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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.: 45902228

TEST MATERIAL (PURITY):BAS 670 H technical (95.8%, Batch No. N 26)

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 Leibold, E. (2003). *Salmonella typhimurium/ Escherichia coli* Reverse Mutation Assay (Standard Plate Test and Preincubation Test) with BAS 670 H. Experimental Toxicology and Ecology BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany; Laboratory Project Identification 40M0124/984235, Document No. 2003/1014309; Study Completion Date: January 28, 2003. Unpublished MRID NUMBER: 45902228

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

EXECUTIVE SUMMARY: In independently performed microbial mutagenicity assays (MRID No. 45902228), 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) of BAS 670 H (95.8%, Batch No. N 26) in the standard plate test and five concentrations (4-2500 µg/plate) in the preincubation modification of the plate test in the presence and absence of S9 activation. Owing to a weak positive response in strain TA98, both the plate incorporation (2 trials) and preincubation (1 trial) techniques were repeated using concentrations ranging from 3000-7000 µg/plate-S9. 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.

In the plate incorporation assay, BAS 670 H was cytotoxic to the majority of *Salmonella* strains and *E. coli* WP2 *uvr*A at 5000 µg/plate -S9 and to all strain at ≥2500 µg/plate +S9. Slight (~1.4- and 1.5-fold) increases in reversion of strain TA98 were recorded at 2500 and 5000 µg/plate -S9,

respectively. With S9 activation, no increases were seen. There was also no indication of a mutagenic response in other strains at any noncytotoxic level either with or without S9 activation.

Results from the preincubation assay are in good agreement with the earlier findings and show that the test material was cytotoxic at the highest nonactivated concentration tested (2500 µg/plate -S9), slightly more cytotoxic in the presence of S9 (reduced revertant, background lawn of growth and/or cell titres) ≥ 500 µg/plate. Similarly, an increase (1.7X) in revertant colonies of TA98 were also seen in the absence of S9 activation at 2500 µg/plate. In the S9-activated plate incorporation assay, the highest level tested (5000 µg/plate) was cytotoxic to all of the *Salmonella* strains and *E. coli* WP2 *uvr*A. The test material was generally more cytotoxic in the presence of S9 activation. There was also no indication of a mutagenic response in other strains at any noncytotoxic level either with or without S9 activation.

In additional tests with TA98, a weak dose-related response was revealed in the first repeat trial plate incorporation assays at all levels ranging from 1.9-fold at 3000 µg/plate to 5.2-fold at 7000 µg/plate (DMSO mutant count = 29 mutants). In the second repeat trial, all mutant colony counts were increased 2-fold compared to the solvent control (DMSO mutant count = 31) but the effect was not dose-related. In contrast to the findings of weak mutagenicity in the plate incorporation assay, no cells survived at ≥4000 µg/plate in the repeat preincubation trial but a 1.6-fold increase was calculated at 3000 µg/plate.

Under all conditions and trials, the nonactivated and S9-activated positive controls for both procedures induced the expected marked mutagenic responses in the corresponding tester strain.

Based on these considerations, it was concluded that there was confirmed evidence of a mutagenic response in S. typhimurium TA98 in the nonactivated portion of both the plate incorporation and preincubation assays. The effect was, however, observed at high concentrations (≥3000 µg/plate–plate incorporation and ≥2500 µg/plate–preincubation). **It was further concluded that the mutagenic effect was likely due to impurities in the test article because: 1) the response was seen at high concentrations including and exceeding the limit dose, 2) bacterial gene mutation assays conducted with other lots of the test material were negative up to the limit dose (see MRID Nos. 45902225 through 45902227, and 3) the active ingredient (a.i.) used in the current study has the lowest percentage of purity (95.8% versus 97.7 to 99.3% a.i. for the other lots).**

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
Description: Light brown crystals
Lot/batch number: N 26
Purity: 95.8%
Stability: The report indicated that a comparable batch of the test material (Batch No. N14, see MRID No. 45902225) was found to be stable in dimethyl sulfoxide (DMSO) over a period of 4 hours.
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. 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 27.4 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.

