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Fungicide and Herbicide Toxicological Evaluation Section,
Health Evaluation Division Date 8/30/05

TXR: 0052097

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: *Salmonella typhimurium/Escherichia coli*--mammalian microsome mutagenicity assay; OPPTS 870.5100 [§84-2]; OECD 471, 472

DPBARCODE: D292904 SUBMISSION NO.:

PC CODE: 123009 TOX. CHEM. NO.: None MRID No.: 45902229

TEST MATERIAL (PURITY): Reg. No. 388 010 (99.3%, Batch No. N3)

COMPOSITION/SYNONYM(S): None

CITATION: Engelhardt, G. and Hoffmann, H.D. (2001). *Salmonella typhimurium/ Escherichia coli* Reverse Mutation Assay (Standard Plate Test and Preincubation Test) with Reg. No. 388 010. Experimental Toxicology and Ecology BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany; Laboratory Project Identification 40M0362/984231, Document No. 2001/1021847; Study Completion Date: May 29, 2001. Unpublished MRID NUMBER: 45902229

SPONSOR: BASF Corp., Agricultural Products, Research Triangle Park, NC

EXECUTIVE SUMMARY: In independently performed microbial mutagenicity assays (MRID No. 45902229), histidine-deficient (*his*$^-$)strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, and TA100) and tryptophan-deficient (*trp*$^-$) *Escherichia coli* strain WP2 *uvrA* were exposed to Reg. No. 388 010 (99.3%, Batch No. N3) for 48-72 hours to five concentrations (20-5000 µg/plate or 10-1000 µg/plate) in the standard plate test and five concentrations (2-500 µg/plate) in the preincubation modification of the plate test in the presence and absence of S9 activation. The S9 fraction was derived from Aroclor 1254 induced Sprague Dawley rat livers and the test material was delivered to the test system in dimethyl sulfoxide (DMSO); the appropriate solvent and positive controls were included.

Reg. No. 388 010 was cytotoxic to all of the *Salmonella* strains and *E. coli* WP2 *uvr*A, causing a reduction in revertant colonies, the background lawn of growth and/ or the cell titres at 5000 µg/plate +/-S9 and 300 -1000 µg/plate +S9 (plate incorporation). In the preincubation assay, reduced revertant colonies were observed for the majority of strains at ≥250 µg/plate +/-S9. Nonactivated and S9-activated positive controls induced the expected mutagenic response in the corresponding

tester strain. **There was, however, no indication of a mutagenic response in any strain up to cytotoxic levels either with or without S9 activation.**

The study is classified as **Acceptable/Guideline** and satisfies the requirements for FIFRA Test Guideline 84-2 for microbial gene mutation mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Reg. No. 388 010
   Description: Light yellow powder
   Lot/batch number: N 3
   Purity: 99.3%
   Stability: The report indicated that the test article was found to be stable in dimethyl sulfoxide (DMSO) over a period of 4 hours.
   CAS number: 223646-24-0
   Structure: Not provided
   Solvent used: DMSO
   Other comments: The test material was stored at room temperature.

2. Control Materials:

   Negative: None

   Solvent/final concentration: DMSO/0.1 mL per plate

   Positive:

Nonactivation:	
N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	5.0 µg/plate TA1535, TA100
4-Nitro-o-phenylenediamine (4-NPDA)	10.0 µg/plate TA98
9-Aminoacridine (9-AA)	100.0 µg/plate TA1537
4-Nitroquinoline-N-oxide (4-NQO)	5.0 µg/plate *E. coli* WP2 *uvr*A
Activation:	
2-Aminoanthracene (2-AA)	2.5 µg/plate all Salmonella strains
	60.0 µg/plate *E. coli* WP2 *uvr*A

3. Activation: S9 derived from adult male Sprague-Dawley (200-300 g)
_x_ Aroclor 1254 _x_ induced _x_ rat _x_ liver
__ phenobarbital __ noninduced __ mouse __ lung
__ none __ hamster __ other
__ other __ other

The S9 homogenate was prepared by the performing laboratory, had a protein content of 32.8 mg/mL and was assayed prior to use for its ability to convert the reference mutagen, benzo[a]pyrene to its reactive metabolites.

S9 mix composition:

Component:	Amount/mL
Phosphate buffer, pH 7.4	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test Organism Used: *S. typhimurium* strains
__ TA97 _x_ TA98 _x_ TA100 __ TA102 __ TA104
_x_ TA1535 _x_ TA1537 _ TA1538
list any others: *E. coli* WP2 *uvr*A

Source: The *Salmonella* tester strains were obtained from KNOLL Aktiengesellschaft and *E. coli* WP2 *uvr*A was obtained from Merck.

