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

007009

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDOM

DEC 21 1987

Subject: Cryolite, Toxicology Chapter of the Registration Standard

To: Joanne Edwards PM
Registration Division (TS-767)

From: William Woodrow Ph.D. 12-21-87
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Attached is the Toxicology Chapter of the Registration Standard for Cryolite. This standard has been reassigned to Dr. Kocialski due to the retirement of Dr. Woodrow. The following portions of this chapter are available on Lexitron disk. You may obtain a copy from Dr. Zendzian, the Tox Branch Registration Standard coordinator.

A. Toxicology Summary
B. Toxicology Profile
C. Data Gaps
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E. Toxicological Issues
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Zendzian
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Toxicology Chapter

of the

Cryolite

Registration Standard

Prepared By

William Woodrow Ph.D
Microbiologist
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A. TOXICOLOGY SUMMARY

Cryolite is the common name for sodium fluoaluminate or sodium aluminofluoride (given variously as Na_3AlF_6, $3NaF.AlF_3$, AlF_6Na_3).

Acute toxicity tests show a low order of toxicity when cryolite was tested by the oral, dermal, or inhalation routes; however, mild to moderate ocular irritative properties were exhibited. Cryolite was shown not be a dermal irritant, and was determined not to be a dermal sensitizing agent. The Agency does not require additional acute toxicity data.

Dog (MRID No. 00157999) and rat (MRID No. 00158000) cryolite subchronic 90-day feeding studies did not establish no effect dosage levels for fluoride accumulation in bones. The significance or lack of significance of this observation was not addressed by the registrant and these studies were classified as Supplementary Data. However, if the issue of fluoride accumulation in the bone could be put to rest both studies would be considered acceptable and NOELs established; 50 ppm for the rat and 10,000 ppm for the dog. At present cryolite rat and dog subchronic feeding study data gaps do exist due to a lack of information concerning the potential effects of bone fluoride, and a lack of established no effect dose levels (NOELS) for fluoride accumulation in animal bones.

Current cryolite chronic toxicity data gaps include: chronic systemic toxicity studies in two species, rodent and nonrodent; chronic oncogenicity studies in two species, the rat and mouse are preferred; a two generation reproduction study; and a rabbit teratology study and a rat metabolism study. An acceptable cryolite rat teratology study has been submitted to the Agency, which indicated no teratogenic potential; a NOEL for maternal and fetal toxicity was greater than 3000 mg/kg, the highest dose tested.

An Ames Salmonella/Microsome Mutagenic Assay, a DNA Repair Evaluation Assay of Kryocide and an In Vivo Cytogenic Evaluation of Kryocide Technical all demonstrated no cryolite mutagenic potential, and fulfills the Agency's mutagency data requirements.

The initial registration standard issued for cryolite indicated that there was no crop residue data as well as no toxicological data base for cryolite. The data required as a result of the first registration standard was limited to residue data, acute toxicity studies, a metabolism study, a subchronic study, a teratology study, and mutagenicity studies with the stipulation that if the exposure indicated by cryolite crop residue data was extensive and depending upon the findings in the required toxicology studies additional subchronic and chronic studies would be required. The recent Residue Chemistry Branch review states that real residues are found in raw agricultural commodities and that exposure will be extensive. Considering the information available, the Agency has reserved the requirement for long-term

toxicity studies. Essentially the compound lacks toxicity with the exception of floride deposition in the bone. The Registrant has been required to address issue of floride deposition in the technical literature as it pertains to long-term toxicty.

B. TOXICOLOGY PROFILE

81 Series Acute Toxicity and Irritation Studies

81-1 - Acute Oral

Sufficient data are available to show that technical Cryolite has a low order of acute oral toxicity to mammals (MRID No. 00138096). The acute oral LD_{50} to rats is greater than 5.0 g/kg. Toxicity Category IV.

81-2 - Acute Dermal

Sufficient data are available to show that technical Cryolite has a low order of acute dermal toxicity (MRID No. 00128107). The acute dermal LD_{50} for rabbits is greater than 2.1 g/kg. Toxicity Category III.

