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Fungicide and Herbicide Toxicological Evaluation Section,
Health Evaluation Division Date 8/3/05

TXR No. 0052097

DATA EVALUATION RECORD

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

DPBARCODE: D292904 SUBMISSION NO.:

PC CODE: 123009 TOX. CHEM. NO.: None MRID No.: 45902302

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* Unscheduled DNA Synthesis (UDS) Assay With BAS 670 H in Primary Rat Hepatocytes. Department of Toxicology of BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany; Laboratory Project Identification 81M0124/984184, Document No. 1999/11404; Study Completion Date: October 12, 1999. Unpublished MRID NUMBER: 45902302

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

EXECUTIVE SUMMARY: In independently performed unscheduled DNA synthesis (UDS) assays (MRID 45902302), primary rat hepatocyte cultures were exposed to BAS 670 H (97.7%, Batch No. N 14) in dimethyl sulfoxide (DMSO) at concentrations ranges of 10-5000 µg/mL (Trial 1) or 78.125-2500 µg/mL (Trial 2) for 18-20 hours. Hepatocytes exposed to 0, 50, 100, 500 or 1000 µg/mL (Trial 1) or 0, 312.5, 625, 1250 or 2500 µg/mL (Trial 2) were scored for UDS using the autoradiographic procedure (^{3}H-thymidine incorporation). Solvent and positive (2-acetylaminofluorene) controls were included.

BAS 670 H precipitated at 5000 µg/mL and was cytotoxic at ≥3750 µg/mL. The solvent and positive control values were appropriate. **There was no evidence that BAS 670 H induced UDS, as determined by radioactive tracer procedures [nuclear silver grain counts] at any concentration tested.**

This study is classified as **Acceptable/Guideline** and the study satisfies the requirements for FIFRA Test Guideline [OPPTS 870.5550 (§84-2)] for *in vitro* UDS data.

(1)

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

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: Untreated cells in Williams medium E supplemented with 200 mM L-glutamine and antibiotics (WME).
   Solvent control/Concentration: DMSO/ 10 µL WME
   Positive control/Concentration: 2-Acetylaminofluorene (2-AAF) was prepared in DMSO at a final concentration of 1 µg/mL

3. Test Compound Concentrations Used:

   Preliminary cytotoxicity test: 0.0, 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, 500.0, 1000.0, 2500.0 and 5000 µg/mL.

   UDS assay:
   Trial 1: 0.0, 10.0, 50.0, 100.0, 500.0, 1000.0, 2500.0, 3750.0 and 5000 µg/mL

   Trial 2: 0.0, 78.125, 156.25, 312.5, 625.0, 1250.0 and 2500.0 µg/mL

4. **Media:** The culture medium was WME; WMEC = WME supplemented with 10% fetal calf serum.

5. **Test cells:** Primary hepatocytes were obtained from the livers of healthy male Wistar rats purchased from Boehringer Ingelheim Pharma KG, Germany.

6. **Cell preparation:**

   a. **Perfusion technique:** Rats were anesthetized with Metofane by inhalation and livers were perfused with EGTA (ethylene glycol-bis (β-aminoethylether) N,N,N',N'-tetraacetic acid) followed by collagenase solution (50,000 U collagenase-IV).

   b. **Hepatocyte harvest/culture preparation:** Following perfusion, livers were removed, rinsed with WMEC and filtered through sterile gauze and centrifuged. The supernatant

3

was removed and cell pellets were resuspended in WMEI; cell viability was determined by trypan blue exclusion. Cultures were prepared by seeding 4 x 10^5 viable cells into 1.9 cm^2 wells in a multiwell culture dish. Four wells/test group (without coverslips) were prepared for the determination of cytotoxicity. At least six wells/test group were prepared with coverslips for autoradiography. Cultures were incubated for ~2 hours (attachment period) at 37°C in a humidified atmosphere with 5% CO_2.

B. TEST PERFORMANCE

1. **Cytotoxicity assay:** Following the attachment period, the media was removed and replaced with 0.2 mL WMEI. The desired concentration of test material was added to four wells per dose group and after 18 to 24 hours of incubation, cytotoxicity was determined by lactate dehydrogenase (LDH) release or lactate concentration. Cells with abnormal morphology were recorded.

2. **UDS assay:**

   a. **Treatment:** Growth medium in the six-well dishes was replaced with medium containing the desired concentration of test material, solvent or positive controls and 5 µCi/mL ^{3}H-thymidine (specific activity = ~0.185 MBq/mL). Cultures were incubated for 18 - 20 hours, washed twice with Hanks balanced salt solution or WMEI. Cells attached to the coverslips were fixed in ethanol/ acetic acid (3 : 1) and air dried.

   b. **Preparation of autoradiographs/grain development:** The dried coverslips were mounted cell-side-up on microscope slides, the slides were dipped in Kodak NTB-2 photographic emulsion, air-dried overnight and then stored in the dark in the presence of a drying agent for 3 - 12 days at -20°C and developed in Kodak D-19 developer. Slides were fixed in Agfa Acidfix, washed with water and stained with methyl green-pyronine Y. Slides were coded before evaluation.

   c. **Grain counting:** Twenty-five to 50 cells per slide, 100 cells per dose were evaluated for UDS activity. The net nuclear grain (NNG) count was determined by counting the number of grains over the nucleus and subtracting the average number of grains in two to three nuclear-sized regions of the corresponding cytoplasm. The mean NNG count and the percentage of cells in repair, defined as nuclei with five or more net nuclear grains, was calculated for each test material dose and for the solvent and positive controls.

   d. **Evaluation criteria:**
   Assay Acceptability: Criteria for an acceptable assay included: 1) hepatocyte viability of at least 70% in isolated cells; 2) NNG counts for the negative and solvent control within the range of historical control data (see MRID 45902302, p.42); and 3) NNG counts for the positive control should show "clearly positive results (≥40% of the cells in repair)", and be within the range of historical positive control data (see MRID 45902302, p.44).

