cause.

Gross necropsies of the P2 and P3 animals performed on the 0% (Control), 0.103% and 0.308% levels after completion of the second and third breeding cycle did not reveal any consistent gross changes when compared to the controls. Other parameters were unremarkable.

The lactation indices for the 0% (Control) of the F1A and F1B litters and the F2A were considered to be below the accepted historical values (90%) observed in the laboratory rat. Without acceptable control values, no conclusion can be made on the validity of the lactation indices at the 0.103% and 0.308% levels (Table 5).

In the same regard, while the F3A 0% (Control) lactation index was similar to the accepted historical values, the test values were depressed. Because of the variation seen in this cycle and the variations noted in the previous generations, no conclusion can be drawn on the validity of the lactation indices (Table 5).

For the F2B and F3B litters, the test group lactation indices were similar to the 0% (Control) groups values (Table 5).

Except for the lactation indices, the appearance, size, weight and other measured parameters of all filial generations were comparable among the test and control groups.

The organs and viscera of representative third generation weanlings (F3B) sacrificed from the 0% (Control), 0.103% and 0.308% levels showed no gross abnormalities.

F. CONCLUSIONS:

Male and female albino rats were administered the test compound at levels of 0% (Control), 0.103%, 308% and 1.03% ad-mixed in the feed and evaluated for reproductive performance during 3 generations in each of 2 litters.

In the pre-mating period of the 1.03% P1 generation, a decrease in weight gain occurred in both sexes and food efficiency was decreased in the females.

The testes at 1.03% in the P1 generation were grossly atrophied as evidenced by a severe decrease in the organ weight and organ/body weight ratio. A decrease in the number of corpora lutea was indicative of a decrease in ovulation. No litters were produced at 1.03% when the test males were mated with test females.

P1 test females at 1.03% mated with control males resulted in a decrease in the number of litters and pup survival.

The ingestion of the test compound showed no compound related effects on the reproductive performance at 0.103% and 0.308% through 3 parental and 3 filial generations of 2 litters each.

NOEL for systemic and reproductive toxicity - 0.308%
LOEL for systemic and reproductive toxicity - 1.03%

Reviewed by Steven L. Malish, Ph.D. Steven L. Malish 11/6/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 10/30/91
Tox. Branch II, Section IV (H7509C)

009301

DATA EVALUATION REPORT

STUDY TYPE:	Primary Dermal Irritation Study (Guideline 81-5)
MRID NO.:	not issued
TEST MATERIAL:	Sodium tetraborate decahydrate
SYNONYMS:	Borax
SPONSOR:	U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA 92801-6794
TESTING FACILITY:	Food and Drug Research Laboratories, Inc. Route 17C, P.O. Box 107, Waverly, NY 14892
REPORT NUMBER:	Food and Drug Laboratories Report 8403A
TITLE OF THE REPORT:	Primary Dermal Irritation Study of 20 Mule TEAM^{R}, Lot USB-11-84 Sodium Tetraborate Decahydrate in New Zealand White Rabbits.
AUTHORS:	Elizabeth Reagan, A.A.S., Peter Becci, Ph.D.
REPORT ISSUED:	January 23, 1985
QUALITY ASSURANCE:	A quality assurance statement was provided.
CONCLUSIONS:	Under conditions of this study, a primary skin irritation score of 0.00 was obtained when 0.5 gm of the test substance, sodium tetraborate decahydrate, was applied to the unabraded skin of albino rabbits. The test article was considered to be a non-irritant.
CLASSIFICATION:	Core guideline. The study satisfies the guideline (81-5) for a "Primary Skin Irritation Study" in rabbIts.
TOXICITY CATEGORY:	-IV- (no irritaticn or slight edema) as per Federal Register/Vol. 49, No. 188/ Wednesday, September 26, 1984/Proposed Rule

0121

A. MATERIALS:

1. Test Compound:

Chemical:	sodium tetraborate decahydrate
Label:	20 Mule Team^{tm} Borax
Description:	data not available
Purity:	data not available
Stability:	data not available

2. Test Animals:

Species:	albino rabbit
Strain:	New Zealand White
Groups:	1 group of six male animals
Age:	young adult
Weight:	males - 2.13 to 2.45 kg
Source:	LaCrosse Industries, Inc., Schenectady, New York

B. STUDY DESIGN:

All animals were acclimated a minimum of five (5) days. Rabbit chow and water were available ad libitum.

