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

AUG 0 8 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 011001, Boric Acid: Mutagenicity Studies.

Tox. Chem. No.: 109
Project No.: 2-1441
Record No. : S411262

FROM: Melba S. Morrow, D.V.M. MSM 7/28/92
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Mario Fiol, PM 72
Registration Division (H7505C)

THRU: Karl Baetcke, Ph.D.
Chief, Toxicology Branch I
Health Effects Division (H7509C) 8/3/92

Sponsor: U.S. Borax

CONCLUSIONS: We have completed our review of the four studies filed to satisfy the requirements for mutagenicity and have the following comments:

MRID 420389-01; Salmonella typhimurium/ Mammalian Microsome Mutagenicity Assay (Ames)

Boric acid was neither cytotoxic or genotoxic in S. typhimurium when tested at concentrations of 10, 50, 100, 500, 1000 and 2500 ug/plate. The study is acceptable and satisfies the requirements (84-2) for mutagenicity (gene mutation).

MRID 420389-02; Gene Mutations in Cultured Mammalian Cells

Doses of boric acid (1200, 1700, 2450, 3500 and 5000 ug/mL) did not induce a mutagenic response with or without S9 activation. The test material was tested over an appropriate concentration range as evidenced by the marginal cytotoxicity that was observed at 5000 ug/mL (without S9 activation) and at 3500 to 5000 ug/mL (with activation). The study is acceptable and satisfies the guideline criteria (84-2a) for mutagenicity (gene mutation).

MRID 420389-03; <u>In vitro</u> Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

This study is unacceptable. The study may be upgraded once primary data (cytoplasmic and gross nuclear grain counts) are provided to support the study author's conclusions. The author concluded that the apparent increases in net nuclear grains and the percent of cells in repair were due to decreased cytoplasmic grain counts that may have resulted from cytotoxicity and not from increased DNA repair. Additionally, the author stated that there was no increase in the level of DNA synhthesis above those levels that were observed in negative controls. (Guideline 84-4, Other genotoxic effects)

MRID 420389-04; <u>In vivo</u> Micronucleus Assay in Mice

Gavage administration of boric acid to male and female Swiss mice for two consecutive days did not result in a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow cells. The doses tested were 900, 1800 and 3500 mg/kg. There was no evidence of toxicity to the animals or of cytotoxicity to the target tissue. The study is acceptable and satisfies the guideline requirements (84-2b) for a mutagenicity (genetic effects) study.

Copies of the DERs are provided for your reference.

DOC930082

FINAL

DATA EVALUATION REPORT

BORIC ACID

Study Type: Mutagenicity: Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer _____ Date _____
Nancy E. McCarroll, B.S.

Independent Reviewer _____ Date _____
Lynne Haber, Ph.D.

QA/QC Manager _____ Date 7/2-7/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-107
Clement Number: 93-49
Project Officer: James Scott

GUIDELINE § 84: MUTAGENICITY SALMONELLA

MUTAGENICITY STUDIES

EPA Reviewer: Melba Morrow, ~~Ph.D.~~ D.V.M. Signature: Melba Morrow
Review Section II, Date: 7/23/92
Toxicology Branch I/HED (H-7509C)

Acting EPA Section Head: Joycelyn Stewart, Ph.D. Signature: JS
Review Section II, Date: 7/24/92
Toxicology Branch I/HED (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay

EPA IDENTIFICATION Numbers:

Tox Chem. Number:

CASWELL Number: 109

MRID Number: 420389-01

TEST MATERIAL: Boric acid

SYNONYMS/CAS No.: None provided/10043-35-3

SPONSOR: U.S. Borax and Chemical Corp., Anaheim, CA

STUDY NUMBER: 2389-A200-91

TESTING FACILITY: SRI International, Inc., Menlo Park, CA

TITLE OF REPORT: Salmonella/Microsome Plate Incorporation Assay of Boric Acid

AUTHOR: K.R. Steward

REPORT ISSUED: August 12, 1991

CONCLUSIONS--EXECUTIVE SUMMARY: In two independently performed assays, boric acid over a concentration range of 10 to 2500 µg/plate was neither cytotoxic nor mutagenic in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, or TA100 in either the absence or presence of 4 and 10% S9 in the S9 cofactor mix. The study author reported that the test material was soluble up to 50 mg/mL in distilled water; therefore, the maximum dose recommended by Guideline for noncytotoxic, soluble compounds (5000 µg/plate) should have been tested. However, the results were in good agreement with the published finding that boric acid, prepared in water up to 1820 µg/plate or prepared in

dimethyl sulfoxide up to 10,000 µg/plate, was not cytotoxic or genotoxic in S. typhimurium.[1] We conclude, therefore, that the study provided acceptable evidence that boric acid is negative in this bacterial gene mutation assay.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.2a) for genetic effects Category I, Gene Mutations.

A. MATERIALS:

1. Test Material: Boric acid (Granular technical)

Description: White crystals
Identification number: Code date: 1D19D
Purity: >99.0%
Receipt date: April 25, 1991
Stability: Stable at room temperature
Contaminants: None listed
Solvent used: Sterile, deionized water (DH_2O)
Other provided information: The test material was stored at room temperature. Test solutions used in the study were prepared immediately prior to use. The report also indicated that boric acid was soluble in DH_2O at 50 mg/mL.

2. Control Materials:

Negative: None.

Solvent/concentration: DH_2O; not reported but assumed to be 50 µL/plate

Positive:

Nonactivation:

Sodium azide	5	µg/plate TA1535, TA100
2-Nitrofluorene	5	µg/plate TA1538, TA98
9-Aminoacridine	50	µg/plate TA1537

Activation:

2-Aminoanthracene	4	µg/plate TA1535, TA1537
	2	µg/plate TA1538, TA98, TA100

3. Activation: S9 derived from male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

[1]Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E. (1983). Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen 5:3-142.

