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TXR-5771

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

005771

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MAR 11 1987

MEMORANDUM

SUBJECT: PP#6F3399. KRYOCIDE® INSECTICIDE. EPA Registration No. 4581-116. Active Ingredient - Sodium Fluoaluminate 96% [Fluorine not less than 50%] - Request for an Exemption From Tolerances For All Crops Listed in 40 CFR 180.145 Including the New Use on Potatoes

Tox. Chem. No. 264
Accession Nos. 262370
262371
262372

FROM: William S. Woodrow, Ph.D. WSW 3-6-87
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: William H. Miller, PM 16
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C) ABK 3/6/87

Recommendation:

It is recommended that the exemption from tolerances be denied. This recommendation is based in part on each of the following:

- Review of the 90-day rat and dog studies reveal that there is no NOEL for fluoride accumulation in bone, and that the significance or the lack of significance of this observation is not addressed;

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- Gross and microscopic effects were observed in/on the stomach of rats at 5000 ppm LEL thereby establishing the test dose of 50 ppm as the NOEL. The severity of the effects at 5000 ppm were apparently not graded nor reported; and

- Based upon a NOEL of 50 ppm in the rat a total TMRC of 3.0557 mg/day and a safety factor of either 100 or 1000, the percent of the ADI or PADI utilized would either be 203.0% or 2037.0% (see attached printout for TMRC).

Based upon our review and interpretation of the available data:

- The percent of the ADI or PADI is substantially exceeded;

- The submission argues for tolerances rather than an exemption from tolerances;

- The current tolerance level of 7.0 ppm for all commodities needs to be reevaluated; and

- The data base does not support additional new uses (i.e., potatoes).

Additionally, regulatory requirements for tolerances or an exemption from tolerances, or a food additive petition are identical (see 40 CFR 158.135).

The Toxicology Branch (TB) believes that the registrant has not made a case for exempting KRYOCIDE® from tolerances. The exemption from the requirement of tolerances is not warranted and the recommendation by TB is that the exemption be denied.

TB also notes that a 28-day range-finding study was submitted but not reviewed. The dose levels for the 28-day dog study and the 90-day dog study were so nearly identical that TB decided to review only the 90-day study. However, the 28-day dog feeding study is cataloged in the branch records.

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Reviewed by: William S. Woodrow, Ph.D.
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: Albin B. Kocialski, Ph.D.
Section VII, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Rat, Subchronic 90-Day TOX. CHEM. NO.: 264

Accession Number: 262372

Test Material: Kryocide® Insecticide (Kryocide)

Synonyms: Cryolite, Sodium Fluoaluminate

Study Number(s): 6120-100

Sponsor: Pennwalt Corporation

Testing Facility: Hazleton Laboratories America, Inc.

Title of Report: Subchronic Toxicity Study with Kryocide in Rats

Author(s): Robert H. Weltman, Ph.D.

Report Issued: November 27, 1985

Conclusions: NOEL = 50 ppm for effects other than fluoride accumulation in bone. LEL = 5000 ppm for stomach findings at the macro- and micropathological levels. There was no NOEL for fluoride accumulation in bone. The LEL for fluoride accumulation in bone was 50 ppm (LDT).

Classification: Core-Supplementary. Based upon the accumulation of fluoride in bone.

Special Review Criteria: None

A. Materials:

1. Test compound: Kryocide, Description: white, crystalline powder, Batch No. (not given), Purity 96%.

2. Test animals: Species: rat (albino), Strain: Crl:CD $(SD)^{Br}$, Age: 4 weeks, Weight: 116.8-132.4, Source: Charles River Labs.

B. Study Design: Ninety-one-day treatment, twenty-eight-day recovery period, for ten rats/sex from high-dose test group.

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

Test Group	Dose in Diet (ppm)	Male	Female
1. Control	0	40	40
2. Low (LDT)	50	40	40
3. Mid (MDT)	5000	40	40
4. High (HDT) including 128-day recovery: 10/sex (basal diet after 100 days treatment)	50,000	50	50
5. Sentinel*	0	30	30

*Sentinel animals fed control diet for 39 days and discarded.

The hematology, clinical chemistry, and urinalysis were performed prior to study initiation, at 45 and 90 days, and at 128 days on a satellite group of 10 animals/sex/group.

2. Diet preparation - Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at weekly intervals. Stability and homogeneity were also determined.

Results - (diet analyses)

Date	Calculated as Cryolite (Uncorrected) 50 ppm	5000 ppm	50,000 ppm
12/7/84 Week 1	43.8	4045	42,462
14-Day Stability	45.0	4345	43,819
12/12/84 Week 2	43.8	4120	45,336
12/20/84 3	48.0	4164	44,422
12/27/84 4	52.4	4285	44,896
1/2/85 5	48.1	4296	44,240
1/9/85 6	43.0	4635	45,970

(cont'd)

Date		Calculated as Cryolite (Uncorrected) 50 ppm	5000 ppm	50,000 ppm
1/16/85	7	41.2	4725	45,361
1/23/85	8	43.3	4503	38,844
1/30/85	9	46.6	4489	40,117
2/6/85	10	43.3	4504	41,484
2/13/85	11	43.0	4418	46,055
2/20/85	12	52.0	4709	41,276
2/28/85	13	53.8	4548	40,173

The test diet analysis for cryolite recovery ranged from 70 to 85 percent (uncorrected values), which is satisfactory.