On the day prior to dosing, the back of each rabbit was clipped free of fur with electric clippers. Care was taken not to abrade the skin.

The test article, moistened with saline, was applied to each of two (2) intact skin sites at a level of 0.5 gm per site. The test sites were on either side of the spinal column.

Following application, each test site was occluded with a one inch square gauze patch and covered with Blenderm^{R} tape.

Four (4) hours post dose the patches were removed. The exposure site was wiped with clean gauze to remove as much non-absorbed article as possible.

One-half hour after unwrapping the exposed sites were examined and scored separately for both erythema and edema on a grades scale of 0 to 4 in accordance with the Skin Reaction Code on page 4. The exposure sites were again examined and scored at 28, 52 and 76 hours after application.

The scoring interpretation is presented on page 5.

Animals were also observed for systemic toxicological effects.

C. DATA EVALUATION:

Irritation scores for the exposure sites for each animal were recorded separately at each time interval - 4.5, 28, 52 and 76 hours after test article application

for erythema/eschar and edema formation.

The cumulative values for each animal were then used to calculate the mean primary dermal irritation score for all animals in the study.

D. RESULTS:

All animals at all time periods had cummulative scores of 0.00 for erythema/ eschar and edema formation (Table 1). The test substance was calculated to have a mean primary irritation score of 0.00 and considered to be a non-irritant (see page 5).

No systemic toxicological effects were noted.

Table 1

Cumulative Irritation Scores

Observation	Time[a]	Score[b]
Erythema/ Eschar	4.5 hr	0.00
	28	0.00
	52	0.00
	76	0.00
Edema	4.5 hr	0.00
	28	0.00
	52	0.00
	76	0.00

[a] hours after application of the test substance

[b] see skin reaction code, p. 4.

E. SUMMARY:

A mean primary irritation scores of 0.00 was obtained when 0.5 gm of the test substance, sodium tetraborate decahydrate, was applied to the unabraded skin of albino rabbits. The test substance was considered to be a non-irritant,

Skin Reaction Code[a]

	Value
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

[a] Draize, J. H., Woodard, G. and Calvery, H. O., Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes, J. Pharm. & Exp. Ther. 82, 337 (1944).

Scoring Interpretation[a]

Mean Primary Irritation Score (Range of Values)	Description Rating
0.00 - 0.09	Non-Irritating
0.10 - 0.50	Minimally Irritating
0.51 - 1.50	Slightly Irritating
1.51 - 3.00	Mildly Irritating
3.01 - 5.00	Moderately Irritating
5.01 - 6.50	Severely Irritating
6.51 - 8.00	Extremely Irritating

[a] Modification of J. Soc. Cosmet. Chem., Vol. 13, No. 6, 1962, p. 281-289.

Reviewed by: Steven L. Malish, Ph.D. Steven L. Malish 10/23/91[1]
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E A Doyle 10/24/91
Head Section II, Tox. Branch IV (H7509C)

