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TXR No. 0052097

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice; OPPTS 870.5395 [§84-2]; OECD 474

DPBARCODE: D292904 SUBMISSION NO.:

PC CODE: 123009 TOX. CHEM. NO.: None MRID No.: 45902234

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). Cytogenetic Study *In Vivo* With BAS 670 H in the Mouse Micronucleus Test After Two Intraperitoneal Administrations. Department of Toxicology of BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany; Laboratory Project Identification 26M0124/984175, Document No. 1999/11683; Study Completion Date: November 29, 1999. Unpublished MRID NUMBER: 45902234

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

EXECUTIVE SUMMARY: In an *in vivo* mouse micronucleus assay (MRID No. 45902234), groups of five male NMRI mice received two intraperitoneal (IP) injections of 375, 750 and 1500 mg/kg (equivalent to 370, 630 and 1130 mg/kg, based on analytical means) of BAS 670 H (97.7%, Batch No. N 14) suspended in aqueous 0.5% carboxymethylcellulose. Dose administration was separated by 24 hours. Bone marrow cells were collected 24 hours after the second administration and were examined for micronucleated polychromatic erythrocytes (MPEs). The use of only male mice was based on the findings of a preliminary study showing death at ≥1750 mg/kg in both sexes and no clear systematic differences between both sexes at lower doses. Cyclophosphamide and vincristine, administered once via IP with a 24 hour sacrifice, served as the positive controls for structural (clastogenic) and numerical (aneugenic) aberrations, respectively.

Piloerection and squatting posture were seen in all dose groups 1 hour after dosing. Additional signs seen in the high dose animals included general poor health. Squatting posture and/or piloerection reappeared 1 hour after receiving the second administration of the high dose but was resolved 2 days after dosing. Although these clinical signs were considered transient, BAS 670 H was tested up to

(1)

a dose in excess (i.e, 1130 mg/kg x 2 = 2260 mg/kg) of the limit concentration (2000 mg/kg). The positive controls induced the expected high yield of MPEs. **There was, however, no evidence that BAS 670 H was clastogenic or aneugenic.**

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a mouse micronucleus assay (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: Beige powder
   Lot/batch number: N 14
   Purity: 97.7%
   Stability: Reported to be stable in the vehicle, 0.5% Aqueous Carboxymethylcellulose (CMC) at room temperature for 96 hours.
   CAS number: 210631-68-8
   Structure: Not provided
   Vehicle: CMC
   Other provided information: The test material was stored at room temperature.

2. Control Materials:

   Negative/route of administration: None

   Vehicle/final concentration/route of administration: 0.5% CMC twice by intraperitoneal injection (IP) (dosing volume = 10 mL/kg).

   Positive/final concentration/route of administration: Cyclophosphamide (CP) was prepared in "purified" water and was administered once by IP at a final dose of 20 mg/kg and vincristine sulfate (VC) was prepared in "purified" water and was administered once by IP at a final dose of 0.15 mg/kg.

3. Test Compound:

   Route of administration: IP administration once daily for 2 days

   (a) Dose Selection: The selection of the BAS 670 H doses was based on a pretest determination of acute IP toxicity. The authors stated that "Deaths were observed down to a dose of 1750 mg/kg body weight, whereas at 1500 mg/kg body weight no test substance related deaths were observed. Evident clinical signs were observed, however, showing no distinct symptomatic differences between the male and female animals. Thus, only male animals were used for the cytogenetic investigations."

   (b) Micronucleus Test: 375, 750 and 1500 mg/kg

4. Test Animals:

(a) Species: Mouse Strain: NMRI Age: Not Reported
Weight (mean) : 29 g (males) Not used (females)
Source: Charles River Deutschland GmbH, Germany

(b) Number of animals used per dose:

Micronucleus assay:

- Treatment groups: 5 males _ females
- Positive control: 5 males _ females (per positive control)
- Vehicle control: 5 males _ females

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound and vehicle control:
Dosing: __ once X twice (24 hr apart)
____ other (describe): ____
Sampling (after last dose): ____ 6 hr ____ 16 hr
_x_ 24 hr ___ 48 hr ___ 72 hr

(b) Positive control:
Dosing: _x_ once ___ twice (24 hr apart)
____ other (describe): ____
Sampling (after last dose): ____ 6 hr ____ 12 hr
_x_ 24 hr

2. Tissues and Cells Examined:

_x_ bone marrow ____ others (list):

Number of polychromatic erythrocytes (PCEs) examined per animal: 2000
The size distribution of micronuclei in the PCEs, (MPCEs) was also determined (*i.e.*, small micronuclei – d<D/4; large micronuclei -- d≥D/4, where d = diameter of micronucleus and D = cell diameter).

Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: number seen while scoring 2000 PCEs

3. Details of Slide Preparation: At 24 hours post-administration of the second dose of the test material or vehicle and 24 hours after administration of the positive control, animals were sacrificed by an unspecified method. Bone marrow cells were recovered from both femurs, aspirated in fetal calf serum (FCS) and centrifuged. The supernatant was removed

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and cells were mixed with 50 µL of FCS and placed on slides. Slides were stained with eosin- methylene blue (May-Grunwald solution = Wrights solution), counterstained with Giemsa (7.5%), rinsed, mounted with Corbit-Balsam, coded and scored.

