DATA EVALUATION RECORD

5/8/200

MESOTRIONE (Metabolite AMBA)

Study Type: §84-2; Bacterial Reverse Gene Mutation Test

Work Assignment No. 2-1-52 FF (MRID 44505038)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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DATA EVALUATION RECORD

STUDY TYPE: *Salmonella typhimurium* and *Escherichia coli* mammalian activation gene mutation assay

OPPTS Number: 870.5100 OPP GUIDELINE NO.: 84-2

DP BARCODE: D259369 SUBMISSION CODE: S541375

P.C. CODE: 122990 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): AMBA (metabolite of mesotrione, 99%)

SYNONYMS: 4-(Methylsulfonyl) - amino benzoic acid

CITATION: Callander, R.D. (1996). AMBA (2-Amino-4-Methylsulfonyl Benzoic Acid): An Evaluation of Mutagenic Potential Using *Salmonella typhimurium* and *E. coli*. Zeneca's Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report No. CTL/P/5226. Laboratory Study No. YV3941. November 28, 1996. MRID 44505038. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE

EXECUTIVE SUMMARY:

In a microbial reverse gene mutation assay (MRID 44505038), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strains WP2P and WP2P μvrA were exposed in repeat assays to AMBA (99%), a metabolite of mesotrione, in dimethylsulfoxide (DMSO) at concentrations of 0, 100, 200, 500, 1000, 2500, or 5000 μg/plate in the presence and absence of mammalian metabolic activation (±S9). The standard plate incorporation test was used in both assays (-S9) and the initial assay (+S9). The standard plate incorporation test was used in both assays (-S9) and the initial assay (+S9). The liquid preincubation modification was used in the repeat assay (+S9). S9 homogenates for metabolic activation were prepared from phenobarbital/β-naphthoflavone-induced rat livers.

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MBA was tested to the limit dose. AMBA was not mutagenic in any tester strain under all conditions. The positive control substances induced marked increases in revertant colonies in their respective strains with or without activation.

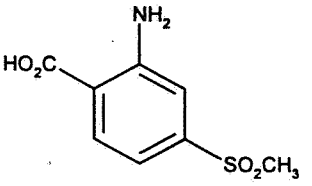
This study is classified as **acceptable (84-2)** and satisfies the FIFRA Test Guideline requirements for an *in vitro* mutagenicity (reverse gene mutation) test.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: AMBA
Description: Yellow solid
Lot/Batch No.: WRC16010-09-01
Purity: 99%
Stability of compound: Not specified. Reported to have been used within the stated expiration date. Stored refrigerated at 5° C.
CAS No.: Not provided.
Structure (Not present in report; presumably copied from Merck Index or other source):



Vehicle: Dimethylsulfoxide (DMSO)
Other comments: DMSO also served as solvent for all positive control substances, except mitomycin C and sodium azide, which were dissolved in water (assumed deionized).

2. Control Materials:
Negative: Vehicle (DMSO) served as negative control
Vehicle/final concentration: DMSO/0.1 mL/plate (plate incorporation method) or 0.02 mL/plate (preincubation method)

Positive:

Nonactivation:

Acridine Mutagen ICR 191	0.5, 1.0 and 2.0 μg/plate	*S. typhimurium* TA1537
Daunomycin HCl (DR)	0.2, 0.5, and 1.0 μg/plate	*S. typhimurium* TA98
N-Ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	0.2, 0.5, and 1.0 μg/plate	*E. coli* WP2P μvraA
Mitomycin C	0.2, 0.5, and 1.0 μg/plate	*E. coli* WP2P

Sodium azide	0.5, 1.0, and 2.0 μg/plate	*S. typhimurium* TA100 and TA1535

Activation:

2-Aminoanthracene (2AA)	0.2, 0.5, and 1.0 μg/plate	*S. typhimurium* TA98 and TA100
	0.5, 1.0, and 2.0 μg/plate	*S. typhimurium* TA1535 and TA1537
	5.0, 10.0, and 20.0 μg/plate	*E. coli WP2P*
	1.0, 2.0, and 5.0 μg/plate	*E. coli* WP2P μvrA

3. Metabolic Activation:

S9 fraction was prepared in the laboratory and was derived from male Alderley Park (Alpk:APfSD) male albino rats:

___ Aroclor 1254	__x__ induced	__x__ rat	__x__ liver
___ phenobarbital	____ non-induced	____ mouse	____ lung
___ none		____ hamster	____ other
___ phenobarbital/ β-naphthoflavone		____ other	

The S9 cofactor solution contained $MgCl_2$ (12 mM), KCl (49.5 mM), NADP (6 mM), and glucose-6-phosphate (7.5 mM), and sodium phosphate buffer at pH 7.4 (150 mM). The S9 mix contained: S9 fraction (10%, v/v), sucrose-Tris-EDTA buffer (23%, v/v), and cofactor solution (65%, v/v). The final concentration of S9 in culture was approximately 2%.