The S9 liver homogenate (lot number 0327) was purchased from Molecular Toxicology, Inc., Annapolis, MD and was checked for sterility, protein content, and the ability to metabolize a known promutagen. The protein content was reported to be 37.8 mg/mL. The S9 cofactor mix was prepared to contain 4 or 10% S9; components are listed below:

S9 mix composition:

Component	Amount/50 mL	
	4% S9-mix	10% S9-mix
0.4 M $MgCl_2$ 1.65 M KCl	1.00 mL	1.00 mL
1 M Glucose 6-phosphate	0.25 mL	0.25 mL
0.1 M NADP	2.00 mL	2.00 mL
0.2 M Sodium phosphate buffer, pH 7.4	25.00 mL	25.00 mL
Sterile H_2O	19.75 mL	16.75 mL
S9	2.00 mL	5.00 mL

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538
list any others:

Test organisms were properly maintained: Yes.
Checked for appropriate genetic markers (rfa mutation, R factor): Yes.

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Six doses (10, 50, 100, 500, 1000, and 2500 µg/mL) were evaluated in the absence and presence of 4% S9 using strain TA100. Three plates were prepared per dose, per condition; positive and negative controls were also assayed.

(b) Mutation assay:

(1) Initial: Six doses (10, 50, 100, 500, 1000, and 2500 µg/plate +/-4% S9); triplicate plates were prepared per dose, per condition.

(2) Confirmatory assay: As above; however, the percentage of S9 in the cofactor mix was increased to 10%.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: __x__ Standard plate test
_____ Pre-incubation (_____) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe)

2. Cytotoxicity/Mutation Assay: Similar procedures were used for the preliminary cytotoxicity and mutation assays. Mixtures containing 2-mL volumes of molten top agar, 0.05 mL of an overnight broth culture of the appropriate tester strain, 0.5 mL of buffer, and the appropriate volume of the solvent, positive control, or test dose were poured over minimal glucose agar plates. For the S9-activated test, 0.5 mL of the appropriate S9 cofactor mix replaced the phosphate buffer; tester strains and test and control solutions were added as described and mixtures were poured over minimal medium. Plates were incubated at 37°C for ~48 hours and were either immediately scored for revertant colonies or were refrigerated and subsequently counted. Means and standard deviations were calculated and the condition of the background lawn of growth was reported. Compounds testing negative in the presence of 4% S9 were reevaluated in the confirmatory assay using 10% S9. The sterility of the test material, S9 mix, and buffer were determined.

3. Evaluation Criteria:

   (a) Assay acceptability: The assay was considered valid if the following criteria were met: (1) the mutant colony counts for the solvent control were within unspecified acceptable limits; (2) the positive controls induced a positive response; (3) the presence of the appropriate genetic markers was verified for each strain; and (4) a sufficient number of noncytotoxic doses were tested.

   (b) Positive response: The test material was considered positive if it caused a reproducible and dose-related increase in the mutant colony counts of one or more strains over at least three consecutive doses.

4. Protocol: See Appendix A

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Results of the preliminary cytotoxicity assay indicated that boric acid was not cytotoxic in strain TA100 at any dose in either the presence or absence of 4% S9. Based on these findings, the mutation assay was conducted with a dose range of 10 to 2500 µg/mL.

2. Mutation Assay: Representative results from the initial and confirmatory mutation assays conducted with boric acid are presented in Tables 1 and 2, respectively. As shown, the test material was neither cytotoxic nor mutagenic at any dose in any strain without S9 activation or with 4 or 10% S9 in the S9 cofactor mix. By contrast the positive controls induced the expected response in the appropriate tester strains without and with the two concentrations of S9.

   Based on the overall results, the study author concluded that boric acid was not mutagenic in this bacterial test system.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with Boric Acid

Substance	S9 Activation[b]	Dose/plate	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Distilled water	-	50 µL	17±2	8±4	35±4	27±3	109±15
	+	50 µL	11±3	9±4	33±5	37±12	118±12
Positive Controls[c]							
Sodium azide	-	5 µg	1021±3	--	--	--	1007±34
2-Nitrofluorene	-	5 µg	--	--	1059±26	693±15	--
9-Aminoacridine	-	50 µg	--	214±42	--	--	--
2-Aminoanthracene	+	2 µg	--	--	990±46	1313±16	1359±109
	+	4 µg	213±2	360±23	--	--	--
Test Material							
Boric acid	-	2500 µg[d]	16±1	12±4	30±11	31±3	118±14
	+	2500 µg[d]	10±6	12±4	30±1	25±5	119±24

[a]Means and standard deviations of counts from triplicate plates
[b]4% S9
[c]Positive controls were dissolved in dimethyl sulfoxide (DMSO); revertant counts were presented for DMSO, but the results for the test material solvent control (distilled water) were selected as representative.
[d]Highest assayed dose; results for lower levels (10, 50, 100, 500, and 1000 µg/plate +/- S9) did not suggest a mutagenic response.

