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

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MAY 24 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cryolite (Kryocide®, EPA Registration No. 4581-116)- Submission of a 21-Day Dermal Study in Response to the Registration Standard for Products Containing Cryolite of April 1988

Tox Chem. No. 264
Project No: 9-2144
Record No: 251708

FROM: William B. Greear, M.P.H. William B. Greear 5/3/90
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Joanne Edwards, RM Team # 74
Registration Branch
Special Review and Reregistration Division (H7508C)

THRU: Marion P. Copley, D.V.M., Section Head
Review Section II, Toxicology Branch I
Health Effects Division (# 7509C)

Conclusions:

The study is classified as Core-Guideline and satisfies the requirement for Guideline Series 82-1. The NOEL is 25 mg/kg based on decreases in body weight gain at 250 and 1000 mg/kg.

Requested Action:

Under a cover letter dated August 31, 1989, Rebecca A. Clemmer of the Pennwalt Corporation has submitted a 21-day dermal study on cryolite in the fulfillment of the requirement as specified in the Registration Standard for Products Containing Cryolite of April 1988. This study is also being submitted on behalf of the Gowan Company.

Considerations:

It was stated "...the final report of this study was issued in the absence of internal review by a Pennwalt toxicologist." Pennwalt contends that the laboratory conducting the study, Battelle Columbus Division, has not accurately assessed the data for the 250 mg/kg/day group. (Battelle considers that the 250

1 of 10

mg/kg/day group to be an effect level based on decreased body weight gain). Pennwalt subsequently had the study reviewed by an independent toxicologist, Vincent J. Piccirillo of NPC, Inc.. Dr. Piccirillo believes that because the body weight gain data were presented as cumulative to Day 1 of the study, normal fluctuations in body weight data as typically seen in mature rabbits during repeated dermal studies were masked. Body weight gain data are indicated in Table 1 of the Data Evaluation Report. This reviewer believes that body weight gain was decreased in males and females in the mid-and high-dose groups, albeit the decrease was less in females than in males.

NOEL = 25 mg/kg
LEL = 250 mg/kg (based on decreases in body weight gain)

Reviewed By: William B. Greear, M.P.H. WBG
Review Section II, Toxicology Branch I
Secondary Reviewer: Marion P. Copley, D.V.M. Marion Copley 5/4/90
Review Section II, Toxicology Branch I

DATA EVALUATION REPORT

Study Type: Guideline Series 82-2
Repeated Dose Dermal
Toxicity: 21-Day

TOX Chem No: 264
MRID No: 412248-01

Test Material: Cryolite

Synonyms: Kryocide, sodium fluoroaluminate, [Cas No: 15096-52-3]

Study No: Battelle Study No: N4900-2001

Sponsor: Pennwalt Corporation
Philadelphia, PA 19102

Testing Facility: Battelle Columbus Division
Columbus, OH 43201-2693

Title of Repcrt: 21-Day Repeated Dose Dermal Toxicity Study of Cryolite in the Rabbit

Authors: G. E. Wilkinson, P.J. Tosca, A. W. Singer, and J. M. Killinger

Report Issued: August 25, 1989

Conclusions: NOEL = 25 mg/kg/day
LEL = 250 mg/kg/day (based on decreases in body weight gain).

In addition at 1000 mg/kg/day, deaths, clinical signs of toxicity (thin appearance, hypoactivity) decreases in body weight gain, anemia and changes in several clinical chemistry parameters occurred.

Classification: Core-Guideline (The study satisfies the requirements for Guideline Series 82-1)

A. Materials:

1. Test Component - Kryocide®; Description: odorless white crystaline powder; Code No: 2202, Lot No: 87.11; Purity: 96%; Contaminants: not provided.

2. Test Animals - Species: rabbit; strain: New Zealand white (SPF); Age: young adults; weight: 2-3 kg; Source: Hazleton Research Product, Denver, PA.

B. Study Design:

1. Animal Assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose Applied Dermally (mg/kg)	Main Study (21-Days) Male	Female
Control	0	5	5
Low	25	5	5
Mid	250	5	5
High	1000	5	5

Twenty male and 20 female rabbits were obtained on April 18, 1989 and were allowed to acclimate to laboratory conditions for eight days. The rabbits were individually housed in stainless steel cages in a room with temperature of 72 $\pm$ 5^0 F and relative humidity of 55 + 15%. A 12-hour on/12-hour off light cycle was employed. The air was exchanged approximately 10 times per hour. Purina Rabbit Chow and water, via an automatic water system, were available ad libitum.

2. Dose Formulation - Cryolite was formulated at three concentrations of 12.5, 125.0 and 5000.0 mg/ml in deionized water. [It was stated that a stability study at the lowest level of 12.5 mg/ml was conducted at Battelle prior to study initiation and it was considered that all three formulations were stable for a period of at least seven days.] Dosing solutions were prepared once weekly. The stability of cryolite formulated at 12.5 mg/ml was determined on Days 0, 1 and 7 after preparation by gravimetry and by fluoride determination using a fluoride ion selective electrode. Homogeneity of each of the doses of cryolite formulated for use in Week 1 was analyzed using a gravimetric procedure.

