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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: APR 2

SUBJECT: Registration Standard and Data Waiver; Chloroneb Fungicide, EPA Reg. No. 352-312, 344, and 386. CASWELL No. 198

FROM: Carlos A. Rodriquez CAR 4/1/82
Review Section #1
Toxicology Branch/HED (TS-769)

TO: Mr. Henry Jacoby, PM #21
Registration Division (TS-767)

THRU: Robert B. Jaeger, Section Head RBJ 4/5/82
Review Section #1
Toxicology Branch/HED (TS-769)

Action Request: Review data in response to Registration Standard requests.

Recommendations:

Based on HED memo (A. Barton, 3/19/82) to RD (J. Akerman), subject: "Chloroneb Data Requirements", TB supports the conclusions identified on the first page of that memo as regards EPA Registrations for chloroneb.

Submission:

Addendum to Two Year Feeding Study with Chloroneb (Fungicide 1823) (Hazleton Laboratories America, Inc., Project No. 201-204, 5/18/81).

This addendum presents the incidence by group and sex, of neoplastic and non-neoplastic lesions in rats sacrificed at 12 months and at termination.

From the terminal sacrifice tissues were examined from the 2500 ppm level and the 2 controls. Only thyroid, liver and unusual lesions were examined in the 100 and 500 ppm groups.

178

Incidence of Non-Neoplastic Lesions
Two-Year Feeding Study in Rats
Terminal Sacrifice

001659

Males (Examined/Occurred)

	Control A	Control B	100 ppm	500 ppm	2500 ppm
Parathyroid Hyperplasia	19/3	14/6	0	0	19/11

Females (Examined/Occurred)

	Control A	Control B	100 ppm	500 ppm	2500 ppm
Parathyroid Hyperplasia	18/5	20/8	0	0	15/5

Males (Examined/Occurred)

	Control A	Control B	100 ppm	500 ppm	2500 ppm
Thyroid Slight Activity	19/10	19/12	18/2	22/17	19/12

Females (Examined/Occurred)

	Control A	Control B	100 ppm	500 ppm	2500 ppm
Thyroid Slight Activity	20/15	22/18	20/11	17/12	20/16

At 12 months a slightly increased thyroid activity in the high dietary level test rats had been suggestive of a compound effect. No relation was evident at 24 months between compound ingestion and the degree of thyroid activity. The morphologic characteristics of the thyroid glands of the test rats were similar to those found in the control group.

2

5

After ingestion of the test diets for 24 months, no relation was evident between the degree of parathyroid hyperplasia in the test animals and those found in control rats. The highest dietary level (2500 ppm) showed a marked growth suppression and reduced food consumption in the female group and moderate growth suppression in the male group.

NOEL = 500 ppm

LEL = 2500 (marked growth suppression and reduced food consumption in the females, and moderate growth suppression in the male group).

Classification: Core-Minimum Study.

1. Mutagenic activity of chloroneb (91.3% Technical) (Benzene 1,4-dichloro-2, 5-dimethoxy) in the Samonella/microsome assay.

Haskell Laboratory for Toxicology and Industrial Medicine, Report No. 147-81, February 26, 1981.

4 histidine requiring strains of Salmonella typhimurium were used in this mutagenesis assay. Both non-activation and activation by S-9 mixture (derived from supernatant of homogenized rat liver) were tested. The procedure used is similar to the method described by Ames, et, al (Mutation Research 31: 347-364, 1975).

The S-9 homogenate, a 9,000 x g supernatant was prepared from 8 to 9 week old male Charles River CD rats given 500 mg of Aroclor 1254/kg five days before sacrifice.

The dose range employed for the evaluation of this compound was from 0 ug to 500 ug per plate.

Positive (known mutagens) and negative (solvent) controls were included in all assays.

Results:

a. Activation Assay:
The test material was mutagenic in strain TA 100 in the presence of activation system. Concentrations (10 and 25 ug per plate) gave significantly greater revertant frequencies ($p < 0.01$) than controls and a significant positive linear dose response was obtained.

