EPA Reviewer: Nancy E. McCarroll Signature: Nancy E. McCarroll
Toxicology Branch 1/HED (7509C) Date: 07/27/05
Secondary Reviewer: Irving Mauer, Ph.D. Signature:
Registration Action Branch 3/HED (7509C) Date: 8/30/05
PMRA Reviewer: Michael Honeyman Signature:
Fungicide and Herbicide Toxicological Evaluation Section,
Health Evaluation Division Date 8/31/05

TXR No. 0052097

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro mammalian cytogenetics: chromosome aberration assay in Chinese hamster lung (V79) cells; OPPTS 870.5375 [§84-2]; 473

DPBARCODE: D292904 SUBMISSION NO.:

PC CODE: 123009 TOX. CHEM. NO.: None MRID No.: 45902232

TEST MATERIAL (PURITY): BAS 670 H (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). *In Vitro* Chromosome Aberrations Assay With BAS 670 H in V79 Cells. Department of Toxicology of BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany; Laboratory Project Identification 32M0124/984174, Document No. 1999/11688; Study Completion Date: December 7, 1999. Unpublished MRID NUMBER: 45902232

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

EXECUTIVE SUMMARY: In independently conducted *in vitro* chromosome aberration assays (MRID No. 45902232), Chinese hamster lung (V79) cells were exposed for 4 hours to BAS 670 H (97.7%, Batch No. N 14) at concentrations ranging from 225-3600 µg/mL with or without S9 activation in the first trial or 1800-3600 µg/mL with S9 activation (Trial 2). Cells treated with 900, 1800 or 3600 µg/mL (Trial 1) or 1800, 2700 or 3600 µg/mL (Trial 2) were harvested 18 hours after initiation of treatment and metaphases were analyzed for structural or numerical chromosome aberrations. 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; the appropriate solvent and positive controls were included.

Compound insolubility was reported at ≥ 1800 µg/mL; the study authors stated that 3600 µg/mL was equivalent to the limit concentration but this level was not cytotoxic. The positive controls induced the expected high yield of metaphases with chromosome aberrations.

(1)

Trial 1: No significant increases in the frequency of structural chromosome aberrations were seen at any nonactivated level. In the presence of S9 activation, however, significant ($p<0.01$) increases, compared to the concurrent control, in the percentage of cells with aberrations (including and excluding gaps) and exchanges were observed at 3600 µg/mL +S9. These values (10% cells with aberrations and 8% exchanges vs 3.5% and 2.0%, respectively for the concurrent solvent control cultures) were also outside of the DMSO historical control range of the reporting laboratory (*i.e*, 0-5% cells with aberrations and 0-3 % exchanges).

Trial 2: Based on the findings from Trial 1, only the S9-activated phase of the assay was repeated and concentrations of 1800, 2700 and 3600 µg/mL were selected. Significant ($p<0.01$) clastogenic activity was only recorded at the highest dose tested (HDT), 3600 µg/mL, and the significant increases occurred for the percent cells with abnormal chromosome morphology (8.5 % vs. 1.0% for DMSO) and chromosome exchanges (6.0% vs. 0.5% for DMSO). However, nonsignificant but concentration-related increases were also scored at lower levels of the test material (4% ↑ in cells with aberrations and 1.5% ↑in exchanges at 2700 µg/mL and 2.5% ↑ in cells with aberrations and 1.0% ↑in exchanges at 1800 µg/mL).

It should also be noted that the results of this study were confirmed in a later study using comparable test material levels from a different batch of BAS 670 H with a higher purity (see MRID No. 45902233).

Based on these findings, BAS 670 H induced a clastogenic response in the presence of S9 activation with significant effects recorded only at an insoluble limit concentration.

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an in vitro mammalian cell cytogenetic assay (84-2).

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

(2)

[redacted]

Activation: S9 derived from 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 in house.

S9 mix composition:

Component:	Concentration
Phosphate buffer, pH 7.4	15 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10% (all trials)

4. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: 0, 100, 500, 1000, 1500, 2000 and 3600 µg/mL – 4-hour exposure, 18-hour harvest (nonactivated and S9-activated treatment groups) and a continuous 18-hour exposure with cell harvest at the end of treatment (nonactivated treatment groups only).

(b) Cytogenetics assays: Two trials of the cytogenetic assay were performed; concentrations used are listed below:

Trial 1: 0, 225, 450, 900, 1800 and 3600 µg/mL +/-S9 (4-hour treatment and 18-hour cell harvest).

