UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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DEC 17 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Portia Jenkins, PM # 12
Insecticides/Rodenticides Branch
Registration Division TS-767C

THRU: K. Bruce Jaeger, Section Head
Rev. Sec. # 1/Toxicology Branch
Hazard Evalauation Division TS-769C 12/9/87

THRU: Dr. T. M. Farber, Chief
Toxicology Branch
Hazard Evalauation Division TS-769C

FROM: D. Ritter, Toxicologist DLR 12-9-87
Rev. Sec. # 1/Toxicology Branch
Hazard Evalauation Division TS-769C

Caswell #: 374.
TOX Project #: 7-0957.

Subject. EPA Reg. # 11678-4: COTNION-M; Azinphos Methyl, Technical. Review of data.

We noted in our most recent review of this application (D. Ritter, 10/30/87) that certain mutagenicity data were being reviewed by Dr. Chen of this Branch.

Dr. Chen's review is attached.

He concluded that Cotnion-M was non-mutagenic in an assay of Histidine Auxotrophs of Salmonella typhimurium (Ames Assay). The study was unacceptable because of data deficiencies.

006526

1 of 10

84-2 - Salmonella Mutagenicity Test

Reviewed by: John H.S. Chen 12/2/87
Section I, Toxicology Branch (TS-769C)
Tertiary Reviewer: I. Mauer 12/3/87
Section VI, Toxicology Branch (TS-769C)
Reviewed by Section Head: R.B. Jaeger 12/4/87
Section I, Toxicology Branch (TS-769C)

006526

DATA EVALUATION REPORT

Study Type: Gene Mutation in Bacteria

TOX. CHEM. NO.: 374

Accession No.: 402801-7

MRID NO.:

Test Material: Cotnion-M (100% Purity)
(Guthion)

Synonyms:

Study Number (s): MAK/141/AZN

Sponsor: MAKHTESHIM-AGAN (AMERICA) INC.

Testing Facility: Life Science Research Israel Ltd., Ness Ziona, Israel

Title of Report: Assessment of Mutagenic Potential in Histidine Auxotrophs of Salmonella typhimurium (The Ames Test)

Author(s): Z. Evenchik

Report Issued: May 6, 1987

Conclusions:

Cotnion-M was nonmutagenic to TA1535, TA1537, TA1538, TA100, and TA98 strains of Salmonella typhimurium either with or without metabolic activation at the concentrations tested (2 through 160 ug/plate).

Concentrations tested: 2, 10, 40, 80, and 160 ug/plate

Classification of Data: Unacceptable

(Deficiency: reported results for determining the upper limit of Cotnion-M in this study were inadequate)

006526

Title of Report: Assessment of Mutagenic Potential of Cotnion-M in Histidine Auxotrophs of Salmonella typhimurium (The Ames Test)
LSRI Report No.MAK/141/AZN, May 6, 1987

I. Materials and Methods

1. Test Material

The test material, Cotnion-M (100% Purity), was stored at -4°C and protected from light. Solutions were made in DMSO and were freshly prepared for each experiment.

2. Test Organisms

Five histidine-requiring strains of Salmonella typhimurium (TA1535, TA1537, TA1538, TA100 and TA98), which were checked and confirmed for appropriate amino acid requirement and characteristic spontaneous reversion rate, were provided by Dr. Bruce Ames in October, 1984.

3. Preliminary Toxicity Test

Six concentrations of the test material (2.5, 25, 125, 625, 1250 and 2500 ug/plate) were used in this study. All tubes were inoculated with an overnight culture of strain TA98 (0.1 ml) and overlaid onto minimal medium plates. After 48 hours of incubation at 37°C., the plates were examined for the number of revertants and the presence of a background lawn of non-revertant colonies; toxicity of the test compound was shown by the decline in the number of spontaneous revertants or the absence/thinning of the background lawn. The highest dose level for this Ames test was chosen according to the results obtained in the preliminary toxicity test.