4. Test Organisms:

S. typhimurium strains	*E. coli* strains
___TA97 __x__TA98 __x__ TA100	__x__ WP2P
___TA102 ___TA104 __x__TA1535	__x__ WP2P μvrA

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___TA1537 ___TA1538	

Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, r factor)? Yes

5. Test Compound Concentrations Used

Mutagenicity assays
Initial mutagenicity test:

Conducted with tester strains TA98, TA100, TA1535, TA1537, WP2P, and WP2P μvrA at concentrations of 0, 100, 200, 500, 1000, 2500, and 5000 μg/plate (±S9) using the standard plate incorporation method.

Conducted with tester strains TA98, TA100, TA1535, TA1537, WP2P, and WP2P μvrA at concentrations of 0, 100, 200, 500, 1000, 2500, and 5000 μg/plate (±S9). Liquid preincubation conditions (+S9) and standard plate incorporation conditions (-S9) were used.

The test substance at all concentrations was tested in triplicate, the positive controls were tested in duplicate, and the vehicle controls were tested in quintuplicate.

B. TEST PERFORMANCE

1. Type of Assay

x	Standard plate incorporation test
	Liquid preincubation (60) minutes
	"Prival" modification (i.e., azo-reduction method)
	Spot test
	Other (describe)

2. Protocol

Bacteria (number of cells/mL unspecified) from overnight cultures were

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stored at room temperature until use. The test substance and the positive controls were diluted in DMSO (or deionized water for mytomycin C and sodium azide) to specified concentrations. For the standard plate incorporation test, melted top agar (2 mL) supplemented with 0.5 mM histidine/0.5 mM biotin for *S. typhimurium* (or 0.5 mM tryptophan for *E. coli*), S9 mix or sucrose buffer (0.5 mL), the tester strain (0.1 mL), and the test or control article (0.1 mL) were combined in bijou bottles, mixed, then poured rapidly over minimal glucose agar plates. After solidification of the agar, the plates were inverted and incubated for 3 days in the dark at 37° C. For the preincubation method, S9 mix or sucrose buffer (0.5 mL), the tester strain (0.1 mL), and the test or control article (0.02 mL for 2-aminoanthracene; made up to 0.1 mL with phosphate buffered saline) were combined in bijou bottles and incubated for 60 minutes at 37° C with shaking. Melted top agar (2 mL) was then added to each bottle, and the cultures were mixed, poured onto agar plates, and incubated as described above.

Following a 3-day incubation, the plates were evaluated for growth inhibition (reduction of background lawn) and total revertant colony numbers. Revertant colonies were counted using an automatic colony counter.

3. Evaluation Criteria

(a) Assay validity: The assay was considered valid if both of the following were met for each strain: (i) the concurrent solvent control data were acceptable; and (ii) the positive control data showed unequivocal positive responses.

(b) Positive response: The test material was considered mutagenic if one or both of the following criteria were met: (i) there was a statistically significant, dose-dependent increase in the number of revertants, and (ii) at least one dose level induced a revertant frequency ≥ 2-fold the concurrent vehicle control value. A result that met one or both of the above criteria, but was not reproducible was considered equivocal.

(c) Statistical analysis: Data were analyzed using a one-tailed student's t-test (Ehrenberg, 1984). For each dose level, the corresponding probability was computer-generated using the appropriate degrees of freedom. Significance was established at

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$p < 0.01$. Values of $0.01 \geq p < 0.05$ were considered to indicate a possible effect.

II REPORTED RESULTS

A. ANALYTICAL DETERMINATIONS

Analyses of the dose formulations for stability, homogeneity, and achieved concentrations were not performed.

B. MUTAGENICITY ASSAYS

AMBA was evaluated for mutagenicity using both plate incorporation (±S9) and liquid preincubation conditions (+S9) at six dose levels ranging from 100 to 5000 μg/plate. Reduction of background lawn was observed at 5000 μg/plate (±S9) in the initial assay and at ≥2500 μg/plate (+S9) and 5000 μg/plate (-S9) in the repeat assay. Statistically significant increases ($p < 0.05$ or 0.01) in revertant frequencies were observed at the low or intermediate dose levels in TA98, TA1537, WP2P, and WP2P μvra (-S9); however, these increased revertant frequencies were not dose dependent and reproducible. In the initial and repeat mutagenicity tests, the positive control substances induced marked increases in revertant frequencies in their respective strains. The data for the mutagenicity assays were summarized as mean revertants/plate ∓ S.D. for each strain and condition tested in Study Report Tables 1-6, pages 21-32, and are summarized in Tables 1 and 2 below.