   Positive Response: The test material was considered positive if it induced a concentration -dependent increase in mean NNG, which must exceed zero at one data point and must be accompanied by a ≥20% increase in the percentage of cells in repair.

4

e. **Statistical analysis:** Since the data produced negative findings, no statistical evaluation was performed.

II. REPORTED RESULTS:

A. **ANALYTICAL DETERMINATIONS:** The determination of pH and osmolality of the test material, as well as solubility in the solvent and in culture medium, revealed no appreciable effects on either the pH or osmolality but compound insolubility at 5000 µg/mL.

B. **PRELIMINARY CYTOTOXICITY ASSAY:** Ten concentrations of BAS 670 H ranging from 0.5 to 5000 µg/mL were tested in the preliminary cytotoxicity test. Based on increased LDH activity (240% vs 100% for the solvent) and a decrease in lactate concentration (36% vs 100% for DMSO) at 5000 µg/mL, this concentration was selected as the highest level for the UDS assay.

C. **UDS ASSAY:** Trial 1 examined 8 concentrations ranging from 10 to 5000 µg/mL of the test material. As shown in Table 1, UDS was not measured in hepatocytes exposed to concentrations ≥2500 µg/mL due to cytotoxicity, evident as no or only a few cells, all with abnormal morphology. Results for the remaining levels (50-1000µg/mL) showed no appreciable increase in NNG or the percentage of cells in repair. By contrast, the positive control (1.0 µg/mL 2-AAF) induced marked increases in both NNG and the percentage of cells in repair. Based on these findings, concentrations ranging from 312.5 to 2500.0 µg/mL were assayed in Trial 2. Summarized results from this trial, presented in Table 2, are in agreement with the earlier findings and indicate that BAS 670H did not induce UDS. The solvent and positive control values were appropriate and within the testing laboratory's historical control ranges.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that BAS 670 H was negative in this *in vitro* rat hepatocyte UDS assay.

B. **REVIEWER COMMENTS:** The reviewer agrees with the investigators' conclusion. There was no evidence that BAS 670 H induced UDS activity up to a cytotoxic concentration (>2500 µg/mL) in two trials. A proper experimental protocol was followed and the solvent and positive control values were appropriate. This is an **Acceptable/Guideline** study.

C. **STUDY DEFICIENCIES:** None.

Table 1

DNA repair activity - 1st experiment

Test groups Doses	NG counts Mean[1] ± SD	CG counts Mean[1] ± SD	NNG counts Mean[1] ± SD	Cells in repair (%) NNG ≥ 0	 NNG ≥ 5
Untreated control	6.20 ± 2.62	10.40 ± 2.77	-4.20 ± 2.76	6	0
Vehicle control (DMSO)	6.53 ± 3.48	12.17 ± 3.60	-5.64 ± 3.18	4	0
50.0 µg/ml	6.27 ± 2.77	10.39 ± 3.44	-4.12 ± 3.85	14	0
100.0 µg/ml	5.58 ± 2.70	9.40 ± 3.03	-3.82 ± 3.30	10	0
500.0 µg/ml	5.81 ± 2.93	11.02 ± 3.13	-5.21 ± 3.69	8	0
1,000.0 µg/ml	5.13 ± 2.02	9.52 ± 2.56	-4.39 ± 3.07	7	0
2,500.0* µg/ml	-	-	-	-	-
3,750.0* µg/ml	-	-	-	-	-
5,000.0* µg/ml	-	-	-	-	-
1.0 µg/ml 2-AAF	12.85 ± 7.62	8.48 ± 2.57	4.37 ± 6.81	80	40

NG = nuclear grains
CG = cytoplasmic grains
NNG = net nuclear grains

[1] = mean of 100 cells
SD = standard deviation

* = no evaluation due to evident cytotoxicity, i.e. no / only few cells available with marked morphological changes

Data were extracted from the Study Report (MRID 45902302), Table 1, p. 29.

Table 2

DNA repair activity - 2nd experiment

Test groups Doses	NG counts Mean[1)] ± SD	CG counts Mean[1)] ± SD	NNG counts Mean[1)] ± SD	Cells in repair (%) NNG ≥ 0	 NNG ≥ 5
Untreated control	9.30 ± 3.62	15.98 ± 4.19	-6.68 ± 3.92	5	0
Vehicle control (DMSO)	8.81 ± 4.15	14.23 ± 3.59	-5.42 ± 3.39	5	0
312.5 µg/ml	6.20 ± 2.90	12.62 ± 3.94	-6.42 ± 4.06	3	0
625.0 µg/ml	6.43 ± 2.92	12.61 ± 3.45	-6.18 ± 3.53	3	0
1.250.0 µg/ml	6.77 ± 3.06	13.90 ± 3.86	-7.13 ± 3.93	2	0
2.500.0 µg/ml	8.18 ± 3.53	14.07 ± 3.90	-5.89 ± 3.70	5	0
1.0 µg/ml 2-AAF	25.87 ± 10.35	11.38 ± 3.10	14.50 ± 9.28	98	88

NG = nuclear grains
CG = cytoplasmic grains
NNG = net nuclear grains

1) = mean of 100 cells
SD = standard deviation

Data were extracted from the Study Report (MRID 45902302), Table 2, p. 30.