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

(a) Preliminary Cytotoxicity Assay: Not performed.

(b) Mutation Assays:

Plate Incorporation: Five concentrations (0, 20, 100, 500, 2500 and 5000 µg/plate) were evaluated in the presence and absence of S9 activation with all *Salmonella* tester strains and with *E. coli* WP2 *uvr*A. Triplicate plates were used per strain per dose per condition.

Repeat Plate Incorporation: Five concentrations (0, 10, 30, 100, 300 and 1000 µg/plate) were evaluated in the presence and absence of S9 activation with all *Salmonella* tester strains and with *E. coli* WP2 *uvr*A. Triplicate plates were used per strain per dose per condition. The repeat plate incorporation assay was performed presumably because of cytotoxicity for all strains at 5000 µg/plate in the initial plate incorporation test..

Preincubation Modification: Five concentrations (0, 2, 10, 50, 250 and 500 µg/plate) treated as above for the plate incorporation assay.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: _x_ Standard plate test
   _x_ Pre-incubation (20) minutes at 37 °C
   ___ "Prival" modification
   ___ Spot test
   ___ Other (describe)

2. Protocol: Similar procedures were used for the plate incorporation and preincubation modification to the mutation assay. A 0.1-mL aliquot of the appropriate overnight broth culture of each tester strain, 0.1 mL of the appropriate test material dose, solvent, or positive control and either 0.5 mL of the S9 mix buffer (nonactivated series) or 0.5 mL of the S9-cofactor mix (S9-activated series) were added to tubes containing 2.0 mL volumes of molten top agar supplemented with biotin and histidine (for the *Salmonella* strains) or tryptophan (for *E. coli* WP2 *uvr*A) . For the preincubation modification, reactive mixtures containing the tester strain, test dose, solvent or positive control and the S9 buffer or the S9 mix were preincubated for 20 minutes at 37°C. The top agar was added and the contents of each tube were mixed, poured over minimal medium plates and incubated at 37±2°C for 48-72 hours. At the end of incubation, plates were scored for revertant colonies, background lawns of growth were examined and cell titres from the two highest test concentrations or the vehicle (with S9 activation) were determined. Means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition. Sterility controls were prepared for the top agar, S9 mix, phosphate buffer, solvent and the two highest test material levels.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if (1) the number of spontaneous revertants for each tester strain was within the expected ranges provided by the performing laboratory, (2) the sterility controls were negative, (3) the density of the tester strain cultures was sufficient (i.e, $\geq 10^9$ cells/mL), and (4) the nonactivated and S9-activated positive controls produced mutagenic responses that were within the provided ranges of the performing laboratory. For all historical control ranges see MRID No. 45902229, pp.52-58.

(b) Positive response: The test material was considered positive if it caused a reproducible and dose-related increase in the mean number of revertants per plate of at least one strain. This increase must be at least 2-fold.

C. REPORTED RESULTS:

1. Mutation Assays: Summarized results from the plate incorporation and preincubation assays are presented in Tables 1 and 2. Although a repeat plate incorporation assay was conducted with a lower dose range (2-1000 µg/plate +/-S9), presumably because of cytotoxicity for all strains at 5000 µg/plate +/-S9, we selected the data from the first assay as representative. As shown, Reg No. 388 010 was cytotoxic to all *Salmonella* strains and *E. coli* WP2 *uvr*A at the highest concentration tested (HTC) in both the plate incorporation procedures. Based on cytotoxicity at 5000 µg/plate +/-S9 in the initial and 1000 µg/plate +S9 in th repeat plate tests, the HTC in the preincubation test was lowered to 500 µg/plate +/-S9. As presented in Table 2, slight reductions in revertant colonies of the majority of strains were noted at the HTC both with and without S9 activation in the preincubation test. There was, however, no indication of a mutagenic response in any strain at any level either with or without S9 activation using either procedure. By contrast, all strains responded to the mutagenic action of the appropriate positive controls.

The study author concluded, therefore, that Reg. No. 388 010 was negative in this bacterial test system using both the plate incorporation and the preincubation modification to the standard assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and we concur with the study authors' conclusion that Reg. No. 388 010 was cytotoxic but not mutagenic up to the limit concentration (5000 µg/plate +/-S9) using the plate incorporation and up to a cytotoxic level (500 µg/plate +/-S9) using the preincubation method. We conclude, therefore, that the study is acceptable for microbial gene mutations.