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with Boric Acid

Substance	S9 Activation[b]	Dose/plate	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Distilled water	-	50 µL	19±0	9±3	27±5	26±4	120±26
	+	50 µL	15±2	9±1	29±6	28±4	123±18
Positive Controls[c]							
Sodium azide	-	5 µg	1009±11	--	--	--	1081±13
2-Nitrofluorene	-	5 µg	--	--	1505±112	868±62	--
9-Aminoacridine	-	50 µg	--	182±29	--	--	--
2-Aminoanthracene	+	2 µg	--	--	549±32	459±36	763±83
	+	4 µg	232±3	142±8	--	--	--
Test Material							
Boric acid	-	2500 µg[d]	15±4	6±2	22±8	20±7	106±14
	+	2500 µg[d]	13±1	7±2	24±6	27±6	102±12

[a]Means and standard deviations of counts from triplicate plates
[b]10% S9
[c]Positive controls were dissolved in dimethyl sulfoxide (DMSO); revertant counts were presented for DMSO, but the results for the test material solvent control (distilled water) were selected as representative.
[d]Highest assayed dose; results for lower levels (10, 50, 100, 500, and 1000 µg/plate +/- S9) did not suggest a mutagenic response.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that over the range of doses selected for study (10 to 2500 µg/mL +/- 4 or 10% S9), boric acid was neither cytotoxic nor mutagenic. No rationale was presented for limiting the highest dose to 2500 µg/plate. Since the test material was reported to be soluble in DH_2O up to 50 mg/mL, the high dose recommended by Guideline for noncytotoxic soluble compounds (5000 µg/plate) could have been easily tested. Nevertheless, the results of this study are in good agreement with the published finding of an interlaboratory investigation showing that boric acid prepared in H_2O up to 1820 µg/plate or prepared in DMSO up to 10,000 µg/plate was not cytotoxic or genotoxic in S. typhimurium strains TA1535, TA1537, TA98, or TA100.[2] We conclude, therefore, that the study provided acceptable evidence that boric acid is not a mutagen in the S. typhimurium/mammalian microsome plate incorporation assay. In addition, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the responses induced by the positive controls both with and without S9 activation.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 9, 1991).

F. CBI APPENDIX: Appendix A, Protocol, CBI Appendix pp. 2-8; Appendix B, Materials and Methods, CBI pp. 8-16.

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.2a) for genetic effects Category I, Gene Mutations.

[2]Haworth, et al. (1983). Environ. Mutagen. 5:3-142.

APPENDIX A

PROTOCOL
CBI Appendix pp. 2-8

RID 5117-93 BORIC ACID
TOX REVIEW 009666

Page_____ is not included in this copy.

Pages 12 through 28 are not included.

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____ Identity of product inert ingredients.

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DOC 930083
FINAL
009661

DATA EVALUATION REPORT

BORIC ACID

Study Type: Mutagenicity: Gene Mutation in Cultured Mammalian Cells
(Mouse Lymphoma Cells)

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer _____ Date _____
Nancy E. McCarroll, B.S.

Independent Reviewer _____ Date _____
Lynne Haber, Ph.D.

QA/QC Manager _____ Date 7/22/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-107
Clement Number: 93-50
Project Officer: James Scott

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

MUTAGENICITY STUDIES

EPA Reviewer: Melba Morrow, ~~Ph.D.~~ D.V.M
Review Section II,
Toxicology Branch I/HED (H-7509C)

Signature: Melba Morrow
Date: 7/25/92

EPA Section Head: Joycelyn Stewart, Ph.D.
Review Section II,
Toxicology Branch I/HED (H-7509C)

Signature:
Date:

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Gene mutation in cultured mammalian cells (mouse lymphoma cells)

EPA IDENTIFICATION Numbers:

Tox Chem. Number:

Caswell Number: 109

MRID Number: 420389-02

TEST MATERIAL: Boric acid

SYNONYMS/CAS No.: None provided/10043-35-3

SPONSOR: U.S. Borax and Chemical Corp., Anaheim, CA

STUDY NUMBER: 2389-G300-91

TESTING FACILITY: SRI International, Menlo Park, CA

TITLE OF REPORT: Mouse Lymphoma Cell Mutagenesis Assay ($tk^{+/-}$ - $tk^{-/-}$) of Boric Acid

AUTHOR: C.J. Rudd

REPORT ISSUED: August 23, 1991

CONCLUSIONS--EXECUTIVE SUMMARY: Doses of boric acid ranging from 1200 to 5000 µg/mL with and without S9 activation did not induce a mutagenic response in two independently performed mouse lymphoma forward mutation assays. Doses of 5000 µg/mL -S9 and 3500 and 5000 µg/mL +S9 were marginally cytotoxic. The findings indicated, therefore, that boric acid was tested over an appropriate concentration range and found to be nonmutagenic in this test system.

STUDY CLASSIFICATION: Acceptable. The study satisfies guideline requirement (§84.2a) for genetic effects Category I, Gene Mutations.

It is, however, recommended that in the future, either primary data or sufficient survival data be provided to allow verification of the reported results.

A. MATERIALS:

1. Test Material: Boric acid (Granular technical)

Description: White crystals
Identification Number: Code date: 1D19D
Purity: >99%
Receipt date: April 25, 1991
Stability: Stable at room temperature
Contaminants: None listed
Solvent used: RPMI - 1640 supplemented with 0.1% pluronic F68, 0.22 mg/mL sodium pyruvate, and antibiotics (R_{op}) or R_{op} + 5% horse serum (R_{5p}).
Other provided information: The test material was stored at room temperature. Test solutions used in the study were prepared immediately prior to use.

2. Control Materials:

Negative: None

Solvent/final concentration: R_{op} or R_{5p}

Positive:
Nonactivation: (Concentrations, solvent): Hycanthone methane sulfonate (HMS) was prepared in dimethyl sulfoxide (DMSO) to yield final concentrations of either 6.25 or 5 µg/mL.

Activation: (Concentration, solvent): 3-Methylcholanthrene (3-MC) was prepared in DMSO to yield a final concentration of 5 µg/mL.

3. Activation: S9 derived from adult male Fischer-344

x Aroclor 1254/1242	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 liver homogenate (lot number 1001) was purchased from Molecular Toxicology, Inc., Annapolis, MD; the protein content was listed as [illegible] mg/mL.

