DATA EVALUATION RECORD

BAS 510 F/128008

STUDY TYPE: *IN VIVO* MAMMALIAN CYTOGENETICS - MICRONUCLEUS ASSAY IN MOUSE BONE MARROW CELLS; [OPPTS 870.5395 (§84-2)[

MRID 45404916

05/10/2002

Prepared for

Health Effects Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
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Prepared by

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Task Order No. 02-06

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(1)

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DATA EVALUATION RECORD
TXR#:0050193

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in the mouse; OPPTS 870.5395 [§84-2]; OECD 474.

PC CODE: 128008

DP BARCODE: D278384
SUBMISSION NO.: S604279

TEST MATERIAL (PURITY): BAS 510 F (94.4% a.i.)

SYNONYMS: No others were provided.

CITATION: Engelhardt, G. and H.D. Hoffmann (1999) Cytogenetic study *in vivo* with BAS 510 F in the mouse micronucleus test after two intraperitoneal administrations. Department of Toxicology of BASF Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, FRG. Laboratory Project ID: 26M0179/974095, BASF Registration Document Number: 1999/11048. August 16, 1999. MRID 45404916. Unpublished

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC 27709-3528

EXECUTIVE SUMMARY: In a mouse bone marrow micronucleus assay (MRID 45404916), 5 NMRI male mice/dose were treated via two intraperitoneal injections 24 hours apart with BAS 510 F (94.4% a.i., batch # N 37) at doses of 0, 500, 1000 or 2000 mg/kg bw. Bone marrow cells were harvested 24 hours after the second treatment. The vehicle was 0.5% carboxymethyl cellulose.

There were signs of toxicity during the study. A preliminary toxicity test with two i.p. injections of 2000 mg/kg test material in male and female mice showed squatting posture, piloerection and poor general state but no mortality. No sex differences were seen, therefore, males only were used in the micronucleus assay. In the micronucleus assay, the same clinical signs seen in the preliminary toxicity test were seen during the first four hours following both the first and second injections. All mice in the low dose group appeared normal 24 hours after each injection while all mice in the mid- and high-dose groups showed squatting posture at this time after the first injection and piloerection at this time after the second injection. The frequencies of micronucleated PCEs in the 500, 1000 and 2000 mg/kg groups were 1.4‰, 1.3‰ and 1.2‰, respectively, compared to the solvent control value of 1.2‰. There were no statistically

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significant differences between groups. Likewise, there were no statistically significant differences between groups in the frequency of micronucleated NCEs. Of the micronuclei that were observed in PCEs, virtually all were small micronuclei. The PCE/NCE ratios indicated no bone marrow cytotoxicity. BAS 510 F was tested to a toxic limit dose. The solvent and positive controls (cyclophosphamide and vincristine sulfate) induced the appropriate responses within the testing laboratory's historical control ranges.. **There was no statistically significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any dose.**

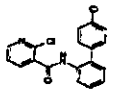
This study is classified as **Acceptable/Guideline.** It satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: BAS 510 F

Description:	White powder
Lot/Batch #:	N 37
Purity:	94.4% a.i.
CAS # of TGAI:	Not provided
Structure:	
Solvent Used:	0.5% Carboxymethyl cellulose (CMC)
Storage:	Room temperature

2. Control materials:

Negative control (if not vehicle) :	None	**Final Volume:**	**Route:**
Vehicle:	CMC	**Final Volume:** 20 mL/kg	**Route:** i.p.
Positive controls :	Cyclophosphamide	**Final Dose(s):** 20 mg/kg	**Route:** i.p.
	Vincristine sulfate	0.15 mg/kg	i.p.

3. Test animals:

Species:	Mouse
Strain:	NMRI
Age/weight at study initiation:	Age not provided / mean weight 29.1 g
Source:	Charles River Deutschland GmbH
No. animals used per dose	5 males; 0 females (females also were treated in preliminary toxicity assay)
Properly Maintained?	Yes

4. Test compound administration:

	Dose Levels	Final Volume	Route
Preliminary:	2000 mg/kg x 2	20 mL/kg body weight	i.p.
Main Study:	500, 1000, 2000 mg/kg x 2	20 mL/kg body weight	i.p.

B. TEST PERFORMANCE:

1. Treatment and sampling times:

a. Test compound:

Dosing:		once	x	twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	x	24 hr		48 hr		72 hr
Other:										

b. Negative and/or vehicle control:

Dosing:		once	x	twice (24 hrs apart)				Other		
Sampling (after last dose)		6 hr		12 hr	x	24 hr		48 hr		72 hr
Other:										

c. Positive control:

Dosing:	x	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	x	24 hr		48 hr		72 hr
Other:										

2. Tissues and cells examined:

Bone marrow:	x
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	the number found while screening 2000 PCEs
Other (if other cell types examined, describe):	–

3. **Details of slide preparation:** Both femurs were removed from each animal, the epiphyses were cut off and the bone marrow flushed out into a centrifuge tube with about 2 mL per femur of fetal calf serum (FCS) at 37°C. The cell suspension was mixed, centrifuged at 300 x g for five minutes, the supernatant removed and the cell pellet resuspended in about 50 µL fresh FCS. One drop of the cell suspension was dropped onto clean microscope slides and smears prepared using the ground edge of a slide. Slides were air dried, stained in eosin and methylene blue solution for five minutes, rinsed in purified water, held in fresh purified water for two or three minutes and then stained in 7.5% Giemsa solution for 15 minutes. The slides were then rinsed twice in purified water, clarified in xylene and mounted using Corbit-Balsam. Slides were coded prior to analysis.