009301

DATA EVALUATION REPORT

STUDY TYPE:	Primary Eye Irritation Study (Guideline 81-4)
MRID:	not issued
TEST MATERIAL:	Sodium tetraborate decahydrate
SYNONYMS:	Borax
SPONSOR:	U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA 92801-6794
TESTING FACILITY:	Food and Drug Research Laboratories, Inc. Route 17C, P.O. Box 107 Waverly, NY 14892
STUDY NO.	FDRL Study No. 8403A
TITLE OF REPORT:	Primary Eye Irritation Study of 20 MULE TEAM^{tm}, Lot No. USB-11-84 Sodium Tetraborate Decahydrate in New Zealand White Rabbits.
AUTHORS:	Elizabeth L. Reagan, A.A.S. and Peter J. Becci, Ph.D.
REPORT ISSUED:	February 8, 1985
QUALITY ASSURANCE:	Quality assurance documentation was provided.
CONCLUSIONS:	Under conditions of this study, severe irritation was evidenced in a primary eye irritation study performed without a washout in albino rabbits using sodium tetraborate decahydrate.
CLASSIFICATION:	Core - guideline. The study satisfies the requirements (81-4) for a "Primary Eye Irritation Study."
TOXICITY CATEGORY:	I (Corrosive); corneal opacity not reversible within 7 days (40 CFR 156.10, 79).

A. MATERIALS:

1. Test Compound:

Chemical:	Sodium tetraborate decahydrate
Label:	20 Mule Team, Lot No. USB-11-84, Sodium tetraborate decahydrate, 12/84.
Description:	powder
Stability:	not given
Purity:	not given

2. Test Animals

Species:	albino rabbit
Strain:	New Zealand White
Groups:	One group of 6 rabbits
Age:	not available
Sex:	1 male, 5 females
Weight:	male 2.44 kg; females 2.32 - 2.71 kg.
Source:	LaCrosse Industries, Inc. Schenectady, NY

B. STUDY DESIGN:

Animals were acclimated a minimum of 5 days and examined daily. Food and water were provided ad libitum.

On the day prior to dosing, the eyes of each of 6 rabbits were examined with sodium fluorescein and an ultraviolet lamp. Only those animals whose eyes were free of irritation and corneal lesions were used.

One hundred milligrams (100 mg) of the test article was instilled as a solid in one (1) eye of each rabbit by gently pulling the lower lid away from the eyeball to form a cup into which the test article was deposited. The lids were then held together for one second and the animal was returned to its cage.

With the untreated eye serving as a control, the eyes were examined and the ocular response graded for each animal at 1, 24, 48 and 72 hours post-treatment using the Draize Scale for the scoring of ocular lesions (p. 7).

If the injury persisted, the eyes were further examined and scored at 4 days and every 3 days, thereafter, until all signs of reversible irritation subsided or until day 21.

Classification of the test article was based on the one animal that elicited the most severe ocular response (p. 8).

C. RESULTS:

The results following application of the test material to the eyes of albino rabbits were presented in Table 1 and 2.

The treated eyes from all 6 rabbits showed mild iritis and mild to moderate conjunctitis characterized by erythema, edema and discharge. The mucous membrane of the eyelid appeared blistered. Burned and/or necrotic areas of the conjunctiva were noted during the first 10 days of the observations.

Mild to moderate corneal opacity was also noted in all 6 rabbits. Corneal changes disappeared by day 10.

All signs diminished in intensity with time (Table 1, 2).

The corresponding control eye of all animals was unremarkable throughout the study.

Based on the scoring and classification systems presented on p. 7 and 8, the test article was rated as a severe irritant.

D. CONCLUSIONS: Under conditions of this study, severe irritation was evidenced in a primary eye irritation study performed without a washout in albino rabbits using sodium tetraborate decahydrate.

Table 1

Individual Rabbit Eye Irritation Scores[a,b]