The summary of the mutagenic activity is as follows:

Strain	Condition	Induction Ratio					Revertants/ Mole
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	
TA 100	With Activation	1.6	1.5	1.1	1.3	1.4	0.82

3

6

b. Without metabolic activation the test material at concentrations of 0, 1, 5, 25, 50, 100 or 500 ug per plate did not produce mutagenic activity as revealed by this test in the Salmonella strains TA-1535, TA-1537, TA-98 or TA-100.

Classification: Acceptable

In Vitro Cytogenetic Assay Measuring Chromosomes Aberrations Frequenecies In Chinese Hamster Ovary Cells (CHO) cells.

Litton Bionetics, Inc., Project No. 20990, May 19,1981.

The objective of this test was to measure the ability of Chloroneb (Benzene 1, 4- dichloro-2, 5-dimethoxy-) to induce chromosome aberrations at a number of concentrations in a series of in vitro cell assays employing chinese hamster ovary cells with and without metabolic activation.

Results:

The test compound chloroneb (12921) did not induce a meaningful increase in chromosome aberrations and is considered negative in this test under the conditions of the assays.

Classification: Acceptable

The Hepatocyte Primary Culture/DNA Repair Assay on Chloroneb (13921) Using Rat Hepatocytes in Culture.

Naylor Dana Institute, November 15, 1981, NDI Nos. 092381CT and 092981CT.

Procedure:

The methodology for this assay has been published by William, G.M. (1976 and 1977). The hepatocyte primary culture/DNA repair test employs freshly isolated liver cells to detect the DNA damaging potential of chemicals by measuring of DNA repair synsthesis.

Freshly prepared rat hepatocyte primary cell cultures from adult male F344 rats were used as the target mammalian cell.

Chloroneb was dissolved in dimethlsulfoxide (DMSO). This stock solution was dissolved in Williams medium to concentrations of 1.0, 0.1, 0.01, 0.001 and 0.0001 mg/ml, then assayed in the HPC/DNA Repair Assay. Paralell run dimethylsulfoxide (DMSO) and fluorene served as negative controls and 2-aminofluorene (2AF) served as positive controls.

Results:

Compound chloroneb (13921) when assayed twice in the HPC/DNA Repair Assay at the highest non-toxic dose (0.1 mg/ml) and lower doses (0.01, 0.001, and 0.0001 mg/ml) did not induce repair.

Negative controls (DMSO and Fluorene) gave negative results.

Positive control (2 AF) did induce repair.

Classification: Acceptable

Chines Hamster Ovary Cell Assay For Mutagenicity (Haskell Laboratory, Report No. 834-81, December 16, 1981)

Test Compound:

Chloroneb (1,4-dichloro-2, 5-dimethoxybenzene)

The chinese hamster ovary cells were used in this mutagenesis assay. Both non-activation and activation by S-9 mixture (derived from supernatant of homogenized rat liver) were tested. The assay is an adaptation of a method largely developed by A.W. Hsie at Oak Ridge National Laboratory.

Results:

The statistical analysis of the test data did not indicate mutagenic activity in the presence of an activation system.

In the absence of activation system eight trails were conducted (trails 1 and 2) the statistical analysis indicated mutagenic activity. These observations could not be reproduced in three subsequent set of trials.

Conclusion:

The test sample was not considered mutagenic in the absence of the activatior system.

Classification: Acceptable

Acute Oral Toxicity (LD_{50}) with Chloroneb (1, 4-Dichloro-2, 5-Dimethoxybenzene (65% W.P.) (Haskell Laboratory, Report No. 157-65, October 11, 1965).

After overnight fast, 10 rats/sex/dose level were administered by oral intubation dose levels of 7500 and 11,000 mg/kg of the test material as a 30% suspension in peanut oil (based on active ingredient). Survivors were sacrificed 14 days later; gross pathology was conducted on two survivors from each group. Animals that died were also necropsied. Mortalities and clinical signs were recorded.

