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TXR No. 0052097

## DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mammalian cells in culture gene mutation assay in Chinese hamster ovary cells; OPPTS 870.5300 [§84-2]; OECD 476

DPBARCODE: D292904

SUBMISSION NO.:

PC CODE: 123009

TOX. CHEM. NO.: None

MRID No.: 45902230

TEST MATERIAL (PURITY): BAS 670 H (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 Hoffmann, H.D. (2000). *In Vitro* Gene Mutation Test With BAS 670 H in CHO Cells (HPRT Locus Assay). Experimental Toxicology and Ecology BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany; Laboratory Project Identification 50M0124/984183, Document No. 2000/1018642; Study Completion Date: November 10, 2000. Unpublished MRID NUMBER: 45902230

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

EXECUTIVE SUMMARY: In independently performed *in vitro* mammalian cell gene mutation assays (MRID No. 45902230), Chinese hamster ovary (CHO) cells were exposed to BAS 670 H (95.8%, Batch No. N 26) at six concentrations ranging from 93.75 to 3000 µg/mL without or with S9 activation (30% S9 in the S9 mix) in the first trial and 93.75 to 3000 µg/mL without S9 activation or 78.13 to 2500 µg/mL with S9 activation (10% S9 in the S9 mix) in the second trial. Cells were treated for 4 hours and cloned for mutant selection in both trials. The S9 was derived from Aroclor 1254-induced Sprague Dawley rat livers, and the test material was delivered to the test system in dimethyl sulfoxide; appropriate negative and positive controls were included.

BAS 670 H was insoluble and cytotoxic at 3000 µg/mL -S9 (36.5% cell survival) and at 3000 µg/mL+S9. (0% cell survival). Findings with the positive controls confirmed the sensitivity of the

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test system to detect mutagenesis. There was, however, no indication that BAS 670 H induced a mutagenic response, either in the presence or absence of S9 activation.

The study is classified as **Acceptable/Guideline** and satisfies the requirements for an *in vitro* mammalian forward gene mutation study (84-2).

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

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I. MATERIALS AND METHODSA. MATERIALS:

1. Test Material: BAS 670 H  
Description: Yellow brown powder  
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/1%

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in culture medium (Ham's F12 medium) to yield a final concentration of 300 µg/mL.

Activation (concentrations, solvent): Methylcholanthrene (MCA) was prepared in culture medium (Ham's F12 medium) to yield a final concentration of 10 µg/mL.

3. Activation: S9 derived from male Sprague-Dawley  
 Aroclor 1254    induced    rat    liver  
 phenobarbital    noninduced    mouse    lung  
 none    hamster    other  
 other    other

The S9 homogenate was prepared in house.

S9 mix composition:

| <u>Component:</u>        | <u>Concentration</u>           |
|--------------------------|--------------------------------|
| Phosphate buffer, pH 7.4 | 50 $\mu$ M                     |
| Glucose-6-phosphate      | 5 mM                           |
| NADP                     | 4 mM                           |
| KCl                      | 30 mM                          |
| MgCl <sub>2</sub>        | 10 mM                          |
| CaCl <sub>2</sub>        | 10 mM                          |
| S9                       | 30% (Trial 1)<br>10% (Trial 2) |

4. Test Cells: Mammalian cells in culture

- mouse lymphoma L5178Y cells  
 Chinese hamster ovary (CHO) cells  
 V79 cells (Chinese hamster lung fibroblasts)  
 other (list):

Source: Not reported.

Properly maintained? Yes.Periodically checked for mycoplasma contamination? Yes.Periodically checked for karyotype stability? Not reported.Periodically "cleansed" against high spontaneous background? Yes.5. Locus Examined: thymidine kinase (TK)Selection agent:  
(give concentration)

- bromodeoxyuridine (BrdU)  
 fluorodeoxyuridine (FdU)  
 trifluorothymidine (TFT)

 hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)Selection agent:  
(give concentration)

- 8-azaguanine (8-AG)  
 10  $\mu$ g/mL 6-thioguanine (6-TG)

 Na<sup>+</sup>/K<sup>+</sup> ATPaseSelection agent:  
(give concentration) ouabain other (locus and/or selection agent; give details): None

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6. Test Compound Concentrations Used:

- (a) Preliminary cytotoxicity assay: Nine concentrations (1, 5, 10, 50, 100, 1000, 2000 and 3600 µg/mL) were evaluated with and without S9 activation.
- (b) Mutation assays: Two independent trials were performed as follows:
- Trial 1: Six concentrations (93.75, 187.5, 375.0, 750.0, 1500.0, and 3000.0 µg/mL) were assayed without and with S9 activation (30%); cells exposed to all levels were cloned.
  - Trial 2: As above for Trial 1 with the exception that cells were exposed to S9-activated (10%) concentrations of 78.13, 156.25, 312.5, 625.0, 1250.0 or 2500.0 µg/mL. CHO cells treated with all nonactivated and S9-activated concentrations were cloned.