4. Statistical Methods: The data were evaluated for statistical significance by the Wilcoxon Test (one-sided) using the MUKERN (BASF) Aktiengesellschaft program at p values of 0.05 and 0.01.

5. Evaluation Criteria:
a. Assay Validity: The assay was considered acceptable if: 1) the values obtained for the vehicle control were within the provided historical range of the performing laboratory (MRID No. 45902234, pp. 46-50) and 2) both positive controls induced significant increases in MPEs. Historical positive control data were also furnished by the performing laboratory (MRID No. 45902234, pp. 52-55).

b. Positive Response: The test material was considered positive if there was a dose-related and significant increase in the frequency of MPEs, and the MPE counts exceed both the concurrent vehicle control and the historical vehicle control ranges.

C. REPORTED RESULTS:

1. Analytical Determinations: Analysis of dosing solution prepared for the micronucleus assay revealed that the actual concentrations contained 37.2, 63.1 or 113.1 mg/mL of the intended doses, 375, 750 or 1500 mg/kg, respectively (see MRID 45902234, pp. 26, 27).

2. Micronucleus Assay:

(a) Animal observations: Piloerection and squatting posture were seen in all animals 1 hour after administration of all doses; piloerection persisted for 4 hours and recurred after compound administration on day 2. In addition to these observations, high-dose males showed signs of poor general health 1 hour postdosing; squatting posture and/or piloerection reappeared 1 hour after receiving the second administration but was resolved 2 days after dosing. The study authors concluded that despite the 75% recovery rate of the highest test dose, toxicity was evident; therefore, the maximum tolerated dose (MTD) 0f 1500 mg/kg was applied.

(b) Bone marrow analysis: Summarized data from the micronucleus assay are presented in Tables 1 and 2 and indicate that exposure of male mice to the two IP doses of BAS 670 H did not result in alteration in the PCE:NCE rate or significantly increased the frequency of bone marrow cells with MPEs. By contrast, the positive controls (20 mg/kg CP, 0.15 mg/kg VC) induced marked and significant ($p<0.01$) genotoxic responses.

From the overall results, the study authors concluded that BAS 670 H did not cause chromosome damage in this study.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted. We do, however, disagree with the study authors' claim that an MTD was reached. Clinical signs were transient and generally resolved within 2 days of dosing. Although BAS 670 H was evaluated up to a high dose (1500 mg/kg x 2) that produced only transient signs of clinical toxicity, the HDT (1130 mg/kg x 2) exceeded the limit dose for this test system (2000 mg/kg). There was no evidence of a cytotoxic or genotoxic effect on the target cell. The sensitivity of the test system to detect a positive response was clearly demonstrated by the significant findings obtained with the positive controls (20 mg/kg CP; 0.15 mg/kg VC). We conclude, therefore, that the study provided acceptable evidence that BAS 670 H is neither clastogenic nor aneugenic in this *in vivo* test system when assayed in excess of the acute limit dose.

E. STUDY DEFICIENCIES: NONE

Table 1. Summarized Results of the Mouse Micronucleus Assay With BAS 670 H
Polychromatic: Normochromatic Erythrocytes Ratios

BASF/ZNI-TOXICOLOGY MICRONUCLEUS TEST

PROJECT-NO. : 26H0124/984175
SUBSTANCE NAME : BAS 670H
SPECIES : NMRI MOUSE
SUMMARY TABLE (MALES): Polychromatic and normochromatic erythrocytes

		Interval: 24 hours Total No. of PCE+S	NCE+S	MN [o/oo] in PCE+S	NCE+S
Vehicle 0.5% CMC	:	10000	5686	1.3	0.4
375 mg/kg	:	10000	4911	1.3	0.4
750 mg/kg	:	10000	4735	1.2	0.6
1500 mg/kg	:	10000	5009	1.9	1.6
CPP 20 mg/kg	:	10000	4921	14.5**	1.0
VCR 0.15 mg/kg	:	10000	5212	62.7**	2.1

WILCOXON TEST (ONE-SIDED) : *: p <= 0.05, **: p <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE VEHICLE CONTROL GROUP

Data were derived from Table 1, p. 37 of MRID No. 45902234.

Table 2. Summarized Results of the Mouse Micronucleus Assay With BAS 670 H
Micronucleated Polychromatic Erythrocytes

BASF/ZNT-TOXICOLOGY MICRONUCLEUS TEST

PROJECT-NO. : 26M0124/984175
SUBSTANCE NAME : BAS 670H
SPECIES : BMBL MOUSE
SUMMARY TABLE (MALES): Polychromatic erythrocytes: differentiation between small and large micronuclei

		Interval: 24 hours Total No. of PCE's	Cells (o/oo) with MN.d<D/4	MN.d->D/4
Vehicle 0.5% CMC	:	10000	1.3	0.0
375 mg/kg	:	10000	1.3	0.0
750 mg/kg	:	10000	1.2	0.0
1500 mg/kg	:	10000	1.9	0.0
CPP 20 mg/kg	:	10000	14.4**	0.1
VCR 0.15 mg/kg	:	10000	55.7**	7.0**

WILCOXON TEST (ONE-SIDED) : *: P <= 0.05 **: P <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE VEHICLE CONTROL GROUP

Data were derived from Table 2, p. 38 of MRID No. 45902234.