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

009361

MAR 17 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Barium Metaborate/Busan 11-M1
Mutagenicity Assay-Salmonella

TO: Bill Crutchfield
PM Team Reviewer (72)
Reregistration Branch, SRRD (H7508C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/5/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 3/10/92
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 3/12/92
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant: Buckman Laboratories, Inc.
Chemical: Barium metaborate
Synonym: Busan 11-M1
Project No.: 2-1354
Caswell No.: 071
Record No.: none. Case: 818581; Submission: S410723
Identifying No.: 011101
DP Barcode: D173846
MRID No.: 421326-01
Action Requested: PLEASE EXPEDITE.

Comment: The Registrant has submitted a new study (cover letter dated 12/16/91): Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with a Confirmatory Assay, which was given the HED Project # 2-1354. This action was dated as sent 1/31/92 and in to HED on 2/14/92 (received by this reviewer on 2/27/92) marked EXPEDITE. PLEASE NOTE: Attached to this study, but not listed on the "bean sheet", was an acute study on Bobwhite (not TB II's purview) marked 6(a)(2) data. TB II re-routed this study to the appropriate section. TB II does not consider the mutagenicity study listed above to be 6(a)(2) data, nor in need of an EXPEDITE. The study has been reviewed, and the DER is attached.

139

Under the conditions of the study, exposure to Busan 11-M1 did not cause a positive response with any of the tester strains (TA98, TA100, TA1535, TA1537, and TA1538) with and without metabolic activation at dose levels up to 5000 µg/plate. This study is classified Acceptable, and it satisfies the guideline requirement [84-2(b)(1)] for a mutagenicity study (gene mutation).

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/5/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: Byron Backus, Ph.D. Byron T. Backus 3/5/92
Section II, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity-Salmonella typhimurium reverse mutation (Ames) assay

MRID NO.: 421326-01 TOX. CHEM. NO.:071

TEST MATERIAL: Busan 11-M1

SYNONYMS: barium metaborate

STUDY NUMBER: TA081.501014

SPONSOR: Buckman Laboratories, Inc.

TESTING FACILITY: Microbiological Associates, Inc.

TITLE OF REPORT: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) With a Confirmatory Assay.

AUTHORS: Richard HC San and Sheri J Olson

REPORT ISSUED: 12/3/91

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, exposure to Busan 11-M1 did not cause a positive response with any of the tester strains (TA98, TA100, TA1535, TA1537, and TA1538) with and without metabolic activation at dose levels up to 5000 µg/plate.

Classification: Acceptable. This study satisfies the guideline requirements [84-2(b)(1)] for a mutagenicity study (gene mutation).

A. MATERIALS

1. Test Compound: Busan 11-M1; Description: white powder; Batch #: Lot #: 19769; Purity: 94.3%.

2. Control Materials: Negative: dimethylsulfoxide (DMSO)
Solvent/final Concentration: dimethylsulfoxide (DMSO)/50 µL
Positive: (a) Non-activation:

2-Nitrofluorene	1.0 µg/plate TA98, TA1538
9-Aminoacridine	75 µg/plate TA1537
Sodium azide	1.0 µg/plate TA100, TA1535
(b) Activation:	
2-Aminoanthracene	1.0 µg/plate all strains

3. Activation: S9 derived from:

X Aroclor 1254	X induced	X rat	X liver
___phenobarbital	___non-induced	___mouse	___lung
___none		___hamster	___other
___other		___other	

S9 was (batch) prepared from male Sprague-Dawley rats induced with a single i.p. injection of Aroclor 1254 (500 mg/kg), 5 days prior to sacrifice, and stored at ≤-70 C. Each bulk preparation of S-9 was assayed for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100. The S-9 mix was prepared immediately before use and contained 10% microsomal enzymes, 5 mM glucose-6-phosphate, 4 mM β-nicotinamide-adenine dinucleotide phosphate, 8 mM $MgCl_2$, and 33 mM KCl in a 100 mM phosphate buffer at pH 7.4.

4. Test Organisms: The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, TA1537, TA1538. These were properly maintained and checked for appropriate genetic markers.

Histidine Mutation			Additional Mutations		
*his*G46	*his*C3076	*his*D3052	LPS	Repair	R-factor
TA1535	TA1537	TA1538	rfa	Δ*uvr*B	-
TA100		TA98	rfa	Δ*uvr*B	+R

5. Test compound concentrations used
Non-activated conditions:100, 333, 1000, 3333, and 5000 µg/plate
Activated conditions: 100, 333, 1000, 3333, and 5000 µg/plate