Results - The purity of the test material was determined to be approximately 96% cryolite based on the fluoride content of aliquots of the formulation that were weighed after drying for 16 hours at 160^{0}C. There was considerable variability in the concentration of cryolite in the samples of the 12.5 mg/ml formulation of cryolite. Dried sample weights ranged from 10.2 to 15.0 mg cryolite/ml. The concentration of cryolite in the aliquots ranged from 10.7 to 15.1 mg/ml using the fluoride ion-selective electrode. Gravimetric determinations were conducted at all three levels by taking samples at three sample

positions after vigorously mixing the samples for 10 to 15 minutes. The amounts found for the 12.5, 125 and 500 mg/ml levels ranged from 12.60 - 12.68, 124.8 - 126.0 and 503.2 - 504.3 mg/ml, respectively.

3. Test Material Application - Prior to application of the test material approximately 10% of the body surface from the inter-scapular region extending down the back to the rump was clipped free of hair. The initial clipping was conducted 1-3 days prior to application of the test material. Subsequent clippings were made as needed, but usually on a weekly basis. The test material was spread over the clipped area and held in contact with the skin for a period of six hours under porous gauze dressing and non-irritating tape. The dressing was covered with an elastic Stockinette®. After removing the dressing, the treatment site was cleaned with a paper towel dampened with deionized water. Applications were made five times per week for a three-week period (15 applications).

4. Statistics - Normally distributed data, e.g. body weights, organ weights and/clinical pathology data, were analyzed by analysis of variance and pairwise comparisons using Dunnett's test. Bartlett's test was used to determine if a non-homogeneous variance existed. Then a nonparametric test (Wilcoxin's T statistic) was used.

5. Quality Assurance examinations were made on several occasions from 3/24/89 to 8/15/89. The QAU statement was signed by Ramona A Mayer on 8/24/89.

6. Good Laboratory Practice - A statement that the study was conducted in compliance with 40 CFR Part 160 was signed by Gary E. Wilkinson on August 25, 1989.

C. Methods and Results

1. Observations - All animals were observed twice daily for clinical signs of toxicity and mortality.

Results - Three of five males and one of five females in the high-dose group died or were terminated in a moribund condition prior to the scheduled termination. Two males in the high-dose group had a thin appearance on days 14, 15 and/or 16. Four males and one female in the high-dose group exhibited no feces on one or more occasions from day 8 to day 15. Three males in the high-dose group were hypoactive on one or more occasions from day 9 to 17.

2. Body Weight - Individual animal body weights were determined on days -2, 1, 5, 12, 19 and 22. Males and females in the high-dose groups exhibited reduced body weights on days 5, 12, 19 and 22 when compared to the controls. Body weight gain

was reduced in males and females in the mid-and high-dose groups on days 5, 12, 19 and 22 when compared to controls. Males appeared to be more sensitive than females with respect to body weight gain. (See Table I.)

3. Blood was collected from the medial ear artery on day 22 prior to necropsy from all animals. Additional samples were obtained from 2 males in the high-dose group on day 14 and from another 2 males in the high-dose group on day 17. The CHECKED (X) parameters were determined.

a. Hematology

X		X	
X	Hematocrit; (HCT)	X	Total plasma protein (TP)
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)
X	Erythrocyte Morphology		

Results - Males in the high-dose group had increases in the platelet and reticulocyte counts, decreases in the eythrocyte counts, hemoglobin, hemotocrit and MCV. There were slight decreases in the leukocyte counts and a slight increase in the segmented neutrophil count in males in the high-dose group. Females in the high-dose group exhibited slight increases in the platelet count and segmented neutrophil count and a slight decrease in the lympohocyte counts, erythroycyte count, hemoglobin and hematocrit. Examination of erythrocyte morphology revealed anisocytosis and microcytic, hypochromic polychromasia in one high-dose male (M35) and polychromasia in one high-dose female (F27). (See Table II.)

b. Clinical Chemistry

X		X	
	Electrolytes		Other
X	Calcuim	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose

	Enzymes		
	Alkaline phosphatase	X	Total bilirubin
	Cholinesterase	X	Total protein
	Creatinine phosphokinase		Triglycerides
X	Sorbitol dehydrogenase		Thyroxine (T4)
	Lactic acid dehydrogenase		Triiodothyronine (T3)
X	Serum alanine aminotransferase (also SGPT)		
X	Gamma - glutamyl transpeptidase		
	Serum aspartate aminotransferase (also SGOT)		

Results - Males in the mid-and high-dose groups had glucose levels that were slightly elevated. Levels of 118, 115, 131 and 131 mg/dl glucose were observed in the male control, low, mid, and high-dose groups, respectively. Glucose was significantly elevated in females in the high-dose group. Levels 118, 115, 122, and 140 mg/dl glucose were observed in the female control, low, mid-and high-dose groups, respectively. One male (M35) in high-dose group exhibited slight decreases in the albumin and total protein and increases in aspartate aminotransferase, alanine aminotransferase and sorbitol dehydrogenase. Serum calcium and inorganic phosphorous were slightly decreased in this male.

