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Date 8/30/05

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 Unpublished MRID NUMBER: 45902230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 of 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.

I. MATERIALS AND METHODS

A. 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

_x_ Aroclor 1254	_x_ induced	_x_ rat	_x_ liver
___ phenobarbital	___ noninduced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

The S9 homogenate was prepared in house.

S9 mix composition:

Component:	Concentration
Phosphate buffer, pH 7.4	50 µM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	30 mM
$MgCl_2$	10 mM
$CaCl_2$	10 mM
S9	30% (Trial 1) 10% (Trial 2)

4. Test Cells: Mammalian cells in culture

__ mouse lymphoma L5178Y cells
X 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)

X hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection agent:
(give concentration)
_____ 8-azaguanine (8-AG)
10 µg/mL 6-thioguanine (6-TG)

__ Na^+/K^+ ATPase
Selection 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 nonactivted 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 $3x10^5$ 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 10^6 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 x 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 ≥2500.0 µg/mL. Although the pH of medium containing ≥2500.0 µg/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 ≥2500.0, there was no clear effect on pH or osmotic pressure at any other nonactivted or S9-activated concentration.

2.. Preliminary Cytotoxicity Assay: Nine concentrations of the test material (1–3600 µg/mL) were evaluated with and without S9 activation. No cells survived treatment with the highest dose tested with and without S9 activation. (3600 µg/mL with and without S9 activation.). For the remaining concentrations, relative survival (RS) was ≥68 or 86% at 2000 µg/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 µg/mL+/-S9.

3. Mutation Assays: Compound precipitation was seen at 3000 µg/mL+/ -S9 and at 2500 µg/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 µg/mL was 36.5% without S9 activation and 0% with S9. For the remaining concentrations (93.75-1500 µg/mL +/S9), RS was ≥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 µg/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 µg/mL EMS-S9; 10 µg/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 µg/mL - S9) and to cytotoxic concentration without or with S9 activation (3000 µg/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 µg/mL EMS -S9; 10 µg/mL MCA +S9). We conclude, therefore. that BAS 670 H is negative in this cultured mammalian cell gene mutation assay.

E. STUDY DEFICIENCIES: None.

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_1 (survival) (4 h after treatment; approx. 200 cells/flask seeded)				CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)			
			Cells flask 1	Cells flask 2	Cloning efficiency (%) Abs.	Cloning efficiency (%) Rel.	Cells flask 1	Cells flask 2	Cloning efficiency (%) Abs.	Cloning efficiency (%) 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	95.5	158	168	100.4	104.3
	B	326,300	140	178			236	241		
187.50 µg/ml	A	287,200	130	168	81.3	99.9	165	180	91.3	94.8
	B	357,300	175	177			192	193		
375.00 µg/ml	A	292,700	128	154	71.9	88.3	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	297,600	177	185			206	225		
3,000.00 µg/ml	A	228,400	30	45	29.7	36.5	176	191	98.9	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	208,400	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 with BAS 670 H Trial 1-Cytotoxicity Data

Cytotoxicity data - 1st experiment with S-9 mix[1]; 4-hour exposure period

Test groups / Doses		Cell density (cells/ml) at 1st sub-culture	CE_1 (survival) (4 h after treatment; approx. 200 cells/flask seeded) Cells flask 1	Cells flask 2	Cloning efficiency (%) Abs.	Rel.	CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded) Cells flask 1	Cells flask 2	Cloning efficiency (%) 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	384,700	187	200	94.4	94.0	144	156	91.5	96.5
	B	388,800	175	183			198	234		
187.50 µg/ml	A	381,400	177	210	98.4	98.0	170	187	98.2	104.6
	B	392,800	195	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			161	185		

[1] = S-9 fraction : cofactors = 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 with H-Tr 1-Mu Freq BAS 670 i a l tation uency

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

Test groups / Doses		Number of colonies[a]						Mutant frequency (per 10^6 cells)	
								Not corrected	Corrected[b]
Vehicle control (DMSO)	A	0	0	0	0	0	1	6.39	6.03
	B	1	2	3	4	5	7		
93.75 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	11.95	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 EMS	A	82	91	94	96	99	101	302.78	340.68
	B	82	82	83	90	92	95		

[a] = number of colonies 7 days after seeding - 300.000 cells/flask into selection medium

[b] = 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

BAS H-Tr l-Mu o n uenc

Mutant frequency - 1st experiment with S-9 mix[1]; 4-hour exposure period

Test groups / Doses		Number of colonies[a]						Mutant frequency (per 10^6 cells)	
								Not corrected	Corrected[b]
Vehicle control	A	2	3	3	5	6	6	9.45	10.09
(DMSO)	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	3.06	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[c]	A	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-		
10.00 µg/ml MCA	A	18	23	27	34	34	35	117.50	130.51
	B	33	35	38	46	47	53		

[a] = number of colonies 7 days after seeding - 300,000 cells/flask into selection medium

[b] = correction on the basis of the absolute cloning efficiency 2 at the end of the expression period

[1] = S-9 fraction : cofactors = 3 7

[c] = Due to evident cytotoxic effects the cultures were not continued.

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