4. Pour-Plate Assay for Mutagenesis

The assay was based on the plate incorporation method described by Ames et al. (Ames et al., Mutation Res. 31: 347-364, 1975). The overnight cultures provided approximately 2×10^9 organisms/ml which were used as the standard bacterial suspensions. The mutagenicity of Cotnion-M was evaluated by the Ames test in the presence or absence of metabolic activation. Mutations were quantified on duplicate plates for each strain by counting the His^+ revertant colonies after 48 hours of incubation on a selective agar plate. Appropriate positive control compound for each strain and solvent control were run cocurrently with the test compound.

5. In-vitro Metabolic Activation System

The mammalian metabolic activation system consisted of rat liver homogenate (S-9) from phenobarbital sodium and 3-methylcholanthrene-treated male CD rats and the cofactor solution described by Ames et al (1975). The S-9 mix (4%) contained the following components: S-9, 2 ml; 0.1M NADP, 2 ml; 1M glucose-6-phosphate, 0.25 ml; 0.4M $MgCl_2$/1.65M KCl, 1 ml; 0.1M NaH_2PO_4-Na_2HPO_4 Buffer (pH 7.4), 25 ml; H_2O, 19.75 ml.

6. Statistical Analysis

006526

If the compound is mutagenic, it would demonstrate at least two-fold increase of the number of revertant colonies over the control value and also exhibited a dose-related increase in the number of histidine-independent colonies.

II. Reported Results

1. Preliminary Toxicity Test (Table 1 attached)

Following the exposure to an overnight TA98 culture with six concentrations of Cotnion-M (2.5, 25, 125, 625, 1250, and 2500 ug/plate) on a selective minimal agar plate for 48 hours at 37°C., inhibition of bacterial growth observed as the decline in the number of spontaneous revertants or the absence/thinning of the background lawn was noted at 625, 1250, and 2500 ug/plate. Therefore, the highest dose level of Cotnion-M selected for the Ames Test was determined to be 160 ug/plate with and without metabolic activation.

2. Ames Test (Tables 2, 3, 4, 5, and 6 attached)

No significant increases in the number of revertant colonies (less than 2-fold) over concurrent control value were obtained for any of the tester strains following exposure to the test compound (i.e., 2, 10, 40, 80 and 160 ug/plate) either in the presence or absence of S-9 mix.

III. Evaluation and Recommendation

1. The specific procedures for confirming the genotypes of TA1535, TA1537, TA1538, TA100 and TA98 strains of Salmonella typhimurium, which were based on the individual sensitivity test recommended by the Ames test, are acceptable.

2. The spontaneous revertant colonies for each of the five tester strains of Salmonella typhimurium are found within the normal ranges of revertant colonies recommended by the Ames test (Ames et al., Mutation Res. 31: 347-364, 1975)

3. The strain specific control compounds (4-nitro-o-phenylenediamine, sodium azide and ICR-191) and the positive control (2-aminoanthracene) to ensure the efficacy of the activation system have given strongly the positive responses as expected.

4. Although the cytotoxicity of the test compound against TA98 culture was observed at 625 ug/plate in the reported preliminary toxicity test (Table 1), there was no cytotoxicity evidenced either by reducing the number of spontaneous revertants or by thinning the background lawn at 160 ug/plate. Therefore, it is questionable whether an appropriate, upper limit of Cotnion-M (i.e., between 160 and 625 ug/plate) was chosen for this study.

5. According to the acceptable procedures for the Ames Salmonella/Mammalian Microsome Mutagenicity Test recommended by EPA (EPA Health Effect Test Guidelines 56/6-83-001), the high-dose level of test material must demonstrate some evidence of cytotoxicity in any of the treated cultures. The study is unacceptable in the present form. However, the study may be upgraded on resolution of the reporting deficiency.

4

006526

Azinphos-methyl RIN: 7365-92

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