In both trials, all concentrations were tested in duplicate.

B. TEST PERFORMANCE:1. Cell Treatments:

- (a) Cells were exposed to the test compound, solvent, or positive controls for: 4 hours (nonactivated) 4 hours (activated)
- (b) After washing, cells were cultured for 7 days (expression period) before cell selection.
- (c) After expression, cells seeded at 3x10<sup>5</sup> cells/plate (6 plates/culture) were cultured for 7 days in selection medium to determine numbers of mutants, and cells seeded at 200 cells/plate (2 plates/culture) were cultured for 7 days without selection medium to determine cloning efficiency (CE).

2. Statistical Analysis: The authors indicated that due to negative results, the data were not evaluated statistically.

3. Evaluation Criteria:

- a. Assay Validity: The assay was considered acceptable if 1) the average CE of the negative and solvent control was at least 50%; 2) the background mutation frequency (MF) for the solvent control did not exceed 0-15 mutants x 10<sup>6</sup> cells and 3) the positive

controls induced "clearly increased" MFs. Historical spontaneous MFs and MFs for the positive control groups were provided (see MRID No. 45902230 pp. 50-53).

b. Positive response: The test material was considered positive if it induced a reproducible and dose-related increase in the "corrected" MF that was  $> 0-15$  mutants  $\times 10^6$  cells. Corrected MF was uncorrected MF divided by the absolute CE times 100.

### C. REPORTED RESULTS:

1. Analytical Determinations: The solubility, pH and osmolality of the test material in culture medium was determined for all concentrations used in the preliminary cytotoxicity test and the mutation assays. Results for the mutation assays indicated that the test material precipitated at levels  $\geq 2500.0$   $\mu\text{g/mL}$ . Although the pH of medium containing  $\geq 2500.0$   $\mu\text{g/mL}$  +S9 was lowered compared to control (6.1-6.5 vs. 7.1-7.3) and the osmotic pressure was lowered 4-8% at  $\geq 2500.0$ , there was no clear effect on pH or osmotic pressure at any other nonactivated or S9-activated concentration.
2. Preliminary Cytotoxicity Assay: Nine concentrations of the test material (1-3600  $\mu\text{g/mL}$ ) were evaluated with and without S9 activation. No cells survived treatment with the highest dose tested with and without S9 activation. (3600  $\mu\text{g/mL}$  with and without S9 activation.). For the remaining concentrations, relative survival (RS) was  $\geq 68$  or 86% at 2000  $\mu\text{g/mL}$  with or without S9 activation, respectively. Based on these results, Trial 1 of the mutation assay was conducted with concentrations of 93.75-3000  $\mu\text{g/mL}$  +/- S9.
3. Mutation Assays: Compound precipitation was seen at 3000  $\mu\text{g/mL}$  +/- S9 and at 2500  $\mu\text{g/mL}$  +S9. Results from the first trial were selected as representative and are presented in Tables 1-4. As shown in Tables 1 and 2, RS at 3000  $\mu\text{g/mL}$  was 36.5% without S9 activation and 0% with S9. For the remaining concentrations (93.75-1500  $\mu\text{g/mL}$  +/- S9), RS was  $\geq 94.0\%$ . There was no appreciable increase in the MF at noncytotoxic levels (Tables 3 and 4). Based on these findings, comparable nonactivated levels were selected for evaluation in Trial 2 and the dose range for the S9-activated phase of testing was lowered (78.13-2500  $\mu\text{g/mL}$ ). Results from Trial 2 were in good agreement with the data from Trial 1 and indicated that the test material was not mutagenic. In both trials, the positive control (300  $\mu\text{g/mL}$  EMS-S9; 10  $\mu\text{g/mL}$  MCA) induced marked increases in the MFs in both trials.