Trial 2: 0, 1800, 2700 and 3600 µg/mL +S9 (4-hour treatment and an 18- hour cell harvest).

5. Test Cells: Chinese hamster lung V79 cells obtained from an unspecified source and were grown in MEM with glutamine, 10% fetal calf serum and antibiotics

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Yes.

Cell line or strain periodically check for karyotype stability? Yes.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BAS 670H
   Description: Beige powder
   Lot/batch number: N 14
   Purity: 97.7%
   Stability: Reported to be stable in the solvent, dimethyl sulfoxide (DMSO) at room temperature for at least 4 hours.
   CAS number: 210631-68-8
   Structure: Not provided
   Solvent: DMSO
   Other provided information: The test material was stored at room temperature.

2. Control Materials:
   Negative control: None

   Solvent/final volume: DMSO/ 50 µL

   Positive controls:

   (a) Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was dissolved in minimal essential medium with Earle's salts (MEM) at a final concentration of 350 µg/mL.

   (b) Activation (concentrations, solvent): Cyclophosphamide (CP) was dissolved in (MEM) at a final concentration of 0.5 µg/mL.

TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound, solvent or positive controls for:
4 hours (nonactivated) 4 hours (activated)

(b) Cells exposed to the test material, solvent or positive control were fixed:
18 hours postinitiation of treatment (nonactivated)

18 hours postinitiation of treatment (activated)

Cytogenetic Assay:

(a) Treatment: Cultures containing 3-8x10^4 cells were seeded into each chamber of Quadriperm dishes and incubated for 24-30 hours at 37°C. Prepared cells were exposed either in the presence or absence of S9 activation for 4 hours to the selected test material doses, negative, solvent or the positive controls (EMS-S9; CP +S9). Duplicate cultures were prepared for each experimental dose and the positive and solvent controls. Cultures were washed, resuspended in fresh medium containing 10% FCS and incubated for 14 hours. Colcemid (final concentration, 0.2 µg/mL) was added 2 -3 hours before cell harvest.

Cell harvest/staining: Culture medium was removed and cells were swollen with 0.4% KCl, and fixed in methanol: acetic acid (3:1). Slides containing the cells were removed from the Quadripem chambers, air-dried, stained with 7.5% Giemsa and coded.

Metaphase analysis: The mitotic index (MI) was determined from the proportion of mitotic cells per 1000 cells for all treatment and solvent control cultures. If possible, 200 metaphase (100 cells per culture) were analyzed for structural and/or numerical (*i.e.*, polyploid and aneuploid cells) chromosome aberrations from the treated and solvent control cultures; 100 metaphases were scored for the positive control groups.

Cell Counts: To determine cytotoxicity, additional cell cultures, treated as described above, were examined for growth inhibition by counting the number of cells.

Statistical analysis: The percentage of cells with aberrations was evaluated using Fisher's exact test (one-sided) with Bonferroni-Holm correction at p values of 0.05 and 0.01. Statistical analysis of the data was performed with and without gaps.

Evaluation criteria:

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Assay acceptability : The assay was considered acceptable if: 1) the proportion of cells with structural chromosome aberrations in the negative or solvent control fell within the historical range of the performing laboratory (see MRID No. 45902232, p.63) and 2) the positive controls induced significant increases in the number of cells with structural chromosome aberrations. For positive historical control data provided by the performing laboratory, see MRID No. 45902232, p.66.

Positive response: The test material was considered positive if there was a dose-related and significant increase in the number of structural chromosome aberrations that exceeded the concurrent solvent control range as well as the historical negative control range

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 chromosome aberration assays. Results indicated that the test material was insoluble at levels ≥1500.0 µg/mL in the solvent but remained in solution in culture medium up to 2000 µg/mL and precipitated at 3600 µg/mL. There was no clear effect on the pH or osmotic pressure at any nonactivated or S9-activated concentration.

2. Preliminary Cytotoxicity Test: There were no clear effects on the MI, cell count or cell morphology at any level either with or without S9 activation after 4-hours of treatment and only a slight decrease (~30%) in the cells counted after 18 hours of continuous treatment with 3600 µg/mL. Based on these findings, Trial 1 of the cytogenetic assay was performed with a concentration range of 225 to 3600 µg/mL +/-S9 using a 4-hour exposure and an 18-hour cell harvest. .

