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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Sodium Bromide - Toxicology Data Submitted Under Accession Nos. 406708-08, -09, -10
EPA ID No. 8622-UL

TOX Chem No.: 750A
TB Project No.: 8-0971
RD Record No.: 224866

FROM: Irving Mauer, Ph.D., Geneticist
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TO: Jeff Kempter/Barbara A. Pringle, PM Team 32
Antimicrobial Program Branch
Registration Division (TS-767C)

THRU: Judith W. Hauswirth, Ph.D., Acting Branch Chief
Toxicology Branch I (IRS)
Health Effects Division (TS-769C)

Registrant: Ameribrom, Inc.
New York City

Request:

Review and evaluate the following three mutagenicity studies, all performed at the Huntington Research Centre (HRC), Cambridgeshire, England:

1. Ames Test - "AMES METABOLIC ACTIVATION TEST TO ASSESS THE POTENTIAL MUTAGENIC EFFECT OF SODIUM BROMIDE" HRC No. DSB 4/98137, May 20, 1988 (EPA Accession No. 406708-08).

2. Cytogenetics In Vitro - "SODIUM BROMIDE TECHNICAL GRADE METAPHASE CHROMOSOME ANALYSIS OF HUMAN LYMPHOCYTES CULTURED IN VITRO" HRC No. DSB5/88447, June 7, 1988 (EPA Accession No. 406708-09).

3. UDS In Vitro - "ASSESSMENT OF UNSCHEDULED DNA REPAIR SYNTHESIS IN MAMMALIAN CELLS AFTER EXPOSURE TO SODIUM BROMIDE (TECHNICAL GRADE)" HRC No. DSB 6/88454, June 8, 1988 (EPA Accession No. 406708-10).

TB Conclusions

These studies were assessed as follows (detailed reviews are attached):

Study Type	Reported Results	TB Evaluation
1. Ames Test	Negative	Acceptable
2. Cytogenetics	Negative	Acceptable
3. UDS	Negative	Acceptable

Attachments

Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I, IRS/HED (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Acting
Chief, Toxicology Branch I, IRS/HED (TS-769C)

DATA EVALUATION REPORT

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I. SUMMARY

TB Project No.: 8-0971
Caswell No.: 750A
MRID No.: 406708-08
Shaughnessy No.: 013907

Study Type: Mutagenicity - Reverse Mutation in Bacteria (Ames Test)

Chemical: Sodium Bromide

Sponsor: Ameribrom, New York City

Testing Facility: Huntingdon Research Centre (England)

Title of Report: Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Sodium Bromide.

Authors: E. Jones and L.A. Wilson

Study Number: DSB 4/88137

Date of Issue: May 20, 1988

TB Conclusions:

Negative for mutagenicity. No increase in revertent colonies was found in cultures of five Ames tester strains exposed to test substance at concentrations up to 5000 ug/plate (limit dose), either in the presence or absence of metabolic activation.

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

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A. Test Material - Sodium bromide

Description: White granular powder
Batch (Lot): 7320
Purity (%): 98
Solvent/carrier/diluent: Water

B. Test Organisms - Bacteria

Species: Salmonella typhimurium LT2
Strains: TA1535, TA1537, TA1538, TA98, TA100 (all his$^-$)

C. Study Design (Protocol) - Although a formal protocol was not included in the final report, the assay was carried out according to OECD Guideline No. 471 as well as authoritative (published) methodology.

Attestations of compliance to FIFRA GLPs and a Quality Assurance audit were included.

D. Procedures/Methods of Analysis - Following preliminary toxicity testing, triplicate cultures of each strain were exposed to 5 concentrations of test substance, both in the absence and presence of a metabolic activation system consisting of Aroclor 1254-stimulated rat liver microsomal homogenate (S9), plus generating cofactors (S9-mix). Appropriate negative controls (medium, water, S9), and positive control substances known to have specific mutagenic activity for each strain,* were included in each test. Following 72 hours incubation, plates were examined for toxicity (bacterial lawn), and revertent colonies enumerated, using an electronic colony counter. The entire assay was repeated once.

Results:

In preliminary toxicity (range-finding) testing, background bacterial lawns in all strains were intact, and no toxicity was evident at doses of test substance up to 5000 ug/plate (Report Table 1). Therefore, 5000 ug/plate

*N-ethyl-N'-nitro-N-nitrosoguanidine, 5 ug/plate, in nonactivated TA1535, TA100.
9-aminoacridine, 80 ug/plate in nonactivated TA1537.
2-nitrofluorene, 1-2 ug/plate, in nonactivated TA1538, TA98.
2-aminoanthracene, 0.5 to 2.0 ug/plate (depending on specific strain) under activation conditions in all strains.

