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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: VANCIDE TH: hexahydro-1,3,5, triethyl-s-triazine
EPA ID # 1965-55, Record # 257349

To: James Wilson/John Lee PM # 31
Disinfectants Branch
Registration Division (H7507C)

Tox Chem No 481 B
Proj No 0-0449

From: Joycelyn E. Stewart, Ph.D. 9/27/90
Section II, Toxicology Branch I
Health Effects Division (H7509C)

Thru: Marion Copley, D.V.M. Marion F Copley 10/1/90
Section Head, Section II, Toxicology Branch I
Health Effects Division (H7509C)

Registrant: R.T. Vanderbilt, Inc
Norwalk, Connecticut

Action Requested: Review mouse micronucleus study submitted in response to the Antimicrobial Data Call-In Notice.

Conclusion: The data provided demonstrated that under the study conditions hexahydro-1,3,5, triethyl-s-triazine did not cause an increase in micronucleated polycharomatic erythrocytes in mouse bone marraow cells. However, the study is classified Unacceptable because transport of the chemical to the bone marrow cells was not demonstrated, and the chemical was not characterized as to its identitiy, purity and stability as required under Subdivision F Guidelines of the Federal Insecticide, Fungicide, and Rodenticide Act.

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Reviewed by: Joycelyn Stewart, Ph.D. 4/1/93
Section II, Tox. Branch I,(H-7509C)
Secondary reviewer: Irving Mauer, Ph.D. 4/24/93
Tox. Branch I (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity- Mouse Micronucleus TOX. CHEM. NO.: 481B

MRID NUMBER: 413215-01 PROJ. NO.: 0-0449

TEST MATERIAL: Hexahydro-1,3,5,triethyl-s-triazine

SYNONYMS: Vancide TH

STUDY NUMBER(S): T8796. 122010

SPONSOR: R.T. Vanderbilt Company, Inc
Norwalk, Connecticut, 06856

TESTING FACILITY: Microbiological Associates, Inc
Bethesda, Maryland

TITLE OF REPORT: Micronucleus Cytogenetic Assay in Mice
(Final Report).

AUTHOR(S): Putman,D.L., and Milhorn, J.M.

REPORT ISSUED: 11/15/1989

CONCLUSION:

Oral administration of hexahydro-1,3,5, triethyl-s-triazine at doses of 0, 25, 125, and 225 mg/kg did not cause an increase in micronucleated polychromatic erythrocytes in male and female ICR mice. The highest dose tested caused an increased incidence of mortality in the treated mice. The study is classified Unacceptable pending characterization of the test chemical as to its identity, purity, and stability, as required by Subdivision F Guidelines. Additionally, transport of the test chemical to the bone marrow was not established.

Classification: Unacceptable

MATERIALS:

Hexahydro-1,3,5, triethyl-s-triazine (Lot M-TH-8K-327), described as a clear colorless liquid, was the test chemical. The test chemical was not further characterized.

Triethylenemelamine (TEM) lot 80386, obtained from Polysciences, Inc., Warrenton, PA, dissolved in sterile water was the reference mutagen. Male and female ICR mice, 6-8 weeks old, obtained from Harlan Sprague-Dawley, Inc., Frederick, MD, were the test animals.

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METHODS:

a. Toxicity Study

A preliminary study was performed to determine the 7 day acute oral LD_{50}, as well as the bone marrow toxicity of the test compound. The animals were assigned as follows:

Test Group	Dose mg/kg gavage	Main Study 7 days M	F	Interim Sac 48 hours M	F
1. Cont	0	5	5	5	5
2. Low	88	5	5	5	5
3. Mid I	133	5	5	5	5
4. Mid II	200	5	5	5	5
5. High	300	5	5	5	5

The test and control compounds were administered to each mouse orally by gavage at the rate of 10 ml/kg of body weight. Five animals/ sex/group were observed daily for seven days for toxic signs and mortality. Body weights were recorded immediately prior to and on days 1 and 3 after dose administration. The LD_{50} was calculated by probit analysis.

Five animals/sex/group were sacrificed 48 hours post compound administration, and bone marrow collected from the femurs and analysed for a shift in polychromatic erythrocytes/total erythrocytes. A statistically significant decrease in the ratio of polychromatic to normochromatic erythrocytes was regarded as indicative of bone marrow cell toxicity.