Rabbit Number (sex)	Time Days	Cornea Opacity (area)	Iris	Conjunctiva Ery.	Conjunctiva Chemosis	Conjunctiva Dis.	Score***
044951 (M)	1 hr	0 (0)	1	2	4*	3	23
	24	1 (2)	1	2+	2*	2	29
	48	1 (2)	1	2+	2*	1	25
	72	1 (1)	1	2+	2*	1	20
	4 days	1 (1)	1	2+	2*	2	22
	7	1 (1)	1	2+	2	2	22
	10	0 (0)	1	1+	2	2	15
	21	0 (0)	0	0	1	0	2
044977 (F)	1 hr	0 (0)	1	2	3*	3	21
	24	1 (4)	1	2+	3*	3	41
	48	1 (3)	1	2+	2*	2	32
	72	1 (1)	1	2+	2*	2	22
	4 days	1 (1)	1	2+	2	2	22
	7	0 (0)	1	1+	1	1	11
	10	0 (0)	0	0	0	0	0
044978 (F)	1 hr	0 (0)	1	2	4*	3	23
	24	1 (3)	1	2+	3*	2	34
	48	1 (1)	1	1+	2*	1	18
	72	1 (1)	1	1+	2*	1	18
	4 days	0 (0)	1	1+	2	1	13
	7	0 (0)	0	0+	1	0	2
	10	0 (0)	0	0	0	0	0

Table 1 (Cont.)

Individual Rabbit Eye Irritation Scores

Rabbit Number (sex)	Time Days	Cornea Opacity (area)	Iris	Conjunctiva Ery.	Conjunctiva Chemosis	Conjunctiva Dis.	Score
044980 (F)	1 hr	0 (0)	1	2	3*	2	19
	24	1 (4)	1	2+	3*	2	39
	48	1 (3)	1	2+	2*	2	32
	72	1 (2)	1	2+	2	1	25
	4 days	1 (1)	1	2+	2	1	20
	7	1 (1)	1	2+	1	1	18
	10	0 (0)	1	0	1	0	7
	13	0 (0)	0	0	0	0	0
044983 (F)	1 hr	0 (0)	1	2	4*	3	23
	24	1 (2)	1	2	3*	3	36
	48	1 (1)	1	1	2*	2	20
	72	1 (1)	1	1	2*	2	20
	4 days	1 (1)	1	1	2	2	20
	7	1 (1)	1	1	1	0	14
	10	0 (0)	0	0	0	0	0
044986 (F)	1 hr	0 (0)	1	2	4*	2	21
	24	1 (1)	1	2	4*	3	28
	48	1 (1)	1	2	3*	3	26
	72	0 (0)	1	2	3*	2	19
	4 days	0 (0)	1	2	3*	2	19
	7	0 (0)	1	2	2	1	15
	10	0 (0)	1	0	1	0	7
	13	0 (0)	0	0	0	0	0

[a] Adapted from p. 13 thru 15 of the original report.

[b] see attached scale for scoring ocular lesions (p. 7).
* Mucous membranes of the eyelid blistered; burned areas throughout the conjunctiva.
+ Necrotic areas of conjunctiva
Ery. = erythema, Dis. = discharge

*** Total score is the sum of the following 3 subtotals
(a) degree of opacity x area involved x 5
(b) iris scores x 5
(c) sum of scores for erythema, chemosis and swelling x 2

Total possible score = 110.

Table 2

Mean Irritation Scores[a]

Time	Score*
1 hr	21.7
24	34.5
48	25.5
72	20.7
4 Days	19.3
7	13.7
10	4.8
13	2.2
16	1.0
19	0.3
21	0.3

[a] Adapted from p. 6 of the original report.
* See attached scale for scoring ocular lesions (p. 7).

Scale for Scoring Ocular Lesions

(1) Cornea

(A) Opacity-degree of density (area most dense taken for reading)

No Opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

(B) Area of cornea involved

One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

A X B X 5 Total Maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A X 5 Total Maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) X 2 Total Maximum = 20

---

* Draize, John H., Woodard, Geoffrey, and Calvery, Herbert O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes," J. Pharm. & Exp. Ther. 82, 377 (1944).

Classification of Test Article Based on Eye Irritation Properties[a,b]

Inconsequential Irritation - Exposure of the eye to the test article under the specified conditions causes no significant ocular changes. No staining with fluorescein can be observed. Any changes that occur clear within 24 hours and are no greater than those caused by isotonic saline under the same conditions.

