DATA EVALUATION RECORD

5/9/2000

MESOTRIONE (ZA 1296)

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

Work Assignment No. 2-1-52 II (MRID 44373526)

Prepared for

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U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

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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 Number: §84-2

DP BARCODE: D259369 SUBMISSION CODE: S541375
P.C. CODE: 122990 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): ZA 1296 (mesotrione, 98.1% a.i.)

SYNONYMS: 2-[4-(Methylsulphonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione

CITATION: Callander, R.D. (1993). ZA 1296 - An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli*. Zeneca's Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report No. CTL/P/4206. Laboratory Study No. YV3205. December 22, 1993. MRID 44373526. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE

EXECUTIVE SUMMARY:
In a microbial reverse gene mutation assay (MRID 44373526), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strains WP2P and WP2P *uvrA* were exposed to ZA 1296 (98.1% a.i.) in sterile deionized water in repeat tests at concentrations of 0, 100, 200, 500, 1000, 2500, or 5000 µg/plate in the presence and absence of mammalian metabolic activation (±S9). Both the standard plate incorporation test (±S9) and the liquid preincubation modification (+S9) were performed. S9 homogenates for metabolic activation were prepared from phenobarbital/ß-naphthoflavone-induced rat livers. The standard strain-specific mutagens served as positive controls.

ZA 1296 was tested to the limit dose. ZA 1296 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.

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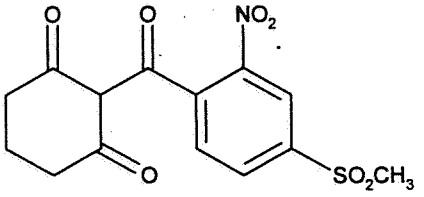
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
   Description: Light beige solid
   Lot/Batch #: P6
   Purity: 98.1% a.i.
   Stability of compound: Not specified. It was stated that the sample was used within the stated expiration date, based on information from the Sponsor.
   CAS #: 104206-82-8
   Structure (Not present in study report; presumably copied from independent sources):



   Vehicle: Sterile deionized water
   Other comments: DMSO served as solvent for all positive control substances, except mitomycin C and sodium azide, which were dissolved in sterilized deionized water.

2. Control Materials
   Negative: Sterile deionized water served as negative control
   Vehicle/final concentration: Deionized water/0.1 mL/plate

   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 *uvrA*
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 TA 100
	0.5, 1.0, and 2.0 µg/plate	*S. typhimurium* TA1535 and TA 1537
	5.0, 10.0, and 20.0 µg/plate	*E. coli* WP2P
	1.0, 2.0, and 5.0 µg/plate	*E. coli* WP2P *uvrA*

3. Metabolic Activation

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

____ Aroclor 1254 ____ phenobarbital ____ none __x__ phenobarbital/ ß-naphthoflavone	__x__ induced ____ non-induced	__x__ rat ____ mouse ____ hamster ____ other	__x__ liver ____ lung ____ 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 (67%, 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 ___ TA102 ___ TA104 __x__ TA1535 __x__ TA1537 ___ TA1538	__x__ WP2P __x__ WP2P uvrA

Properly maintained? **Yes**

Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test Compound Concentrations Used

Mutagenicity Assays

Initial mutagenicity test: The test was conducted first using tester strain TA 98. The test was then conducted using the other five tester strains: TA100, TA1535, TA1537, WP2P,

and WP2P *uvrA*. All six strains were tested at concentrations of 0, 100, 200, 500, 1000, 2500, and 5000 µg/plate (±S9) using the standard plate incorporation method.