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - The following procedure was utilized in analyzing the numerical data: $p < 0.05$ for an analysis of variance. Dunnett's t-test was used to compare means if group means differed significantly by the analysis of variance.

5. Quality assurance was adequate.

C. Methods and Results:

1. Observations - Animals were inspected two times daily for signs of toxicity and mortality.

Individual values for hematology, clinical chemistry, and urinalysis data for baseline, 45-day, 90-day, and 128-day recovery values were measured.

Fluoride (bone) content was measured at pretest and days 7, 15, 45, and 90, and 128. Fecal collection for fluoride analysis was conducted at pretest, 1 week prior to necropsy and day 128.

Gross and histopathological examinations were made on 91-day animals and on recovery animals. Those animals dying intercurrently were examined grossly.

Results - Toxicity

Mortality (survival)

No test animals showed signs of toxicity or illness; the only observed changes occurred in rat teeth, which all appeared pale in color.

Three males died on test in the 50,000 ppm group. The only alterations from scheduled terminal sacrifices were one male and one female, which were sacrificed in a moribund condition from the 50,000 ppm dose level.

2. Body weight - Animals were weighed weekly throughout the study.

   Results - Males showed statistically reduced body weights at the 5000 ppm level from week 1 through week 6, and from week 1 through week 13 at the 50,000 ppm dose level. Female rats showed statistically reduced body weights at week 2 for the 5000 ppm dose level, and from week 1 through week 6 and week 8 through week 10 at the 50,000 ppm dose level.

3. Food consumption and compound intake - Consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data.

   Results - Food consumption -
   Compound intake -

   A statistically significant reduction in food consumption occurred for male rats at week 1 through week 4 at the 50,000 ppm dose level while females showed statistically increased food consumption at weeks 11 and 13 for the same dose level.

   From the tester's report:

	Compound Consumption (13 weeks) mg/kg/day ppm Kryocide		
	50	5000	50,000
Males, mean	3.8	399.2	4172.3
Females, mean	4.5	455.9	4758.1

4. Ophthalmological examinations were performed apparently once, for "dullness and opaqueness," on all animals.

   Results - Apparently the only ocular examinations were conducted pretest; eyes were checked for ocular discharge and for dull/opaque eyes.

5. Blood was collected before treatment and at 45 and 90 days (128 days HDT only) for hematology and clinical analysis from 10 animals per sex per group. The CHECKED (X) parameters were examined.

a) Hematology

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

Results - Hemoglobin values for male rats were statistically low at the 5000 and 50,000 ppm doses, both at 45 and 90 days. Hematocrit values for males were statistically low at 45 days for the 5000 ppm and 50,000 ppm groups, and were low at 90 days. Platelet values for male rats at the 50,000 ppm dose level were high at 45 and 90 days.

Female rats showed statistically lower hemoglobin values at the 50,000 ppm dose level at 45 and 90 days, and lower hematocrit values at the 5000 and 50,000 ppm dose levels at 90 days only. Female platelet values were statistically elevated at the 90-day sampling at the 50,000 ppm dose level only.

b) Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase	X	Total protein
	Cholinesterase		Triglycerides
	Creatinine phosphokinase	X	Fluoride
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		

Results - Male rats showed mean statistically lower values for total protein and albumin at the 5000 and 50,000 ppm dose levels, and a lowered total bilirubin

value at the 50,000 ppm dose level when sampled at 45 days. Male rats also showed statistically lower total protein and total bilirubin values for the 50,000 ppm dose at 90 days. At 45 days, only creatinine and phosphorous values were statistically significantly increased at 5000 and 50,000 ppm while sodium was decreased.

Female rats showed statistically reduced total protein values at the 5000 and 50,000 ppm dose levels at both 45 and 90 days. At 90 days only, females showed a statistically elevated AST/SGOT value at the 5000 ppm dose level, and elevated ALT/SGPT values at all three dose levels - 50, 5000, and 50,000 ppm.

6. Urinalysis - Urine was collected from fasted animals at 45, 90, and 128 days. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
-	Volume 45 days only	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen
		X	Fluoride

Results - Urinalysis values for male rats were comparable to those of control animals. Female rats showed statistically elevated specific gravity urine values at the 5000 and 50,000 ppm dose levels at 45 days only, and lowered urine volume values during the same sampling period for the 5000 and 50,000 ppm dose levels.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
	Tongue	X	Aorta	XX	Brain (3 levels)
X	Salivary glands	XX	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic n.)

X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	X	Adrenals
X	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	X	Parathyroids
XX	Liver	XX	Epididymides	X	Thyroids
	Gallbladder	X	Prostate		Other
X	Pancreas		Seminal vesicle	X	Bone
	Respiratory	X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Corpus	X	All gross lesions and masses
		X	Cervix		

Results

a) Organ weight

The male rat Group IV mean terminal body weight was statistically significantly lower at the high-dose level (50,000 ppm) as were the absolute mean liver and kidney (R & L) organ weights for Groups III and IV (5000 and 50,000 ppm).