[illegible] mix composition:

Component	Concentration mL of Culture Medium
NADP	[illegible] mM
[illegible]	[illegible] mM
[illegible]	[illegible]

4. Test Cells: Mammalian cells in culture

__x__ mouse lymphoma L5178Y cells
_____ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Identity of the cell line was confirmed by cytogenetic analysis.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

__x__ thymidine kinase (TK)
selection agent: _____ bromodoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)
5 µg/mL trifluorothymidine (TFT)

_____ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentration) _____ 6-thioguanine (6-TG)

_____ Na+/K+ATPase
Selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentration Used:

(a) Cytotoxicity assay: The report indicated that a cytotoxicity assay was performed with test doses up to 5 mg/mL.

(b) Mutation assay:

(1) Initial test: 1200, 1700, 2450, 3500, and 5000 µg/mL +/-S9

(2) Confirmatory test: As above

B. TEST PERFORMANCE:

1. Cell Treatment:

a Cells exposed to test compound for:
__4__ hours (nonactivated) __4__ hours (activated)

b Cells exposed to positive controls for:
__4__ hours (nonactivated) __4__ hours (activated)

(c) Cells exposed to negative and/or solvent controls for: 4 hours (nonactivated) 4 hours (activated)

(d) After washing, cells cultured for 2 days (expression period) before cell selection

(e) After expression, $3x10^6$ cells/culture were incubated for 11 to 12 days in selection medium to determine numbers of mutants and 600 cells/culture were incubated for 11 to 12 days in nonselective cloning medium to determine cloning efficiency.

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria:

(a) Assay validity: For the assay to be considered valid, the following criteria must be satisfied: (1) the average cloning efficiency (CE) of the solvent control must exceed 50%; (2) the mutation frequency (MF) of the solvent control must be between 20 and $100x10^{-6}$; (3) the MF of the positive controls must be 3-fold higher than the corresponding solvent control value; (4) and the test substance must be evaluated to a cytotoxic level, the limit of solubility, or a maximum applied concentration of 5000 µg/mL.

(b) Positive response: The test material was considered positive if it induced a reproducible, dose-related increase in the MF that exceeded 2 times the MF of the solvent control at one or more doses with ≥10% total survival.

4. Protocol: See Appendix A

C. REPORTED RESULTS: The outcome of the preliminary cytotoxicity assay was not reported. Representative results from the summarized data presented for the two independently performed mouse lymphoma forward mutation assays with boric acid are present in Table 1. The findings from the two trials were in good agreement and indicated that nonactivated 5000 µg/mL and S9-activated 3500 and 5000 µg/mL were marginally cytotoxic. Without S9 activation, there was no evidence of a mutagenic response at any dose. Although a doubling of the background MF occurred at 5000 µg/mL +S9 in the initial trial, the 2-fold increase was confined to this dose and not reproduced in the confirmatory trial. The finding is, therefore, insufficient to conclude that boric acid was mutagenic. We were, however, unable to verify any of the summarized results or recalculate the MFs since the survival data (i.e., viable colonies) necessary to perform these calculations were not furnished.

Based on the overall results, the study author concluded that boric acid was not mutagenic in this test system.

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TABLE 1. Representative Results of the Mouse Lymphoma Forward Mutation Assays with Boric Acid

Substance	Dose/mL	S9 Activation	Average Percent Relative Growth[a]	Average Mutant Colonies[a]	Average Percent Relative Cloning Efficiency[a]	Average Percent Relative Total Growth[a]	Mutation Frequency per 10^6 Survivors[a] ±SD
Solvent Control							
	--	-[b]	100	84	100	100	54±10
	--	-[c]	100	76[d]	100	100	42±1[d]
	--	+[b]	100	42	100	100	29±10
	--	+[c]	100	74	100	100	36±7
Positive Control[e]							
Hycanthone methanesulfonate	6.25 µg	-[b]	40	612	34	14	1091±167
	5.00 µg	-[c]	54	288	34	19	501±21
3-Methylcholanthrene	5.00 µg	+[b]	58	518	58	34	632±97
	5.00 µg	+[c]	56	378	43	24	436±81
Test Material							
Boric acid	5000 µg[e]	-[b]	78	90	113	88	50±22
	5000 µg[e]	-[c]	82	57[f]	82	67	41±5
	3500 µg[e]	+[b]	80	48	84	68	41±13
	5000 µg	+	81	76	92	75	58±2
	3500 µg[e]	+[c]	79	72	84	67	41±6
	5000 µg	+	74	84	85	62	47±3

[a]Average values from triplicate solvent control cultures and duplicate test group and positive control cultures.
[b]Results from the initial test.
[c]Results from the confirmatory test.
[d]One of the three replicate cultures was contaminated; results are the average of two cultures.
[e]Findings for lower levels (1200, 1700, 2450, and 3500 µg/mL -S9 and 1200, 1700, and 2450 µg/mL +S9) did not suggest a mutagenic response.
[f]One of the replicate selection medium plates was contaminated; results are the average of 5 plates.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess, in agreement with the study author, that boric acid was not mutagenic in this test system. In all trials, the test material was assayed to levels that were marginally cytotoxic but failed to induce a reproducible mutagenic response. As previously stated, we were unable to verify the reported values; however, the overall results suggest that the study was properly conducted and well controlled. In addition, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response of the target cell to the nonactivated (6.25 and 5 µg/mL HMS) and S9-activated (5 µg/mL 3-MC) positive controls. We conclude, therefore, that the study provided acceptable evidence that boric acid was not mutagenic in this cultured mammalian cell system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 23, 1991).