From the overall findings, the study author concluded that BAS 670 H was not mutagenic in this test system.

- D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the study was properly conducted and that the investigators interpreted the data correctly. BAS 670 H was evaluated in independently performed CHO/HPRT cell assays up to insoluble

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levels in the absence or presence of S9 activation (3000  $\mu\text{g/mL}$  - S9) and to cytotoxic concentration without or with S9 activation (3000  $\mu\text{g/mL}$ : 36.5% relative cell survival -S9 and 0% cell survival +S9) but failed to induce a mutagenic response. Additionally, the sensitivity of the test system to detect a mutagenic effect was clearly demonstrated by the results obtained with the positive controls (300  $\mu\text{g/mL}$  EMS -S9; 10  $\mu\text{g/mL}$  MCA +S9). We conclude, therefore, that BAS 670 H is negative in this cultured mammalian cell gene mutation assay.

E. STUDY DEFICIENCIES: None.

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Table 1. Summarized Results of the Nonactivated CHO/HPRT Mutation Assay with  
 BAS 670 H-Trial 1- Cytotoxicity Data

Cytotoxicity data - 1st experiment without S-9 mix; 4-hour exposure period

| Test groups            | Doses | Cell density (cells/ml) at 1st sub-culture | CE <sub>1</sub> (survival) (4 h after treatment; approx. 200 cells/flask seeded) |               |                        |       | CE <sub>2</sub> (viability) (at the end of the expression period; approx. 200 cells/flask seeded) |               |                        |       |
|------------------------|-------|--|--|---------------|------------------------|-------|---|---------------|------------------------|-------|
|                        |       |  | Cells flask 1  | Cells flask 2 | Cloning efficiency (%) |       | Cells flask 1   | Cells flask 2 | Cloning efficiency (%) |       |
|                        |       |  |  |               | Abs.                   | Rel.  |   |               | Abs.                   | Rel.  |
| Vehicle control (DMSO) | A     | 250,800                                    | 145  | 168           | 81.4                   | 100.0 | 160   | 181           | 96.3                   | 100.0 |
|                        | B     | 225,400                                    | 134  | 204           |                        |       | 209   | 220           |                        |       |
| 93.75 µg/ml            | A     | 245,200                                    | 151  | 152           | 77.7                   | 96.5  | 158   | 168           | 100.4                  | 104.3 |
|                        | B     | 326,300                                    | 140  | 178           |                        |       | 236   | 241           |                        |       |
| 187.50 µg/ml           | A     | 287,200                                    | 130  | 168           | 81.3                   | 93.3  | 165   | 180           | 91.3                   | 94.8  |
|                        | B     | 357,300                                    | 175  | 177           |                        |       | 192   | 193           |                        |       |
| 375.00 µg/ml           | A     | 292,700                                    | 128  | 154           | 71.9                   | 86.3  | 173   | 176           | 99.4                   | 103.2 |
|                        | B     | 337,700                                    | 139  | 156           |                        |       | 221   | 225           |                        |       |
| 750.00 µg/ml           | A     | 295,500                                    | 115  | 182           | 82.2                   | 101.0 | 178   | 194           | 111.0                  | 115.3 |
|                        | B     | 348,000                                    | 160  | 200           |                        |       | 257   | 259           |                        |       |
| 1,500.00 µg/ml         | A     | 277,200                                    | 166  | 212           | 95.0                   | 116.7 | 137   | 146           | 89.3                   | 92.7  |
|                        | B     | 297,800                                    | 177  | 185           |                        |       | 206   | 225           |                        |       |
| 3,000.00 µg/ml         | A     | 228,400                                    | 30   | 45            | 29.7                   | 36.5  | 176   | 191           | 96.9                   | 102.7 |
|                        | B     | 254,100                                    | 67   | 95            |                        |       | 209   | 215           |                        |       |
| 300.00 µg/ml EMS       | A     | 239,900                                    | 123  | 145           | 64.8                   | 79.6  | 159   | 164           | 90.2                   | 93.7  |
|                        | B     | 206,400                                    | 111  | 139           |                        |       | 196   | 202           |                        |       |

Data were extracted from Study Report,, Table 5, p. 38 MRID No. 45902230.