Organ-to-terminal-mean-body-weight ratios were statistically significantly less for Groups III and IV male livers. Group IV male and Group IV female hearts, and Group IV female ovaries, were statistically significantly increased.

Decreased terminal body weights, absolute organ weights, and organ weight to body and brain weight ratio may well have been attributed to the nutritional status of the animals as a result of the concentration of the compound used, as all values returned to the normal range during the recovery period.

b) Gross pathology

Macroscopic observations included:

Kidneys - Three male and two female high-dose rats showed rough kidney surface; these effects were considered to be compound related. Seven, three, and three male rats showed large pelvises at the low, medium, and high doses, respectively, while two, one, and three large pelvises were observed at the zero, low-, and high-dose levels for female rats. This effect was not dose related, and since two untreated females showed large pelvises, it is unlikely that this finding was compound related.

Stomach - Macroscopic stomach findings were listed as follows:

Sex / Group		Male				Female			
		1	2	3	4	1	2	3	4
Stomach (St)	Number examined:	30	30	30	26	30	30	30	29
	Not remarkable:	29	30	9	1	30	30	12	1
Thickened Wall-Nonglandular		0	0	4	15	0	0	5	19
Contents-Dark		0	0	2	5	0	0	0	0
Raised Area(s)/Focus(I)		0	0	10	8	0	0	7	8
Thickened Wall-Glandular		0	0	9	6	0	0	9	4
Light Focus(I)/Area(s)-Nonglandular		0	0	3	0	0	0	0	0
Dark Focus(I)/Area(s)-Glandular		1	0	5	4	0	0	0	0
Red Focus(I)/Area(s)-Glandular		0	0	4	0	0	0	1	0

Dose-related, compound-related stomach macroscopic effects are apparent in table presented above, for mid- and high-dose male and female treated rats.

Liver - A low, scattered incidence of 1 or 2/30, 2/30, 1/26, or 1/29 for liver macroscopic observations including light coloration, large size, depressed or red focal areas, accentuated lobular pattern or light focal areas in treated or untreated animals were not compound related.

One mid-dose male rat showed a large liver (1 of 30 rats). Three control rats showed depressed liver focal areas, red focus, or an accentuated lobular pattern, respectively. Two low-dose treated male rat livers showed accentuated lobular patterns, while one female rat at the same dose level showed the same liver manifestation. One mid-dose and two high-dose females showed diffusely light livers.

Duodenum - Thickened duodenum walls in one male mid-dose and one male high-dose rat were observed.

Testes - Small testes were observed for one rat at each of the low, mid, and high dose levels, and small epididymes were noted for one rat at the low-dose level.

Uteri - Fluid-filled uteral lumens were noted in several untreated and treated rats.

Lymph nodes - Treated and untreated male and female rats showed scattered incidence of large mandibular lymph nodes; one female rat showed diffuse red mandibular/cervical lymph nodes at the mid-dose level.

One high-dose male and one low-dose female rat showed peritoneal masses.

c) Microscopic pathology

1) Non-neoplastic

Lungs - 2/30 males and 1/30 female mid-dose rats showed pneumonitis, and one low-dose female showed lung granulomas.

Kidneys - A scattered incidence of kidney microscopic findings in treated and untreated female rat kidneys was observed. These findings included cortical fibrosis, regenerative tubules, pelvic dilation, tubular dilation, mineralization, chronic progressive nephropathy, focal mononuclear filtration, microabscesses, and chronic pyelitis. These findings were not dose or compound related.

Heart - A low, scattered incidence of microscopic heart degenerative cardiomyopathy was observed in treated and untreated male and female rats.

Liver - A high incidence of chronic liver inflammation was observed in treated and untreated male and female rats at all dose levels; one male rat showed microscopic liver congestion at the mid-dose level, one male rat showed acidophil cell focus at the high-dose level, and two female rat livers showed fatty changes at the mid-dose level.

Thyroid - Approximately similar incidences of thyroid cysts occurred in both male and female rats at the high-dose level.

Adrenals - Two high-dose males and one control male rat showed adrenal medulla missing, and one high-dose female showed adrenal cortical vacuolization.

Pancreas - Untreated male rats showed a low incidence of chronic pancreas inflammation, pigmentation, or islet cell hyperplasia. Two high-dose female rats showed chronic inflammation or ocinar atrophy of the pancreas.

Stomach - Summary mean stomach microscopic findings are presented in the table below:

Groups		Males				Females			
		I	II	III	IV	I	II	III	IV
Stomach (ST)	Number examined:	21	0	21	26	20	0	18	28
	Not remarkable:	21	0	3	0	19	0	0	0
--Lymphoid Focus(I), Submucosal		0	0	7	11	0	0	16	16
--Epidermal Hyperplasia		0	0	10	11	0	0	15	15
--Hyperkeratosis/Acanthosis		0	0	10	11	0	0	15	15
--Erosion/Ulceration		0	0	1	0	0	0	0	0
--Atrophy, Mucosa		0	0	2	13	0	0	2	10
--Inflammation, Chronic Submucosa		0	0	15	20	0	0	17	27

Mid- and high-dose male and female rat stomachs showed a relatively high incidence of various microscopic findings, according to the data shown above. The severity of the lesions were not graded or reported.