Table 1. Summary of Initial Mutagenicity Assay Results

Dose (μg/plate)	Mean Revertants/Plate[a]											
	TA98		TA100		TA1535		TA1537		WP2P		WP2P μvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Standard Plate Test												
Vehicle Control	19.0	20.4	94.6	106.0	15.8	20.4	2.6	3.4	33.4	47.2	101.4	122.3
100	24.0*	17.7	90.3	108.7	13.3	16.7	2.3	2.7	27.7	39.7	113.7	99.0
200	22.0	24.3	84.3	104.7	12.3	19.7	3.3	4.0	26.0	40.7	119.3*	108.7
500	21.7	19.3	90.0	120.3	14.7	17.7	3.0	2.7	30.0	47.7	105.3	99.0
1000	23.3*	20.0	88.7	121.0	19.0	21.0	3.7*	3.0	26.3	42.3	102.7	106.0
2500	22.7*	20.5	96.7	110.0	13.0	13.7	3.0	4.0	32.0	39.3	106.7	105.3
5000	19.3	20.7	80.3	111.3	12.0	23.7	2.3	4.3	25.7	35.0	82.0	54.7
Positive Control[b]	132.0**	516.0**	346.0**	484.0**	244.5**	119.5**	29.0**	17.5**	55.0*	73.0*	197.0**	428.5**

a Means of up to five plates.

b Mean revertants/plate reported for the lowest concentration tested.

* $p < 0.05$**, $p < 0.01$

Table 2. Summary of Repeat Mutagenicity Assay Results

Dose (μg/plate)	Mean Revertants/Plate											
	TA98		TA100		TA1535		TA1537		WP2P		WP2P μvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Standard Plate Test (±S9) with Liquid Pre-incubation (+S9 only)												
Vehicle Control	20.6	20.4	94.6	96.2	15.8	24.8	4.2	6.2	27.6	50.6	108.2	151.2
100	23.3	21.0	100.3	104.0	14.3	20.0	2.3	5.7	26.7	42.3	112.3	160.0
200	25.3*	18.3	85.7	104.0	13.7	24.7	3.0	7.7	35.0*	48.7	121.0	158.0
500	21.3	19.0	88.7	110.3	18.0	28.0	3.3	6.3	31.0	42.3	98.3	155.7
1000	25.0	17.7	101.0	102.3	14.3	26.3	3.3	4.0	38.7*	51.3	110.3	147.0
2500	19.0	19.3	104.3	108.0	11.7	31.3	4.0	10.3	30.0	38.7	84.7	123.3
5000	22.3	13.3	93.3	101.3	9.0	22.0	3.7	8.3	28.7	44.7	75.7	134.7
Positive Control[b]	57.0**	166.5**	402.0**	452.5**	170.0**	74.0**	13.0**	16.0*	84.0**	76.0**	243.0**	515.5**

a Means of up to five plates.
b Mean revertants/plate reported for the lowest concentration tested.
* $p < 0.05$**, $p < 0.01$.

III. DISCUSSION/CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS

Under the conditions of this study, AMBA was considered negative for mutagenicity with or without S9 activation.

B. REVIEWER'S DISCUSSION

AMBA, tested up to the limit dose of 5000 μg/plate in two independent assays using four *Salmonella typhimurium* strains and two *E. coli* strains, was cytotoxic at ≥ 2500 μg/plate (+S9) and 5000 μg/plate (-S9), did not induce increases in revertant colonies with or without S9 activation, under plate incorporation or liquid preincubation conditions. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls.

C. STUDY DEFICIENCIES

Data on the analysis of dose formulations for actual concentration were not submitted. However, as the test compound was tested up to the limit dose with toxicity (reduced background lawn) at the highest two dose levels, this deficiency is not expected to alter the conclusions of this review. Several minor deficiencies noted in the study that are not considered to affect the validity of the study results are:

- Data for the test compound stability (reported to be available from the sponsor) and homogeneity were not provided.
- The number of cells plated per culture was not reported.
- Historical vehicle control ranges were not provided.
- No explanation was provided as to why the preincubation method was used only with S9 activation.

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