Moderate Irritation - Exposure of the eye to the test article under the specified conditions causes minor, superficial, and transient changes of the cornea, iris, or conjunctiva as determined by external examination with fluorescein staining. The appearance at the 24-hour or subsequent grading of any of the following changes is sufficient to characterize a response as moderate irritation: opacity of the cornea (other than a slight dulling of the normal luster), hyperemia of the iris, or swelling of the conjunctiva. Any changes that are seen clear within 7 days.

Substantial Irritation - Exposure of the eye to the test article under the specified conditions causes significant injury to the eye, such as loss of the corneal epithelium, corneal opacity, iritis (other than a slight injection), conjunctivitis, pannus, or bullal. The effects clear within 21 days.

Severe Irritation or Corrosion - Exposure of the eye to the test article under the specified conditions results in the same types of injury as in the previous category and in significant necrosis or other injuries that adversely effect the visual process. Injuries persist for 21 days or more.

a) Principles and Procedures for Evaluating the Toxicity of Household Substances, Washington, 1977, National Academy of Sciences, committee for the Revision of NAS Publication 1138.

b) Classification of the test article is based on the one animal that elicits the most severe ocular response.

Reviewed by: Steven L. Malish, Ph.D. Steven L. Malish 11/1/91
Tox. Branch II, Section II (H7509C)
Secondary Reviewer: Elizabeth A Doyle, Ph.D. Elizabeth A Doyle 10/29/91
Head Section II, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Primary Eye Irritation Study (81-4)
MRID:	not issued
TEST MATERIAL:	Sodium tetraborate decahydrate
SYNONYMS:	Borax
SPONSOR:	U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, Anaheim, CA 92801-6794
TESTING FACILITY:	Hill Top Research, Inc., Miamiville, Ohio , 45147
TITLE OF REPORT:	Acute Application of Sample 713-123B to the Eyes of Rabbits
AUTHORS:	Carol L. Estep, B.S., Richard H. Teske, D.V.M.
REPORT NO.:	S-152B
REPORT ISSUED:	May 16, 1968
QUALITY ASSURANCE:	Quality assurance documentation was not provided.
CONCLUSIONS:	Under condition of this study, sodium tetraborate decahydrate was considered to be an eye irritant in albino rabbits when no irrigation (wash out) procedure was used after test compound administration. Although the irritant effects decreased during the study, minimal corneal opacity was still evident at the day 14 termination. Minimal irritation occurred in animals whose eyes were irrigated after administration of the test article. Eye irritation in these animals was judged unremarkable at the 14-day study termination.
CLASSIFICATION:	Core - minimum. This study satisfies the requirements (81-4) for a "Primary Eye Irritation Study". The study was deficient in the fact that, 1) the method of eye examination was not stated and 2) the duration of the observation period was not long enough to assess the reversibility of the eye effects.

TOXICITY CATEGORY: I Corrosive; corneal opacity not reversible within 7 days (40 CFR 156.10, 79, 1989). This category was based on animals that did not have the eyes irrigated (wash out) after test compound administration.

A. MATERIALS:

1. Test Compound:

Chemical:	Sodium tetraborate decahydrate
Label:	713-123B
Description:	white crystalline solid, no odor
Stability:	not given
Purity:	not given

2. Test Animals:

Species:	albino rabbit
Strain:	not given
Groups:	Two (2) groups of 6 animals each
Age/Sex	not given
Weight:	not given
Source:	not given

B. STUDY DESIGN: One hundred milligrams (100 mg) of the sample was instilled as a solid into the right eye of each of 12 albino rabbits. The left eye was untreated and served as the control. Six of the 12 animals received no further treatment.

The treatment eye of the remaining 6 rabbits were irrigated with 20 ml of lukewarm water approximately 4 seconds after application of the test material.

