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

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APR 18 1990

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Malathion - Tox Data Submitted under MRID No. 41389301
EPA ID No. 241-208

Chemical (Caswell) No.: 535
RD Record No.: 260,402
HED Project No.: 0-0775/0775A

FROM: Irving Mauer, Ph.D., Geneticist 3/30/90
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Health Effects Division (H7509C)

Registrant:

American Cyanamid (in conjunction with A/S Cheminova), Princeton, NJ.

Request

Review and evaluate the following mutagenicity study, submitted on behalf of the Malathion Reregistration Force, No. 57875:

(84-4) Test for Chemical Induction of Unscheduled DNA Synthesis in Rat Primary Hepatocyte Cultures by Autoradiography with AC 6,601, unpublished study No. 0125-5100 performed by SITEK Research Labs., Rockville MD, Final Report issued December 22, 1989 (EPA MRID 41389301).

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TB Conclusion:

The study is ACCEPTABLE in demonstrating this lot of malathion (designated AC 6015-136B) was negative for inducing UDS (a form of DNA damage/repair) in rat hepatocyte cultures exposed up to cytotoxic levels, 0.12 to 0.16 uL/mL (corresponding to approximately 150 to 200 ug/mL). A detailed review is attached to this memorandum.

Attachment (DER)

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Reviewed by: Irving Mauer, Ph.D., Geneticist 3-30-90
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D, Chief
Toxicology Branch I - IRS (H7509C)

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DATA EVALUATION REPORT

I. SUMMARY

Study Type: 84-4 Mutagenicity - DNA damage/repair in vitro (Rat HPC/UDS)

MRID No.: 413893-01
ID No.: 241-208
RD Record No.: 260,402
Caswell No.: 535
Project No.: 0-0775/0775A

Chemical: Malathion

Synonyms: AC 6,601

Sponsor: American Cyanamid (associated with A/S Cheminova), Princeton, NJ

Testing Facility: SITEK Research Laboratories, Rockville, MD

Title of Report: Test for Chemical Induction of Unscheduled DNA Synthesis in Rat Primary Hepatocyte Cultures by Autoradiography with AC 6,601.

Authors: Kamela J. Pant

Study Number: SITEK No. 0125-5100

Date of Issue: December 22, 1989

TB Conclusions:

Negative for inducing unscheduled DNA synthesis (UDS) in primary rat hepatocyte cultures treated up to cytotoxic levels (0.12 to 0.16 uL/mL, equivalent to approximately 150 to 200 ug/mL).

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material: AC 6,601 (Malathion, from American Cyanamid)

Description: Clear, pale yellow liquid
Batch (Lot): AC 6015-136B
Purity (%): 94.0
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism: Primary hepatocyte cultures

Species: Rat
Strain: Sprague-Dawley
Age: (Adult)
Weights - males (only): 200-325 g
Source: Charles River Laboratories

C. Study Design (Protocol) - This study was designed to assess the genotoxic (DNA damage/repair) potential of malathion when administered in vitro to rat primary hepatocyte cultures (HPC), by determining unscheduled DNA synthesis (nuclear silver grain counts). A copy of the protocol employed is appended to this DER (from APPENDIX I of the investigator's FINAL REPORT) as ATTACHMENT I.

A statement affirming compliance with Agency GLPs was provided. A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Hepatocytes isolated from a male rat (by standardized, referenced, methods employed by experts for this type of assay) were treated for 18 hours with test article in a preliminary toxicity test, employing 10 evenly spaced dose levels ranging from 0.08 through 100 uL/mL, applied to duplicate cultures at each dosage. Following treatment, trypsinized cultures were scored for viability (by exclusion of trypan blue), and relative cell survivals (RCS) calculated by comparing treated to solvent (DMSO) controls. From the results of this range-finder, six dose levels of malathion were selected for the main (UDS) assay.

