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

008169

NOV 27 1990

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Vancide TH - Toxicity Data Submitted Under MRID Nos. 413434-01 and -02
EPA ID No. 1965-55

Chemical (Caswell) No.: 481B
RD Record No.: 266,392
HED Project No.: 0-1456

FROM: Irving Mauer, Ph.D., Geneticist
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THRU: Karl P. Baetcke, Ph.D., Chief 11/16/90
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Registrant: R.T. Vanderbilt, Norwalk, CT

1 of 20

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Request

Review and evaluate the following mutagenicity studies, both performed by Microbiological Associates, Inc. (MAI), Bethesda, MD:

1. [Volume 2 of Submission, for Guideline 84-2, Ames Test (EPA MRID No. 413434-01)]: SALMONELLA/MAMMALIAN MICROSOME PLATE INCORPORATION MUTAGENICITY ASSAY (AMES TEST) WITH A CONFIRMATORY ASSAY.

2. [Volume 3 of Submission, for Guideline 84-4, Other Genotoxic Effects (EPA MRID No. 413434-02)]: UNSCHEDULED DNA SYNTHESIS IN RAT PRIMARY HEPATOCYTES.

TB Conclusions

These studies have been judged as follows (detailed reviews are appended to this memorandum):

Study 1 (Ames)	Dose-related POSITIVE in activated TA98 and TA100 (100-333 ug/plate), in repeat trials.	ACCEPTABLE
Study 2 (UDS):	Dose-related POSITIVE in primary rat hepatocytes treated at 0.03 and 0.06 uL/mL.	ACCEPTABLE

Attachments (DERs)

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

11/22/90

DATA EVALUATION REPORT

003169

I. SUMMARY

Study Type: Mutagenicity - Ames Assay

MRID (Acc.) No.: 413434-01
ID No.: 1965-55
RD Record No.: 266,392
Caswell No.: 481B
Project No.: 0-1456

Chemical: Vancide TH [1,3,5-triethylhexahydro-s-triazine]

Sponsor: R.T. Vanderbilt, Norwalk, CT

Testing Facility: Microbiological Associates, Inc. (MAI) Bethesda, MD

Title of Report: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) With a Confirmatory Assay.

Authors: R.H.C. San and C. Kruel

Study Number: (MAI) T8796.501014

Date of Issue: November 28, 1989

TB Conclusions:

Positive for gene reversions in two of the five Salmonella his⁻ strains employed in Ames Testing, but only at elevated concentrations (100 to 333 ug/plate) in the presence of rat S9 activation.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - Hexahydro-1,3,5-triethyl-s-triazine (Vancide TH)

Description: Clear colorless liquid
Batch (Lot): M-TH-8K-327
Purity (%): (Not stated)
Solvent/Carrier/Diluent: Water (DW)

B. Test Organism - Bacterial cell cultures

Species: Salmonella typhimurium LT2

Strains: TA98, TA100, TA1535, TA1537, TA1538 (all his$^-$ auxotrophs)

Source: Dr. Bruce Ames, UCal (Berkeley)

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of Vancide TH when administered in vitro to his$^-$ strains of Salmonella typhimurium, according to a protocol (provided in the Final Report) based upon validated (published) methodology.

Statements of Quality Assurance measures (inspections/audits), as well as adherence to Good Laboratory Practice were both provided.

D. Procedures/Methods of Analysis - Following a dose range-finding study with strains TA98 and TA1535 (10 concentrations of test article ranging from 6.7 to 5000 ug/plate), triplicate cultures from all five tester strains were exposed to graded doses of Vancide TH, both in the absence (-S9) and presence (+S9) of mammalian metabolic activation provided by the microsomal fraction of liver homogenates prepared from pretreated male Sprague-Dawley rats pretreated with Aroclor 1254, plus NADP(H)-generating cofactors. In addition to solvent (DW) controls, other cultures were exposed to strain-specific mutagens* to serve as positive controls.

After 48 hours incubation, revertent colonies (his$^+$) were counted (automatically, or manually in the event of interfering precipitant), and mean numbers of reverents

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*Without activation (-S9): TA98/TA1538, 2-nitrofluorene (1.0 ug/plate); TA100/TA1535, sodium azide (1.0 ug/plate); TA1537, ICR-191 (2.0 ug/plate).
With activation (+S9): All strains, 2-aminoanthracene (0.5 ug/plate).

per test plate compared to solvent control values. A substance is considered positive by this lab if it causes a dose-related response in treated cultures, with at least a doubling of control values.

