DATA EVALUATION RECORD

BAS 510 F/128008

STUDY TYPE: OTHER GENOTOXICITY: UNSCHEDULED DNA SYNTHESIS IN PRIMARY RAT HEPATOCYTES/MAMMALIAN CELL CULTURES; OPPTS 870.5550[§84-2]

(MRID 45404917)

Prepared for 5/10/2002

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DATA EVALUATION RECORD
TXR#: 0050193

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes/ Mammalian Cell Cultures; OPPTS 870.5550 (*in vitro*)[§84-2]; OECD 482 (*in vitro*)

PC CODE: 128008 **DP BARCODE:** D278384
SUBMISSION NO.: S604279

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

SYNONYMS: None provided

CITATION: Engelhardt, G. and H.D. Hoffmann (2000) *In vitro* unscheduled DNA synthesis (UDS) assay with BAS 510 F in primary rat hepatocytes. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, FRG. Laboratory Project ID: 81M0179/974096, BASF Registration Document Number: 2000/1011413. April 14, 2000. MRID 45404917. Unpublished

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

EXECUTIVE SUMMARY: In repeat unscheduled DNA synthesis assays (MRID 45404917), primary rat hepatocyte cultures were exposed to BAS 510 F (94.4% a.i., Batch # N 37) in DMSO at concentrations of 0, 5, 10, 50, 100, 250, 500, 750, and 1000 µg/mL for 18 - 20 hours in the first UDS experiment. Due to excess cytotoxicity, the first experiment was repeated at BAS 510 F concentrations of 0, 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, 250.0, and 500.0 µg/mL for 18 - 20 hours. A second experiment was conducted at BAS 51 F concentrations of 1.563, 3.125, 6.250, 12.500, 25.000, and 50.000 µg/mL for 18 - 20 hours.

BAS 510 F was tested up to cytotoxic concentrations. An upper dose of 500 µg/mL was chosen for the first UDS experiment based on results of a preliminary cytotoxicity test using lactate dehydrogenase activity and lactate concentration as the measure of cytotoxicity. A precipitate was seen in the culture medium at test material concentrations of 50 µg/mL and higher. The test material did not change the pH or osmolality of the culture medium. Cytotoxicity was greater than expected in the initial experiment and the cells were not evaluated for UDS. In the repeat first experiment, BAS 510 F concentrations of 1, 5, 10 and 50 µg/mL were evaluated for UDS.

Concentrations of 100 - 500 µg/mL were excessively cytotoxic and were not evaluated. There was no evidence of induced UDS (all net nuclear grain counts were well below zero) or any increase in the number of cells in repair (net nuclear grain counts ≥ 5) compared to the solvent control in the first experiment. BAS 510 F concentrations of 6.25, 12.50, 25.00 and 50.00 µg/mL were evaluated in the second UDS experiment. The results confirmed those of the first experiment, with no induction of UDS (all net nuclear grain counts were well below zero) or increases in the percentage of cells in repair. The mean net nuclear grain counts were -5.74 ± 4.44 and 29.01 ± 15.00 for the solvent and positive controls, respectively, in the first experiment. Comparable values in the second experiment were -4.40 ± 3.62 and 10.24 ± 10.45 for the solvent and positive controls, respectively. The control values were within the testing laboratory's historical control ranges. **There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced.**

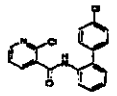
This study is classified as **Acceptable/Guideline**. It satisfies the guideline requirement for Test Guideline OPPTS 870.5550; OECD 482/486 for other genotoxic mutagenicity data.

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.
Compound Stability:	Not provided
CAS # of TGAI:	Not provided
Structure:	
Solvent Used:	Dimethylsulfoxide (DMSO)
Storage:	Room temperature

2. Control materials:

Negative control:	Culture medium	
Solvent:	DMSO	**Concentration:** 20 µL/culture
Positive control /solvent:	2-Acetylaminofluorene/unspecified solvent	**Concentration:** 1.0 µg/mL

3. Test compound concentrations used:

Preliminary cytotoxicity test: 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, 500.0 µg/mL

UDS assays:

First experiment: 5, 10, 50, 100, 250, 500, 750, 1000 µg/mL
First experiment repeat: 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, 250.0, 500.0 µg/mL
Second experiment: 1.563, 3.125, 6.250, 12.500, 25.000, 50.000 µg/mL