Spinal Cord - One male and one female rat at the high-dose level showed basophilic granules on spinal cord vertebrae.

Testes - One male control rat showed unilateral digospermia of the testes, and one each low-dose, mid-dose, and high-dose rat showed bilateral degenerative atrophy of the testes. One incidence of lack of epididymal contents was apparent in a low-dose rat.

Mandibular/cervical lymph nodes - A relatively low incidence of microscopic lymphoreticular hyperplasia of the mandibular/cervical lymph nodes occurred in untreated and treated male and female rats.

Incisors - Male and female rats displayed a relatively low incidence of a variety of findings upon microscopic examination of incisors at the high-dose level which included: ameloblast basic granules, dentin basophilic granules, enamel basophilic granules, mandibular basophilic granules, and predention basophilic granules.

Peritoneal cavity - One male and one female rat displayed adipose tissue inflammation with fibrosis at the high- and low-dose levels, respectively.

Microscopic; recovery sacrifice animals

Lung - One high-dose male lung showed pneumonitis.

Kidneys - A low incidence of several microscopic kidney findings in male rats at the high-dose level included: cortical fibrosis scars, regenerative tubules, tubular dilation, and focal mononuclear infiltration.

Heart - Degenerative cardiomyopathy was evident in two male hearts and one female heart at the high-dose level.

Liver - Five male live and one female liver at the high-dose level displayed chronic inflammation.

Thyroid - Three high-dose female thyroid glands displayed ultimobrachial cysts.

Adrenals - High-dose female rats displayed one medulla missing (two rats) and one high-dose female displayed acinar atrophy.

Stomach - One high-dose female showed stomach ulceration and erosion, and one high-dose male showed chronic inflammation of the stomach submucosa. Note that 10 female and 10 male stomachs were examined; it seems apparent upon comparison of the high- and mid-dose male and female incidence of stomach findings at terminal sacrifice that upon cessation of treatment, most of the treatment-related findings disappeared.

Uterus - One high-dose female displayed a dilated uterus.

Mandibular/cervical lymph nodes - One high-dose male rat displayed lymphorecticular hyperplasia of the mandibular/cervical lymph nodes.

2) Neoplastic

No neoplastic findings were found in either male or female rats.

D. Fluoride Analyses (Quoted from the tester's report)

Blood - Male and Female Blood Separately Pooled

Study Day		Fluoride All Dose Levels (ppm)
-3	males and females	< 1.0
45	males and females	< 1.0
90	males	< 3.0
	females	< 4.0
128	(recovery) males and females	< 1.0

Urine - Male and Female Urine Separately Pooled

Males

		Fluoride (ppm)				
Study Day		0	50	5000	50,000	Recovery
45	Mean	-	2.6	59.9	54.2	
	SD	-	-	45.6	28.95	
	N	-	1	10	10	
90	Mean	3.6	5.0	50.7	49.9	
	SD	1.79	1.94	42.55	23.57	
	N	5	8	10	10	
128	Mean					10
	SD					4.75
	N					10

Females

45	Mean	-	2.6	33.8	69.7	
	SD	-	-	13.75	34.83	
	N	-	1	10	10	
90	Mean	3.1	3.7	33.6	60.3	
	SD	0.81	1.1	27.8	35.42	
	N	4	5	10	10	
128	Mean					4.9
	SD					3.29
	N					8

Femur - Summary of Male and Female Mean Fluoride lues

Day of Study		Fluoride (ppm) 0	50	5000	50,000	Recovery
Males						
-3	Mean	19.0	-	-	-	-
	SD	1.84	-	-	-	-
	N	2	-	-	-	-
7	Mean	27.2	67.0	787.5	1040.0	-
	SD	6.72	17.39	328.80	28.28	-
	N	2	2	2	2	-
15	Mean	37.6	109.0	1125.0	1410.0	-
	SD	0.49	13.44	148.49	98.99	-
	N	2	2	2	2	-
45	Mean	42.9	177.0	2646.7	2826.6	-
	SD	4.15	11.36	283.61	210.08	
	N	3	3	3	3	
90	Mean	75.9	251.0	2943.3	3236.7	-
	SD	2.70	23.52	739.08	185.02	
	N	3	3	3	3	
128	Mean	-	-	-	-	2760.0
	SD	-	-	-	-	169.71
	N	-	-	-	-	2
Females						
-3	Mean	21.6	-	-	-	-
	SD	0.21	-	-	-	-
	N	2	-	-	-	-
7	Mean	29.9	85.8	990.0	1160.0	-
	SD	4.45	5.73	127.28	98.99	-
	N	2	2	2	2	-
15	Mean	56.3	123.0	2030.0	1835.0	-
	SD	1.98	21.21	961.67	49.50	-
	N	2	2	2	2	-
45	Mean	56.1	235.7	3050.0	3156.7	-
	SD	3.74	13.65	460.33	453.25	-
	N	3	3	3	3	-

Femur - Summary of Male and Female Mean Fluoride Values (cont'd)