81-3 - Acute Inhalation

Sufficient data are available to show that technical Cryolite has a low order of acute toxicity by the inhalation route (MRID No. 00128108). The acute inhalation LC_{50} for rats was greater than 2.06 mg/L but less than 5.03 mg/L. Toxicity Category III.

81-4 - Primary Eye Irritation

Sufficient data are available to show that technical Cryolite produces mild-to-moderate eye irritation in rabbits (MRID No. 00128106). Toxicity Category III.

81-5 - Primary Dermal Irritation

Sufficient data are available to show that technical Cryolite is not a skin irritant (MRID No. 00128106). Toxicity Category IV.

81-6 - Dermal Sensitization

Sufficient data are available to show that technical Cryolite is not a dermal sensitizer (MRID No. 00138099).

81-7 - Acute Delayed Neurotoxicity

No data are available on the acute neurotoxic effects of Cryolite. This test is required only for compounds which are organophosphate inhibitors of cholinesterase or related to such

inhibitors or metabolites of such inhibitors. Cryolite is not an organosphosphate compound, therefore a study is not required.

82 Series Subchronic Testing

82-1 - Subchronic Oral

No acceptable data are available on the subchronic oral toxicity of technical Cryolite in rodents and nonrodents. This data requirement may be waived based on the requirement for chronic studies in the rodent and the nonrodent or provision of rational for waiving the chronic studies.

Rat Study

Three groups of 40 rats/sex per group and one group of 50 rats/sex/group (high dose) were dosed, for 90 days, at 0, 50, 5000 and 50,000 ppm Kyrocide in the diet (MRID No. 00158000). The most sensitive observed effect was floride accumulation in the bone with a LEL of 50 ppm (LDT) and no NOEL. Abnormalities of the stomach were observed grossly and histologically at 5000 ppm with a NOEL of 50 ppm. The study was classified supplementary because the lack of a NOEL for floride accumulation in the bone. The registrant has been requested to address this issue.

Dog study

Three groups of 6 dogs/sex per group and one group of 8 dogs/sex/group (high dose) were dosed, for 90 days, at 0, 500, 10,000 and 50,000 ppm Kyrocide in the diet (MRID No. 00157999). The most sensitive observed effect was floride accumulation in the bone with a LEL of 500 ppm (LDT) and no NOEL. Decreased body weight gain and food consumption and hematological abnormalities were observed at 50,000 ppm (LEL) with a NOEL of 10,000 ppm. The study was classified supplementary because the lack of a NOEL for floride accumulation in the bone. The registrant has been requested to address this issue.

82-2 - Subchronic Dermal (21-Day)

No data are available on the subchronic dermal toxicity of Cryolite. A study is required.

82-3 - Subchronic Dermal (90-Day)

No data are available on the 90-day subchronic dermal toxicity of Cryolite. A study is not required under the registered use patterns.

82-4 - Subchronic Inhalation (90-Day)

No data are available on the subchronic inhalation toxicity of Cryolite. A study is not required under the registered use

patterns.

82-5 - Subchronic Neurotoxicity

No data are available on the subchronic neurotoxicity of Cryolite. Since an acute neurotoxicity study is not required and there is no evidence of neurotoxicity in mammalian species, this study is not required.

83 Series Chronic and Long-Term Studies

83-1 - Chronic Toxicity

No data are available on the chronic toxicity of cryolite. Studies are required in rodent and nonrodent species. This data requirement is reserved.

83-2 - Oncogenicity

No data are available on the oncogenic potential of cryolite. Studies are required in two species. This data requirement is reserved.

83-3 - Teratogenicity

Cryolite was administered by gavage to pregnant rats at doses of 750, 1500, and 3000 mg/kg/day. Cryolite did not manifest teratogenicity or fetotoxicity at the highest dose tested. The NOEL for maternal and fetal toxicity was greater than 3000 mg/kg/day. The study (MRID No. 00131352) is acceptable.

No data are available on the teratogenic potential of technical Cryolite in a second species. The requirement for a teratology study in a second species is waived based on the lack of toxicity displayed in the rat teratology study and other studies available.

83-4 - Reproduction

No data are available on the reproductive toxicity of cryolite. A study is required. This data requirement is reserved.