Each rabbit was examined 1 hour after application of the test material and once daily, thereafter, until any observed eye irritation had subsided completely, as judged by two consecutive daily negative readings or for a maximum of 14 days. The rabbits were observed both for eye irritation and for gross signs of systemic toxicity from mucous membrane absorption of the test material.

Scoring of the irritative effects were according to the method of Draize (Lehman, A.J. et al, Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics; Association of Food and Drug Officials of the U.S.; Austin, Texas, 1959). The scores were weighted and combined into a total composite score (see attached scoring scale, p. 7).

C. RESULTS: The results following application of the test material to the eyes of albino rabbbits were presented in Table 1 for animals whose eyes were not irrigated and in Table 2 for animals whose eyes were irrigated.

The treated eyes from all 6 rabbits in the non-irrigated groups showed mild iritis and mild to moderate conjunctivitis characterized by erythema,

edema and discharge when compared to the respective control (Table 1).

Mild to moderate corneal opacity was also noted in 3 rabbits, one of which also exhibited pannus (Table 1).

The irritative effects diminished in intensity and in 4 animals completely subsided by the end of the 14 day observation period. One of the 2 remaining rabbits showed minimal corneal opacity and the second animal showed mild conjunctival erythema on the 14th day. Controls appeared unremarkable (Table 1).

In rabbits whose eyes were irrigated following application of the test substance, mild erythema was noted in 6 of 6 animals, mild edema in 5 of 6 rabbits and mild to moderate discharge in 4 of 6 rabbits. No effects were seen in the control animals (Table 2).

Irritative effects diminished in intensity and completely subsided within 2 to 9 days. No corneal opacity or iritis was noted in any rabbit in the irrigated group when compared to the controls.

There was no indication in any rabbit of systemic toxicity from mucous membrane absorption of the test material.

D. SUMMARY: The acute irritative effects of sodium tetraborate decahydrate was evaluated in both irrigated and non-irrigated groups of 6 albino rabbits each.

In the non-irrigated eyes, the test compound produced mild iritis and mild to moderate conjunctivitis in 6 our of 6 rabbits. Mild to moderate corneal opacity was noted in 3 rabbits, one of which also showed pannus formation. The signs diminished in intensity with time.

In rabbits whose eyes were irrigated following the test compound administration, mild conjunctivitis evidenced by edema, erythema and discharge were noted. These signs diminished in intensity and completely subsided within 2 to 9 days following application. There was no signs of of corneal opacity or iritis in the irrigated group.

There was no indication of systemic toxicity from mucous membrane absorption of the test material.

E. CONCLUSIONS: Under condition of this study, sodium tetraborate decahydrate was considered to be an eye irritant in albino rabbits when no irrigation (wash out) procedure was used after test compound administration. Although the irritant effects

decreased during the study, minimal corneal opacity was still evident at the day 14 termination.

Minimal irritation occurred in animals whose eyes were irrigated after administration of the test article. Eye irritation in these animals were judged unremarkable at the 14-day study termination.

Table 1

Eye Irritation Scores in Albino Rabbits without Irrigation[a]

