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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.: 45902225

TEST MATERIAL (PURITY):BAS 670 H technical (= Reg.-Nr. 375 080) (97.7%, Batch No. N 14)

COMPOSITION/SYNONYM(S): Methanone [3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)-

CITATION: Engelhardt, G. and Hoffmann, H. D. (1999). *Salmonella typhimurium/ Escherichia coli* Reverse Mutation Assay (Standard Plate Test and Preincubation Test) with BAS 670 H (= Reg. -Nr. 375 080). Department of Toxicology BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany; Laboratory Project Identification 40M0124/984210, Document No. 1999/11499; Study Completion Date: October 29, 1999. Unpublished MRID NUMBER: 45902225

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

EXECUTIVE SUMMARY: In independently performed microbial mutagenicity assays (MRID No. 45902225), histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, and TA100) and tryptophan-deficient (*trp*$^-$) *Escherichia coli* strain WP2 *uvrA* were exposed for 48-72 hours to five concentrations (20-5000 µg/plate) in the standard plate test and five concentrations (4-2500 µg/plate) of BAS 670 H (= Reg. No. 375 080) (97.7%, Batch No. N 14) 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.

BAS 670 H (= Reg. -Nr. 375 080) was cytotoxic to all *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 (plate incorporation) or 2500 µg/plate +/-S9 (preincubation). Nonactivated and S9-activated positive controls induced the expected mutagenic response in the corresponding tester

(1)

strain. **There was, however, no indication of a mutagenic response in any strain at any level up to cytotoxic concentrations 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: BAS 670 H (= Reg.-Nr. 375 080)
Description: Beige powder
Lot/batch number: N 14
Purity: 97.7%
Stability: Reported to be stable at room temperature until May 2000. The stability of the test material in the solvent dimethyl sulfoxide (DMSO) over a 4-hour period was reported to have been verified analytically.
CAS number: 210631-68-8
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-phenylendiamine (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)

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 28.39 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.

Preincubation Modification: Five concentrations (0, 4, 20, 100, 500 and 2500 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 100-µL 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. 45902225, pp.45-51.

(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. Overall, the findings from both assays agree and indicate that BAS 670 H (= Reg. -Nr. 375 080) was cytotoxic to all *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 (plate incorporation) or 2500 µg/plate +/-S9 (preincubation). There was, however, no indication of a mutagenic response in any strain at any noncytotoxic level either with or without S9 activation. By contrast, all strains responded to the mutagenic action of the appropriate positive controls.

The study author concluded, therefore, that BAS 670 H (= Reg. -Nr. 375 080) 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 BAS 670 H (= Reg. -Nr. 375 080) was cytotoxic but not mutagenic up to the limit concentration (5000 µg/plate +/-S9) using the plate incorporation and up to a cytotoxic level (2500 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	–	21 ± 3	127 ± 12	10 ± 3	26 ± 2	25 ± 1
BAS 670 H (= Reg.-Nr. 375 080)	20	–	19 ± 4	106 ± 11	10 ± 2	23 ± 3	21 ± 6
	100	–	19 ± 2	114 ±12	9 ± 1	20 ± 1	23 ± 3
	500	–	21 ± 3	93 ±10	9 ± 2	20 ± 1	23 ± 1
	2500	–	18 ± 3	104 ± 8	8 ± 1	21 ± 3	30 ± 7
	5000	–	8 ± 1*	72 ±13 *	4 ± 1*	16 ± 4*	18 ± 8
MNNG	5	–	556 ± 36	838 ± 71			
4-NPDA	10	–				704 ± 61	
9-AA	100	–			657 ± 57		
4-NQO	5	–					1053 ± 54
DMSO	0.1mL	+	20 ±1	126 ± 13	10 ± 2	32 ± 5	29 ± 5
BAS 670 H (= Reg.-Nr. 375 080)	20	+	23 ± 4	117 ± 2	9 ± 1	31± 3	26 ± 4
	100	+	17 ±3	125 ± 6	6 ± 1	25 ± 5	23 ± 4
	500	+	16 ± 2	124 ± 16	7 ± 2	25 ± 1	25 ± 6
	2500	+	5 ± 2	140 ± 6	6 ± 2	37 ± 3	21 ± 3
	5000	+	3 ± 2*	128± 16*	4 ± 1*	34 ± 4*	17 ± 3
2-AA	2.5	+	153 ±10	1175 ± 43	174 ± 14	861 ± 40	
	60	+					253 ± 22

Data summarized from MRID 45902225, Tables 1 - 5, pages 29 - 33
* = 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	-	21 ± 3	125 ± 3	9 ±1	25 ± 1	30 ± 2
BAS 670 H (= Reg.-Nr. 375 080)	4	-	22 ± 1	120 ±20	10 ± 2	22 ± 2	27 ± 2
	20	-	20 ± 1	107 ± 6	8 ± 3	23 ± 4	27 ± 5
	100	-	22 ± 3	118 ± 19	8 ± 1	25 ± 1	26 ±3
	500	-	21 ± 1	111 ± 1	7 ± 1	22± 2	24 ± 5
	2500	-	15 ± 4	40 ± 8	7 ± 2	20 ± 2	19 ± 2
MNNG	5	-	1318 ± 29	1198 ± 57			
4-NPDA	10	-				796± 20	
9-AA	100	-			419± 16		
4-NQO	5	-					728± 44
DMSO	0.1mL	+	18 ± 1	120 ± 6	11± 2	28 ± 3	27 ± 4
BAS 670 H (= Reg.-Nr. 375 080)	4	+	17 ± 1	135 ± 6	7 ± 3	27 ± 3	24 ± 5
	20	+	17 ± 2	126 ± 24	8 ± 2	22 ± 2	22 ± 3
	100	+	16 ± 4	109 ± 2	7 ± 1	22 ± 5	20 ± 3
	500	+	13 ± 3	106 ± 6	7 ± 1	21± 2	21 ± 5
	2500	+	7 ± 1*	90 ± 9*	5 ± 1*	15± 2*	12 ± 2*
2-AA	2.5	+	103 ± 9	588 ± 30	106 ± 7	538 ± 32	
	60	+					229 ± 17

Data summarized from MRID 45902225, Tables 6 - 10, pages 35 -39
* =Reduced titer background lawn of growth
MNNG =N-methyl-N'-nitro-N- nitrosoguanidine 9-AA = 9-Aminoacridine
4-NPDA = 4-Nitro-1,2-phenylenediamine 4-NQO = 4-Nitroquinoline-N-oxide
2-AA = 2-Aminoanthracene