DATA EVALUATION REPORT

PEBULATE

STUDY TYPE: *IN VITRO* MAMMALIAN CYTOGENETICS ASSAY IN HUMAN LYMPHOCYTES (84-2)

4/13/1999

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

Task Order No. 98-18L

Primary Reviewer: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: ____________ Date: ____________
Secondary Reviewers: B. L. Whitfield, Ph.D.	Signature: ____________ Date: ____________
Robert H. Ross, M.S., Group Leader	Signature: ____________ Date: ____________
Quality Assurance: Lee Ann Wilson, M.A.	Signature: ____________ Date: ____________

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: Nancy E. McCarroll ______________________, Date ________
Toxicology Branch 1/HED (7509C)
EPA Secondary Reviewer: Yung Yang, Ph.D. ______________________, Date ________
Toxicology Branch I /HED(7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian cytogenetics assay in human lymphocytes OPPTS 870.5375 [§84-2].

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

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

CITATION: Randall, V. and J. M. Mackay (1990)Pebulate: An evaluation in the in vitro cytogenetic assay in human lymphocytes. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report number, CTL/P/2826, January 9, 1990. MRID No. 41556802. Unpublished.

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

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics chromosomal aberration assay (MRID No. 41556802), cultures of primary human lymphocytes from one male and one female donor were exposed to pebulate (97.1% w/w) in dimethyl sulfoxide (DMSO). Cultures from the male donor were treated at concentrations of 15, 75, or 150 µg/mL in the absence and presence of an exogenous metabolic activation system (S9-mix). Cultures from the female donor were treated at concentrations of 15, 75, or 100 µg/mL in the absence of S9-mix and at concentrations of 15, 75, or 150 µg/mL in the presence of S9-mix. The S9 preparation was obtained from Aroclor 1254 induced male rat liver.

Pebulate was tested up to cytotoxic concentrations (100 or 150 µg/mL -S9; 150µg/mL +S9) as determined by reduced mitotic index or virtual absence of metaphase cells at the highest concentrations tested. No statistically or biologically significant increases in chromosomal aberration frequencies were observed at any pebulate concentration in either donor in the presence or absence of S9-mix. **There was no evidence that pebulate induced a treatment-related induction of chromosomal aberrations over background.**

This study is classified as acceptable (guideline). It satisfies the requirement for FIFRA Test Guideline 84-2 for in vitro 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: Pebulate

Description: amber liquid
Lot/Batch #: not given
Purity: 97.1% w/w
Stability of compound: not given
CAS #: unknown
Structure: not given
Solvent used: dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored at room temperature, protected from light. The frequency of dosing solution preparation was not reported.

2. Control materials

Negative: Culture Media (RPMI)
Solvent/final concentration: DMSO/5 µL/mL
Positive/final concentration:
Nonactivation - Mitomycin C/1.0 µg/mL
Activation - Cyclophosphamide/50 µg/mL

3. Activation

S9 derived from Male Alpk:APfSD:

_x_ Aroclor 1254	_x_ induced	_x_ rat	_x_ liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other

The S9 liver homogenate was prepared by the performing laboratory and contained the following components:

Concentration in S9-mix (dissolved in deionized water, final pH = 7.5)

Na_2HPO_4	75 mM
KCl	25 mM
Glucose-6-phosphate	4 mM
NADP, sodium salt	3 mM
$MgCl_2$	6 mM
S9	50%

Note: 200µL of the S9 mix were added to 10 mL of culture medium.

4. Test compound concentrations used:

Preliminary cytotoxicity tests: 10 to 5000 µg/mL with and without activation were initially assayed. Since no metaphases were observed at or above 250 µg/mL, a repeat cytotoxicity test was performed using 15, 50, 75, 100, 150, 200, or 250 µg/mL with and without activation.

Main cytogenetic test: Cultures treated in the repeat cytotoxicity test were used for the main cytotogentic assay as follows:

Without S9-mix: Cultures from the male donor treated with 15, 75 or 150 µg/mL were examined for chromosome aberrations. Cultures from the female donor treated with 15, 75 or 100 µg/mL were examined for chromosome aberrations.

With S9-mix: Cultures from both donors treated with 15, 75 or 150 µg/mL were examined for chromosome aberrations.

5. Test cells: Human lymphocytes derived from one male and one female donor were used in the study. Peripheral blood was obtained by venepuncture on the days that cultures were initiated from healthy non-smoking donors with a known low incidence of chromosomal aberrations in their peripheral blood lymphocytes. Cultures were started by adding 0.5 mL of whole blood and 0.5 mL of a solution of phytohemagglutinin (0.1 mg/mL) to 9.0 mL of RPMI-1640 (Dutch modification) medium supplemented with 10% fetal bovine serum, 1.0 IU/mL heparin, 100 IU/mL penicillin and 100 µg/mL streptomycin.