Day of Study		Fluoride (ppm) 0	50	5000	50,000	Recovery
Females						
90	Mean	87.1	299.3	3533.3	3413.3	-
	SD	5.99	26.27	456.54	729.47	-
	N	3	3	3	3	-
128	Mean	-	-	-	-	3805.0
	SD	-	-	-	-	190.9
	N	-	-	-	-	2

Feces - Summary of Male and Female Mean Fluoride Values

Day of Study		Fluoride (ppm) 0	50	5000	50,000	Recovery
Males						
-3	Mean	16.2	-	-	-	-
	SD	0.35	-	-	-	-
	N	2	-	-	-	-
7	Mean	192.5	75.3	15,520.0	64,700.0	-
	SD	108.19	19.80	13,123.90	7212.49	-
	N	2	2	2	2	-
15	Mean	38.7	288.0	6170.0	57,450.0	-
	SD	9.90	193.75	1555.63	3181.98	-
	N	2	2	2	2	-
45	Mean	15.4	72.3	5900.0	63,533.3	-
	SD	5.27	7.16	1048.43	5352.98	-
	N	3	3	3	3	-
90	Mean	21.7	65.2	6410.0	50,966.7	-
	SD	0.75	14.15	528.49	4427.57	-
	N	3	3	3	3	-
128	Mean	-	-	-	-	69.5
	SD	-	-	-	-	34.65
	N	-	-	-	-	2

Feces - Summary of Male and Female Mean Fluoride Values (cont'd)

Day of Study		Fluoride (ppm) 0	50	5000	50,000	Recovery
Females						
-3	Mean	17.9	-	-	-	-
	SD	-	-	-	-	-
	N	1	-	-	-	-
7	Mean	32.3	99.3	7285.0	51,800.0	-
	SD	12.62	12.3	1067.73	10,465.18	-
	N	2	2	2	2	-
15	Mean	152.5	116	9765.0	61.350.0	-
	SD	170.41	1.41	3019.35	8697.41	-
	N	2	2	2	2	-
45	Mean	21.5	133.2	6296.7	59,400.0	-
	SD	0.42	83.01	2692.07	11,384.64	-
	N	3	3	3	3	-
90	Mean	21.5	68.8	6886.7	48,433.3	-
	SD	6.25	55.59	1157.86	8561.74	-
	N	3	3	3	3	-
128	Mean	-	-	-	-	459.7
	SD	-	-	-	-	535.00
	N	-	-	-	-	2

Feces - Summary of Male and Female Mean Kryocide Values (Parent Compound Chemical)

Day of Study		Kryocide (ppm) 0	50	5000	50,000	Recovery
Males						
-3	Mean	29.8	-	-	-	-
	SD	0.64	-	-	-	-
	N	2	-	-	-	-
7	Mean	354.6	138.7	28,588.0	119,177.5	-
	SD	199.26	36.49	24,174.57	13,285.83	-
	N	2	2	2	2	-

Feces - Summary of Male and Female Mean Kryocide Values (Parent Compound Chemical) (cont'd)

Day of Study		Kryocide (ppm) 0	50	5000	50,000	Recovery
15	Mean	71.3	530.4	11,365.0	105,822.5	-
	SD	18.24	356.88	2865.20	5861.21	-
	N	2	2	2	2	-
45	Mean	28.3	133.2	10,868.0	117,028.7	-
	SD	9.68	13.16	1931.41	9859.90	-
	N	3	3	3	3	-
90	Mean	34.0	120.0	11,807.0	93,880.7	-
	SD	1.36	26.12	973.47	8155.61	-
	N	3	3	3	3	-
128	Mean	-	-	-	-	128.0
	SD	-	-	-	-	63.78
	N	-	-	-	-	2
Females						
-3	Mean	33.0	-	-	-	-
	SD	-	-	-	-	-
	N	1	-	-	-	-
7	Mean	72.6	182.9	13,419.0	95,415.5	-
	SD	4.88	22.63	1967.17	19,276.44	-
	N	2	2	2	2	-
15	Mean	280.9	213.6	17,987.0	113,006.5	-
	SD	313.96	2.62	5562.10	16,020.92	-
	N	2	2	2	2	-
45	Mean	39.6	245.3	11,598.3	109,415.0	-
	SD	0.78	152.92	4958.99	20,970.34	-
	N	3	3	3	3	-
90	Mean	39.6	126.8	12,685.0	89,214.3	-
	SD	11.57	102.40	2132.65	15,771.17	-
	N	3	3	3	3	-
128	Mean	-	-	-	-	846.8
	SD	-	-	-	-	985.42
	N	-	-	-	-	2

End of quotation

E. Discussion:

Three males died at the HDT and one male and one female were each sacrificed at the HDT.

1. Fluoride Analysis - Negligible blood concentrations of fluoride were detected throughout the experimental period. The majority of the test compound was excreted in the feces with a comparatively negligible amount found in the urine. Dose-related concentrations of fluoride were detected in male and female femurs at all dose levels at all time periods. Concentrations were cumulative with time at all dose levels. The significance of this kind of fluoride accumulation in bone is not known. There is no NOEL for fluoride concentration in rat femurs.