In the main study, groups of 15 male and female mice were administered doses of 0, 25, 125 and 225 mg/kg of Vancide TH in sterile distilled water by gavage. An additional group of three males and three females was administered 225 mg/kg of the test compound and kept for replacement purposes in case some of the treated animals died before the scheduled sacrifice time. Five per sex were administered 0.25 mg/kg of triethylenemelamine, the positive control compound i.p. Five per sex of the negative control and test groups were sacrificed 24, 48, and 72 hours post dosing. Positive controls were sacrificed 24 hours post dosing.

b. Micronucleus Assay

Both femurs were dissected out, and a smear was made from each femur on a slide containing a drop of fetal bovine serum. The prepared smears were air dried, stained with May-Gruenwald-Giemsa stain and mounted on coverslips. Smears were coded and examined by light microscopy to determine the incidence of micronucleated cells per 1000 polychromatic cells per animal, the ratio of poly-

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chromatic cells to normochromatic cells for each animal, and the number of micronucleated normochromatic cells.

EVALUATION CRITERIA

The test was considered valid if the mean incidence of polychromatic micronucleated erythrocytes did not exceed 5/1000 in the negative (vehicle) control group, and the incidence of polychromatic micronucleated erythtocytes in the positive control was significantly increased relative to the negative control.

The compound was determined to be positive in the micronucleus test if a significant increase in the incidence of micronucleated polychromatic erythrocytes was observed compared to the controls. The positive response should be dose related or must be observed at a single dose level at adjacent sacrifice times.

STATISTICAL ANALYSIS

The statistical significance of the data was determined using Kastenbaum-Bowman tables, which are based on the binomial distribution. The level of significance was 5%.

QUALITY ASSURANCE

A signed and dated quality assurance statement was included in the submission.

RESULTS

In the preliminary study, six high dose animals (2M, 4F) died on study. Clinical signs were: ruffled fur, diarrhea, lethargy, distended abdomen, crusty eye, decreased body weight gain in the high dose animals. In high dose males, the body weight decrement was 12.8% on day 3. One 200 mg/kg female female had a swollen shoulder, lethargy, ruffled fur, and crusty eye. The LD_{50} was calculated to be 331 mg/kg.

Four females designated for the bone marrow toxicity study (2, 200 mg/kg and 2, 300 mg/kg) died prior to the scheduled sacrifice. There were no significant differences in the ratio of polychromatic erythrocytes to total erythrocytes in cells harvested from males and females 48 hours post compound administration. The high dose for the micronucleus test was set at 225 mg/kg.

In the micronucleus assay, four high dose animals died before the scheduled sacrifice time and were replaced by animals from the replacement group which had been treated with 225 mg/kg of the test chemical. No increase over the controls in the number of micro-

nucleated polychromatic erythrocytes or micronucleated normochromatic erythrocytes were observed in the treated males or females at any sampling time, and there were no compound related changes in the p/n ratio. The positive control substance, triethylenemelamine, caused a significant increase in the incidence of micronucleated polychromatic erythrocytes ($p < 0.05$). The results obtained are shown in the attached table.

DISCUSSION AND CONCLUSIONS

Oral administration of hexahydro-1,3,5,triethyl-s-triazine to male and female ICR mice at doses of 0, 25, 125, and 225 mg/kg did not cause an increase in micronucleated polychromatic erythrocytes. Administration of the positive control chemical resulted in a significant increase in the incidence of micronucleated polychromatic erythrocytes. The data presented support the investigators' conclusion that the chemical did not appear to be clastogenic under the conditions of this study.

Toxicology Branch notes that the $LD_{50}/7$ (dose required to kill 50% of the animals within 7 days after administration) was reported to be 331 mg/kg. Based on the data presented, Toxicology Branch believes that the LD_{50} is < 300 mg/kg. Since no cytotoxicity was evident at the clinially lethal dose, the company needs to demonstrate that the test compound was transported to the target tissue. In addition, the compound needs to be characterized as to its purity and stability in accordance with Subdivision F Guidelines in order to be considered Acceptable.

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Representative Results in the Mouse Micronucleus Study Using Hexahydro-1,3,5,triethyl-s-triazine

	Exposure Time (Hrs)	PCE/Total Erythrocytes	Micronucleated Polychromatic Erythrocytes/1000 PCE's Mean ±S.D.
		Males	
Distilled H_2O	24	0.57	2.6 ±2.30
	48	0.63	1.0 ±1.41
	72	0.54	1.2 ±0.84
TEM 0.25 mg/kg	24	0.53	32.2±9.01*
Vancide TH 225 mg/kg	24	0.56	1.4 ±1.14
	48	0.58	0.4 ±0.89
	72	0.59	1.0 ±0.71
		Females	
Distilled H_2O	24	0.61	1.6 ±2.07
	48	0.60	0.6 ±0.89
	72	0.62	2.0 ±1.22
TEM 0.25 mg/kg	24	0.55	32.4±1.52*
Vancide TH 225 mg/kg	24	0.56	0.4 ±0.89
	48	0.58	1.2 ±1.79
	72	0.59	0.6 ± 0.89

* Significantly different from control $p < 0.05$
Kastenbaum-Bowman Tables