4. **Media:**
Incubation medium: Williams medium E, incomplete (WMEI) - Williams medium E supplemented with 1.0% (v/v) L-glutamine (200 mM) and 0.1% (v/v) gentamycin sulfate (50 mg/mL);
Attachment medium: Williams medium E, complete (WMEC) - WMEI supplemented with 10% (v/v) fetal calf serum (FCS);
Labeling medium: WMEI supplemented with 0.5% (v/v) ^{3}H-thymidine solution (final concentration about 5 µCi/mL)
5. **Test cells:** Mammalian cells in culture/primary rat hepatocytes. The hepatocytes were obtained from healthy male Wistar rats ((Chbb: THOM; SPF), Boehringer Ingelheim Pharma KG, FRG). Mean weight of the rats used was 269 g.
6. **Cell preparation:** Hepatocytes were isolated by a procedure based on the method described by Butterworth et al. (1987)[1].

a. **Perfusion technique:** Few details were provided. Rats were anaesthetized with Metofan® and the livers profused with ethylene glycol-bis(β-amino ethyl ether) N,N,N',N'-tetraacetic acid (EGTA) solution followed by collagenase solution.

b. **Hepatocyte harvest/Culture preparation:** The hepatocyte harvest was not described. Isolated hepatocytes were washed with 20 mL WMEI at about 20°C and mixed well, filtered through sterile gauze, centrifuged at 500 rpm for five minutes, the supernatant discarded and the cell pellet resuspended in about 20 mL WMEI. Cell viability was determined by the trypan blue exclusion method. Cell cultures were established by adding about 400,000 viable hepatocytes in 2 mL of attachment medium to at least four wells/test group without coverslips for cytotoxicity determination and to at least six wells/test group with coverslips for UDS determination (autoradiography). After a two hour attachment period at 37°C in a 5% CO_2, ≥ 90% humidity atmosphere, the medium was replaced by fresh WMEI medium to remove the unattached cells.

B. TEST PERFORMANCE:

1. **Cytotoxicity assay:** The preliminary cytotoxicity assay was conducted at eight BAS 510 F concentrations ranging from 0.1 to 500.0 µg/mL. Hepatocyte cultures were incubated in the presence of test material for 18 - 20 hours at 37°C in a 5% CO_2, ≥ 90% humidity atmosphere and cytotoxicity then determined by lactate dehydrogenase (LDH) release and lactate concentration in the culture medium. The pH and osmolality of the treatment medium was measured and solubility of the test material in the solvent and in culture medium was determined.

2. **UDS assay:**

a. **Treatment:** Following the attachment period, the culture medium was replaced with 1.8 mL of labeling medium and 0.2 mL of test material, solvent control or positive control solution.

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[1]Butterworth, B.E., Ashby, J., Bermudez, E., Casciano, D., Mirsalis, J., Probst, G. and Williams, G. (1987). A protocol and guide for the *in vitro* rat hepatocyte DNA repair assay. Mutation Res. 189, 113-121.

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The cells were incubated at 37°C in a 5% CO_2, ≥ 90% humidity atmosphere for an 18 - 20 hour treatment and labeling period.

b. **Preparation of autoradiographs/Grain development:** After the treatment and labeling period, the cells were washed twice with Hank's Balanced Saline Solution or WMEI, fixed on the coverslips with ethanol/acetic acid (3 : 1, v/v) for at least 30 minutes, rinsed 2 - 4 times with distilled water and air-dried. The coverslips were mounted cell side up on glass slides using Corbit-Balsam and dried overnight. They were then coated with KODAK NTB-2 photographic emulsion at about 37°C for 5 - 10 seconds, dried at room temperature in the dark for about 16 hours and then stored in the dark with a desiccant at -20°C for 3 - 12 days. The slides were warmed to room temperature in the dark and then developed with KODAK D-19 at about 15°C, fixed in Agfa Acidofix for about 5 minutes, washed in water for 5 - 10 minutes and stained with methyl green-pyronine Y.

c. **Grain counting:** Grain counts were obtained from a total of 100 cells/dose group using two or three slides per group, 25 - 50 cells displaying good morphology per slide. The slides were coded prior to analysis. Counting was done using an automatic image analyzer (ARTEK). The nuclear grain count (number of grains over the nucleus) and the cytoplasmic grain count (number of grains over a nucleus-sized area of cytoplasm averaged from two or three nucleus sized areas adjacent to the nucleus) were determined for each cell. The net nuclear grain count of each cell was calculated by subtracting the cytoplasmic grain count from the nuclear grain count. The mean nuclear and cytoplasmic grain counts and the mean net nuclear grain counts were calculated for each treatment group. The percentage of cells in repair (cells showing net nuclear grain counts ≥ 0 and also those showing net nuclear grain counts ≥ 5 were recorded.

d. **Evaluation criteria:** The testing laboratory's criteria for an acceptable assay require clearly negative results in the untreated and solvent controls and clearly positive results in the positive control (≥ 40% cells in repair), all within the historical control ranges; isolated hepatocyte viability should be at least 70%; evidence of possible cytotoxicity should be clear after incubation for 18 - 20 hours and "cell material of one animal sufficient for all test groups". The last criterion was quoted because its meaning is unclear to the reviewer.