84 Series Mutagenicity

84-2 Mutagenicity Tests

Technical Cryolite was tested for mutagenic activity in the Ames Assay and was determined to be negative. The study (MRID No. 00128113) was found to be acceptable.

Technical Cryolite was tested for genotoxic effects and was found to be negative in the DNA repair test using Escherichia coli. The study (MRID No. 00128114) was acceptable.

Technical Cryolite was tested in an in vivo cytogenetics assay using rats. No structural chromosome aberrations were observed at the highest dose tested. The study (MRID No. 00128115) is acceptable.

C. DATA GAPS

Cryolite is registered for raw agricultural commodity uses (40 CFR 180.145) and therefore, the following Guideline toxicology studies are required for registration.

81-1 - Acute Oral
81-2 - Acute Dermal
81-3 - Acute Inhalation
81-4 - Primary Eye Irritation
81-5 - Primary Dermal Irritation
81-6 - Dermal Sensitization

82-1 - Subchronic Oral, (rodent and nonrodent)
82-2 - Subchronic Dermal (21-Day)
82-3 - Subchronic Dermal (90-Day)
82-4 - Subchronic Inhalation

83-1 - Chronic Toxicty, (rodent and nonrodent)
83-2 - Oncogenicity, two species
83-3 - Teratogenicity, two species
83-4 - Reproduction
84-2 - Mutagenicity Tests
85-1 - Metabolism

Based on this assessment of the required toxicology data base for cryolite the following Guideline toxicity studies have been identified as data gaps and are required.

82-1 - Subchronic Oral, (rodent and nonrodent) This data requirement may be waived based on the requirement for chronic studies in the rodent and nonrodent or provision of rational for waiving the chronic studies.
82-2 - Subchronic Dermal (21-Day)

83-1 - Chronic Toxicity, (rodent and nonrodent)[a]
83-2 - Oncogenicity, two species[a]
83-4 - Reproduction, 2-Generation[a]

85-1 - Metabolism Study

a. The data requirement is reserved.

D. ADI REASSESSMENT

The 40 CFR 180.145 indicates that a tolerance of 7.0 ppm of combined fluorine is established for residues of the insecticidal fluorine compounds cryolite and synthetic cryolite (sodium aluminum fluoride) for many raw agricultural commodities. This data review has determined that rodent and nonrodent chronic toxicity studies, a reproduction study and teratology study in a second species are not available and that the available 90-day subchronic rat (MRID No. 00158000) and dog (MRID No. 00157999) failed to established a NOEL for fluoride accumulation in the bone. Consequently, there are no studies on which to calculate a NOEL and ADI. However, if the issue of fluoride accumulation in the bone could be resolved then a provisional acceptable daily intake (PADI) could be established using the 90-day rat study (MRID No. 00158000) and a NOEL of 50 ppm.

E. TOXICOLOGICAL ISSUES

Subchronic oral administration of Kryocide containing 96 percent sodium fluoaluminate to rats resulted in dose-related fluoride accumulation in male and female femurs that was cumulative with time at all dose levels.

Administration of 96 percent sodium fluoaluminate orally for 90 days to dogs resulted in statistically significant increases in sternebrae and femur fluoride levels by which the end of 90 days were both dose-related and cumulative with time in males and females. A NOEL was not established.

The significance or lack of significance of fluoride accumulation in bone is not known at present and remains as an unresolved issue. The Registrant has been requested to address this issue by an evaluation of the information available in the technical literature and/or consulation with experts. The requirements for chronic, oncogenic and reproductive studies have been reserved and will be reconsidered in light of this evaluation.