Additional Trials: Three additional trials (2 plate incorporation and one preincubation) were performed with strain TA98 exposed to five concentrations (0, 3000, 4000, 5000, 6000 and 7000 µg/plate -S9) treated as above for the nonactivated 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 either 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, $\geq 10^8$ 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. 45902228, pp.48-54.

(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:

- Plate Incorporation Assay: Findings from the plate incorporation assay are presented in Table 1 and indicate that BAS 670 H was cytotoxic to the majority of *Salmonella* strains and *E. coli* WP2 *uvr*A at 5000 µg/plate -S9 and to all strain at ≥2500 µg/plate +S9. As note in Table 1, slight (~1.4- and 1.5-fold) increases in reversion of strain TA98 were recorded at 2500 and 5000 µg/plate -S9, respectively. With S9 activation, no increases were seen. There was also no indication of a mutagenic response in other strains at any noncytotoxic level either with or without S9 activation. All strains responded to the mutagenic action of the appropriate positive controls.

- Preincubation Assay: Results from the preincubation assay (Table 2) are in good agreement with the earlier findings and show that the test material was cytotoxic at the highest nonactivated concentration tested (2500 µg/plate -S9) and slightly more cytotoxic in the presence of S9 (reduced revertant, background lawn of growth and/or cell titres at ≥ 500 µg/plate). Similarly, an increase (1.7X) in revertant colonies of TA98 was seen in the absence of S9 activation at 2500 µg/plate. For the remaining strains, no evidence of a mutagenic response was observed in the treatment groups, and all strains responded to the mutagenic action of the appropriate positive control.

- Additional Tests: Owing to the slight but less than 2-fold increases in revertant colonies of strain TA98, three additional trials (2 plate incorporation and 1 preincubation assays) were conducted in the absence of S9 activation with strain TA98 exposed to 3000 to 7000 µg/plate. As shown in Table 3, a weak dose-related response was revealed in the first repeat trial plate incorporation assay at all levels ranging from 1.9-fold at 3000 µg/plate to 5.2-fold at 7000 µg/plate compared to the solvent control (DMSO mutant colony count = 29 mutants). In the second repeat, all mutant colony counts were increased ≥2-fold compared to the solvent control (DMSO mutant count = 31) but the effect was not dose-

related. In contrast to the findings of weak mutagenicity in the plate incorporation repeat tests. no cells survived at ≥4000 µg/plate in the preincubation repeat, however. a 1.6-fold increase was calculated at 3000 µg/plate (50 revertant colonies vs. 32 in the DMSO control).

Therefore, The study author concluded that BAS 670 H was "a weakly mutagenic agent", and the effect was probably due to impurities.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and we concur with the study authors' conclusion that BAS 670 H, Batch N 36 was weakly mutagenic in *Salmonella* TA98 at high nonactivated concentrations that included the limit dose. The response was confined to the nonactivated portion of the assays suggesting that the mutagenic component was possibly detoxified by the exogenous metabolic activation system. We also agree with the study authors' claim that the positive response was likely due to impurities in the test material for several reasons: 1) mutagenic activity was limited to high test material concentrations; 2) a series of other microbial gene mutation assays conducted by the registrant's laboratory were negative when other lots of the same test article were assayed at comparable doses and under comparable test condition (see MRID Nos. 45902225 through 45902227) and 3) the active ingredient (a.i.) used in the current study had the lowest percentage of purity (95.8% versus 97.7 to 99.3% a.i. for the other lots). Based on these considerations, we conclude that the study is acceptable for microbial gene mutations and it is plausible that the weak mutagenic effect seen in this study was due to impurities.