Rabbit Number	Time Days	Cornea Opacity (area)	Iris	Conjunctiva Ery.*	Conjunctiva Swell.	Conjunctiva Dis.	Score[b]
13	1 hr	0 (0)	0	1	3	2	12
	1 day	0 (0)	1	1	2	1	13
	2	0 (0)	1	1	1	1	11
	4	0 (0)	1	1	1	0	9
	8	0 (0)	0	1	1	0	4
	14	0 (0)	0	1	0	0	2
14	1 hr	0 (0)	0	1	3	2	12
	1 day	0 (0)	1	1	2	2	15
	2	0 (0)	1	1	1	0	9
	4	0 (0)	0	1	1	0	4
	8	0 (0)	0	1	1	0	4
	14	0 (0)	0	0	0	0	0
15	1 hr	1 (1)	0	1	3	2	17
	1 day	1 (1)	1	1	1	1	16
	2	1 (1)	1	1	1	1	16
	4	1 (2)	1	1	1	1	21
	8	1+(3)	0	1	1	1	21
	14	1+(1)	0	0	0	0	5
16	1 hr	0 (0)	0	1	2	2	10
	1 day	1 (1)	1	1	1	2	18
	2	1 (1)	1	1	1	1	16
	4	1 (1)	0	1	1	1	11
	8	0 (0)	0	1	0	0	2
	14	0 (0)	0	0	0	0	0
17	1 hr	0 (0)	0	1	3	3	14
	1 day	1 (1)	1	1	2	2	20
	2	1 (1)	0	1	1	2	11
	4	0 (0)	0	1	1	1	6
	8	0 (0)	0	1	1	0	4
	14	0 (0)	0	0	0	0	0
18	1 hr	0 (0)	0	1	2	2	10
	1 day	0 (0)	1	1	1	1	11
	2	0 (0)	0	2	1	0	6
	4	0 (0)	0	1	1	0	4
	8	0 (0)	0	1	0	0	2
	14	0 (0)	0	0	0	0	0

[a] adopted from Table 1 (p. 27-28) in the original report
[b] see attached scale for scoring ocular lesions (p. 6)
* erythema concentrated in the lower conjunctiva.
\+ Pannus
Ery. = erythema; Swell. = swelling; Dis. = discharge

Table 2

Eye Irritation Scores in Albino Rabbits After Irrigation[a]

Rabbit	Time	Cornea	Iris	Conjunctiva			Score[b]
Number	Days	Opacity (area)		Ery.	Swell.	Dis.	
19	1 hr	0 (0)	0	1	1	1	6
	1 day	0 (0)	0	1	0	0	2
	2	0 (0)	0	0	0	0	0
	4	0 (0)	0	0	0	0	0
20	1 hr	0 (0)	0	1	1	0	4
	1 day	0 (0)	0	1	1	0	2
	2	0 (0)	0	0	0	0	0
	4	0 (0)	0	0	0	0	0
21	1 hr	0 (0)	0	1	1	2	8
	1 day	0 (0)	0	1	0	1	4
	2	0 (0)	0	0	0	0	0
	4	0 (0)	0	0	0	0	0
22	1 hr	0 (0)	0	1	3	2	12
	1 day	0 (0)	0	1	1	1	6
	2	0 (0)	0	1	1	0	4
	4	0 (0)	0	1	0	0	2
	8	0 (0)	0	0	0	0	0
23	1 hr	0 (0)	0	1	0	0	2
	1 day	0 (0)	0	1	0	0	2
	2	0 (0)	0	1	0	0	2
	4	0 (0)	0	0	0	0	0
24	1 hr	0 (0)	0	1	2	2	10
	1 day	0 (0)	0	1	1	1	6
	2	0 (0)	0	1	1	1	6
	4	0 (0)	0	1	0	0	2
	10	0 (0)	0	0	0	0	0

[a] Adopted from Table 2 (p. 29) in the original report
[b] see attached scale for scoring ocular lesions (p. 6)
Ery. = erythema; Swell. = swelling; Dis. = discharge

Scale for Scoring Ocular Lesions

(1) Cornea

(A) Opacity-degree of density (area most dense taken for reading)

No Opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

(B) Area of cornea involved

One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

A X B X 5 Total Maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A X 5 Total Maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) X 2 Total Maximum = 20