2. Hematology - Statistically significant lower male rat hemoglobin and hematocrit values were observed at the 5000 and 50,000 ppm levels at 45 and 90 days. Female rats showed statistically significant lower hemoglobin values for the 50,000 ppm dose levels at 45 and 90 days. Female rats at the 5000 ppm and 50,000 ppm dose levels also showed significant lower hematocrit values at 90 days.

3. Body Weights - Apparently dose-related, statistically significant reductions in male and female body weights were observed. Male rats showed reduced weight from week 1 through week 6, and females at week 2 for the 5000 ppm dose level. At the 50,000 ppm dose, males showed reduced body weights from week 1 through week 13, and females from week 1 through week 6, and week 8 through week 10.

4. Gross Pathology - Male and female rats exhibited several macroscopic stomach findings at the 5000 and 50,000 ppm dose levels which included: thickened walls, dark contents, raised focal areas, glandular thickened walls, nonglandular light focal areas, glandular dark focal areas, red glandular focal areas.

5. Microscopic Pathology - Mid- and high-dose male and female rats displayed a number of histopathologic stomach findings at the 5000 and 50,000 ppm dose levels including: submucosal lymphoid focus,

epidermal hyperplasia, hyperkeratosis/acanthosis, erosion/ulcerative, mucosal atrophy, and chronic submucosal inflammation.

NOEL and LEL: A no-observable-effect-level of 50 ppm for effects other than fluoride accumulation in bone can be established. The LEL for effects other than fluoride accumulation in the bone is 5000 ppm based upon quantitative gross and microscopic findings in the stomach. There was no NOEL for fluoride accumulation in bone. The LEL for fluoride accumulation in bone was 50 ppm.

Classification: Core-Supplementary.

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Section VII, Toxicology Branch (TS-769C)
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DATA EVALUATION REPORT

Study Type: 90-Day Dog Feeding TOX. CHEM. NO.: 264

Accession Number: 262371

Test Material: Kryocide 96W (97.3% ai) Batch 8401

Synonyms: Cryolite, Sodium Fluoaluminate

Study Number: WIL-75007

Sponsor: Pennwalt Corporation

Testing Facility: WIL Research Labs

Title of Report: 90-Day Dietary Study in Dogs with Kryocide

Author(s): Not named

Report Issued: January 1986

Conclusions:

Classification: Core-Supplementary. Based on the accumulation of fluoride in bone. The NOEL for male and female dogs was 10,000 ppm cryolite for effects other than fluoride accumulation in bone. The LEL was 50,000 ppm (the HDT), based on decreased body weight, body weight gain, food consumption, and hematology. There was no NOEL for fluoride accumulation in bone. The LEL for fluoride accumulation in bone was 500 ppm (LDT).

A. Materials:

1. Test compound: Cryolite; Description white powder; Batch No. 8401; Purity 97.3%. Contaminants not listed.

2. Test animals: Species: dogs; Strain: AKC Beagles; Age: 3-5 months; Weight: 6150 to 9285; Source: Marshall Research Animal, Inc., North Rose, NY.

B. Study Design:

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

Test Group	Dose in Diet (ppm)	Main Study 3 Months Male	Main Study 3 Months Female	Interim Sac. 45 Days Male	Interim Sac. 45 Days Female
1. Control	0	6	6	1	1
2. Low (LDT)	500	6	6	1	1
3. Mid (MDT)	10,000	6	6	1	1
4. High (HDT)	50,000	8	8	1	1

2. Diet preparation: Diet was prepared weekly and samples saved for later analysis. Diet was analyzed for stability, homogeneity, and concentration.

Selected samples only were analyzed.

Intended Dose (ppm)	Analyzed Sample Range (ppm)	Percent of Theoretical (Uncorrected)
50,000	35,885 - 40,468	72 - 81
10,000	7606 - 8760	76 - 88
500	371 - 463	74 - 93
0	18 - 22	

3. Animals received 400 g/day of food (2 hr/day) and water was given ad libitum.

4. Statistics: The statistics utilized in analyzing the numerical data was a two-tailed test for minimum significance at 5 percent.

5. Quality assurance was adequate (21 inspections for GLP were made throughout the study and reported to the study director).

C. Methods and Results:

1. Observations: Animals were inspected daily for signs of toxicity and mortality.

Results

Mortality (survival): All animals survived.

One high-dose (50,000 ppm) male showed dorsal head hair loss, another high-dose male had a missing tooth, upper right jaw.

One female at each of 0, 10,000, and 50,000 ppm doses displayed clear ocular discharge, right eyes.

One female each at 0 and 10,000 ppm and two females at 50,000 ppm displayed similar discharge from left eyes.

2. Body weight: Weighed weekly throughout the study.

Results

Males — Low dose (500 ppm) - no effect.

Mid dose (10,000 ppm) - body weights not statistically significantly different, but slightly depressed from week 1 through week 6 only.

High dose (50,000 ppm) - not statistically different, but depressed from week 1 throughout remainder of experiment (week 13).

Females — Low dose - no effect.

Mid dose - not statistically different; weight slightly depressed from week 7 through week 11 only.

High dose - not statistically different, but weights depressed from week 6 until the experiment termination.

Body weight gains

Males — Low dose - decreased at week 1.

Mid dose - decreased at week 1. Statistically significant.