F. CRYOLITE

TABLE A
GENERIC DATA REQUIREMENTS FOR CRYOLITE

Data Requirement	Composition[1]	Use Patterns[2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3]
§158.135 Toxicology					
ACUTE TESTING					
81-1 - Acute Oral - Rat	TGAI	A,B,H	Yes	MRID #00138096	No
81-2 - Acute Dermal	TGAI	A,B,H	Yes	MRID #00128107	No
81-3 - Acute Inhalation - Rat	TGAI	A,B,H	Yes	MRID #00128108	No
81-4 - Eye Irritation - Rabbit	TGAI	A,B,H	Yes	MRID #00128106	No
81-5 - Dermal Irritation - Rabbit	TGAI	A,B,H	Yes	MRID #00128106	No
81-6 - Dermal Sensitization-Guinea Pig	TGAI	A,B,H	Yes	MRID #00138097	No
81-7 - Acute Delayed Neurotoxicity	TGAI	A,B,H	No		No[4]
SUBCHRONIC TESTING					
82-1 - 90-Day Feeding					No[5]
- Rat	TGAI	A,B,H	No		No[6]
- Dog	TGAI	A,B,H	No		Yes
82-2 - 21-Day Dermal	TGAI	A,B,H	No		No[7]
82-3 - 90-Day Dermal	TGAI	A,B,H	No		No[7]
82-4 - 90-Day Inhalation	TGAI	A,B,H	No		No[8]
82-5 - 90-Day Neurotoxicity	TGAI	A,B,H	No		

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TABLE A
GENERIC DATA REQUIREMENTS FOR CRYOLITE

Data Requirement	Composition[1/]	Use Patterns[2/]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/]
§158.135 Toxicology (cont'd)					
CHRONIC TESTING					
83-1 - Chronic Toxicity					
- Rodent	TGAI	A,B,H	No	--	Yes[9/]
- Nonrodent	TGAI	A,B,H	No	--	Yes[9/]
83-2 - Oncogenicity Study					
- Rat	TGAI	A,B,H	No	--	Yes[9/]
- Mouse	TGAI	A,B,H	No	--	Yes[9/]
83-3 - Teratogenicity					
- Rat	TGAI	A,B,H	Yes	MRID No. 00131352	No
- Rabbit	TGAI	A,B,H	No	--	No[10/]
83-4 - Reproduction	TGAI	A,B,H	No	--	Yes[9/]
MUTAGENICITY TESTING					
84-2 - Gene Mutation	TGAI	A,B,H	Yes	MRID No. 00128113	No
84-2 - Chromosomal Aberration	TGAI	A,B,H	Yes	MRID No. 00128114	No
84-2 - Other Mechanisms of Mutagenicity	TGAI	A,B,H	Yes	MRID No. 00128115	No

TABLE A
GENERIC DATA REQUIREMENTS FOR CRYOLITE

Data Requirement	Composition[1/]	Use Patterns[2/]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/]
§158.135 Toxicology (cont'd)					
SPECIAL TESTING					
85-1 - General Metabolism	PAI or PAIRA	A,B,H	No		Yes

1/ Composition: TGAI Technical Grade Active Ingredient; PAI = Pure Active Ingredient; PAIRA = Pure Active Ingredient, Radiolabeled; Choice = Choice of several test substances determined on a case-by-case basis.

2/ The use patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Nonfood; C = Aquatic, Food Crop; D = Aquatic, Nonfood; E = Greenhouse, Food Crop, F = Greenhouse, Nonfood; G = Forestry; H = Domestic Outdoor; I = Indoor; IP = Industrial Preservative.

3/ Unless otherwise specified data must be submitted no later than six months after publication of this Standard.

4/ This test is required only for compounds which are organophosphate inhibitors of cholinesterase, or related to such inhibitors or metabolites of such inhibitors. Cryolite is not an organophosphate, therefore, a study is not required.

5/ This requirement may be waived based on the requirement for a chronic feeding study in the rat or provision of rational for waiving the chronic study

6/ This requirement may be waived based on the requirement for a chronic feeding study in the dog or provision of rational for waiving the chronic study.

7/ This study is not required under the registered use patterns.

8/ Since an acute neurotoxicity study is not required for this compound and there is no evidence of neurotoxicity in mammalian species, this study is not required.

9/ This data requirement is reserved, and may be waived, pending the results of an evaluation of the technical literature concerning the relevence of the floride accumulation in bone observed in the subchronic studies.

10/ This data requirement is waived considering the lack of toxicity demonstrated by the compound a 5 gm/kg in the rat teratology study and the minimla toxicity observed in other species.

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G. CRYOLITE

Bibliography

157999 Hagen, C.; Strouse, J. (1986) 90-Day Dietary Study in Dogs with Kryocide: Final Report: Project No. WIL-75007. Unpublished study prepared by WIL Research Laboratories, Inc. 563 p.