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was selected at the highest test concentration; together with four lower doses separaced by half-$\log_{10}$ intervals (i.e., 5, 50, 500, ug/mL).

In neither of the two assays was there any increase over controls in mean revertent counts in any tester strain at any dose level, either in the presence or absence of metabolic activation (S9). Negative control counts were within published background ranges* (TA1535 = 10-15; TA1537 = 9-14; TA1538 = 11-13; TA98 = 19-29; TA100 = 110-140), and cultures treated with positive control substances responded appropriately (4 to 100 times negative control counts).

The authors concluded that sodium bromide showed no evidence of mutagenicity in Ames testing.

TB Evaluation:

ACCEPTABLE. The study was conducted with adequate procedures in repeat experiments, and the negative results generated are judged to be valid.

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*deSerres, F.J.; Shelby, M.D. (1979) Mutation Res. 61:159.

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DATA EVALUATION REPORT

I. SUMMARY

TB Project No.: S-0971
Caswell No.: 750A
MRID No.: 406708-09
Shaughnessy No.: 013907

Study Type: Mutagenicity - Cytogenetics in vitro (CA in HLC)

Chemical: Sodium Bromide

Sponsor: Ameribrom, New York City

Testing Facility: Huntington Research Centre (England)

Title of Report: Sodium Bromide Technical Grade - Metaphase Chromosome Analysis of Human Lymphocytes Cultured In Vitro.

Authors: P.C. Brooks and L.C. Akhurst

Study Number: DSB 5/88447

Date of Issue: June 7, 1988

TB Conclusions:

Negative for chromosome aberrations at test concentrations up to 5000 ug/mL (limit dose), both in the presence and absence of metabolic activation.

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - Sodium bromide technical

Description: White crystalline solid
Batch (Lot): 7320
Purity (%): 99
Solvent/carrier/diluent: Water

B. Test Organism - Primary lymphocyte cultures

Species: Human volunteers

C. Study Design (Protocol) - No formal protocol was included, but the study was conducted according to OECD Guideline No. 473, as well as authoritative (published) methodology.

Statements of compliance with FIFRA GLPs and Quality Assurance audit were included in the Final Report.

D. Procedures/Methods of Analysis - Human lymphocytes were established in culture with the required mitotic-stimulating agent, phytohemagglutinin, and 1 mL aliquots dispensed in multiwell tissue culture plates. After 48 hours incubation, test article was added to duplicate cultures at 10 concentrations (ranging from 9.77 to 5000 ug/mL), both in the absence and presence of a metabolic activation system consisting of Aroclor 1254-stimulated rat liver microsomal homogenate (S9) plus appropriate cofactors (S9 mix). The medium in S9-supplemented cultures was replaced after a 2-hour treatment. All cultures were harvested 22 hours later, and microscope slide preparations of colchicine-treated cells scored for mitotic index (number of metaphases per 1000 nuclei), an indirect measure of toxicity.

A second set of lymphocyte cultures was treated in duplicate with test article after 48 hours initial incubation. Again, S9-supplemented cultures were exposed for only 2 hours, then fresh medium replaced the treatment cocktail for an additional 20-hour incubation. Known clastogens served as positive controls: ethylmethanesulfonate (EMS, 1000 ug/mL) for the nonactivated series, and cyclophosphamide (CP, 20 ug/mL) for the activated series. All cultures were then harvested at 22 hours, cells again arrested in metaphase by colchicine treatment, microscope slides (5 per culture) prepared by conventional techniques, and 100 metaphases per culture examined under an oil immersion objective (total magnification, 1000X) for chromosome aberrations.

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Results:

The test article was apparently not toxic, since no significant alterations in mitotic indices were found at any concentration up to 5000 ug/mL in the preliminary range-finding test. In the absence of activation, mean percent of dividing (metaphase) cells ranged from 7.8 to 11.7 with no dose-relationship, compared to the water (solvent) control mean of 9.8 percent, while in S9-supplemented cultures, the test range was again a flat 7.8 to 11.6 percent compared to the control value of 7.7 percent (Report Table 1). Hence, the highest dose selected for the main assay was the maximum achievable concentration, 5002 ug/mL, together with two lower doses of 2501 and 5002 ug/mL.