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	–	16 ± 1	111 ± 9	10 ± 2	28 ± 2	29 ± 5
BAS 670 H Batch N 26	20	–	19 ± 4	107 ± 5	7 ± 2	25 ±5	33 ± 7
	100	–	16 ± 1	104 ± 4	8 ± 2	22 ± 2	31 ± 1
	500	–	16 ± 3	104 ± 5	5 ± 2	25 ± 2	29 ± 4
	2500	–	15 ± 2	96 ± 7	3 ± 1	38 ± 3	25 ± 2
	5000	–	9 ± 2	111 ± 16	5 ± 4	40 ± 2**	26 ± 2
MNNG	5	–	722 ± 61	674 ± 38			
4-NPDA	10	–				562 ± 139	
9-AA	100	–			350 ± 14		
4-NQO	5	–					657 ± 89
DMSO	0.1mL	+	18 ± 2	106 ± 7	8 ± 2	31 ± 5	37 ± 3
BAS 670 H Batch N 36	20	+	13 ± 3	114 ± 5	6 ± 1	28 ± 2	30 ± 6
	100	+	13 ± 3	107 ± 4	9 ± 3	29 ± 2	38 ± 6
	500	+	13 ± 4	102 ± 2	7 ± 2	27 ± 6	34 ± 3
	2500	+	11± 3	86 ± 14	5 ± 2	33 ± 6	21 ± 7
	5000	+	4 ± 2*	49 ± 11*	5 ± 2*	24 ± 3*	13 ± 4*
2-AA	2.5	+	106 ± 7	647 ± 49	104 ± 3	575 ± 36	
	60	+					213 ± 7

Data summarized from MRID 45902228, Tables 1 - 5, pages 29 - 33
*=Reduced background lawn of growth
** = 1.5-fold increase in revertant colonies
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	–	17 ± 2	110 ± 18	11 ± 2	31 ± 3	30 ± 3
BAS 670 H Batch N26	4	–	16 ± 2	124 ± 12	7 ± 2	33 ± 4	34 ± 6
	20	–	15 ± 5	117 ± 27	9 ± 2	29 ± 6	27 ± 3
	100	–	10 ± 1	77 ± 6	7 ± 2	28 ± 3	27 ±1
	500	–	11 ± 3	83 ± 12	7 ± 3	29 ± 3	29 ± 1
	2500	–	7 ± 2	87 ± 4	5 ± 3	54 ± 2**	32 ± 5
MNNG	5	–	621 ± 44	566 ± 49			
4-NPDA	10	–				633 ± 20	
9-AA	100	–			342 ± 42		
4-NQO	5	–					618 ± 69
DMSO	0.1mL	+	17 ± 2	109 ± 8	9± 2	38 ± 10	29 ± 6
BAS 670 H Batch N26	4	+	15 ± 1	109 ± 9	6 ± 1	37 ± 3	26 ± 1
	20	+	19 ± 2	109 ± 7	7 ± 1	32 ± 3	30 ± 9
	100	+	14 ± 3	98 ± 6	9 ± 1	19 ± 3	23 ± 3
	500	+	11± 4	103 ± 10	8 ± 1	25 ± 5	22 ± 4
	2500	+	6 ± 4*	90 ± 7*	4 ± 0*	24 ± 4	16 ± 4
2-AA	2.5	+	126 ± 11	562 ± 55	139 ± 14	609 ± 40	
	60	+					234 ± 22

Data summarized from MRID 45902228. Tables 6 - 10, pages 35 -39
* =Reduced background lawn of growth
** = 1.7-fold increase in revertant colonies.
MNNG =N-methyl-N`-nitrosoguanidine 9-AA = 9-Aminoacridine
4-NPDA = 4-Nitro-0-phenylendiamine 4-NQO = 4-Nitroquinoline-N-oxide
2-AA = 2-Aminoanthracene

TABLE 3.: Repeat Plate Incorporation and Preincubation Mutation Assay

Treatment	Dose (µg/plate)	S9 (10%)	Mean number of revertants per plate (triplicate plating)					
			Salmonella TA98					
			Plate Incorporation				Preincubation	
DMSO	0.1 mL	–	29 ± 8	FI	31 ± 3	FI	32 ± 3	FI
BAS 670 H Batch N26	3000	–	57 ± 16	1.9 X	69 ± 6	2.2 X	50 ± 7	1.6 X
	4000	–	67 ± 3	2.3 X	76 ± 5	2.5 X	0*	
	5000	–	113 ± 36	3.8 X	78 ± 21	2.5 X	0*	
	6000	–	126± 30	4.3 X	67 ± 4	2.2 X	0*	
	7000	–	153± 16	5.2 X	61 ± 14	2.0 X	0*	
4-NPDA	10	–	686 ± 62		854 ± 40		660 ± 51	

Data summarized from MRID 45902228. Tables 11 - 13. pages 40-42
* =Reduced background lawn of growth
FI = Fold Increase
4-NPDA = 4-Nitro-o-phenylendiamine