F. CBI APPENDICES: Appendix A, Protocol, CBI Appendix pp. 2-7; Appendix B, Materials and Methods, CBI pp. 8-15.

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.2a) for genetic effects Category I, Gene Mutations.

APPENDIX A

PROTOCOL
CBI Appendix pp. 2-7

RIN 5117-93 BORIC ACID
TOX REVIEW 009666

Page_____ is not included in this copy.

Pages 37 through 51 are not included.

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____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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GUIDELINE § 84: MUTAGENICITY UDS

EPA Reviewer: Melba Morrow, DVM, Ph.D.
Review Section II,
Toxicology Branch I/HED (H-7509C)
Signature: Melba S Morrow
Date: 1/28/92

Acting EPA Section Head: Joycelyn Stewart, Ph.D.
Review Section II,
Toxicology Branch I/HED (H-7509C)
Signature: JS
Date: 1/29/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in primary rat hepatocytes.

EPA IDENTIFICATION Numbers:

Caswell. Number: 109

MRID Number: 420389-03

TEST MATERIAL: Boric acid

SYNONYMS/CAS NUMBER: None provided/10043-35-3

SPONSOR: U.S. Borax and Chemical Corp., Anaheim, CA

STUDY NUMBER: 2389-V500-91

TESTING FACILITY: SRI International, Menlo Park, CA

TITLE OF REPORT: Evaluation of the Potential of Boric acid to Induce Unscheduled DNA Synthesis in In Vitro Hepatocyte DNA Repair Assay Using the Male F-344 Rat

AUTHOR: J. P. Bakke

REPORT ISSUED: August 23, 1991

CONCLUSIONS--EXECUTIVE SUMMARY: No conclusions can be reached from the two independently performed primary rat hepatocyte unscheduled DNA synthesis assays conducted with doses of boric acid ranging from 5 to 5000 µg/mL. We are unable to determine the relevance of the dose-related increases in net nuclear grains and/or percentages of cells with ≥5 net nuclear grains (i.e., cells in repair) observed at 1000 and 5000 µg/mL in Trial 1 and over the narrower range of concentrations examined in Trial 2 (250, 500, 1000, 2500, 3800, and 5000 µg/mL). Although the net nuclear grains counts at these levels were negative values, the extremely low background counts (-12.6 and -15.7 net nuclear grains in Trials 1 and 2, respectively) for the solvent control cultures (Williams' Medium E) confounded the interpretation of the findings. The study author claimed that the apparent increases in net nuclear grains and percentage of cells in repair was due to decreased cytoplasmic grain counts,

which he speculated was a result of cytotoxicity and not increased DNA repair. The primary data (cytoplasmic and gross nuclear grain counts) necessary to support this statement were not provided. We assess, therefore, that without these data, the study results can not be fully assessed.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms. However, the study may be upgraded if the primary data are submitted and the independent evaluation of this data supports the study author's conclusions.

A. MATERIALS:

1. Test Material: Boric acid (Granular technical)

   Description: White crystalline solid
   Identification no.: Code date: 1D19D
   Purity: >99.0%
   Receipt date: April 25, 1991
   Stability: Not reported
   Contaminants: None listed
   Solvent used: Culture medium: Williams' Medium E (WME) supplemented with 2 mM glutamine and antibiotics.
   Other provided information: The test material was stored at room temperature. Test solutions were prepared immediately before use.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the livers of male Fischer-344 rats purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN.

3. Control Substances: WME served as the solvent control. 2-Acetylaminofluorene (2-AAF) at 3.0 and 0.5 µg/mL was used as the positive control; hepatocytes exposed to 0.5 µg/mL were scored.

4. Medium: WME; WME+: WME with 10% fetal bovine serum.

5. Test Compound Concentrations Used:

   (a) Trial 1: The ten doses initially evaluated were 0.5, 1, 5, 10, 50, 100, 250, 500, 1000, and 5000 µg/mL. Hepatocytes exposed to levels ranging from 10 to 5000 µg/mL were scored.

   (b) Trial 2: Ten levels were assayed (5, 10, 50, 100, 250, 500, 1000, 2500, 3800, and 5000 µg/mL); cells treated with all doses were scored.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion techniques: Rats (one/experiment) were anesthetized with sodium pentobarbital and the livers were perfused with an unspecified collagenase solution. Livers were combed to release hepatocytes: no further details were provided.

(b) Hepatocyte harvest/culture preparation: Recovered cells ($6x10^5$ cells/mL) were seeded onto coverslips in 6-well culture dishes containing WME+, and allowed to attach for 1.5-2 hours in a 37°C, CO_2 incubator. Unattached cells were removed; viable cells were fed WME containing 10 µCi/mL [^{3}H] thymidine.

2. UDS Assay:

(a) Treatment: Triplicate cultures were exposed to each of the selected test material doses, the solvent control (culture medium), or the positive control (2-AAF), for ≈19 hours. Treated hepatocytes, attached to coverslips, were washed, exposed to 1% sodium citrate, fixed in glacial acetic acid:ethanol (1:3), and mounted.

(b) Preparation of autoradiographs/grain development: Slides were coated with Kodak NTB-2 emulsion, exposed for 7 days at -20°C, developed, and stained with 1% methyl-green pyronin Y.

(c) Grain counting: Slides were visually evaluated for cytotoxicity (dead or morphologically altered cells) before coding and counting. The nuclear grains of at least 90 morphologically normal cells (30/coverslip) from a randomly selected area of each slide were counted. Cytoplasmic background counts were determined by counting two nuclear-sized areas over the most heavily labeled areas adjacent to the nucleus. Net nuclear grain counts were determined by subtracting the higher cytoplasmic background count from the nuclear grain count. The percentage of cells in repair (cells with ≥5 net nuclear grains) was also calculated.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if the solvent control data were within unspecified historical ranges, and if the positive control had >5 net nuclear grain counts and a significant increase in the percent of cells with ≥5 net nuclear grains. The definition of significance was not reported; no statistical methods were used.

