# DATA EVALUATION REPORT

4/13/1999

PEBULATE

STUDY TYPE: SALMONELLA/MAMMALIAN ACTIVATION;
GENE MUTATION (84-2)

Prepared for

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

Prepared by

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Task Order No. 90-18J

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# DATA EVALUATION RECORD

STUDY TYPE: Salmonella/mammalian activation gene mutation assay; OPPTS 870.5265 [§84-2]

DP BARCODE: D247841
P.C. CODE: 041403

SUBMISSION NO.: S546073
TOX. CHEM. NO.: 710

TEST MATERIAL (PURITY): Tillam® (97.3%)

SYNONYMS: Pebulate

CITATION: Majeska, J. B. (1987) Mutagenicity Evaluation in *Salmonella typhimurium*. Stauffer Chemical Company, 400 Farmington Avenue, Farmington, CT 06032. Report No. T-12888. June 19, 1987. MRID No. 41556803. Unpublished.

SPONSOR: Stauffer Chemical Company, Agricultural Chemical Division, 400 Farmington Avenue, Farmington, CT 06032.

EXECUTIVE SUMMARY: In independently performed reverse gene mutation assays in bacteria (MRID No. 41556803), strains TA 1535, TA 1537 and TA 98 of *Salmonella typhimurium* were exposed to Tillam (Pebulate, 97.3%) in dimethyl sulfoxide (DMSO) at concentrations of 0.0375, 0.0750, 0.1500, 0.3000, or 0.6000 µL/plate in the presence and absence of mammalian metabolic activation in the two main mutation assay trials. In a preliminary mutation assay, *S. typhimurium* strain TA 100 was initially exposed to 0.020 to 10.0 µL/plate +/-S9 and was subsequently treated with doses of 0.0375-0.600 µl/plate +/-S9. The S9 fraction was derived from Aroclor 1254 induced Sprague-Dawley rat liver.

Tillam was tested up to an acceptable concentration since Tillam precipitated upon addition to top agar at ≥0.313 µL/plate and appeared as a visible chemical residue. In addition, cytotoxicity (decreased colonies and microcolonies present) was noted at ≥0.625 µL/plate +/-S9. The positive and DMSO controls induced the appropriate responses in the corresponding strains. **There was no evidence that Tillam induced an increase in mutant colonies over background in any of the *Salmonella* strains with or without metabolic activation.**

This study is classified as acceptable (guideline). It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

# I. MATERIALS AND METHODS

## A. MATERIALS

1. Test material: Tillam (Pebulate)
   Description: amber liquid
   Lot/Batch #: 4921-20-18
   Purity: 97.3%
   Stability of compound: not given; expiration date 04/88
   CAS #: not given
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: The test material was stored at ≈20°C, protected from light. Dosing solutions were prepared immediately prior to use; analytical determinations were conducted to verify actual concentrations used in the study. Osmolality and pH measurements were also performed.

2. Control materials

   Solvent/final concentration: DMSO/50 µL/plate
   Negative: untreated bacteria
   Positive:
   Nonactivation:
   Sodium azide __10__ µg/plate TA100, TA1535
   2-Nitrofluorene __10__ µg/plate TA98
   ICR-191 __5__ µg/plate TA1537

   Activation:
   2-Aminoanthracene _5_ µg/plate (TA 98, TA 100, TA 1535) or _40_ µg/plate (TA 1537)

3. Activation: 50 µL/plate

   S9 (Lot No. EHC-0476-25) was prepared by the performing laboratory and was derived from Aroclor 1254 induced Sprague-Dawley rat liver.

   S9 mix composition final concentration:
   S9 fraction: 100 µL/mL
   $MgCl_2$: 8 mM
   KCl: 34 mM
   NADP: 4 mM
   Glucose-6-phosphate: 5 mM
   Phosphate buffer: 100mM

4. Test organisms: *S. typhimurium* strains

| | | | |
|---|---|---|---|
| ___ TA97 | _x_ TA98 | _x_ TA100 | ___ TA102 |
| ___ TA104 | _x_ TA1535 | _x_ TA1537 | ___ TA1538 |

Properly maintained? Y
Checked for appropriate genetic markers (rfa mutation, R factor)? Y

5. Test compound concentrations used:

Preliminary range-finding mutation assay: Ten doses (0.020, 0.039, 0.078, 0.156, 0.313, 0.625, 1,25, 2.5, 5.0, or 10.0 µL/plate) were tested with and without S9 activation using strain *S. typhimurium* TA 100. Positive and negative controls were included.