4. Sacrifice and Necropsy - A complete necropsy was performed on all animals that died prior to terminal sacrifice, sacrificed in a moribund condition or on those at termination. The following organs were weighed from only those animals sacrificed at termination: liver, kidney and gonads. The following tissues were collected at necropsy and processed for histological examination: gross lesions, skin application site, skin (ventral surface) non-application site, liver and kidneys.

Results

a. Gross Necropsy - No gross lesions were found that could be related to treatment.

b. Organ Weights - Absolute and relative organ weights were comparable among the control and treated groups.

c. Microscopic Pathology - Minimal renal tubular epithelial degeneration (necrosis) was present in four male and four female animals in the high-dose group. This lesion was not observed in any of the rabbits in the control, low-or mid-dose groups.

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D. Discussion

Three males and one female in the high-dose groups died or were sacrificed in a moribund condition. Several males in the high-dose group had a thin appearance and were hypoactive. Death as well as the observed clinical signs of toxicity were attributed to treatment. Males and females in the high-dose group exhibited reduced body weights on days 5, 12, 19 and 22 when compared to controls. Body weight gain of males (oin particular) and females in the mid-and high-dose groups were reduced on day 5, 12, 19 and 22 when compared to controls. One male in the high-dose group (M35)* exhibited increases in the platelet and reticulocyte count and decreases in the erythrocyte count, hemoglobin, hematocrit and MCV. There were also slight decreases in the leukocyte and lymphocyte counts and a slight increase in the segmented neutrophile count. The male also exhibited anisocytosis and microcytic, hypochromic polychromasia. Females in the high-dose group exhibited slight reductions in hematocrit (hemoglobin, erythrocyte count and lymphocyte count. There was also slight increases in the platelet count and segmented neutrophile count. One female in the high-dose group also exhibited polychromasia. Glucose was significantly elevated in females in the high-dose group and was slightly elevated in males in the mid-and high-dose group. One male (M35) in the high-dose group exhibited decreases in albumin and total protein and increase in alanine aminotransferase, aspartate aminotransferase and sorbitol dehydrogenase. Serum calcium and inorganic phosphorus were also slightly decreased in this male.

NOEL = 25 mg/kg
LEL = 250 mg/kg (based on decreases in body weight gain).

[Pennwalt has argued that the NOEL is 250 mg/kg rather than 25 mg/kg. The NOEL of 25 mg/kg was designated by the Battelle reviewer based on decreased body weight gain. A second independent toxicologist, Dr. Piccuillo, contends that the NOEL is 25 mg/kg because day to day body weight gain is quite variable in the rabbit and the Battelle reviewer based his decision on cumulative body weight gain. This reviewer believes that the data would support a NOEL of 25 mg/kg <u>not</u> 250 mg/kg.]

TABLE I - Cumulative Mean Body Weight Gain (8)[1]

Group	Day of Study 5	12	19	22
		Male		
Control	32.4	139.6	203.4	169.2
Low	54.8	166.8	293.0	262.4
Mid	-163.8*	101.4	183.0	84.0
High	-378.2*	-678.8*	-385.0*	-408.5*
		Female		
Control	77.6	209.6	327.6	269.0
Low	91.4	162.6	304.8	216.4
Mid	48.8	130.6	249.8	162.4
High	-217.4*	-110.5*	-45.8*	-156.0*

*- significantly different from controls at p=0.05
1 Values obtained from Table A-6 p.41

TABLE II - Selected Hematology Values

Group	RBC[1]	HCT[2]	HGB[3]	MCV[4]	Platelets[5]	Retics[6]
			Males			
Control	6.95	44.6	14.4	64.2	419.0	1.84
Low	6.70	43.4	14.2	64.8	376.6	2.12
Mid	6.36	41.9	13.5	66.2	482.8	2.48
High	5.37**	28.2**	9.4*	53.0**	894.0**	4.70*
			Females			
Control	6.92	44.7		64.6	475.8	2.12
Low	6.60	42.4		64.3	410.8	1.65
Mid	6.10	42.1		69.3	423.7	3.67
High	6.14*	40.3		65.8	624.0	2.55

*-significantly different from controls at p=0.05
**-significantly different from controls at p=0.01
1-erythrocyte count X 10^6/microlites
2-percent
3-g/deciliter
4-volume in femtoliters
5-platelets X 10^3microlites
6-percent
TOXCHM2.64 (Disk 1)