(b) Positive response: The test material was considered positive if it induced a mean net nuclear grain count of >5 grains/nucleus at any dose.

4. Protocol: See Appendix A

C. REPORTED RESULTS:

1. Solubility Determinations: The solubility of the test material was determined using culture medium, ethanol, and DMSO as the solvents. The report indicted that boric acid was "most soluble" in culture medium. Accordingly, culture medium was chosen as the solvent and a concentration range of 0.5 to 5000 µg/mL of the test material was evaluated in Trial 1. Neither the pH nor the osmotic pressure of the treatment medium containing boric acid were determined. Considering the physical properties of test material (i.e., low pH, low molecular weight, and dissociation into ionic forms), it is not unreasonable to have expected these data to be present. However, the study author did not report a color change in the treatment medium, we assumed, therefore, that the addition of boric acid did not alter the pH. Additionally, since 1.9% aqueous boric acid is isotonic,[1] 5000 µg/mL would not increase the osmotic pressure of the treatment medium.

2. UDS Assay: Representative results from both trials of the UDS assay conducted with boric acid are presented in Table 1. As shown, net nuclear grain counts for the solvent control in both trials were well below the expected background rates reported by numerous investigators (overall range for the cited studies was -5 to 1 net nuclear grains).[2,3,4] Our reviewers are, therefore, unable to determine the relevance of the dose-related decline in the negative net nuclear grains counts observed in both trials at levels ≥250 µg/mL. The study author stated that the apparent increase in net nuclear grains was due "solely to decreased cytoplasmic counts, probably as a result of cytotoxicity, not of increased DNA repair." Cytoplasmic grain counts were, however, not presented to support this statement. The study author further stated that there was no absolute cytotoxic effect at the highest level (5000 µg/mL). We acknowledge that in the absence of overt morphological changes in the hepatocytes, cytoplasmic grain counts can decrease as a consequence of cytotoxicity while gross nuclear counts tend to remain constant or decrease slightly. The verification that this type of cytotoxic response was produced can only be obtained from a full review of the primary data.

In addition, our reviewers have concerns regarding the increases in cells with ≥5 net nuclear grains that were reported at 1000 and 5000 µg/mL in Trial 1 and at 250 to 5000 µg/mL in Trial 2, which the

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[1]Merck Index 1989; Eleventh Edition.

[2]Casciano, D.A., and Gaylor, D.W. (1983). Statistical criteria for evaluating chemicals as positive or negative in the hepatocyte/DNA repair assay. Mutat. Res. 112:81-86.

[3]Mitchell, A.D., Casciano, D.A., Meltz, M.L., Robinson, D.E., San, R.H.C., Williams, G.M., and Von Halle, E.S. (1983). Unscheduled DNA Synthesis Tests. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123:363-410.

[4]Probst, G.S., McMahon, R.E., Hill, L.E., Thompson, C.Z., Epp, J.K., and Neal, S.B. (1981). Chemically-Induced DNA Synthesis in Primary Rat Hepatocyte Cultures: A Comparison With Bacterial Mutagenicity Using 218 Compounds. Environ. Mutagen. 3:11-32.

TABLE 1. Representative Results of the Unscheduled DNA Synthesis (UDS) Rat Hepatocyte Assays with Boric Acid

Treatment	Dose/mL	Trial 1		Trial 2	
		Net Nuclear Grains±S.E.[a]	Percent cells with ≥5 Net Nuclear Grains	Net Nuclear Grains±S.E.[a]	Percent cells with ≥5 Net Nuclear Grains
Solvent Control					
Culture medium	--	-12.6±0.9	3	-15.7±0.4	1
Positive Controls					
2-Acetylaminofluorene	0.5 µg	21.0±4.4	80	15.1±0.8	77
Test Material					
Boric acid	100 µg[b]	-12.1±2.2	2	-12.2±0.9	1
	250 µg	-11.8±1.6	3	-10.3±3.1	4
	500 µg	-8.3±0.7	2	-5.2±0.7	8
	1000 µg	-8.5±2.2	8	-4.8±1.2	7
	2500 µg	NT[c]		-1.0±0.8	13
	3800 µg	NT		-1.9±1.0	12
	5000 µg	-2.5±0.4	6	-1.1±1.1	14

[a]Means and standard errors of the count of 90 cells; thirty cells from triplicate slides were analyzed.

[b]Results for lower doses (10 and 50 µg/mL in Trial 1 and 5, 10, and 50 µg/mL in Trial 2) did not suggest a genotoxic effect.

[c]NT - Not tested

study author also claimed was probably associated with cytotoxicity. While our reviewers have seen artificial increases in net nuclear counts as a function of cytotoxicity, an increased rate of cells in repair is an unusual finding.

Based on the overall results, the study author concluded, however, that "boric acid tested negative in the in vitro rat hepatocyte DNA repair assay."

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that no conclusions can be reached from the two independently performed primary rat hepatocyte UDS assays conducted with boric acid. The extremely low net nuclear grain counts for the solvent control cultures suggest that cytoplasmic counts were high probably as a result of technical problems with the staining procedures or the method used to select cytoplasmic grain counting areas.