Table 2. Summarized Results of the CHO/HPRT S9-Activated Assay

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Cytotoxicity data - 1st experiment with S-9 mix<sup>1)</sup>; 4-hour exposure period

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| Test groups            | Doses | Cell density (cells/ml) at 1st sub-culture | CE <sub>1</sub> (survival) (4 h after treatment; approx. 200 cells/fask seeded) |               |                        | CE <sub>2</sub> (viability) (at the end of the expression period; approx. 200 cells/fask seeded) |               |               |                        |       |
|------------------------|-------|--|---|---------------|------------------------|--|---------------|---------------|------------------------|-------|
|                        |       |  | Cells flask 1   | Cells flask 2 | Cloning efficiency (%) |  | Cells flask 1 | Cells flask 2 | Cloning efficiency (%) |       |
|                        |       |  |   |               | Abs.                   | Rel.   |               |               | Abs.                   | Rel.  |
| Vehicle control (DMSO) | A     | 389.500                                    | 198   | 215           | 100.4                  | 100.0  | 181           | 189           | 94.8                   | 100.0 |
|                        | B     | 340.700                                    | 181   | 209           |                        |  | 188           | 200           |                        |       |
| 93.75 µg/ml            | A     | 364.700                                    | 187   | 200           | 94.4                   | 94.0   | 144           | 156           | 91.5                   | 96.5  |
|                        | B     | 368.800                                    | 175   | 183           |                        |  | 198           | 234           |                        |       |
| 187.50 µg/ml           | A     | 381.400                                    | 177   | 210           | 98.4                   | 98.0   | 170           | 187           | 98.7                   | 104.6 |
|                        | B     | 392.800                                    | 185   | 205           |                        |  | 202           | 234           |                        |       |
| 375.00 µg/ml           | A     | 384.200                                    | 200   | 218           | 100.2                  | 99.8   | 165           | 166           | 98.9                   | 104.3 |
|                        | B     | 382.700                                    | 183   | 200           |                        |  | 201           | 259           |                        |       |
| 750.00 µg/ml           | A     | 366.500                                    | 197   | 211           | 104.4                  | 104.0  | 190           | 193           | 96.4                   | 101.7 |
|                        | B     | 398.100                                    | 213   | 214           |                        |  | 190           | 198           |                        |       |
| 1,500.00 µg/ml         | A     | 365.200                                    | 180   | 206           | 95.3                   | 94.9   | 188           | 194           | 106.9                  | 112.8 |
|                        | B     | 384.000                                    | 177   | 199           |                        |  | 222           | 251           |                        |       |
| 3,000.00 µg/ml         | A     | 43.800                                     | 0   | 0             | 0.0                    | 0.0  | -             | -             |                        |       |
|                        | B     | 59.900                                     | 0   | 0             |                        |  | -             | -             |                        |       |
| 10.00 µg/ml MCA        | A     | 392.200                                    | 180   | 186           | 92.8                   | 92.4   | 178           | 205           | 91.2                   | 96.2  |
|                        | B     | 387.400                                    | 179   | 197           |                        |  | 181           | 185           |                        |       |

<sup>1)</sup> = S-9 fraction : collectors = 3 7

Data were extracted from Study Report, Table 6, p. 39 MRID No. 45902230.

Table 3. Summarized Results of the CHO/HPRT Nonactivated Assay

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Mutant frequency - 1st experiment without S-9 mix; 4-hour exposure period