E. STUDY DEFICIENCIES: None.

TABLE 1.: Plate Incorporation Mutation Assay							
Treatment	Dose (µg/plate)	S9 (10%)	Mean number of revertants per plate (triplicate plating)				
			Salmonella				E. coli
			TA1535	TA100	TA1537	TA98	WP2 uvrA
DMSO	0.1 mL	–	19 ± 2	110 ± 6	9 ± 1	31 ± 1	35 ± 1
Reg. No. 388 010	20	–	17 ± 7	115 ± 11	8 ± 1	24 ± 4	36 ± 3
	100	–	16 ± 2	114 ± 13	7 ± 2	21 ± 3	36 ± 1
	500	–	13 ± 1	123 ± 8	6 ± 4	17 ± 3	36 ± 7
	2500	–	13 ± 2	109 ± 4	6 ± 2	12 ± 2	28 ± 4
	5000	–	8 ± 4*	94 ± 23*	6 ± 1*	13 ± 2*	16 ± 4*
MNNG	5	–	952 ± 115	954 ± 122			
4-NPDA	10	–				782 ± 69	
9-AA	100	–			350 ± 43		
4-NQO	5	–					626 ± 39
DMSO	0.1mL	+	19 ± 2	107 ± 4	10 ± 2	34 ± 4	40 ± 5
Reg. No. 388 010	20	+	13 ± 5	105 ± 8	7 ± 1	20 ± 4	40 ± 4
	100	+	16 ± 3	129 ± 20	12 ± 3	23 ± 1	36 ± 6
	500	+	15 ± 3	118 ± 2	12 ± 3	22 ± 3	32 ± 4
	2500	+	12 ± 3	107 ± 24	7 ± 2	19 ± 1	28 ± 3
	5000	+	11 ± 1*	65 ± 2*	6 ± 1*	16 ± 1*	16 ± 4*
2-AA	2.5	+	201 ±13	1038 ± 28	151 ± 39	583 ± 46	
	60	+					284 ± 7

Data summarized from MRID 45902229, Tables 1 - 5, pages 30 - 34
* = Reduced background lawn of growth
** = Mutagenic
MNNG =N-methyl-N`-nitro-N-nitrosoguanidine 9-AA = 9-Aminoacridine
4-NPDA = 4-Nitro-o-phenylendiamine 4-NQO = 4-Nitroquinoline-N-oxide
2-AA = 2-Aminoanthracene

TABLE 2.: Preincubation Mutation Assay							
Treatment	Dose (µg/plate)	S9 (10%)	Mean number of revertants per plate (triplicate plating)				
			Salmonella				E. coli
			TA1535	TA100	TA1537	TA98	WP2 uvrA
DMSO	0.1 mL	–	18 ± 4	108 ± 5	10 ± 1	34 ± 2	32 ± 3
Reg. No. 388 010	2	–	16 ± 4	99 ± 8	9 ± 2	27± 1	28 ± 2
	10	–	15 ± 1	93 ± 17	8 ± 2	25 ± 5	32 ± 6
	50	–	12 ± 3	100 ± 13	8 ± 1	22 ± 1	31 ±2
	250	–	11 ± 2	86 ± 6	7 ± 2	22 ± 1	26 ± 2
	500	–	9 ± 1	78 ± 3	6 ± 2	18 ± 4	27 ± 6
MNNG	5	–	826 ± 29	894 ± 144			
4-NPDA	10	–				919 ± 38	
9-AA	100	–			567 ± 26		
4-NQO	5	–					520 ± 63
DMSO	0.1mL	+	18 ± 1	107 ± 8	9± 2	32 ± 4	28 ± 1
Reg. No. 388 010	2	+	13 ± 2	102 ± 1	8 ± 1	29 ± 3	25 ± 3
	10	+	11 ± 3	102 ± 2	7 ± 0	26 ± 4	22 ± 2
	50	+	10 ± 2	109± 3	6 ± 2	27 ± 3	23 ± 3
	250	+	12 ± 2	96 ± 14	7 ± 2	24 ± 3	17 ± 3
	500	+	10 ± 1	64 ± 6	7 ± 0	17 ± 5	15 ± 1
2-AA	2.5	+	116 ± 16	607 ± 33	112 ± 13	520 ± 7	
	60	+					206 ± 2

Data summarized from MRID 45902229. Tables 11 - 15, pages 41 -45
* =Reduced background lawn of growth
MNNG =N-methyl-N'-nitro-N-nitrosoguanidine 9-AA = 9-Aminoacridine
4-NPDA = 4-Nitro-o-phenylendiamine 4-NQO = 4-Nitroquinoline-N-oxide
2-AA = 2-Aminoanthracene