Repeat mutagenicity test: Conducted with tester strains TA98, TA 100, TA1535, TA 1537, WP2P, and WP2P *uvrA* 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
X	Liquid preincubation (60) minutes
	"Prival" modification (i.e. azo-reduction method)
	Spot test
	Other (describe)

2. Protocol

Aliquots (0.1 mL) of bacteria (number of cells/mL unspecified) from overnight cultures were stored at room temperature until use. The test substance and the positive controls were diluted in the appropriate vehicle 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 0.1mL 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 all of the following were met for each strain: (i) the concurrent solvent control data were acceptable; (ii) the positive control data showed unequivocal positive responses; and, (iii) at least the lowest test compound dose did not show evidence of toxicity, and at least three test doses did not show significant toxicity (i.e. significant loss of background growth and/or reductions in colony numbers).

(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 $p<0.01$. Values of $0.01 \leq p<0.05$ 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

ZA 1296 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 the background lawn was observed at 5000 µg/plate (±S9). Statistically significant increases ($p<0.05$ or 0.01) in revertant frequencies were observed in TA 100, TA 1535, TA 1537, and WP2P *uvrA*, ±S9; however, these increased frequencies were either not dose-dependent or not 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 1-8, pages 16-29, and are summarized in Tables 1 and 2 below.

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Table 1. Summary of Initial Mutagenicity Assay Results

Dose (µg/plate)	Mean revertants/plate[a]											
	TA98		TA100		TA1535		TA1537		WP2P		WP2P *uvrA*	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Standard plate test												
Vehicle control	17.6	21.0	91.6	101.0	9.4	12.6	6.8	4.8	39.4	57.2	101.8	151.2
100	16.7	17.0	87.7	105.3	8.3	12.0	5.0	5.0	32.0	52.3	94.3	151.3
200	18.0	19.3	89.3	97.0	8.0	16.7*	7.0	6.0	30.0	48.7	89.7	149.5
500	19.0	16.5	84.3	107.3	6.7	17.3*	6.0	6.0	30.0	53.0	93.0	146.0
1000	19.0	20.3	80.0	131.5*	4.3	11.3	5.7	8.7	31.0	44.0	98.5	128.7
2500	20.0	19.3	78.3	142.3**	4.3	17.3	4.3	8.0	37.7	51.0	98.7	134.0
5000	20.3	26.3	88.3	106.3	5.7	9.7	2.0	12.7**	24.7	43.3	117.3*	127.3
Positive control[b]	1151.5**	102.0**	294.5**	277.5**	224.5**	105.0**	46.0**	24.5**	154.5**	80.0**	256.5**	383.5**

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[a]											
	TA98		TA100		TA1535		TA1537		WP2P		WP2P *uvrA*	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Standard plate test (-S9) or liquid pre-incubation method (+S9)												
Vehicle control	21.2	26.4	86.2	92.0	11.6	10.8	5.2	9.3	28.8	51.8	86.2	110.4
100	18.0	25.3	79.0	90.3	7.7	11.7	7.0	9.0	23.3	42.0	71.3	105.0
200	23.0	20.0	86.0	89.0	8.7	15.0*	4.3	7.7	27.3	42.7	87.7	105.0
500	20.7	20.0	90.0	101.7	14.0	13.7	9.0	7.3	21.3	36.0	76.0	110.0
1000	20.7	24.7	93.3	92.3	11.7	15.3	6.3	6.3	22.7	29.0	79.7	112.3
2500	23.3	22.7	105.0**	95.3	12.3	17.0*	9.7*	7.0	22.0	33.0	72.3	76.3
5000	24.0	28.7	113.3**	44.0	10.3	9.3	7.3	3.0	24.0	20.3	96.3	88.3
Positive control[b]	472.0**	154.0**	179.0**	278.5**	199.0**	90.5**	49.5**	40.5**	112.0**	77.5**	196.0**	210.0**

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, ZA 1296 was considered negative for mutagenicity with or without S9 activation.

B. Reviewer's Discussion

ZA 1296, tested up to the limit dose of 5000 µg/plate in two independent assays using four *S. typhimurium* strains and two *E. coli* strains, 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 and toxicity (reduction of background lawn) was observed at this dose, 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 pre-incubation method was used only with S9 activation.