Results

11,000 mg/kg

Male rats - salivation, chewing and pawing motions, chinrubbing, weight losses 1-4 days, poorly formed feces, deaths 2-3 days after dosing (2/10).

Female rats - chinrubbing and pawing motions, weight losses 1-4 days, poorly formed feces, stained perineal area, death 4 days after dosing (1/10).

7500 mg/kg

Male rats - slight hyperemia one day after dosing. No deaths.

Female rats - slight weight losses initially. No deaths.

Gross Pathology at 11,000 mg/kg: possibly blood in bladder of one male.

LD_{50} = > 11,000 mg/kg

Classification: Core-Minimum

Acute Skin Absorption Toxicity in Rabbits with Chloroneb (75% a.i.) (Haskell Laboratory, Report No. 153-64, Dec. 4, 1964)

Six male albino rabbits weighing between 2981-3213 gms had their hair removed from their backs. The test material was applied to their backs at a dose level of 5,000 mg/kg or a 70% aqueous paste. The entire area was covered with wet cheese cloth and impervious film. The animls were restrained in stocks for 24 hours after which the mateial was washed off. After 14-day observation period, they were sacrificed and tissues saved for microscopic examination. Body weight, food consumption, clinical signs of toxicity were recorded - Necropsy examination was performed.

6

9

001659

Results:

Four rabbits consumed less a mount of food and water the day after treatment. Mild initial weight loss was observed.

Necropsy revealed no gross pathological changes.

Microscopic examination of tissues has not been completed.

LD_{50} = > 5,000 mg/kg/day (male rabbits)

Classification: Core-Minimum

TB does not need an evaluation in females since the LD_{50} is greater than 5,000 mg/kg/day. An LD_{50} determination in females is not believed to be of toxicological significance in this particular study.

Ten - Dose Subacute Oral Study in Rats with Chloroneb (Haskell Laboratory, Report No. 33-63, February 20, 1964).

The test material identified (INK-1823-8: 100% active) was administered daily by intragastric intubation at a dose level of 3400 mg/kg/day (30% suspension in peanut oil) to each of three Charles River-CD male rats, five times a week for two weeks, after which the animals were killed. Similarly the test material identified (INK-1823-16; 75% W.P.) was administered at a dose level of 5000 mg/kg/day (25% suspension in peanut oil) to each of six Charles River-CD male rats. Animals were killed after treatment and after 14-day recovery period.

Results:

3400 mg/kg/day

First Week - no toxic signs
- no deaths

Second Week - salivation during dosing
- no deaths. No pathological changes.

5,000 mg/kg/day

First Week - weight loss, orange discoloration of perineal area, slight diarrhea, discomfort, small amount of dark urine.

Second Week - weight loss, orange discoloration of perineal area, slight diarrhea, discomfort, small amount of dark urine, weakness and semi-prostration, two animals died after 8th dose.

Recovery Period - orange discoloration of perineal area during first week, rate of weight gain more rapid than that of controls.

Animals dead or killed after 10 dose - congestion of organs, injury to blood cells and blood forming organs (spleen and bone marrow), minor changes in kidney, large liver weights and cells with irregularities in cytoplasm and nucleus.

Pathologic changes - large liver weights and cells with irregularities in cytoplasm and nucleus, pigment in Kupffer cells.

Classificaiton: Supplementary

Primary Skin Irritation and Senzitization Tests in Guinea Pigs with Chloroneb (INK-1823-16) (Haskell Laboratories, Report No. 23-64, Feb. 20, 1964)

The test material (50% and 10% active) was applied to the intact and abraded skin of 10 male albino guinea pigs as a suspension in 1% aqueous Duponol PT for 24 hours for irritation.

In the skin sensitization test the material (50% and 10% active), was applied as a suspension in 1% aqueous "Duponol") PT to the abraded skin of 10 male guinea pigs, three times a week for three weeks. Two weeks later the animals were challenge with the test material.

Results: Inconclusive

Irritation: Inconclusive

Classification: Supplementary (no individual scores; test method not adequately described; scoring method not described.

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78