E. Results - From the results of the range-finder (Report Tables 1 and 2), the following maximum doses for the initial main assay were selected, based on moderate to severe cytotoxicity:

Strains	S9	Concentration (ug/plate)
TA98/TA1537/TA1538	-/+	100/333
TA100/TA1535	-/+	333/500

In addition, four or five lower concentrations (including at least three nontoxic doses) were chosen.

Three mutagenicity assays were run (B1, B2, and B3), data from individual cultures collected in Report Tables 3 through 26, and summarized in Report Tables 27, 28, and 29 (these latter appended to this DER).

In Experiment B1, equivocal but apparently positive responses for Vancide treatment (as defined above by this lab's criteria) were recorded in activated cultures of TA100 and TA1535 at 333 ug/plate (2.7 and 2.1X solvent controls) but not at the HDT (500 ug/plate), which was cytotoxic to both strains (Report Table 27, attached). As well, a positive was registered in S9-supplemented TA98 at the HDT, 333 ug/plate (2.4-fold). Since cytotoxicity was not obtained (nor evidence for mutagenicity) in the battery of his^- strains treated in the absence of activation, a second trial (B2) was run, but at the following (enhanced) dose schedules:

TA98: 333, 200, 100, 33, and 10 ug/plate
TA1535: 500, 333, 100, 33, and 10 ug/plate
TA1537: 500, 333, 100, 33, 10, and 6.7 ug/plate
TA1538: 500, 333, 100, 67, 33, and 10 ug/plate

In Experiment B2, cytotoxicity was found at the higher doses of Vancide applied to each of these nonactivated strains but, except for an equivocal response in TA98 treated at 100 ug per plate (2X control), no increases in revertents at any other dose (Report Table 28, appended).

In a further (confirmatory) trial (again at enhanced doses of Vancide), definitively positive dose-related responses were recorded for both TA100 and TA98 under activated conditions, but not in any other strain.

In all trials, positive controls registered the appropriate increases in reversions, many-fold greater than background values.

The authors concluded that under conditions employed for Ames testing in this lab, the test article was positive in cultures of strains TA98 and TA100 supplemented with microsomal enzymes (S9-metabolic activation).

F. TB Evaluation - ACCEPTABLE. In adequate testing under appropriate controls, the test article was demonstrably positive for reverse gene mutation in the two pKM101 plasmid-containing repair-deficient strains of the Ames Salmonella battery of testers, but only at elevated concentrations in the presence of S9 activation.

Attachment (Summary Data Tables)

ATTACHMENT I
(Data Tables)

VANCIDE TOXR 008169

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

11/14/90

DATA EVALUATION REPORT

001169

I. SUMMARY

Study Type:	Mutagenicity - Other genotoxic effects (DNA damage/repair in mammalian cells)	MRID (Acc.) No.: 413434-02 ID No.: 1965-55 RD Record No.: 266,392 Caswell No.: 481B Project No.: 0-1456

Chemical: Vancide TH [1,3,5-triethylhexahydro-s-triazine]

Sponsor: R.T. Vanderbilt, Norwalk, CT

Testing Facility: Microbiological Associates, Inc. (MAI) Bethesda, MD

Title of Report: Unscheduled DNA Synthesis in Rat Primary Hepatocytes.

Author: R.D. Curren

Study Number: (MAI) T8796.380010

Date of Issue: December 1, 1989

TB Conclusions:

POSITIVE for unscheduled DNA synthesis (UDS, a measure of DNA damage/repair) as determined by dose-related increases in net nuclear grain counts at concentrations of 0.03 and 0.06 uL/mL.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - Hexahydro-1,3,5-triethyl-s-triazine (Vancide TH)

Description: Clear colorless liquid
Batch (Lot): M-TH-8K-327
Purity (%): (Not stated)
Solvent/Carrier/Diluent: Water (DW)/Aqueous t.c. medium (WME)

B. Test Organisms - Rodent primary hepatocyte cultures

Species: Rat
Strain: Fischer-344 (liver cells)
Age: "Adult" (males only)
Source: Charles River Labs, Raleigh, NC

C. Study Design (Protocol) - This study was designed to assess the genotoxic (DNA damage/repair) potential of Vancide TH when administered in vitro to primary rat hepatocytes, and unscheduled DNA synthesis (UDS) determined by autoradiographic methods according to an enclosed protocol based upon published procedures.