0128107 Hansen, K.; Mills, V.; Beck, L.; et al. (1981) Acute Dermal Toxicity Study: Kryocide Insecticide (N.B. 84-146-2B): Rabbits: Project No. 1685-C; Project No. 1136. Rev. rept. (Unpublished study received February 10, 1983 under 4581-116; prepared by Elars Bioresearch Laboratories, Inc. and Westpath Laboratories, Inc., submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA; CDL:071392-B)

131352 Harris, S.; Holson, J.; Barnett, W.; et al. (1983) Final Report for a Tertology Study of Kryocide Insecticide in Albino Rats: PNW/SAI 1182008. (Unpublished study received August 10, 1983 under 4581-116; prepared by Science Applications, Inc., submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA; CDL:250968-D)

0138096 Hazleton Laboratories America, Inc. (1983) Acute Oral Toxicity--Method, Summary, Pathology--Raw Data Attached: Kryocide: RT Lab No. 814515. (Unpublished study received December 29, 1983 under 4581-116; submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA; CDL:252071-A)

0138097 Hazleton Laboratories America, Inc. (1983) Dermal Sensitization Study in Guinea Pigs--Closed Patch Technique: Kryocide: RT Lab No. 814516. (Unpublished study received December 29, 1983 under 4581-116; submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA; CDL:252071-B)

0123108 Meclar, F.; Knapinski, P. (1981) Acute Inhalation Toxicity Study in Rats: Kryocide 96W Insecticide (NB 84-103-4): LBI Project No. 22098. Final rept. (Unpublished study received February 10, 1983 under 4581-116; prepared by Litton Bionetics, Inc., submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA; CDL:071392-C)

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0128114 Putman, D.; Moore, W.; Schechtman, L. (1981) Activity of T1693 in a DNA Repair Test Using Escherichia coli: MA Project No. T169104 Final rept. (Unpublished study received February 10, 1983 under 4581-116; prepared by Microbiological Assoc. and Genetic Toxicology Testing Service, submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA: CDL:071392-J)

0128115 Putman, D.; Moore, W.; Schechtman, L. (1981) Activity of T1693 in the in vivo Cytogenetics Assay in Rodents: MA Study No. T1693.112. Final rept. (Unpublished study received February 10, 1983 under 4581-116; prepared by Microbiological Assoc. and genetic Toxicology Testing Service, submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA; CDL:071392-K)

0128113 Putman, D.; Parmar, A.; Schectman, L. (1981) Activity of Kryocide in the Salmonella/Microsomal Assay for Bacterial Mutagencity: MA Study No. T1693.102. Final rept. (Unpublished study received February 10, 1983 under 4581-116; prepared by Microbiological Assoc. and Genetic Toxicology Testing Service, submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA; CDL:071392-I)

0128106 Ralston Purina Co. (1981) Acute Oral Toxicity--Method, Summary, Pathology; Primary Dermal Irritation--Method, Summary: Primary Eye Irritation--Method, Summary: Kryocide Insecticide NB 84-146-2B: Rats, Rabbits: RT Lab No. 880531. (Unpublished study received February 10, 1983 under 4581-116; submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA; CDL:071392-A)

00128109 Walter, M.; Nier, K.; Hepler, D.; et al. (1982) Twenty-eight Day Range Finding and Palatability Study with Kryocide Insecticide in Rats: Project No. 1821. (Unpublished study received February 10, 1983 under 4581-116; prepared by Elars Bioresearch Laboratories, Inc. and Westpath Laboratories, submitted by Agchem Div., Pennwalt Corp., Philadelphia, PA; CDL:071392-D)

0158000 Weltman, R. (1985) Subchronic Toxicity Study with Kryocide Insecticide in Rats: Final Report: Study No. 6120-100. Unpublished study prepared by Hazleton Laboratories America, Inc. 684 p.

II.