It was, however, noteworthy that while the net nuclear counts for the treatment groups were negative values, the overall results for both trials did suggest a dose-related increase in UDS. Our reviewers have discounted treatment-condition effects (i.e., pH and osmolality) as a cause for the increased nuclear grain counts (see Section C.1). It is, therefore, conceivable that boric acid induced a marginal genotoxic response that was masked by the overall poor quality of the staining procedure. The study author claimed that the increases in net nuclear grains were associated with decreases in cytoplasmic background counts resulting from cytotoxicity. These data should, therefore, have been submitted to support this statement. Based on our reviewers' experience with this test system, cytotoxicity-related declines in cytoplasmic counts without overt cytotoxic effects in the hepatocytes can occur. However, increases in cells in repair as a function of cytotoxicity is an unusual finding. Even with adjustments made for decreased cytoplasmic grains, the increasing number of cells in repair, particularly in Trial 2, suggests that a sizable portion of the nuclei must have been very heavily labeled. If technical problems with autoradiography resulted in heavily labeled cells, the ability of the reader to distinguish artifact from nuclear grains is questionable and raises additional concerns regarding the overall performance of the study.

Based on the above considerations, we assess that the study is unacceptable. It is, nevertheless, possible that submission of the primary data for review will satisfy the above concerns and allow the study to be upgraded.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 23, 1991).

F. CBI APPENDICES: Appendix A, Protocol, CBI Appendix pp. 2-6; Appendix B, Materials and Methods, CBI pp. 8-12.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms.

APPENDIX A

PROTOCOL
CBI Appendix pp. 2-6

RID 5117-93 BORIC ACID
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Pages 61 through 71 are not included.

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DOC 930085

FINAL

DATA EVALUATION REPORT

BORIC ACID

Study Type: Mutagenicity: In Vivo Micronucleus Assay in Mice

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer _____ Date 7/22/92
Nancy E. McCarroll, B.S.

Independent Reviewer _____ Date 7/23/92
Lynne Haber, Ph.D.

QA/QC Manager _____ Date 7/22/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-107
Clement Number: 92-52
Project Officer: James Scott

GUIDELINE § 84: MUTAGENICITY MICRONUCLEUS

MUTAGENICITY STUDIES

EPA Reviewer: Melba Morrow, ~~Ph.D.~~ DVM
Review Section II,
Toxicology Branch I/HED (H-7509C)

Signature: Melba S Morrow
Date: 7/28/92

Acting EPA Section Head: Joycelyn Stewart, Ph.D.
Review Section II,
Toxicology Branch I/HED (H-7509C)

Signature:
Date:

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice

EPA IDENTIFICATION Numbers:

Tox Chem. Number:

Caswell Number: 109

MRID Number: 420389-04

TEST MATERIAL: Boric acid

SYNONYMS/CAS NO.: None provided/10043-35-3

SPONSOR: U.S. Borax and Chemical Corp., Anaheim, CA

STUDY NUMBER: 2389-C400-91

TESTING FACILITY: SRI International, Menlo Park, CA

TITLE OF REPORT: Bone Marrow Erythrocyte Micronucleus Assay of Boric Acid in Swiss-Webster Mice

AUTHOR: K.G. O'Loughlin

REPORT ISSUED: August 19, 1991

CONCLUSIONS--EXECUTIVE SUMMARY: The oral gavage administration of 900, 1800, or 3500 mg/kg boric acid to male or female mice for 2 consecutive days did not cause a significant increase in the frequency of micronucleated polychromatic erythrocytes (MPEs) in bone marrow cells harvested 24 or 48 hours after the final dosing. Similarly, there was no evidence of overt toxicity in the treated animals or cytotoxic effects on the target organ. Based on these findings, we concluded that boric acid was adequately tested and found to be nongenotoxic in the mouse micronucleus assay.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2b) for genetic effects Category II, Structural Chromosome Aberrations.

A. MATERIALS:

1. Test Material: Boric acid (Granular technical)

Description: White crystalline solid
Identification number: Code date: 1D19D
Purity: >99.0%
Receipt date: April 25, 1991
Stability: Stable at room temperature
Contaminants: None listed
Solvent used: Sterile deionized water (DH_2O)
Other provided information: The test material was stored at room temperature. Dosing solutions were prepared immediately before use.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: DH_2O at a dosing volume of 10 mL/kg/day was administered by oral gavage for 2 consecutive days.

Positive/final concentration/route of administration: Urethane was dissolved in DH_2O and administered by oral gavage at 300 mg/kg/day for 2 consecutive days to male mice only.

3. Test Compound:

Route of administration: Oral gavage (2 administrations separated by 24 hours)

Dose levels used:

- Range-finding study: 225, 450, 900, 1800, and 3500 mg/kg/day (3 males and 3 females, per dose)
- Micronucleus assay: 900, 1800, and 3500 mg/kg/day (5 males and 5 females, per dose, per sacrifice time)

4. Test Animals:

(a) Species: mouse Strain: Swiss-Webster Age (at dosing): ≈9 weeks (males); ≈13 weeks (females)
Weight range (at dosing): 31.7-34.8 g (males); 24.4-26.7 g (females)
Source: Charles River Laboratories, Portage, MI

(b) Number animals used per dose: 10 males; 10 females (vehicle and treatment groups)
10 males (positive control group)

Note: Dosing was based on individual body weights.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
Dosing: _____ once __x__ twice (24 hr apart)
_____ other (describe): _____
Sampling (after final dose): _____ 6 hr _____ 12 hr
__x__ 24 hr __x__ 48 hr _____ 72 hr

(b) Vehicle control:
Dosing: _____ once __x__ twice (24 hr apart)
_____ other (describe): _____
Sampling (after final dose): __x__ 24 hr __x__ 48 hr
_____ 72 hr

(c) Positive control:
Dosing: __x__ once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after final dose): __x__ 24 hr __x__ 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

__x__ bone marrow _____ others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: __1000__
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: __200__ (PCEs + NCEs)

3. Details of Slide Preparation: At 24 and 48 hours after the final administration of the test material, the appropriate groups of animals were sacrificed by cervical dislocation.