3. Cytogenetic Assay:

Trial 1: In agreement with the preliminary cytotoxicity data, BAS 670 H had no clear effect on the MI, cell count or cell morphology at any level either with or without S9 activation. Summarized results from the metaphase analysis for structural chromosome damage of the nonactivated and S9-activated cytogenetic assay (Trial 1) are presented in Tables 1 and 2, respectively. As shown, no significant increase in the frequency of structural chromosome aberrations was seen at any nonactivated level. In the presence of S9 activation, however, significant ($p<0.01$) increases, compared to the concurrent control, in the percentage of cells with aberrations (including and excluding gaps) and exchanges were only seen at 3600 µg/mL +S9 (Table 2), these values (10% cells with aberrations and 8% exchanges vs 3.5% and 2.0%, respectively for the concurrent solvent control cultures) were also outside of the DMSO historical control range of the reporting laboratory (*i.e*, 0-5% cells with aberrations and 0-3% exchanges, see MRID 45902232, Appendix 3, pp. 61-64).

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Trial 2: Based on the findings from Trial 1, only the S9-activated phase of the assay was repeated and concentrations of 1800, 2700 and 3600 µg/mL were selected for Trial 2. As shown in Table 3, significant ($p<0.01$) clastogenic activity was only recorded at the highest dose tested (HDT), 3600 µg/mL. Also in agreement with the earlier findings, significant increases occurred for the percent cells with abnormal chromosome morphology (8.5 % vs. 1.0% for DMSO) and chromosome exchanges (6.0% vs. 0.5% for DMSO). Additionally, nonsignificant but concentration-related increases were scored at lower levels of the test material (4% ↑ in cells with aberrations and 1.5% ↑ in exchanges at 2700 µg/mL and 2.5% ↑ in cells with aberrations and 1.0% ↑ in exchanges at 1800 µg/mL). In both trials, no increases in numerical chromosome aberrations were observed.

The positive controls (350 µg/mL EMS -S9; 0.5 µg/mL CP +S9) caused significant ($p<0.01$) increases in the incidence of cells with aberrant chromosome morphology.

The study author concluded from the overall results that BAS 670 H "is a weak chromose-damaging (clastogenic) agent under *in vitro* conditions using V79 cells".

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and we agree with the study authors' interpretation of the results. BAS 670 H was assayed to the limit dose (3600 µg/mL, equivalent to 10mM, as stated by the study authors), which was not cytotoxic but was insoluble, and induced significant increases ($p<0.01$) in both the frequency of structural chromosome aberrations and exchanges in two separate trials. However, the significant clastogenic activity of BAS 670 H was confined to an insoluble limit concentration. Nevertheless, increases in both parameters were also recorded at 1800 and 2700 µg/mL, which would also be near or at the solubility limit. Based on the above considerations, we conclude that the study provided acceptable evidence that BAS 670 H is clastogenic in this *in vitro* mammalian cell cytogenetic assay. It should also be noted that the results of this study were confirmed in a later study using comparable test material levels from a different batch of BAS 670 H with a higher purity (see MRID No. 45902233).

E. STUDY DEFICIENCIES: NONE

(7)

Table 1. Cytogenetic Assay With Chinese Hamster V79 Cells: Nonactivated BAS 670 H
(4- hour treatment and 18-hour harvested)-Trial 1

BASF AG - TOXICOLOGY — ANALYSIS OF CHROMOSOMES — TABLE 1

PROJECT-NO. : 32H0126/984174
SUBSTANCE NAME : BAS 670 H — SUMMARY TABLE

S-9 MIX: WITHOUT — TREATMENT TIME: 4 h — HARVEST TIME: 18 h — 27-OCT-1999

Dose		NO. OF METAPHASES	METAPHASES WITH ABERRATIONS									
			Incl.Gaps		Excl.Gaps		Exchanges		Mul.Aber.		Chr. Dis.		Aneupl.		Polypl.	
			a	%	a	%	a	%	a	%	a	%	a	%	a	%
VEHICLE DMSO	:	200	11	5.5	8	4.0	2	1.0	0	0.0	0	0.0	0	0.0	1	0.5
900 ug/ml	:	200	15	7.5	14	7.0	9	4.5	0	0.0	0	0.0	0	0.0	4	2.0
1800 ug/ml	:	200	16	8.0	8	4.0	6	3.0	0	0.0	0	0.0	1	0.5	1	0.5
3600 ug/ml	:	200	9	4.5	4	2.0	2	1.0	0	0.0	0	0.0	0	0.0	3	1.5
EMS 350 ug/ml	:	100	20	20.0**	20	20.0**	10	10.0**	1	1.0	0	0.0	0	0.0	0	0.0

FISHER'S EXACT TEST (ONE-SIDED) WITH BONFERRONI-HOLM CORRECTION: * P <= 0.05 ** P <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE SOLVENT CONTROL GROUP , BONFERRONI-HOLM CORRECTED FOR EACH TIME

Data were extracted from the Study Report, Table 1, p. 42, MRID No. 45902232.