Properly maintained? Y

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay

An initial range-finding cytotoxicity assay was conducted on blood from both a male and a female donor using a range of ten pebulate concentrations ranging from 10 to 5000 µg/mL. No metaphases were observed in cultures treated with pebulate concentrations of 250 µg/mL and above. Therefore, a further cytotoxicity test was performed with pebulate concentrations of 15, 50, 75, 100, 150, 200, or 250 µg/mL tested with and without S9-mix. About 44 hours after the cell cultures were initiated, the appropriate concentration of pebulate, the solvent or the positive control was added to duplicate cultures from each of the donors. Cultures without and cultures with S9 mix (200 µl of a 1:1 mix of S9 and co-factor solution added at the same time as test agent) were exposed to the test agent for 3 hours at which time the growth medium was changed and incubation continued for the remainder of the 72 hour culture period.

2. Cytogenetic assay

a. Cell treatment

Cells exposed to test compound, solvent, or positive control for 3 hours (nonactivated), 3 hours (activated)

b. Spindle inhibition

Inhibitor used/concentration: colcemid / 0.4 µg/mL
Administration time: 2 hours (before cell harvest)

c. Cell harvest

Cells exposed to test material, solvent or positive control were harvested 25 hours after termination of treatment (nonactivated), 25 hours after termination of treatment (activated).

d. Details of slide preparation

Cultures were transferred to conical centrifuge tubes and cells collected by centrifugation at 400 g for 5 min. Cells were resuspended in 5 mL of 0.075 M KCl at room temperature, an additional 5 mL of the KCl solution was added and the resuspension allowed to stand at room temperature for 12 min. The cells were again pelleted by centrifugation at 400 g for 5 min, the supernatant discarded and the cells fixed in freshly prepared methanol/glacial acetic acid fixative (3:1 v/v) added dropwise with the final volume made up to 10 mL. The fixation procedure was repeated twice. Single drops of cell suspensions were dropped onto clean, moist slides and the slides were air dried. The slides were stained in a filtered 10% solution of buffered Giemsa stain (Gurr's R66) in double deionized water for 7 min, rinsed in water, air-dried and mounted in DPX.

e. Metaphase analysis

No. of cells examined per culture 1000 for mitotic index (MI) determinations and 100 metaphase cells per culture for structural chromosomal aberration determination. Duplicate cultures were used for each test point.

Scored for structural: Y

Scored for numerical: N

Coded prior to analysis: Y

f. Evaluation criteria

Cells were evaluated for structural chromosomal damage according to the criteria recommended by Scott et.al. (1983). The following aberrations were recorded: gaps, breaks, fragments and minutes, multiple damage, interchanges, rearrangements and "others".

g. Statistical analysis

Data were evaluated for statistical significance at $p<0.01$, using Fisher's Exact Test (one sided).

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY: In the initial range-finding test, carried out using pebulate concentrations from 10 to 5000 µg/mL, no metaphases were observed in cultures treated at 250 µg/mL and above. Due to the cytotoxicity of pebulate over the concentration range tested, a further cytotoxicity test was done using seven concentrations of pebulate ranging from 15 to 250 µg/mL. A concentration-related effect on the MI was observed in the presence and absence of exogenous metabolic activation for both donors. Decreased MIs of 69% (with S9-mix)and 70% (without S9-mix) were observed at 150 µg/mL in cultures from the male donor. Decreased MIs of 52% at 150 µg/mL with S9-mix and ≥58% at ≥75 µg/mL without S9-mix were observed in cultures from the female donor. Solvent and positive controls responded appropriately.

B. CYTOGENETIC ASSAY: Based on the above findings, cultures exposed to pebulate concentrations of 15, 75, or 150 µg/mL in the presence of S9-mix (both donors) and in the absence of S9-mix (male donor) or 15, 75, or 100 µg/mL in the absence of S9-mix (female donor) were examined for structural chromosome aberrations. Effects of treatment on the MI and incidence of chromosome aberrations without and with S9-mix are presented in Study Report Tables 1 and 2, respectively (MRID 41556802, pp. 19 and 20--see Attachment). As shown, so statistically or biologically significant increases in the chromosomal aberration frequencies (with or without gaps) were observed in either donor in the presence or absence of S9-mix. The positive and solvent controls responded appropriately.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This study was acceptable. The test material was assayed to concentrations limited by cytotoxicity (100-150 µg/mL -S9; 150 µg/mL +S9) with dose-related reductions in mitotic activity and few metaphases present at the highest concentrations tested. The positive controls, Mitomycin C in the absence of S9-mix and Cyclophosphamide in the presence of S9-mix, and the solvent control gave the expected results. The experimental protocol basically followed the pertinent guidelines.

B. STUDY DEFICIENCIES: Minor: The stability and lot number of pebulate were not provided.

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

STUDY REPORT TABLES 1 and 2,
MRID NO. 41556802, pp. 19 and 20

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