DATA EVALUATION REPORT

PEBULATE

4/13/1999

Study Type: UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES IN VIVO

Prepared for

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

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DATA EVALUATION REPORT

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Rat Hepatocytes/ *in vivo*

DP BARCODE: D247841
P.C. CODE: 041403
SUBMISSION NO.: S546073
TOX. CHEM. NO.: 710

TEST MATERIAL (PURITY): Pebulate (97.1% w/w)

CITATION: Kennelly, J. C. (1990) Pebulate: Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes *In Vivo*. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report Number, CTL/P/3032, August 17, 1990. MRID No. 41614809. Unpublished.

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, Delaware 19897

EXECUTIVE SUMMARY: In an *in vivo* unscheduled DNA synthesis (UDS) assay in rats (MRID No. 41614809), male Alderley Park rats were given a single oral dose of 400 or 800 mg/kg Pebulate in corn oil by gavage. Hepatocytes from these rats were prepared for autoradiographic nuclear grain counting at 4 and 12 hours postdosing. Corn oil served as the negative control and 6-p-dimethylaminophenylazobenzthiazole and N-nitrosodimethylamine served as positive controls.

Pebulate at the assayed doses was not overtly toxic to the test animals or cytotoxic to the target tissue. **Without evidence of toxicity to the treated animals and/or cytotoxicity to the hepatocytes, no conclusions can be reached regarding the potential genotoxicity, if any of the test material.**

This study is classified as an UNACCEPTABLE and does not satisfy the FIFRA Test Guidelines for a UDS assay.

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

A. MATERIALS

1. Test material: Pebulate

   Description: amber liquid
   Lot/Batch #: not given
   Purity: 97.1% w/w
   Stability of compound: not given; expiration date: April 1, 1991
   CAS #: not given
   Structure: not given
   Vehicle used: Corn oil
   Other provided information: The test material was stored at room temperature. Dosing solutions were used within 24 hours of preparation; analytical determinations were not conducted to verify actual concentrations used in the study.

2. Control materials

   Vehicle/final concentration: Corn oil/ 10 mL/kg body weight

   Positive/final concentration: The positive control for the 12-hour cell harvest was 6-p-dimethylaminophenylazobenzthiazole (6BT) at 40 mg/kg. N-nitrosodimethylamine (DMN) at 10 mg/kg was used as the positive control for the 4-hour cell harvest.

3. Test compound concentrations used

   400 or 800 mg/kg body weight

4. Animal dosage

   Dose selection- A previous acute oral toxicity study yielded an LD_{50} of 1750 mg Pebulate/kg body weight in male rats. In that study, deaths were observed at doses as low as 1259 mg/kg. To estimate the maximum tolerated dose (MTD) in the strain of rats used for the UDS study, Pebulate was orally administered at 800 or 1250 mg/kg (dosing volume = 10 mL/kg) to groups of four male animals, followed by a 4-day observation period. No animals died after administration of 800 mg/kg; however, animals treated with 1250 mg/kg showed signs of severe toxicity and were sacrificed. Based on these data, 800 mg/kg was selected as the high dose for the UDS assay.

   Compound administration- Groups of ten male Alderley Park rats were given a single oral dose of 400 or 800 mg/kg Pebulate in corn oil by gavage at a volume of 10 mL/kg body weight. Four animals were administered corn oil by gavage at a volume of 10 mL/kg, while two rats each (positive controls) were administered 40 mg/kg 6BT or 10 mg/kg DMN.

5. Cell preparation

a. Animal preparation

Four or 12 hours after dosing, animals were anesthetized with Fluothane BP and maintained under deep anaesthesia to prevent recovery. Liver perfusion was then carried out as described below.

b. Perfusion technique

The liver was perfused *in situ* for 10-15 minutes at a rate of 40 mL/min with 450 mL of KP1 calcium-free buffer at pH 7.4, followed by perfusion with KH2 buffer with calcium and collagenase solutions added. Perfusion rate was decreased to 20 mL/min. The liver was then cut from the carcass and chopped with scissors until a uniform consistency was obtained. The crude homogenate was diluted with Williams' medium E complete.

c. Hepatocyte harvest/culture preparation

The hepatocyte suspension was filtered through 150 µm nylon bolting cloth with additional Williams' medium E complete to obtain a final volume of 120 mL. Approximately 40 mL of this suspension was centrifuged at 40×g for 2 min. Centrifugation and resuspension steps were repeated; viability was determined by trypan blue exclusion. Hepatocyte viability was 45.5% and 78.8% for the negative control cell preparations.