High dose - decreased weight gain from week 1 through week 11, with statistical significance achieved at weeks 1, 5, and 7.

Females — Low dose - no effect.

Mid dose - body weight gain decreased at weeks 1 and 2.

High dose - sporadically decreased body weight gains; decreased at weeks 1 and 2, decreased at weeks 4 through 6 and weeks 8 and 10.

3. Food consumption and compound intake: Food consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data.

Results: Food consumption was generally comparable to controls for the low-and mid-dose groups of both sexes. However, the high-dose group of both sexes generally showed a greater decrease in food consumption which generally paralleled decreases in body weight gain.

Dose	Compound Consumption (mg/kg/day) Mean Values
500 ppm	17
10,000 ppm	368
50,000 ppm	1692

4. Ophthalmological examinations were performed on all animals prior to study start, and on control and high-dose animals on days 46 and 90.

No ocular lesions were found on day 46. No ocular lesions were found on day 90; however, two male dogs showed a pigment rest on the interior lens capsule. Two female dogs displayed bilateral distichia.

5. Blood was collected before treatment and at 45 days and 90 days for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RCB)	X	Mean corpuscular volume (MCV)
X	Platelet count		

Results: Low-and mid-dose animal hematology values were unaffected by treatment. High-dose males and females at 45 days showed statistically decreased hemoglobin and hematocrit values. At 90 days for both males and females, the following were statistically significantly decreased at the high dose: RBC, Hg, HCT, MCV, and MCH.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium	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	X	Total bilirubin
X	Alkaline phosphatase	X	Total protein
	Cholinesterase	X	Fluoride
	Creatinine phosphokinase	X	A/G ratio
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		

Results: Alkaline phosphatase values were slightly increased for females at mid and high-dose levels at 45 and 90 days, while these values were slightly increased for males at low, mid, and high dose levels at the 45-day sampling period. Low- and high-dose cholesterol values were slightly increased in males at 45 days, and decreased at the 90-day high-dose level (in males).

Globulin levels were slightly increased at all three dose levels for males at 45 days, serum alanine aminotransferase was decreased in high-dose females at 45 days, and high-dose males exhibited slightly decreased albumin values at 90 days.

The lactic dehydrogenase value was elevated at 45 and 90 days for high-dose males, and was elevated at the mid- and high-dose levels for females at both the 45- and 90-day sampling periods.

However, percentage change from control was not great and numerical values were generally not statistically significant. Correlations with histopathology were negative and changes therefore were considered not biologically meaningful.

Urinalysis: Urine was collected from fasted animals twice prior to study initiation, 4 days per sex per group at approximately 45 and 90 days treatment, and

on two dogs in the high-dose group after the 28-day recovery period. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Nitrote
	Sediment (microscopic)	X	Urobilinogen
X	Protein	X	Fluoride
X	Color		
X	Occult blood		

Results: No compound-related effects were observed in the urinalysis at both the 45- and 90-day periods. The 28-day recovery period also proved negative for compound effect on urinalysis results.

7. Sacrifice and pathology: All animals were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta	XX	Brain
X	Salivary glands	XX	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic n.)
X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	X	Adrenals
X	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	X	Parathyroids
XX	Liver	X	Epididymides	XX	Thyroids
X	Gallbladder	X	Prostate		Other
X	Pancreas		Seminal vesicle	X	Bone
	Respiratory	X	Ovaries	X	Skeletal muscle
X	Trachea	XX	Uterus	X	Skin
X	Lung	X	Vagina	X	All gross lesions and masses

8. Fluoride analysis: Dog blood, urine, and bone (sternabrae and femur) fluoride analyses were conducted at 45 days, 90 days, and the 28-day recovery period. (See Addendum - Special Clinical Chemistry Analysis - Tables 1, 2, 3.) The data indicated that very little of the administered dose was found in urine and plasma. The majority of the dose was accumulated into bone at all dose levels with time.

Results

a. Organ weight: Absolute organ weights as well as organ-to-body-weight-ratio and organ-to-brain-weight-ratio were not statistically significant from controls.

b. Gross pathology: No compound-related lesions were observed during the 45-day and 90-day gross necropsy examinations.

Sporadic incidence of various lesions in control and treated animals included:

45-day sacrifice (one animal/sex/dose)

A pituitary cyst was found on a low-dose female and an enlarged firm prostate gland was noted on a mid-dose male.

90-day sacrifice

Sporadic incidence of gross lesions and observations were noted in both control and test animals that included a diverticulated jejunum, dilated pelvises, enlarged red mammary gland, enlarged ovaries, enlarged firm prostate glands, dark and firm spleen, dark red and firm thymus, thickened stomach, and a cervicle lymph node nodule.

c. Microscopic pathology

1. Non-neoplastic: No microscopic, compound-related lesions were observed.

A number of observations were recorded in control and treated animals that fell within the normal spontaneous incidence for control animals.

These noncompound-related microscopic lesions included hemorrhagic mesenteric lymph nodes, thyroglossal duct cysts of parathyroid glands, pituitary gland cysts, congested spleens, gastritic stomach, tubule atrophy of the testis, hemorrhagic thymus, a cell hyperplasia of the thyroid gland, and thyroglossal duct cyst of the thyroid gland.

