DATA EVALUATION RECORD

5/9/2000

MNBA (Metabolite of Mesotrione)

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

Work Assignment No. 2-01-52 EE (MRID 44505037)

Prepared for

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

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Joan L. Harlin, M.S.

Signature: Joan L. Harlin
Date: 3/28/00

Secondary Reviewer:
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 3/28/00

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 03/28/00

Quality Assurance:
Sharon Meyer, Ph.D.

Signature: Sharon L. Meyer
Date: 3/19/00

Disclaimer

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EPA Reviewer: Irving Mauer, Ph.D. Date: 04/14/00
Registration Action Branch 3, HED (7509C)
EPA Work Assignment Manager: Marion Copley, DVM, DABT Date: 5/9/00
Registration Action Branch 1, HED (7509C)

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): MNBA (metabolite of mesotrione, 97%)

SYNONYMS: 2-Nitro-4-methylsulfonyl benzoic acid

CITATION: Callander, R.D. (1996). MNBA: An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli*. Zeneca's Central Toxicology Laboratory (CTL), Alderly Park, Macclesfield, Cheshire, UK. Laboratory Report No. CTL/P/4955. Laboratory Study No. YV3770. April 22, 1996. MRID 44505037. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE

EXECUTIVE SUMMARY:

In a microbial reverse gene mutation assay (MRID 44505037), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strains WP2P and WP2P *uvrA* were exposed in repeat assays to MNBA (97%), 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 liquid pre-incubation modification was used in the repeat assay (+S9). S9 homogenates for metabolic activation were prepared from phenobarbital/ß-naphthoflavone-induced rat livers. The standard strain-specific mutagens served as positive controls.

MNBA was tested to the limit dose. MNBA was negative for mutagenicity in all tester strains under all conditions, except for an equivocal response with tester strain TA100 using the pre-incubation method. A statistically significant increase ($p<0.01$) in mutation frequency was observed in TA100 at 5000 µg/plate (+S9). Since the pre-incubation method was used only in the repeat assay, the reproducibility of the response under these conditions was not determined.

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The positive control substances induced marked increases in revertant colonies in their respective strains with or without activation.

Because of a number of deficiencies listed in Sections III B and C (below), this study is classified as **unacceptable/not upgradable (§84-2)** and does not satisfy 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.

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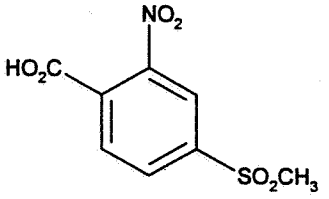
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MNBA
   Description: White solid
   Lot/Batch #: WRC15483-30-1
   Purity: 97%
   Stability of compound: Not specified. Stored at room temperature in the dark.
   CAS #: 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 deionized water.

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 (pre-incubation 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 *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) male 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) in sterile deionized water. 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:

Conducted with tester strains TA98, TA100, TA1535, TA1537, WP2P, and WP2P *uvrA* at concentrations of 0, 100, 200, 500, 1000, 2500, and 5000 µg/plate (±S9). Plate incorporation conditions were used.

Repeat mutagenicity test:

Conducted with tester strains TA98, TA100, TA1535, TA1537, WP2P, and WP2P *uvrA* at concentrations of 0, 100, 200, 500, 1000, 2500, and 5000 µg/plate (±S9). Liquid pre-incubation 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

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 DMSO (or deionized water for mitomycin 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; 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 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 $p<0.01$. 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

MNBA was evaluated for mutagenicity using both plate incorporation and liquid pre-incubation conditions (+S9) and plate incorporation conditions (-S9) at six dose levels ranging from 100 to 5000 µg/plate. Reduction of the background lawn was observed at ≥2500 µg/plate (-S9) in the initial assay and at 5000 µg/plate (-S9) in the repeat assay. The mean number of revertants was statistically significant ($p<0.01$) with TA100 at 5000 µg/plate (+S9); however, the pre-incubation method was used only in the repeat test so it is not known whether the result was reproducible. In the presence or absence of S9 activation, there were no increases in revertant frequencies at any dose level of MNBA in any strain, under either treatment condition. 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 are summarized as mean revertants/plate ± S.D. for each strain and condition 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 *uvrA*	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Standard plate test												
Vehicle control	20.2	20.4	89.2	119.4	7.6	12.8	3.2	3.8	39.2	45.8	91.0	123.0
100	20.7	17.7	88.3	117.3	9.7	9.7	3.3	5.0	35.3	44.0	107.0**	130.7*
200	18.0	21.0	87.3	117.7	5.3	11.0	3.3	4.3	31.0	45.7	87.7	112.0
500	17.7	21.7	81.7	111.7	6.7	12.3	3.7	4.3	21.0	49.7	88.7	122.7
1000	19.3	23.0	83.3	121.0	7.7	10.7	2.7	4.3	22.3	48.7	82.3	127.3
2500	4.7	21.7	43.0	130.0	2.0	11.3	0.7	3.7	19.0	41.0	73.3	130.7
5000	0.0	18.3	0.0	128.0	0.0	9.0	0.0	4.0	7.3	36.0	4.3	92.3
Positive control[b]	103.5**	93.5**	388.5**	276.5**	176.5**	40.0**	22.5**	13.5**	88.5**	78.0**	217.5**	238.0**

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) with liquid pre-incubation (+S9 only)												
Vehicle control	19.8	23.2	82.6	83.6	10.6	20.6	3.2	7.2	31.4	52.4	113.2	163.0
100	19.7	22.7	81.3	82.7	9.3	23.3	3.3	6.3	37.5	48.0	112.5	164.0
200	20.3	21.0	82.0	87.7	9.0	25.0	3.0	6.3	36.0	56.7	117.7	186.3**
500	22.3	26.7	81.7	77.0	9.3	19.3	3.3	5.3	37.0	45.0	115.3	178.3
1000	22.0	25.0	82.7	98.7	12.0	18.3	3.3	6.0	33.7	56.0	105.7	186.7*
2500	18.0	23.7	66.3	83.7	10.7	21.3	2.7	8.0	32.3	51.0	91.0	188.7*
5000	14.3	24.7	4.3	124.7**	0.0	25.3*	1.3	5.3	7.3	52.3	14.7	182.0
Positive control[b]	174.0**	75.5**	396.0**	168.5**	168.5**	50.0**	22.5**	32.5**	102.5**	86.0**	271.5**	322.0**

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

B. Reviewer's Discussion

MNBA, tested up to the limit dose of 5000 µg/plate in two independent assays using four *S. typhimurium* strains and two *E. coli* strains, was cytotoxic at ≥2500 µg/plate (-S9), and did not induce increases in revertant colonies with or without S9 activation, under plate incorporation or pre-incubation conditions, except for an equivocal response with tester strain TA100 at 5000 µg/plate (+S9). A statistically significant increase ($p<0.01$) was observed; however, the pre-incubation method was used only in the repeat test so it is not known whether the result was reproducible. 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 pre-incubation method was used only with S9 activation.

A repeat test using the pre-incubation method was needed, because a statistically significant increase in mutation frequency was observed with TA100 (+S9) using this method.