Under neither activation nor its absence did sodium bromide induce any significant increases in either the proportion of metaphases with chromosome aberrations, or the types of aberrations (Report Table 2, attached to this DER). By contrast, both positive controls caused statistically highly significant increases ($p < 0.001$) in aberrant metaphases.

The authors concluded that sodium bromide showed no evidence of clastogenicity in human lymphocyte cultures.

TB Evaluation:

ACCEPTABLE. The study was conducted with adequate procedures and controls, and the negative results are judged to be valid.

Attachment

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The effect of sodium bromide technical grade on the chromosomes of cultured human lymphocytes

(a) Without metabolic activation

Culture no.	Test agent	Concentration µg/ml	No. cells examined	No. aberrations per 100 cells		Aberrations								Number of aberrant cells			
				Exc. gaps	Inc. gaps	BWF	BF	I	SM	A	GT	P	CHR	Exc. gaps	% Mean	Inc. gaps	% Mean
101	Sterile distilled water (solvent control)	10 µl/ml	100	0	0									0	0.0	0	0.0
102			100	0	0									0		0	
103			100	0	0									0		0	
104			100	0	0									0		0	
111		500.2	100	0	0									0	0.5	0	0.5
112			100	1	1					1				1		1	
113	Sodium bromide technical grade	2501	100	0	0									0	0.5	0	0.5
114			100	1	1	1								1		1	
115		5002	100	1	1				1					1	0.5	1	0.5
116			100	0	0									0		0	
117	Ethylmethane sulphonate (positive control)	1000	100	34	37	16	2	1	2	10	2	1	3	21	19.0***	22	19.5***
118			100	30	30	16	1	4	1	4	4			17		17	

(b) With metabolic activation

Culture no.	Test agent	Concentration µg/ml	No. cells examined	No. aberrations per 100 cells		Aberrations							Number of aberrant cells			
				Exc. gaps	Inc. gaps	BWF	BF	I	SM	A	GT	CHR	Exc. gaps	% Mean	Inc. gaps	% Mean
123	Sterile distilled water (solvent control)	10 µl/ml	100	0	0								0	0.0	0	0.0
124			100	0	0								0		0	
125			100	0	0								0		0	
126			100	0	0								0		0	
133		500.2	100	0	0								0	0.0	0	0.0
134			100	0	0								0		0	
135	Sodium bromide technical grade	2501	100	0	0								0	0.0	0	0.0
136			100	0	0								0		0	
137		5002	100	0	1							1	0	0.0	1	0.5
138			100	0	0								0		0	
139	Cyclophosphamide (positive control)	20	91	48.4	52.7	21	4	10	4	3	2	4	28	27.7***	30	30.4***
140			100	42.0	48.0	30		8		3	1	6	25		28	

Statistical analysis used was Fisher's test
*** P<0.001
Otherwise P>0.05

BWF Chromatid break with fragment
BF Chromatid break without fragment
I Interchange
SM Single minute
A Acentric fragment
GT Greater than 10 aberrations
CHR Chromatid gap

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DATA EVALUATION REPORT

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I. SUMMARY

TB Project No.: 8-0971
Caswell No.: 750A
MRID No.: 406708-10
Shaughnessy No.: 013907

Study Type: Mutagenicity - DNA Damage/Repair In Vitro (UDS in HeLa cells)

Chemical: Sodium Bromide

Sponsor: Ameribrom, New York City

Testing Facility: Huntington Reseacch Centre (England)

Title of Report: Assessment of Unscheduled DNA Repair Synthesis in Mammalian Cells After Exposure to Sodium Bromide (Technical Grade).

Authors: J.M. Henderson and R.J. Proudlock

Study Number: DSB 6/88454

Date of Issue: June 8, 1988

TB Conclusions:

The test article did not induce increased unscheduled DNA synthesis-repair (as measured by net silver grain counts), either in the presence or absence of metabolic activation, even at concentrations causing severe toxicity (25,600 ug/mL).

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - Sodium bromide technical

Description: White crystalline solid
Batch (Lot): 7320
Purity (%): 99.23
Solvent/carrier/diluent: Water

B. Test Organism - Established cell line

Species: Human
Strain: HeLa - S3
Source: FLow Labs (England)

C. Study Design (Protocol) - The assay was conducted according to procedures of the OECD (Guideline No. 482), as well as methodology derived from authoritative (published) articles.