B. Study Design

1. Methodology: The test system was exposed to the test material

via the plate incorporation methodology of Ames *et al.* (1975). Test material dilutions were prepared immediately before use. In the absence of S-9 mix, 100 µL of tester strain and 50 µL of vehicle or test material were added to 2.5 mL of molten selective top agar at 45±2°C. When S-9 mix was required, 500 µL of S-9 mix was added to 2.0 mL of molten selective top agar, and test material/vehicle and tester strain additions were as above. When plating the positive controls, the test material aliquot was replaced by a 50 µL aliquot of the appropriate positive control. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. After the overlay solidified, the plates were inverted and incubated for ≈ 48 hours at 37±2°C. Plates were then counted or stored at 4±2°C until counted. The condition of the bacterial background lawn was evaluated for evidence of test material toxicity by using a dissecting microscope. This toxicity was scored relative to the vehicle (DMSO) control plate, using the criteria and codes listed in Figure 3 (copy appended). Revertant colonies for a given tester strain and activation condition, except for the positive controls, were counted either entirely by automated colony counter or entirely by hand. Plates with sufficient test material precipitate to interfere with automated colony counting were counted manually. For each replicate plating, the mean and standard deviation of the number of revertants per plate was calculated.

2. Criteria for a Valid Test/Evaluation of Results

To be considered valid, the following criteria must be met: "All tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) the deletion of the *uvrB* gene and the characteristic mean number of spontaneous revertants in the vehicle control. Cultures of tester strain TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to 0.6×10^9 cells/ml. The mean of each positive control must exhibit at least a three-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic dose levels are required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) A >50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2) A reduction in the background lawn."

For the test material to be considered positive, "it must cause a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article. Data sets for strains TA1535,

TA1537 and TA1538 will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than three times the mean vehicle control value. Data sets for strains TA98 and TA100 will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than two times the mean vehicle control value."

3. Preliminary cytotoxicity assay: Ten dose levels (6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, and 5000 µg/plate) of the test material were plated, one plate per dose, with an overnight culture of TA100 on selective minimal agar in both the presence and absence of rat microsomal enzymes.

RESULTS

No precipitate or appreciable toxicity was observed at any of the dose levels tested (up to 5000 µg/plate). The maximum dose that was plated in the mutagenicity assay was 5000 µg/plate.

4. Mutagenicity assay: Five dose levels (100, 333, 1000, 3333, and 5000 µg/plate) of test material, appropriate vehicle and positive controls were plated with tester strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence and absence of rat liver microsomal enzymes. All dose levels of test material and vehicle and positive controls were plated in triplicate.

RESULTS

It is reported that no positive responses were observed with tester strains TA98, TA100, TA1537, and TA1538, with and without microsomal enzymes. Although a 1.5-fold non-dose related increase was observed with strain TA98 with metabolic activation at 1000 and 3333 µg/plate, the authors did not evaluate this as positive due to the lack of a dose response (presumably as the increase at 5000 µg/plate was 1.3-fold). TB II notes that all of the test material dose levels displayed an increase (1.1-fold at the 2 lowest levels, 1.5-fold at the next 2 highest levels, and 1.3-fold at the highest level), but that all were less than 3 times the vehicle control value required for a + response. Additionally, the mean numbers of revertants/plate at all dose levels were within the acceptable range (10-50) for a negative control for this strain. Due to unacceptable vehicle control values (not reported in the first experiment), tester strain TA1535 with and without S-9 mix was not evaluated but was retested in a second experiment. It was stated that an evaluation was not possible because the colony selected for culture preparation in the first experiment was not histidine dependent resulting in confluent bacterial growth that precluded evaluation of the plates for mutagenesis.

In the second experiment, no positive responses were observed

with tester strain TA1535 with or without S-9 mix. To confirm all observations, a confirmatory test was conducted.

In the confirmatory test, no positive responses were observed with any of the tester strains, with and without microsomal activation. A 1.7-fold dose-related increase at the 5000 µg/plate (Table 14, copy appended) was observed with tester strain TA100 in the absence of metabolic activation, and this was part of a dose-related trend. However, this was not evaluated as positive since the number of revertants was not 2-fold greater than that of the mean vehicle control value. A 2-fold non-dose-related increase was observed with tester strain TA1537 in the absence of S-9 mix, but this was not evaluated as positive by the Study Director since it was not 3-fold greater than the mean control value.

C. DISCUSSION

The authors stated that all criteria for a valid study had been met (as described in the protocol), with the exception of the titer values for the cultures of tester strains TA1535 used in the second experiment and TA100 used in the confirmatory assay. Since the culture turbidity data and all other strain characterization indicators were acceptable (the vehicle and positive control values and the ampicillin, crystal violet and ultraviolet light sensitivities), the Study Director accepted the data generated with these cultures. Although the criteria for a valid study states that tester strain culture titers "must be greater than or equal to 0.6×10^9", 67% of that required was attained for these two strains and, considering that the numbers of revertants observed for both the vehicle and positive control plates fell within acceptable ranges, TB II agrees that the data can be accepted.

D. CONCLUSION

Under the conditions of the study, Busan 11-M1 did not cause a positive response with any of the tester strains with and without metabolic activation at dose levels up to 5000 µg/plate. This study is classified Acceptable, and it satisfies the guideline requirement [84-2(b)(1)] for a mutagenicity study (gene mutation).

7

RIN 5117-93 Barium Metaborate
Tox Review #009361

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