Chem No. 264 Cryolite | File Last Updated 7-11-85 | Current Date 10-9-87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results	TOX Category	CORE Grade
o Acute oral LD_{50} - rat; Hazleton Lab.; # 814515; 11/10/83	Kryocide	252071 MRID # 00138096	LD_{50} > 5 gm/kg (only level tested)	IV	Minimum 001696
o Acute dermal LD_{50} - rabbit; ELARS Biosearch Labs; # 1685-6; 7/20/81	Kryocide	071392 MRID # 00128107	No mortality LD_{50} > 2.1 g/kg	III	Minimum 003775
o Acute inhalation LC_{50}- rat; Litton Bionetics; # 22098; 6-81	Kryocide	071392 MRID # 00128108	LD_{50} < 5.03 mg/l, > 2.06 mg/l (T.W.A. - time weighted average)	III	Minimum 003775
o Primary eye irritation- rabbit; Raltech Scientific Services; # 880531; 9-21-81	Kryocide	071392 MRID # 00128106	moderate conjunctival redness and chemosis irritation that disappeared within 7 days.	III	Minimum 003775

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Chem No. 264 Cryolite | File Last Updated 7-11-85 | Current Date 10-9-87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results	TOX Category	CORE Grade
o Primary dermal irritation - rabbit; Raltech Scientific Services; # 880531; 9-21-81	Kryocide	071392 MRID # 00128106	P.I. Score= 0.0; not an irritant.	IV	Minimum 003775
o Dermal sensitization - guinea pig; Hazleton Lab.; # 814516; 12/13/83	Kryocide	252071 MRID # 00138097	Negative results.		Minimum 001696
o Mutagenic - ames; Microbiological Assoc.; # T-1693.102; 9-29-81	Kryocide 96%	071392 250968 MRID # 00128113	No mutagenic potential demonstrated		Acceptable
o Mutagenic - DNA repair 3 tests using E. Coli; Microbiological Assoc.; # T-1693.104; 9-21-81	Kryocide	071392 250968 MRID # 00128114	No mutagenic potential demonstrated		Acceptable
o Mutagenic - in vivo cyto-2 genetics assay - rats; Microbiological Assoc.; # T-1693.112; 10-2-81	Kryocide	071392 250968 MRID # 128115	No demonstrated toxic effects or mutagenic potential at highest tested dose.		Acceptable

087869

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
o 90-Day feeding - dog; Wil Res. Lab.; #WIL-75007; 1/86	Kryocide 96W 97.3% ai Batch #8401	262371 MRID # 00157999	Levels tested in beagles - 0,500, 10,000 and 50,000 ppm Systemic NOEL < 500 ppm (fluoride accumulation in bone) at 10,000 ppm - fluoride accumulation in bone at 50,000 ppm - fluoride accumulation in bone, decreased body wt., decreased food consumption; decreased hemoglobin and hematocrit values, RBC,Hg,HCT,MCV and MCH		Supplementary 005771
o 90-Day feeding - rat; Ha.leton Lab.; #6120-100; 11/27/85	Kryocide 96% pure	262372 MRID # 00158000	Levels tested in Charles Riv. CrL; CD(SD)Br strain-0,50,5000 and 50,000 ppm NOEL < 50 ppm (fluoride accumulation in bone) At 5000 ppm-lower hemoglobin and hematocrit values; stomach findings such as: thickened walls, dark contents, raised focal areas, glandular thickened walls, nonglandular light focal areas, glandular dark focal areas and red glandular focal areas, submucosal lymphoid focus, epidermal hyperplasia, hyperkeratosis/acanthosis, erosion/ulcerative, mucosal atrophy and chronic submucosal inflammation.		Supplementary 005771

Chem No. 264 Cryolite | File Last Updated 7-11-85 | Current Date 10-9-87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results	TOX Category	CORE Grade
o Teratology - rat; Science Applications, Inc.; # 1182008; 1/15/83	Kryocide Lot # 86-11-9	071392 250968 MRID # 00131352	Levels tested by gavage- 0, 750, 1500, and 3000 ppm. Teratogenic NOEL > 3000 ppm(HDT) Maternal NOEL > 3000 ppm(HDT) Feto toxic NOEL > 3000 ppm(HDT) Final Report		Core Minimum

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Guideline Reference No. 81-1

Confirmation of Conclusions in the DER

Study Type: Acute Oral Toxicity Evaluation of Kryocide in the Rat

Primary Reviewer: William S. Woodrow

Secondary Reviewer: Edwin Budd

Accession No. (MRID No.): 252071

Test Material: Kryocide, 96.0% pure

Study No.: 814515

Testing Facility: Hazleton Laboratories

Report Issued: November 10, 1983

Conclusion:

No mortality. Animals gained weight during the observation period. No lesions were visible at necropsy. Three males and one female appeared hypoactive on day 1 postexposure.

