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PMRA Reviewer: Michael Honeyman Signature:
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.: 45902226

TEST MATERIAL (PURITY):BAS 670 H technical (99.3%, Batch No. 30786/22)

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. (2002). *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 40M0124/984232, Document No. 2002/1014113; Study Completion Date: November 25, 2002. Unpublished MRID NUMBER: 45902226

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

EXECUTIVE SUMMARY: In independently performed microbial mutagenicity assays (MRID No. 45902226), 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 (99.3% Batch No. 30786/22) 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. 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 was generally cytotoxic to the majority of *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 500 to 5000 µg/plate +/-S9 (plate incorporation) or 500 to 2500 µg/plate +/-S9 (preincubation). Nonactivated and S9-activated positive controls induced the expected mutagenic response in the

(1)

corresponding tester 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.

[redacted]

## I. MATERIALS AND METHODS

### A. MATERIALS:

1. Test Material: BAS 670 H
Description: Light brown crystals
Lot/batch number: 30786/22
Purity: 99.3%
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-phenylene diamine (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 27.49 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 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, $\geq 10^8$ 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. 45902226, pp.47-53.

(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 was cytotoxic to all *Salmonella* strains but TA1535 (plate incorporation only) and *E. coli* WP2 *uvr*A. (preincubation only), causing a reduction in revertant colonies, the background lawn of growth and/ or the cell titres at 500 to 5000 µg/plate +/-S9 (plate incorporation) or 500 to 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 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 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 | – | 16 ± 2 | 106 ± 7 | 9 ± 1 | 26 ± 1 | 33 ± 7 |
| BAS 670 H Batch No. 30786/22 | 20 | – | 16 ± 2 | 113± 22 | 9 ± 0 | 23 ± 2 | 31 ± 2 |
| | 100 | – | 16 ± 3 | 126 ±12 | 8 ± 3 | 21 ± 6 | 27 ± 1 |
| | 500 | – | 16 ± 2 | 95 ±14 | 7 ± 1 | 25 ± 5 | 30 ± 4 |
| | 2500 | – | 13 ± 2 | 97 ± 6 | 8 ± 2 | 18 ± 2 | 27 ± 7 |
| | 5000 | – | 13 ± 2 | 81 ±9 | 4 ± 2 | 5 ± 3 | 22 ± 2 |
| MNNG | 5 | – | 538 ± 32 | 666 ± 36 | | | |
| 4-NPDA | 10 | – | | | | 763 ± 8 | |
| 9-AA | 100 | – | | | 447 ± 20 | | |
| 4-NQO | 5 | – | | | | | 705 ± 24 |
| DMSO | 0.1mL | + | 17 ±1 | 111 ± 6 | 11 ± 3 | 33 ± 2 | 32 ± 3 |
| BAS 670 H Batch No. 30786/22 | 20 | + | 17 ± 2 | 105 ± 12 | 9 ± 2 | 34± 5 | 32 ± 5 |
| | 100 | + | 16 ±4 | 100 ± 3 | 10 ± 3 | 28 ± 3 | 28 ± 4 |
| | 500 | + | 20 ± 5 | 64 ± 3 | 10 ± 3 | 30 ± 4 | 29 ± 3 |
| | 2500 | + | 14 ± 2 | 76 ± 6 | 7 ± 1 | 14 ± 6 | 22 ± 3 |
| | 5000 | + | 11 ± 1 | 50± 7 | 2 ± 1 | 7 ± 3 | 17 ± 3 |
| 2-AA | 2.5 | + | 102 ±32 | 862 ±118 | 107 ± 7 | 772 ± 33 | |
| | 60 | + | | | | | 239 ± 9 |

Data summarized from MRID 45902226, Tables 1 - 5, pages 31 - 35
* = 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 ± 8 | 13 ±3 | 33 ± 4 | 31 ± 4 |
| BAS 670 H Batch No. 30786/22 | 4 | - | 17 ± 3 | 102 ± 8 | 11 ± 1 | 24 ± 3 | 32 ± 5 |
| | 20 | - | 16 ± 2 | 95 ± 8 | 13 ± 3 | 24 ± 4 | 28 ± 3 |
| | 100 | - | 13 ± 4 | 86 ± 12 | 11 ± 4 | 27 ± 8 | 35 ±2 |
| | 500 | - | 11 ± 3 | 81 ± 16 | 10 ± 1 | 24± 2 | 32 ± 7 |
| | 2500 | - | 12 ± 2 | 66 ± 5 | 6 ± 2 | 21 ± 4 | 29 ± 2 |
| MNNG | 5 | - | 534 ± 11 | 920 ± 226 | | | |
| 4-NPDA | 10 | - | | | | 582± 43 | |
| 9-AA | 100 | - | | | 485 ± 50 | | |
| 4-NQO | 5 | - | | | | | 543 ± 19 |
| DMSO | 0.1mL | + | 18 ± 3 | 111 ± 9 | 13± 1 | 34 ± 6 | 37 ± 3 |
| BAS 670 H Batch No. 30786/22 | 4 | + | 15 ± 3 | 100 ± 8 | 12 ± 1 | 27 ± 3 | 34 ± 3 |
| | 20 | + | 17 ± 3 | 104 ± 2 | 10 ± 2 | 31± 4 | 25 ± 4 |
| | 100 | + | 16 ± 4 | 95 ± 7 | 12 ± 3 | 27 ± 4 | 25 ± 4 |
| | 500 | + | 14 ± 3 | 84 ± 16 | 9 ± 3 | 23± 4 | 25 ± 4 |
| | 2500 | + | 9 ± 1 | 48 ± 11 | 4 ± 1 | 16± 5 | 21 ± 4 |
| 2-AA | 2.5 | + | 128 ± 15 | 735 ± 29 | 109 ± 10 | 573 ± 57 | |
| | 60 | + | | | | | 213 ± 45 |

Data summarized from MRID 45902226, Tables 6 - 10, pages 37 -41
* =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