Mutation assays: Two trials were performed with doses of 0.0375, 0.0750, 0.1500, 0.3000, or 0.6000 µL/plate +/-S9 in both trials.

For all mutation assays, triplicate plates per dose per strain per condition were processed.

B. TEST PERFORMANCE

1. Type of Salmonella assay:

_x_ standard plate test
___ pre-incubation (__ minutes)
___ "Prival" modification *(i.e. azo-reduction method)*
___ spot test
___ other [describe]

2. Protocol

Approximately $10^8$ cells from 12-16 hour cultures were added to test tubes containing 2.0 mL of molten soft agar (0.6%) supplemented with 0.5 mM histidine and 0.5 mM biotin. Additionally, the designated concentration of test solution or control substance in 50 µL and either 0.5 mL of phosphate buffer (nonactivation) or 0.5 mL of S9 mix were added. The tube contents were poured onto Vogel-Bonner minimal medium agar plates. Three plates were used for each experimental point and duplicate experiments were performed. The plates were incubated at 37°C for 2 days and the mean and standard deviation of the mean revertant colonies was determined.

3. Evaluation criteria

(a) Assay validity: The assay was considered valid if: 1) the data from the negative, solvent and positive controls were within the unprovided historical control ranges for the performing laboratory; 2) data from at least three test material doses were available for analysis; and 3) in the absence of a cytotoxic effect, the limit of solubility or a maximum dose of 3-10 mg/plate was tested.

(b) Positive response: The assay was considered positive if the test material induced a reproducible and dose-related increase in revertant colonies that was 3-fold higher than the solvent control value for strains TA 1535 or TA 1537, 2-fold higher than the solvent control value for strain TA 100 or 2.5-fold higher than the solvent control value for strain TA 98.

## II. REPORTED RESULTS

### A. Analytical Determinations

The study author stated that the actual concentration of the test substance in stock solutions was within 10% of the nominal values. It was further reported that the test material did not alter the pH or osmolality of the $PO_4$ buffer or S9 mix.

### B. Preliminary Range-finding Mutation Assay

A range-finding assay was done with strain TA 100 with and without metabolic activation at concentrations of 0.020, 0.039, 0.078, 0.156, 0.313, 0.625, 1.250, 2.500, 5.000, or 10.000 µL/plate. At levels ≥0.313 µL/plate +/-S9, Tillam precipitated upon addition to the top agar and a visible chemical residue was observed on plates containing ≥0.625 µL/plate. Also at concentrations ≥0.625 µL/plate, cytotoxicity was indicated for tester strain TA 100 by a decrease in the number of revertant colonies and/or the presence of microcolonies. There was, however, no indication of a mutagenic effect at any noncytotoxic dose.

### C. Mutagenicity assay

Based on the preliminary data, doses of 0.0375 to 0.6000 µL/plate +/-S9 were selected for further study in both trials. Reductions in revertant colony counts and/or the background lawn of growth were observed for all strains at ≥0.1500 µL/plate +/-S9 in both trials. There was, however, no evidence of induced mutant colonies over background in any of the *Salmonella* strains when Tillam was tested with or without metabolic activation over a concentration range of 0.0375 to 0.6000 µL/plate. Mean revertant colony counts of the three plates per experimental point for the four strains are shown in the attachment (MRID No. 41556803, Study Report Tables 1-8, pp. 10-17).

## III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. Tillam (Pebulate) was tested to an acceptably high concentration (limited by precipitation and cytotoxicity) and controls responded appropriately. The experimental protocol followed the pertinent guidelines.

B. STUDY DEFICIENCIES: None identified.

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

STUDY REPORT TABLES 1-8, pp. 10-17
MRID No. 41556803

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