Criteria for a positive response were a dose-related increase in both the mean net nuclear grain counts, with a value greater than zero at one or more test points and in the percentage of cells in repair (NNG ≥ 5) ≥ 20. Results are considered marginal if the percentage of cells in repair is ≥ 3 but < 20 and the dose-related increase in the mean net nuclear grain count approaches but does not exceed zero. Results were considered negative if both the mean net nuclear grain counts and the percentage of cells in repair were within the historical negative control range.

e. **Statistical analysis:** Because the results were clearly negative, no statistical analysis was performed.

II. REPORTED RESULTS:

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 CYTOTOXICITY ASSAY:** Eight concentrations of BAS 510 F ranging from 0.1 to 500.0 µg/mL were tested. The upper dose was based on results from other studies. The mean LDH activity of the solvent control was 226.3 ± 17.1 U/L and remained near this value at test material concentrations through 10 µg/mL. The value at 500 µg/mL rose to 429.8 U/L or 189.9% of the solvent control value. The mean lactate concentration decreased to 65.5% of the solvent control value at 500 µg/mL. The test material did not change the pH or osmolality of the treatment medium compared to the values seen with solvent addition alone. The cytotoxicity observed in the pretest was less than expected, therefore, the upper dose selected for the first UDS assay was 1000 µg/mL.

B. **UDS assay:** Eight concentrations of BAS 510 F ranging from 5 to 1000 µg/mL were tested initially in the first UDS assay; however, the test material was more cytotoxic than expected and cells exposed to concentrations of 50 µg/mL and higher could not be evaluated. The first experiment was repeated at eight concentrations ranging from 0.5 to 500.0 µg/mL. Results of this experiment, based on 100 cells per dose group, are summarized in Table 1 (compiled from MRID 45404917, Tables 1 and 3, pp. 30 and 33).

TABLE 1. Summary of the first UDS assay

Test Groups	NNG Counts (mean ± SD)	Cells in Repair (%) NNG ≥ 5	Relative LDH Activity (%)
Untreated control	-4.87 ± 4.27	0	–
Vehicle control (DMSO)	-5.74 ± 4.44	0	100.0
BAS 510 F (µg/mL)			
0.5	–	–	94.8
1.0	-6.44 ± 3.83	0	102.8
5.0	-6.27 ± 4.86	2	105.7
10.0	-7.25 ± 5.55	1	105.3
50.0	-5.20 ± 4.13	0	151.7
100.0	–	–	156.7
250.0	–	–	140.9
500.0	–	–	133.7
Positive Control (2-AAF)	29.01 ± 15.00	89	–

NNG = net nuclear grains
SD = standard deviation
LDH = lactate dehydrogenase
Mean = mean of 100 cells
2-AAF = 2 acetylaminofluorene (1.0 µg/mL)

BAS 510 F concentrations of 100 - 500 µg/mL were excessively cytotoxic and were not evaluated for UDS.

In the second UDS experiment, BAS 510 F concentrations ranging from 1.563 to 50.000 µg/mL were tested. Results of this experiment, based on 100 cells per dose group, are summarized in Table 2 (compiled from MRID 45404917, Tables 2 and 5, pp. 31 and 35).

TABLE 2. Summary of the second UDS assay			
Test Groups	NNG Counts (mean ± SD)	Cells in Repair (%) NNG ≥ 5	Relative LDH Activity (%)
Untreated control	-4.76 ± 4.30	0	–
Vehicle control (DMSO)	-4.40 ± 3.62	0	100.0
BAS 510 F (µg/mL)			
1.563	–	–	87.3
3.125	–	0	99.8
6.250	-4.59 ± 4.30	1	108.2
12.500	-3.53 ± 3.37	1	113.6
25.000	-3.32 ± 3.14	1	149.2
50.000	-4.61 ± 4.27	0	187.0
Positive Control (2-AAF)	10.24 ± 10.45	69	–

NNG = net nuclear grains
SD = standard deviation
LDH = lactate dehydrogenase
Mean = mean of 100 cells
2-AAF = 2 acetylaminofluorene (1.0 µg/mL)

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that BAS 510 F did not induce UDS in primary rat hepatocytes as tested in this study.

B. **REVIEWER COMMENTS:** The reviewer agrees with the investigators' conclusion. BAS 510 F was tested to a sufficiently high concentration, proper experimental protocol was followed and the solvent and positive control values were appropriate and within the historical control ranges. There was no evidence of UDS induction over the solvent control value at any test material concentration. This is an **Acceptable/Guideline** study.

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