Reviewed by: Steven L. Malish, Ph.D. Steven L. Malish 12/10/91
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 12/13/91
Tox Branch II, Section IV (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Dermal Toxicity Study - Rabbit (81-2)
MRID:	not issued
TEST MATERIAL:	Sodium tetraborate decahydrate
SYNONYMS:	Borax
SPONSOR:	U.S. Borax Research Corporation - Subsidiary of United States Borax & Chemical Corporation, 412 Crescent Way Anaheim, CA 92801-6794
TESTING FACILITY:	Food & Drug Research Laboratories, Inc. Route 17C, P.O. Box 107 Waverly, NY 14892
TITLE OF REPORT:	Acute Dermal Toxicity Study of 20 MULE TEAM, Lot. No. USB-11-84 Sodium Tetraborate Decahydrate in New Zealand White Rabbits
AUTHORS:	Elizebeth L. Reagan, A.A.S.; Peter J. Becci, Ph.D.
REPORT NO.	FDRL Study No. 8403A
REPORT ISSUED:	February 20, 1985
QUALITY ASSURANCE:	Quality Assurance documentation was provided.
CONCLUSIONS:	All animals survived the 15 day post-application observation period. Based on this result, the acute dermal LD_{50} of the test article was considered to be greater than 2.0 gm/kg of body weight.
CLASSIFICATION:	Core - guideline This study satisfies the guideline requirements 81-2 for an "Acute Dermal Toxicity Study - Rabbits".
TOXICITY CATEGORY:	-III- (Dermal LD_{50} greater than 2.0 thru 5.0 gm/kg) as per the Federal Register, Vol 49, No. 188, September 28, 1984, Proposed Rule.

A. MATERIALS:

1. Test Compound: Chemical: Sodium tetraborate decahydrate
Lot: USB-11-84
Label: not available (NA)
Description: NA
Purity: NA
Stability: NA

2. Test Animals: Species: rabbit
Strain: New Zealand White
Groups: One (1) group of ten (10) animals
Age: NA
Weight: NA
Source: Sgarlat's Rabbitry, Harveys Lake, PA

Study Design: All animals were acclimated for a minimum of 5 days. Rabbits were given chow and water ad libitum.

On the day prior to dosing, the back of each rabbit was clipped free of fur with an electric clipper. Body weights were recorded prior to treatment, on day 8 and day 15.

The test article was applied "neat" as a single dose of 2.0 gm/kg of body weight to the intact skin under an occlusive binder. The binder consisted of a layer of plastic wrap and a stockinette sleeve secured in place with masking tape.

After an exposure period of 24 hours, the binders were removed. The exposure sites were gently wiped with clean gauze to remove as much non-absorbed test article as possible. The animals were then observed on the day of dosing and twice (2) daily, thereafter, for the remainder of the study. All external signs of toxicity or pharmacological effects were noted.

All animals sacrified at termination were subjected to gross necropsy.

B. RESULTS: Weight Data:

Animals of both sexes gained weight during the 15 day observation period.

Table 1

Mean Body Weight Data
(Kg)

Day	Males	Females
0	2.20	2.11
8	2.37	2.35
15	2.38	2.60

Observations:

The following were noted throughout the study (Table 2).

Table 2

Daily Observations

Signs	Observed/Total Males	Females
anorexia	1/5	0/5
decreased activity	1/5	0/5
diarrhea	2/5	0/5
soft stools	2/5	0/5
mucus (seen in tray)	1/5	0/5
nasal discharge	0/5	1/5

Necropsy:

Animals were considered to be unremarkable at necropsy.

C. SUMMARY:

Sodium tetraborate decahydrate, Lot No. USB-11-84 was evaluated for acute dermal toxicity in male and female New Zealand White rabbits. The test article was applied to each of ten rabbits at a single dose of 2.0 gm/kg of body weight and observed for symptomatology and mortality for 15 days.

All animals survived the 15 day post-application period. Based on this result, the acute dermal LD_{50} was considered to be greater than 2.0 gm/kg of body weight.

Observations noted in male animals included anorexia, decrease activity, diarrhea, soft stools and excess mucous (seen in tray).

Both sexes gained weight throughout the study.

Animals were unremarkable at necropsy.

D. CONCLUSIONS:

All animals survived the 15 day post-application observation period. Based on this result, the acute dermal LD_{50} of the test article was considered to be greater than 2.0 gm/kg of body weight.