4. **Evaluation criteria:** Criteria for an acceptable assay are slide quality permitting evaluation of at least 2000 PCEs per animal, micronucleated PCE frequency of the vehicle control group within the historical control range and statistically significant increases in the frequencies of micronucleated PCEs in the two positive control groups. The positive controls include a clastogen producing small micronuclei generally < one-fourth the cell diameter and a spindle poison producing large micronuclei generally > one-fourth the cell diameter. Data reported for each group are the number of micronucleated PCEs (both large and small), the number of micronucleated NCEs and the PCE/NCE ratio. Clinical signs are also reported.

Criteria for a positive response are a dose-related and statistically significant increase in the number of micronucleated PCEs in test material treated animals compared to the vehicle control group and frequencies of micronucleated PCEs exceeding both the values of the concurrent and historical negative historical control ranges.

5. **Statistical methods:** The number of micronucleated PCEs in the test material dose groups was compared with that in the vehicle control group using the program system MUKERN (BASF Aktiengesellschaft). The comparison was done using the Wilcoxon test for the hypothesis of equal medians on the relative frequency of micronucleated PCEs of each mouse. Tests were for significance at $p \leq 0.05$ or $p \leq 0.01$. This was an appropriate statistical approach.

II. REPORTED RESULTS:

Stock solutions of BAS 510 F at 25, 50 and 100 mg/mL were analyzed by HPLC and found to be within 90% to 115% of the theoretical concentrations. Stability of a comparable batch of test material (Batch # N26) held in an aqueous solution at room temperature over a 96-hour period was analytically determined.

A. **PRELIMINARY TOXICITY ASSAY:** In the preliminary toxicity assay, an unspecified number of male and female mice were given two i.p. injections of BAS 510 F 24 hours apart at a concentration of 2000 mg/kg body weight. Clinical signs observed in both sexes were squatting posture, piloerection and poor general state. No mortality was seen. The time of appearance of clinical signs and length of observation period were not provided. Based on the results of the preliminary test, 2000 mg/kg was selected as the top dose for the micronucleus assay and, because no sex differences were seen, males only were used.

B. **MICRONUCLEUS ASSAY:** Five male mice per dose were treated via two i.p. injections 24 hours apart with BAS 510 F at concentrations of 500, 1000 or 2000 mg/kg body weight and the bone marrow harvested 24 hours following the second injection. Squatting posture was seen in all mice at all doses in the first hour following both the first and second injections. In addition to squatting posture, piloerection was seen in all mice at all doses during the second through the fourth hour following both the first and second injections. At 24 hours after either the first or second injection, all mice appeared normal in the low dose group, all mice in the mid-dose and high-dose groups still exhibited squatting posture after the first injection and still exhibited piloerection after the second injection.

The frequencies of micronucleated PCEs in the 500, 1000 and 2000 mg/kg groups were 1.4‰, 1.3‰ and 1.2‰, respectively, compared to the solvent control value of 1.2‰. There were no statistically significant differences between groups. Likewise, there were no statistically significant differences between groups in the frequency of micronucleated NCEs with values of 2.0‰, 1.5‰, 0.7‰ and 1.4‰ in the solvent control, 500, 1000 and 2000 mg/kg groups, respectively. Of the micronuclei that were observed in PCEs, virtually all were small micronuclei. The frequency of micronucleated PCEs in the cyclophosphamide positive control was 15‰ and that of the vincristine sulfate positive control was 60.8‰, both statistically significant increases at $p \leq 0.01$. The frequencies of micronucleated NCEs in the

positive controls were similar to the solvent control value. The PCE/NCE ratios showed no bone marrow cytotoxicity at any test material dose. Results of the micronucleus assay are summarized in Appendix Tables 1 and 2 (MRID 45404916, pp. 36 and 37).

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that BAS 510 F did not induce micronuclei in mouse bone marrow cells as tested in this study.

B. **REVIEWER COMMENTS:** The reviewer agrees with the investigators' conclusion. BAS 510 F was tested to a limit value of 2000 mg/kg/day for two days, proper experimental protocol was followed and the solvent and positive control values were appropriate. The use of males only in the micronucleus assay was justified by the results obtained in the preliminary toxicity assay and a single sampling time is acceptable when the test material is administered twice separated by 24 hours between treatments. This is an **Acceptable/Guideline** study.

C. **STUDY DEFICIENCIES:** No study deficiencies were identified.

APPENDIX
(MRID NO. 45404916)

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