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004586

Data Evaluation Record

Study Type: In vitro rat hepatocyte DNA repair assay.

Study Identification: "Rat Hepatocyte Primary Culture/DNA Repair Test."

Lab. performing study:	Pharmakon Research International, Inc. Waverly, PA 18471
Sponsor:	Monsanto Agricultural Products Co. St. Louis, MO. 63167
Study no.:	PK 82-151 (Monsanto)
Project no.:	PH 311-MO-001-82 (Pharmakon)
Accession no.:	071970
Report date:	2/17/83
Submitted to EPA:	9/22/83
Study director:	Robert W. Naismith, Ph.D.
Reviewed By:	D. Stephen Saunders Jr., Ph.D. Toxicologist, Section V TOX/HED (TS-769)
Approved By:	Irving Mauer, Ph.D. Geneticist, Toxicology Branch Hazard Evaluation Division (TS-769)

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Conclusions: No effect of treatment on the rate of thymidine incorporation was apparent at doses of 0.032 to 3.2 ug/well. Doses of 10.6 to 320 ug/well were reported as "cytotoxic", however the criteria for this assessment, or any data obtained from these cells, were not submitted. The purity and method of dose calculation for the test article were not supplied, and it is not clear whether the technical grade of active ingredient was tested in this assay.

Classification: Unacceptable Deficiencies as noted.

Materials

(1) Test chemicals: Acetochlor (MON 097), a "colorless, pale yellow liquid", Lot #NBP1737813, % a.i. not stated.

Positive control: 2-AAF (Aldrich Chem. Co.), % a.i. not stated.

Vehicle control- DMSO (Mallinkrodt, Inc.), purity not stated.

(2) Doses tested: Acetochlor- 0.032 ug/well to 320 ug/well.

vehicle control- DMSO

positive control- 2-AAF, 1×10^{-4}M.

(3) Test system: Rat hepatocytes isolated from the liver of male Fischer-344 rats, obtained from Charles River Breeding Laboratories, Wilmington, Mass., "or any USDA acceptable source".

Methods

A photocopy of the submitted methods is appended. The methods were reviewed and the following points were noted:

1) The criteria for assessing cytotoxicity were not stated.

2) The method for calculating doses was not stated. Since the doses were reported as "ug/well", and the test substance was supplied as a liquid, the density and purity of the test material were required to calculate doses. If the investigators assumed a density of 1.0 and purity of 100%, it should be so stated. Further, since the test material in the in vivo cytogenetics study (Monsanto #HL 83-006) was a brown liquid, with reported purity of 96.3% a.i., it is not clear whether the technical material was tested in the present study since it was described as a "pale yellow liquid".

Results/Discussion

No effect of treatment on the rate of incorporation of ^{3}H-thymidine by hepatocytes in vitro was apparent at doses of 0.032 ug/well to 3.2 ug/well. Doses of 10.6 ug/well and above were reported as cytotoxic, however the criteria for this assessment were not provided, nor were any effects of treatment on thymidine incorporation by these cells reported. The positive control, 2-AAF, induced about a 100-fold increase in incorporation of thymidine, demonstrating that the test system could respond appropiately to a known mutagen. These data are presented in the table below (photocopied from the submitted study report):

Treatment	Concentration	Net Nuclear Grains Triplicate Cultures $\bar{x} \pm$ s.d.
Untreated		0.0 ± 0.1
DMSO		0.2 ± 0.2
2AAF	1×10^{-4} M	52.7 ± 9.5**
MON 097	0.032 ug/well	0. [illegible]
MON 097	0.106 ug/well	0.3 ± 0.4
MON 097	0.32 ug/well	0.7 ± 0.6
MON 097	1.06 ug/well	0.6 ± 1.0
MON 097	3.2 ug/well	0.4 ± 0.5
MON 097	10.6 ug/well	Cytotoxic
MON 097	32.0 ug/well	Cytotoxic
MON 097	106.6 ug/well	Cytotoxic
MON 097	320.0 ug/well	Cytotoxic

**Positive finding. Mean net nuclear grain count of five or greater than the vehicle control.

Classification: Unacceptable Inadequate identification of test article; method of dose calculation not adequately described; criteria for cytotoxicity or data from these cells not submitted.