For the main assay, hepatocytes from another adult male were established on glass coverslips in culture and exposed for 18 hours to 10 uCi/mL tritiated thymidine

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(^{3}H-TdR, sp. act. 20 Ci/mM) together with test article at concentrations of 0.01, 0.02, 0.04, 0.08, 0.12, and 0.16 uL/mL (each in triplicate), or to two solvents, DMSO and ethanol, or to the mutagen, 2-acetylaminofluorene (2AAF, 10 ug/mL). In addition, a set of untreated (culture medium, WME) controls was run concurrently. Following exposure, cells were expanded in hypotonic (1%) sodium citrate, dried and mounted cell side up on standard microscope slides. Slides were then dipped (in the dark) in heated NTB photographic emulsion, dried and then sealed in light-tight microscope slide boxes at 0 to 4 °C. After 8 days, the slide preparations were developed by standard photographic methods, stained with hematoxylin-eosin and mounted permanently under #1 cover glasses.

Coded slides were scored for silver grain counts in 150 randomly selected, normally-appearing cells per test treatment, and net nuclear grain counts calculated [NNG, by subtracting contiguous cytoplasmic, background counts], using an electronic colony counter (Artek 880) equipped with an auxiliary TV monitor attached to the universal microscope scanning the slides. The numbers of nuclei showing 5 or more NNG counts per treatment were also recorded. Finally, the incidence of nuclei exhibiting S-phase (replicative) DNA synthesis among 200 cells per culture was also determined.

For an assay to be considered valid for evaluation, the investigator accepts only those with: 1) An average NNG in negative controls below 5, with less than 20 percent of cells showing NNG of 5 or more; 2) a positive control recording at least 30 percent of cells with NNG of 5 or more, and an average NNG count per cell of 20 or more; and 3) at least 0.2 percent of nuclei in negative control(s) in S-phase DNA synthesis (to indicate no substantive inhibition of DNA synthesis has occurred).

A test compound is considered positive by this laboratory when the average NNG counts are significantly increased by at least 5 grain counts over control, or more than 25 percent of cells scored show a net nuclear grain count of 5 or more at at least two successive doses, or exhibiting a definitive dose-response relationship, with at least one dose level significantly increased over the concurrent control. However, if a single test dose shows a significantly increased grain

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count, but there is no evidence for a positive dose response, the result is considered marginal. On the other hand, the test substance is considered negative in the absence of a dose-response, and when no test dose shows a significant increased count.

E. Results - In the range-finder, malathion doses of 0.31 uL/mL and above were 100 percent toxic; RCS at 0.16 uL was 17.9, and 59 percent at the LDT, 0.08 uL/mL (Report Table 1, attached to this DER). Hence it was decided to select doses for the UDS assay bracketing the effect level in the preliminary test, namely, 0.01, 0.02, 0.04, 0.08, 0.12, and 0.16 uL/mL, but score for UDS only the highest four doses providing viable cells.

The four high (scorable) test doses were found to be 0.02, 0.04, 0.08, and 0.12 uL/mL (corresponding to 25, 50, 100, and 150 ug/mL based on the density of the technical, 1.25 gm/mL). The results (Report Table 2, attached) showed that none of these test doses showed a significantly increased grain count over control in mean NNG, whereas the positive control, 2AAF, induced an appropriately significantly increased silver grain count. The average NNG for the several negative controls (DMSO, EtOH, medium) were not significantly different from each other. Finally, sufficient replicative S-phase DNA synthesis was recorded (1.22 to 1.89%), indicating no significant inhibition of DNA synthesis.

The investigator concluded that this lot of AC 6,601 (malathion) was negative for inducing UDS under conditions of the study.

F. TB Evaluation - ACCEPTABLE

The study appears to have employed adequate procedures and controls such that the negative results generated may be judged valid.

Attachments (Protocol/Data Tables)

RIN 1244-00

Malathion Tox Review # 7864

Page_____ is not included in this copy.

Pages 7 through 23 are not included.

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