Peripheral blood smears were made from the animals used in the range-finding study and bone marrow cells were harvested from the animals in the preliminary study and the micronucleus test. Bone marrow cells were aspirated from one femur with fetal calf serum and centrifuged. Supernatants were discarded and cells were resuspended in the residual fluid. Slides were prepared, fixed in absolute methanol, stained with acridine orange, coded and scored. Using this stain, PCEs will uniformly fluoresce bright orange and are referred to as RNA^+ erythrocytes; NCEs will not take up the acridine orange and are referred to as RNA^- erythrocytes; micronucleated PCEs (MPEs) are RNA^+ erythrocytes with a visible chromatid structure.

4. Statistical Methods: The micronucleus data were evaluated for statistical significance using the Kruskal-Wallis analysis of variance

and the Cochran-Armitage test. Mean animal body weights were analyzed using the Student's t-test.

5. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if (1) the frequency of MPEs for the vehicle control group was within an unspecified normal historical range; (2) the positive control induced a significant positive response; and (3) there were at least 3 surviving animals per sex with a ratio of RNA$^+$ to total erythrocytes ≥0.1 in two or more treatment groups.

(b) Positive response: The test material was considered positive if the frequency of MPEs was significantly ($p<0.05$) higher than the vehicle control in two dose groups, or if a dose-related increase in MPEs was seen.

C. REPORTED RESULTS:

1. Range-finding Study: Based on the results of a solubility test, the study author stated that owing to the physical bulk of the test material, the highest practical dose that could be administered by gavage was 3500 mg/kg/day. Accordingly, groups of 3 male and 3 female mice receive two consecutive daily gavage administrations of 225, 450, 900, 1800, or 3500 mg/kg/day boric acid. Animals were observed daily for signs of compound toxicity and death. Body weights were determined prior to dosing and at sacrifice (48 hours after the last compound administration). At sacrifice, peripheral blood and bone marrow cell were collected and assessed for cytotoxic effects.

No animals died prior to sacrifice. The report stated that all animals appeared normal throughout the study. There was also no adverse effect on body weight or evidence of a cytotoxic response in peripheral erythrocytes or bone marrow cells at any dose in either sex. Therefore, the doses selected for the micronucleus assay were 900, 1800, and 3500 mg/kg/day.

2. Micronucleus Assay:

(a) Animal observations: In agreement with the preliminary results, there was no indication of overt compound toxicity at any dose. The study author stated that on day 2, 1 male at 1800 mg/kg/day had rough fur and a humped back and 1 high-dose male had rough fur. Neither the body weights nor body weight gain for the males and females in the treatment group indicated a compound-related effect.

(b) Micronucleus assay: Representative results for the micronucleus assay are presented in Table 1. No significant increase in the frequency of micronucleus induction was seen in bone marrow cells of male and female mice sampled 24 and 48 hours after the admini-

TABLE 1. Representative Results of the Micronucleus Assay in Mice with Treated with Boric Acid

Substance	Dose/kg/day	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	Mean Percent MPEs ±S.E.	Mean % PCE per NCE ±S.E.
Vehicle Control								
Deionized water	10 ml	24	M	5	5008	14	0.28±0.12	58.55±3.94
			F	5	5007	13	0.26±0.08	57.21±3.36
		48	M	5	5009	9	0.18±0.10	62.93±3.79
			F	5	5007	12	0.24±0.05	54.92±2.23
Positive Control								
Urethane	300 mg	24	M	5	5009	209	4.17±0.55*	61.66±1.93
		48	M	5	5010	56	1.12±0.13*	48.45±4.21
Test Material								
Boric acid	3500 mg[b]	24	M	5	5010	13	0.26±0.07	59.61±5.66
			F	5	5009	7	0.14±0.02	60.54±2.10
		48	M	5	5006	6	0.12±0.04	68.42±3.19
			F	5	5008	8	0.16±0.06	62.37±3.51

[a]Time after oral gavage administration of the final dose
[b]Results for the low- (900 mg/kg/day) and mid- (1800 mg/kg/day) dose groups did not suggest a positive effect.

*Significantly higher ($p<0.01$) than the corresponding vehicle control by Cochran-Armitage test.

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes.

stration of the final dose of 900, 1800, or 3500 mg/kg/day boric acid. PCE:NCE ratios for all treatment groups at both sacrifice times were comparable to the vehicle control values, indicating that boric acid did not suppress hematopoiesis. By contrast, the frequency of MPEs in male mice receiving the positive control (300 mg/kg/day urethane) was significantly increased ($p<0.01$) at both sacrifice intervals.

Based on the overall results, the study author concluded that boric acid was not genotoxic in this in vivo test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study author correctly interpreted the data. The high dose selected for study (3500 mg/kg/day) was limited by the physical properties of the test material; however, the combined dose (7000 mg/kg) exceeded the maximum recommended level (5000 mg/kg) for nontoxic substances in the micronucleus assay. We concluded, therefore, that boric acid was tested up to an appropriate high concentration with no evidence of overt toxicity in the treated animals or cytotoxic effects on the target organ and failed to induce a clastogenic response.

Additionally, the sensitivity of the test system to detect a genotoxic response was demonstrated by the significant ($p<0.01$) results obtained in male mice exposed to the positive control (300 mg/kg/day urethane).

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 19, 1991).

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 11-20; Appendix B, Protocol and Protocol Amendments, CBI Appendix pp. B1-B12.

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.2b) for genetic effects Category II, Structural Chromosome Aberrations.

APPENDIX A

MATERIALS AND METHODS

CBI pp. 11-20

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