Table 2. Cytogenetic Assay With Chinese Hamster V79 Cells : S9-activated BAS 670 H
(4- hour treatment and 18-hour harvested)-Trial 1

BASF AG - TOXICOLOGY — ANALYSIS OF CHROMOSOMES — TABLE 2

PROJECT-NO. : 32M0124/984174
SUBSTANCE NAME : BAS 670 H — SUMMARY TABLE

S-9 MIX: WITH — TREATMENT TIME: 4 h — HARVEST TIME: 18 h — 27-OCT-1999

Dose		No. of Metaphases	Metaphases with Aberrations									
			Incl.Gaps n	%	Excl.Gaps n	%	Exchanges n	%	Mul.Aber. n	%	Chr. Dis. n	%
VEHICLE DMSO	:	200	12	6.0	7	3.5	4	2.0	0	0.0	0	0.0
900 ug/ml	:	200	11	5.5	6	3.0	3	1.5	2	1.0	0	0.0
1800 ug/ml	:	200	10	5.0	5	2.5	1	0.5	0	0.0	0	0.0
3600 ug/ml	:	200	24	12.0	20	10.0*	16	8.0*	0	0.0	0	0.0
CPP 0.5 ug/ml	:	100	17	17.0**	17	17.0**	15	15.0**	0	0.0	0	0.0

Dose		Aneupl. n	%	Polypl. n	%
VEHICLE DMSO	:	0	0.0	1	0.5
900 ug/ml	:	1	0.5	1	0.5
1800 ug/ml	:	0	0.0	3	1.5
3600 ug/ml	:	0	0.0	0	0.0
CPP 0.5 ug/ml	:	0	0.0	0	0.0

FISHER'S EXACT TEST (ONE-SIDED) WITH BONFERRONI-HOLM CORRECTION: *: P <= 0.05, **: P <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE SOLVENT CONTROL GROUP , BONFERRONI-HOLM CORRECTED FOR EACH TIME

Data were extracted from the Study Report, Table 2, p. 43 MRID No. 45902232.

Table 3. Repeat Cytogenetic Assay With Chinese Hamster V79 Cells : S9-activated BAS 670 H
(4- hour treatment and 18-hour harvested)-Trial 2

BASF AG - TOXICOLOGY — ANALYSIS OF CHROMOSOMES — TABLE 13

PROJECT-NO. : 32M0124/984174
SUBSTANCE NAME : BAS 670 H — SUMMARY TABLE

S-9 MIX: WITH — TREATMENT TIME: 4 h — HARVEST TIME: 18 h — 27-OCT-1999

Dose		NO. OF METAPHASES	METAPHASES WITH ABERRATIONS									
			Incl.Gaps n	%	Excl.Gaps n	%	Exchanges n	%	Mul.Aber. n	%	Chr. Dis. n	%	Aneupl. n	%	Polypl. n	%
VEHICLE DMSO	:	200	8	4.0	2	1.0	1	0.5	0	0.0	0	0.0	0	0.0	2	1.0
1800 ug/ml	:	200	7	3.5	5	2.5	2	1.0	0	0.0	0	0.0	0	0.0	0	0.0
2700 ug/ml	:	200	9	4.5	8	4.0	3	1.5	0	0.0	0	0.0	0	0.0	0	0.0
3600 ug/ml	:	200	19	9.5	17	8.5**	12	6.0**	0	0.0	0	0.0	0	0.0	1	0.5
CPP 0.5 ug/ml	:	100	14	14.0**	14	14.0**	14	14.0**	0	0.0	0	0.0	0	0.0	0	0.0

FISHER'S EXACT TEST (ONE-SIDED) WITH BONFERRONI-HOLM CORRECTION: * P <= 0.05 ** P <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE SOLVENT CONTROL GROUP (BONFERRONI-HOLM CORRECTED FOR EACH TIME

Data were extracted from the Study Report, Table 13, p. 55 MRID No. 45902232.