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| Test groups<br>Doses      |   | Number of colonies <sup>a</sup> |    |    |    |    |     | Mutant frequency (per 10 <sup>6</sup> cells) |                        |
|---------------------------|---|---------------------------------|----|----|----|----|-----|--|------------------------|
|                           |   |                                 |    |    |    |    |     | Not corrected                                | Corrected <sup>b</sup> |
| Vehicle control<br>(DMSO) | A | 0                               | 0  | 0  | 0  | 0  | 1   | 6.39   | 6.03                   |
|                           | B | 1                               | 2  | 3  | 4  | 5  | 7   |  |                        |
| 33.75 µg/ml               | A | 0                               | 0  | 1  | 2  | 6  | 7   | 12.78  | 12.44                  |
|                           | B | 3                               | 4  | 4  | 5  | 6  | 8   |  |                        |
| 187.50 µg/ml              | A | 0                               | 0  | 1  | 1  | 1  | 2   | 4.45   | 4.78                   |
|                           | B | 0                               | 1  | 1  | 2  | 3  | 4   |  |                        |
| 375.00 µg/ml              | A | 1                               | 2  | 2  | 2  | 3  | 4   | 6.67   | 6.95                   |
|                           | B | 0                               | 1  | 1  | 2  | 2  | 4   |  |                        |
| 750.00 µg/ml              | A | 1                               | 1  | 2  | 4  | 4  | 7   | 11.65  | 10.84                  |
|                           | B | 2                               | 2  | 3  | 5  | 5  | 7   |  |                        |
| 1,500.00 µg/ml            | A | 0                               | 0  | 0  | 0  | 0  | 0   | 6.39   | 5.93                   |
|                           | B | 2                               | 4  | 4  | 4  | 4  | 5   |  |                        |
| 3,000.00 µg/ml            | A | 1                               | 1  | 3  | 3  | 4  | 4   | 7.50   | 7.72                   |
|                           | B | 1                               | 1  | 1  | 2  | 2  | 4   |  |                        |
| 300.00 µg/ml<br>EMS       | A | 82                              | 91 | 94 | 86 | 99 | 101 | 302.78                                       | 340.68                 |
|                           | B | 82                              | 82 | 83 | 90 | 92 | 95  |  |                        |

<sup>a</sup> = number of colonies 7 days after seeding - 300,000 cells/flask into selection medium  
<sup>b</sup> = correction on the basis of the absolute cloning efficiency 2 at the end of the expression period

Data were extracted from Study Report, Table 1, p. 33 MRID No. 45902230.

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Table 4. Summarized Results of the CHO/HPRT S9-activated Assay

Mutant frequency - 1st experiment with S-9 mix<sup>1)</sup>; 4-hour exposure period

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| Test groups<br>Doses      |   | Number of colonies <sup>a</sup> |    |    |    |    |    | Mutant frequency (per 10 <sup>6</sup> cells) |                        |
|---------------------------|---|---------------------------------|----|----|----|----|----|--|------------------------|
|                           |   |                                 |    |    |    |    |    | Not corrected                                | Corrected <sup>b</sup> |
| Vehicle control<br>(DMSO) | A | 2                               | 3  | 3  | 5  | 6  | 6  | 9.45   | 10.09                  |
|                           | B | 0                               | 0  | 1  | 1  | 2  | 5  |  |                        |
| 93.75 µg/ml               | A | 1                               | 1  | 2  | 2  | 3  | 5  | 8.89   | 9.82                   |
|                           | B | 2                               | 2  | 2  | 3  | 4  | 5  |  |                        |
| 187.50 µg/ml              | A | 1                               | 1  | 1  | 2  | 2  | 3  | 3.89   | 4.14                   |
|                           | B | 0                               | 0  | 1  | 1  | 1  | 1  |  |                        |
| 375.00 µg/ml              | A | 0                               | 0  | 0  | 0  | 1  | 1  | 2.78   | 2.60                   |
|                           | B | 1                               | 1  | 1  | 1  | 1  | 3  |  |                        |
| 750.00 µg/ml              | A | 0                               | 0  | 0  | 0  | 0  | 1  | 3.08   | 3.16                   |
|                           | B | 0                               | 1  | 1  | 2  | 3  | 3  |  |                        |
| 1,500.00 µg/ml            | A | 3                               | 4  | 4  | 4  | 7  | 9  | 15.00  | 14.42                  |
|                           | B | 1                               | 3  | 3  | 4  | 6  | 6  |  |                        |
| 3,000.00 µg/ml*           | A | -                               | -  | -  | -  | -  | -  | -  | -                      |
|                           | B | -                               | -  | -  | -  | -  | -  |  |                        |
| 10.00 µg/ml<br>MCA        | A | 18                              | 23 | 27 | 34 | 34 | 35 | 117.50                                       | 130.51                 |
|                           | B | 33                              | 35 | 38 | 46 | 47 | 53 |  |                        |

- <sup>a</sup> = number of colonies 7 days after seeding - 300,000 cells/fask into selection medium
- <sup>b</sup> = correction on the basis of the absolute cloning efficiency 2 at the end of the expression period
- \* = S-9 fraction : cofactors = 3 7
- \* = Due to evident cytotoxic effects the cultures were not continued.



Data were extracted from Study Report, Table 2, p. 34 MRID No. 45902230.