Acute oral LD_{50} (Kryocide in the rat):

LD_{50} > 5g/Kg
Toxicity Category IV

Classification: Core Minimum Data

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William S. Woodrow 12/16/87

Supervisors Signature: Alan B Konaski 12/16/87

Acute Oral Toxicity Evaluation of Kryocide in the Rat. Sponsor: Pennwalt Corp. Tester: Hazleton Laboratories, Report No. 814515, November 10, 1983.

Test Material:

Kryocide formulation N_aAlF_6 (Sodium fluoaluminate) approximately 96.0% pure.

Detailed formulation (Confidential)

ACTIVE INGREDIENT:
sodium fluoaluminate 96.0%

Guideline Reference No. 81-1

Following a 7-day laboratory acclimation period, 5 male and 5 female Sprague-Dawley rats weighing between 207 and 283 g were dosed by gavage with 5 g/kg bwt each.

The test material was mixed with distilled water to a concentration of 0.5 g/mL, and each animal was administered 10 mL of the kryocide/water mixture.

Each animal was observed for clinical signs of toxicity and mortality at 1.0, 2.5, and 4.0 hours postdosing and once daily for clinical signs and twice daily during a 14-day observation period. Body weights were recorded pretreatment, at 7 days, and at termination.

All animals were subjected to gross necropsy at termination; all abnormalities were recorded.

Results:

No mortality. Animals gained weight during the observation period. No lesions were visible at necropsy. Three males and one female rat appeared hypoactive on day 1 postexposure.

Acute oral LD_{50}, kryocide in the rat:

$LD_{50} > 5$ g/Kg

Toxicity Category: IV

Classification: Core Minimum Data

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Guideline Reference No. 81-2

Confirmation of Conclusions in the DER

Study Type: Acute Dermal Toxicity Evaluation of Kryocide, Rabbit

Primary Reviewer: William S. Woodrow

Secondary Reviewer: William J. Burnam

Accession No.: 071392

Test Material: Kryocide, 96.0% pure

Study No.: 1685-6

Testing Facility: Elars Bioresearch Laboratories

Report Issued: July 20, 1981

Conclusion:

No mortality. All rabbits treated with Kryocide exhibited slightly red and swollen skin at test sites. Most of the rabbits appeared normal throughout the test period.

Dermal LD_{50} > 2.1 g/kg body weight

Toxicity Category: III

Classification: Core Minimum Data

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William S. Woodrow 12/16/87

Supervisors Signature: Alan B. Kowalski 12/16/87

Acute Dermal Toxicity Evaluation of Kryocide, Rabbits. Sponsor: Pennwalt Corp. Tester: ELARS Bioresearch Laboratories. No. 1685-6, July 20, 1981

Test Material:

Kryocide (cryolite), fine white powder (96% pure).

Twenty New Zealand White rabbits (10 males and 10 females) approximately 2 months old were acclimated to laboratory conditions for 18 days prior to testing. The rabbits weighed 2.2 to 3.3 kg at initiation of the study.

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Twenty four hours before dermal treatment, approximately 10 percent of the animals back and flanks were clipped free of hair.

On the day of testing prior to application, all rabbits were weighed to determine dosages, and the exposure sites on all rabbits were abraded.

Five male and five female rabbits received 2.1 g/kg body weight of kryocide, moistened with physiological saline applied under 4" x 4" gauze sponges which were held in place with plastic wrap. The sponges and plastic wrap were taped to the shaved treatment sites, and then the animals were wrapped with elastic tape.

Five male and five female rabbits were similarly treated with physiological saline only, to serve as controls.

Twenty hours following compound application, the treated test sites were uncovered and wiped to remove excess test material. The animals were observed for mortality, skin irritation, and behavioral abnormalities twice daily for a total of 14 days. Body weights were recorded at 0, 7, and at termination (14 days). All rabbits were killed and subjected to gross necropsy; skin samples at the test sites were collected for histopathological examination.