2. Neoplastic: No microscopic evidence of neoplasms was detected.

D. Discussion and Conclusion:

Compound-related toxicity was observed at the high dose tested in male and female beagle dogs when administered in diets containing 0, 500, 10,000, or 50,000 ppm of cryolite for 90 days. Decreased food consumption, body weights, body weight gain, and hematology values at the HDT were observed. The NOEL was 10,000 ppm for these effects and the LEL for these effects was 50,000 ppm. There was no NOEL for fluoride accumulation in the bone. The LEL for fluoride accumulation in bone was 500 ppm. The significance of this kind of fluoride accumulation in bone is not known. There is no NOEL for this dog study.

ADDENDUM (Special Clinical Chemistry Analyses):

Fluoride recovery in dog urine, plasma, and bone sternabrae and femur summary tables are quoted from the tester's report as follows:

Table 1

Dog Urine Fluoride Levels

		Males			Females		
Dose (ppm)	Group Number	Day 45 (ppm)	Day 90 (ppm)	28-Day Recovery (ppm)	Day 45 (ppm)	Day 90 (ppm)	28-Day Recovery (ppm)
0	1 Control	2.80	5.71	NA	3.73	5.10	NA
500	2	6.57	14.27	NA	10.47	19.16	NA
10,000	3	58.9	92.3	NA	44.2	78.7	NA
50,000	4	181.6	161.7	19.8	168.7	161.0	23.0

Pretest Mean Values: Males = 3.04, Females = 3.04
NA = Not applicable

Table 2

Dog Plasma Fluoride Levels

		Males			Females		
Dose (ppm)	Group Number	Day 45 (ppm)	Day 90 (ppm)	28-Day Recovery (ppm)	Day 45 (ppm)	Day 90 (ppm)	28-Day Recovery (ppm)
0	1 Control	0.02	0.09	NA	0.00	0.09	NA
500	2	0.06	0.17	NA	0.00	0.18	NA
10,000	3	0.38	1.04	NA	0.48	1.23	NA
50,000	4	1.52	2.28	0.75	2.11	2.05	0.73

Pretest Mean Values: Males = 0.12, Females = 0.10
NA = Not applicable

Table 3

Fluoride Levels in Lyophilized Sternabrae

Dose (ppm)	Group Number	Males Day 45 (ppm)	Males Day 90 (ppm)	Males 28-Day Recovery (ppm)	Females Day 45 (ppm)	Females Day 90 (ppm)	Females 28-Day Recovery (ppm)
0	1 Control	506.96	237.86	NA	455.54	532.35	NA
500	2	546.72	677.29	NA	455.44	996.02	NA
10,000	3	4144.87	4113.61	NA	2062.31	4445.54	NA
50,000	4	4260.18	7050.64	6775.47	4683.17	7071.71	5145.14

Fluoride Levels in Lyophilized Femur

Dose (ppm)	Group Number	Males Day 45 (ppm)	Males Day 90 (ppm)	Males 28-Day Recovery (ppm)	Females Day 45 (ppm)	Females Day 90 (ppm)	Females 28-Day Recovery (ppm)
0	1 Control	624.39	454.28	NA	593.07	644.97	NA
500	2	715.83	1374.64	NA	797.64	1830.85	NA
10,000	3	3676.91	8080.00	NA	2887.60	10410.41	NA
50,000	4	6177.61	12490.12	7702.97	5537.85	11448.55	7754.70

End of quotation.

Comments Regarding the Fluoride Data Shown in Tables 1, 2, and 3:

Dose-related increases in urine, plasma, and bone fluoride levels were generally apparent in male and female dogs. These increased fluoride levels were higher at 90 days than values determined for the 45-day analyses except for high-dose (50,000 ppm) male and female urine and female plasma fluoride levels.

Twenty-eight-day recovery fluoride values were significantly lower compared to 45- and 90-day top-dose values for male and female urine and plasma analyses.

The top-dose male and female femur and sternabrae fluoride values at the 28-day recovery analyes were somewhat less than the 90-day values; however, they were consistently higher than the 45-day fluoride values.

R:89862:Woodrow:W-1:KENCO:2/11/87:4/20/87:TAR:LF:de
R:89868:Woodrow:W-1:KENCO:2/27/87:3/11/87:LIZ:VO:TAR

R:89863:Woodrow:W-2:KENCO:2/13/87:5/18/87:DEJ:VO:JS:EK:TAR:LF:TAR
R:89865:Woodrow:W-2:KENCO:2/19/87:5/30/87:BJP:LF:BJP
R:89868:Woodrow:W-2:KENCO:2/27/87:3/11/87:LIZ:VO:TAR

MPI	TMRC	% ADI
$$$$$$$$$$ mg/day/60kg	2.4359 mg/day/1.5kg	0.00

Current Action 84-DY-09 [Section 18]

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CROP	Tolerance	Food Factor	mg/day/1.5kg
Potatoes(127)	7.000	5.43	0.56930

MPI	TMRC	% ADI
$$$$$$$$$$ mg/day/60kg	3.0357 mg/day/1.5kg	0.00

DRAFT