B. TEST PERFORMANCE

1. UDS assay

a. Cell cultures

Hepatocytes were plated at 1.5 x 10^5 cells/mL in petri dishes or six-well plates containing coverslips (No. 1½, 25 mm). After an attachment period of 2 hr in 5% CO_2 in air at 37°C, the cells were washed once with Williams' medium E complete. Serum-free Williams' medium (2 mL) containing 3H-thymidine was then added to the cultures. After the cultures were incubated for 4 hr, the media was removed and the cultures washed three times with Williams' medium- 3H-thymidine solution to remove excess radiolabel from the cultures. The cells were then incubated overnight with 3 mL of the same medium.

b. Cell fixation

Cultures were rinsed once with incomplete medium or saline, and then fixed by three 10 minute washes with ethanol/acetic acid (3:1, v/v). Cultures were then washed 4 times with deionized water. Coverslips were air-dried and mounted with DPX.

c. Preparation of autoradiographs/grain development

After air-drying, slides were dipped into Ilford K2 photographic emulsion in water/glycerol, allowed to gel, transferred to a drying cabinet at room temperature for 2-6 hr, sealed in light-tight desiccated boxes and incubated for 14 days at 4°C. Slides were developed with Kodak D-19 developer, fixed with Ilford HYPAM fixer and stained with aqueous eosin Y phloxine.

d. Grain counting

The number of silver grains over the cell nucleus as well as cytoplasmic background counts were measured with an automated image analyzer (AMS 40-10) and the data fed directly into a computer. For each treatment, nuclei of at least 25, but usually 50, morphologically normal cells were counted on each of two slides for each animal.

3. Evaluation criteria

(a) Assay validity: The assay was considered valid if: 1) the cytoplasmic grain count for the vehicle control group was <40; 2) the mean net nuclear grain count for the vehicle control was <0; and 3) the net nuclear grain count for the positive control was ≥5 with at least 20% of the cells in repair.

(b) Positive response: The assay was considered positive if a treatment group induced a reproducible increase in the net nuclear grain count that was ≥5 with at least 20% of the cells in repair.

C. REPORTED RESULTS

No unscheduled deaths occurred and no clinical signs of toxicity were observed following exposure to the high dose (800 mg/kg). Similarly, no treatment-related signs of cytotoxicity were observed in Pebulate-treated cells compared to vehicle controls. No evidence of UDS induction was found at either concentration or time point (the net nuclear grain counts were all negative). Results with the vehicle and positive controls were appropriate. Summary data are presented in Study Report Table 1; MRID 41614809, p. 21 (see Attachment). From the results, the study author stated that Pebulate was not genotoxic in this *in vivo test* system.

D. REVIEWER'S DISCUSSION/CONCLUSIONS

We assess that no conclusions can be reached for this study because of the failure to test a level of Pebulate that was overtly toxic to the treated animals or induced a cytotoxic effect on the target cells (i.e., hepatocytes). We conclude, therefore, that the study is unacceptable and can not be upgraded. The study should be repeated using a high level that clearly demonstrates that the maximum tolerated dose was achieved.

E. STUDY DEFICIENCIES: See above.

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY.
SEE THE FILE COPY

STUDY REPORT TABLE 1, p.21
MRID No. 41614809

SignOff Date:	4/13/99
DP Barcode:	D254687
HED DOC Number:	013311
Toxicology Branch:	TOX1

Table 7. Summary of Short-Term and Intermediate-Term Risks: MOEs for Dermal and Inhalation Scenarios.

Exposure Scenario (Scen. #)	Crop Type/Use	Acres Treated or Amount Handled per Day	Application Rate (lb ai/A)		*Baseline MOE		*PPE MOE		*Engineering Controls MOE	
					Dermal	Inhalation	Dermal	Inhalation	Dermal	Inhalation
Mixer Loader Exposures										
Mixing/Loading Emulsifiable Concentrate for Chemigation (1a)	Tomatoes	350 acres	6 lb ai/acre		1	25	**200**	**250**	NA	NA
			4 lb ai/acre		2	37	**290**	**370**	NA	NA
Mixing/Loading Emulsifiable Concentrate for Soil Injection and Groundboom Application (1b)	Tomatoes (Western Region)	80 acres	10 lb ai/acre		3	65	**510**	**640**	NA	NA
	Sugar Beets, Tomatoes, Tobacco		6 lb ai/acre		5	**110**	**860**	NA	NA	NA
			4 lb ai/acre		8	**160**	**1300**	NA	NA	NA
	Tobacco		3 lb ai/acre		10	**220**	**1700**	NA	NA	NA
Mixing/Loading Emulsifiable Concentrate for Impregnation on Dry Bulk Fertilizer (Closed System) (1c)	Dry Bulk Fertilizer (assuming 40 ten ton trucks loaded per day)	3,200 lb ai/day	4 lb ai/acre	1,000 lb fertilizer/acre	NA	NA	NA	NA	**250**	**230**
		1,600 lb ai/day	4 lb ai/acre	2,000 lb fertilizer/acre	NA	NA	NA	NA	**510**	**470**
Mixing/Loading Fluid Formulation for Combination with Liquid Fertilizer (1d)[a]	Fluid Fertilizer	No Data	10 lb ai/acre	10 gallons fertilizer/acre	No Data	No Data	No Data	No Data	No Data	No Data
		No Data	10 lb ai/acre	20 gallons fertilizer/acre	No Data	No Data	No Data	No Data	No Data	No Data
		No Data	10 lb ai/acre	40 gallons fertilizer/acre	No Data	No Data	No Data	No Data	No Data	No Data