Statements of Quality Assurance measures (inspections/audits) as well as adherence to Good Laboratory Practice were both provided.

D. Procedures/Methods of Analysis - Following preliminary cytotoxicity testing (10 concentrations of Vancide ranging from 0.0003 to 10 uL/mL), coverslip preparations of primary rat hepatocytes were exposed in triplicate for 18 to 20 hours to graded doses of test article in the presence of a constant concentration of tritiated-thymidine (10 mCi/mL ^{3}H-TdR). Cytotoxicity was again assessed in parallel with the assay, as measured by the level of lactic dehydrogenase (LDH) activity present at each dose level (an indirect measure of cell death). Concurrent with solvent controls, other cultures were treated with the genotoxic mutagen, dimethylbenz(a) anthracene (DMBA, 3 and 10 ug/mL) to serve as positive control.

At harvest, the coverslip cell preparations were washed free of test materials, treated with hypotonic 1% sodium citrate (to swell the cells), fixed in Carnoy's (EtOH-glacial HAc), dried and mounted cell-side out onto standard glass microscope slides. These slides were coated (in the dark) with Kodak NTB2 photographic emulsion, stored for 7 days at 4 °C in air- and light-tight boxes, then developed (D-19), fixed, and stained in H&E.

Fifty morphologically normal cells on each of the three coded preparations (= 150 cells per treatment) were scored for UDS by determining net nuclear (silver) grain counts (NNGC = grain count over nuclei minus average count of three adjacent cytoplasmic areas). Scheduled, or replicative, DNA synthesis was differentiated by nuclei blackened by grains ("TNTC," too-numerous-to-count). Nuclei showing evidence of treatment toxicity (e.g., dark/uneven staining, pycnosis, ruptured cell membranes, irregular shape, etc.) were not counted.

Mean (± SD) NNGC were calculated for each treatment and dose level, as well as percent cells in repair (cells with ≥ 5 net grains). These data were analyzed by computer using the Lotus 1-2-3 program, which also provided significant differences of test values from solvent. A test article is considered positive by this laboratory if it induces a dose-related response, with at least one dose producing a significant increase in mean NNGC over solvent control; or, in the absence of a dose-response, significant increases in grain count at at least two successive doses. For an assay to be considered valid for analysis, the positive control must show significantly increased grain counts; and further, the proportion of negative control cells in repair must be < 15 percent, with a NNGC < 1.0.

E. Results - The preliminary cytotoxicity test revealed basic pH shifts (and concomitant interference with LDH determinations) in cultures exposed to 3 and 10 uL/mL Vancide, and dose-related abnormal cell morphology at concentrations as low as 0.03 uL/mL (Report Table 1). Hence, the HDT selected for the UDS assay was 0.1 uL/mL, together with eight lower doses (0.06, 0.03, 0.01, 0.003, 0.001, 0.0003, 0.00001, and 0.00003 uL/mL).

In parallel cultures examined for toxicity determinations, relative toxicity was greater then 60 percent (i.e., relative survival = 39%) at the HDT, 0.1 uL/mL, but gradually dropped toward background at lower concentrations (Report Table 2, appended to this DER). Normal cellular morphologies were observed below 0.06 uL/mL. Cells treated at 0.1 uL/mL Vancide, however, could not be evaluated for UDS due to excessive toxicity, but all the next five lower doses were fully evaluable.

The highest dose of Vancide that could be analyzed, 0.06 uL/mL, induced a significant increase in UDS as measured by a mean NNGC = 7.3 (compared to -1.2 in the WME solvent control) in 65 percent of cells with five or more net grains per cell (Report Tables 3 to 5,

appended here). In addition, a clear dose-response was evident at lower doses. The positive control, DMBA, responded appropriately at both doses applied.

The author concluded that Vancide caused a significant increase in grain count in treated primary rat hepatocyte cultures, used as a measure of unscheduled DNA synthesis/ repair, and thus was considered positive in this test.

F. TB Conclusions - ACCEPTABLE. Although only a single assay was run, the study reported here appeared to have been conducted under adequate conditions and controls, such as to generate valid results, i.e., a demonstrable positive for UDS repair.

Attachments (Data Tables)

ATTACHMENT I
(Data Tables)

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