Statements of compliance with FIFRA, OECD, UK, and Japan's GLPs as well as a Quality Assurance audit were included.

D. Procedures/Methods of Analysis - Cells incubated on glass coverslips in culture dishes containing arginine-deficient media with low serum concentration for 72 hours (to reduce replicative, i.e., S-phase, DNA synthesis) were then exposed to tritiated thymidine (6 ^{3}H-TdR, 0.5 mCi/mL, sp. act. 24 Ci/mmole), simultaneously with test substance at a range of concentrations consisting of 12 serial twofold dilutions from a maximum of 25,600 ug/mL to 12.5 ug/mL, both in the absence and presence of a metabolic activation system consisting of an Aroclor 1254-stimulated male rat hepatic microsomal homogenate (S9) plus cofactors (S9 mix). Mutagens known to be active as DNA damage/repair agents served as positive controls: 4-nitroquinoline (4NQO, 5 concentrations ranging from 0.02 to 0.32 ug/mL) for nonactivated cultures, and 2-aminoanthracene (2AA, 5 concentrations ranging from 2.5 to 40 ug/mL). Negative controls were the test vehicle, water, S-9 mix, and medium.

After 3 hours treatment, coverslips with attached cells were washed, fixed and stained, then processed on glass microscope slides for autoradiography using stripping film (Kodak AR-10) according to standard (referenced) methodology. After exposure in the dark at 4 °C for 13 days, the autographic preparations were developed and fixed by a conventional photographic technique.

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Coded slides were examined microscopically under oil immersion and scored for silver grains over cell nuclei (a measure of unscheduled DNA repair), corrected for incidental incorporation in an equal area of surrounding cytoplasm (to give net grains).

Two separate tests were conducted. Results of both were analyzed statistically by one-way ANOVA. The authors considered a positive response if a reproducible statistically significant increase in grain count per 100 treated cells over solvent control values were recorded.

Results:

In both tests, the HDT of the test compound caused some toxicity, but at no test concentration was there any consistent increase in grain count over solvent control (Report Tables 1 and 2, extracted on following page).

In the second activated test, a small but significant increase was recorded in gross nuclear count at a single concentration, 1600 ug/mL; however, since the cytoplasmic (background) labeling level was comparably high, there was no increase in the corresponding net nuclear grain count. Another isolated small but significant increase in net nuclear grain count was registered in this second activated test at 400 ug/mL, although no corresponding increase was recorded in the gross nuclear grain count. Both these increases were discounted as representing chance variations, since they were unrelated to dose and not reproducible between tests.

By contrast, both positive control agents caused marked increases in grain count.

The authors concluded sodium bromide showed no evidence of DNA damage in this test system, as measured by silver grain counts (unscheduled DNA synthesis).

TB Evaluation:

ACCEPTABLE. The procedures in this study were adequate enough to judge the negative results valid.

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Assay for DNA Repair (UDS) in HeLa Cells
Treated with Sodium Bromide
(Selected Values for Grain Count)[1]

Treatment (ug/mL)	Mean Net Grains/ 100 Nuclei	% Nuclei w > 3 Net Grains	Mean Net Grains/100 Nuclei	% Nuclei w > 3 Net Grains
	TEST - 1			
	-S9		+S9	
Solvent (water)	26	1.9	-57	2.1
Sodium Bromide:				
1600[1]	37	3	-65	1.5
3200	-5	0	-16	3.5
6400	-61	1.5	-95	4.5
12,800	-11	2.0	-84	1.0
25,600	(T)	(T)	-64(T)	1.5(T)
4NQO: 0.02	1481***	100	--	--
-0.32	-3065***			
2AA: 2.5	--	--	37-769***	5.5-79.0
-40.0				
	TEST - 2			
	-S9		+S9	
Solvent (water)	36	2.6	-36	1.5
Sodium Bromide:				
1600[1]	19	2	-70	3
3200	-33	0.5	-4	0.5
6400	-51	0.5	-28	0.5
12,800	-35	0.5	-19	1.5
25,600	-35(T)	1.0	-25	1.0
4NQO: 0.02	1829***	100.0	--	--
-.32	-3663***			
2AA: 2.5	--	--	135*-627***	9.5-65.0
-20.0				
(40.0)			(15)	(3.5)

[1]/Values at lower doses did not differ from solvent control.
(T) = Toxicity.
*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
***Significant at $p < 0.001$.

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