Results:

No mortality. All rabbits treated with kryocide displayed slightly red and swollen skin at test sites. Most of the rabbits appeared normal throughout the entire test period; however, occasional soft stool, diarrhea, or ocular discharge was noted in several of the treated animals. The control rabbits appeared normal.

One of the rabbits treated with kyrocide had pale, pitted kidneys and another in the same group had enlarged mesenteric lymph nodes. One control rabbit had diarrhea and one other had a slightly enlarged spleen.

Histopathologic examination revealed that eight of ten test group animals showed very slight-to-slight acanthosis, fibrosis, hyperkeratosis, and chronic dermal inflammation at the test sites. Dermal lesions were not found in the skin of control animals.

Dermal LD_{50} = > 2.1 g/kg body weight.

Toxicity Category: III

Classification: Core Minimum Data

007009

Guideline Reference No. 81-3

Confirmation of Conclusions in the DER

Study Type: Acute Inhalation Toxicity Evaluation of Kryocide, Rat

Primary Reviewer: William S. Woodrow

Secondary Reviewer: William J. Burnam

Accession No. (MRID No.): 071392

Test Material: Kryocide, 96.0% pure —

Study No.: LBI No. 22098

Testing Facility: Litton Bionetics

Report Issued: June 1981

Conclusion:

Nine of ten animals exposed to T.W.A. of 5.03 mg/L died by day 2 postexposure. Ten animals exposed to T.W.A of 2.06 mg/L - survived.

LC_{50} < 5.03 mg/L, > 2.06 mg/L (T.W.A)

Classification: Core Minimum Data

This reviewer is in general agreement with the conclusions of the DER.

Reviewers Signature: William S. Woodrow 12/16/87

Supervisors Signature: Alan B. Kowalski 12/16/87

Acute Inhalation Toxicity Evaluation of Kryocide, Rats. Sponsor: Pennwalt Corp. Tester: Litton Bionetics. LBI@22098, June 1981

Test Material: Kryocide (cryolite) white powder, 96% pure.

This test was actually conducted in two parts; apparently the first (Expt: #1) inhalation study (April 8, 1981) test material concentration killed 90 percent of the test rats within two days posttreatment. Therefore, a second inhalation exposure (Expt. #2) was conducted on May 21, 1981, using a reduced inhalation exposure level.

One series of particle size measurements were made using an Anderson cascade Impactor during the two different experiments; presumably prior to the first exposure, April 8, 1981.

Five male and five female Charles River CD strain rats weighing between 192.7 and 269.1 g were acclimated to laboratory conditions for 7 days prior to Expt. #1 testing.

Five male and five female Charles River CD strain rats that weighed between 255.4 to 423.8 were acclimated to laboratory conditions for 6 weeks prior to testing in Expt. #2.

The exposure cloud concentrations were generated by blowing dry filtered air through a fluid bed generator into the exposure chamber. The exposure chamber for both inhalation tests consisted of a 30 liter glass cylinder.

The exposed rats were observed frequently on the day of exposure and twice daily for toxic signs and mortality throughout the 14 day observation period. Test animals were weighed on days 0, 2, 3, 4, 7, and 14 postexposure. Necropsies were performed on all animals that died during exposure and on survivors at experiment termination.

Results:

Mortality: Group 1 (5.03 mg/L T.W.A.*)

	Day of Death
5/5 males	1
4/5 females	2

Group 2 (2.06 mg/L T.W.A.)

0/5 males
0/5 females

---

*Time Weighted Average.

The actual cloud concentrations were determined on an hourly basis by withdrawing cloud samples (during the 4-hour exposure periods) through filters in measured volumes. The gain in filter weight was divided by sample volume to determine actual concentrations per liter of exposure chamber atmosphere (telecon communication between Woodrow and P.J. Knapinski of Litton Bionetics).

Mortality: Nine of ten animals exposed to T.W.A. of 5.03 mg/L (group 1) died by day 2 postexposure. One survivor was sacrificed at termination (14 days).

All 10 animals in group 2 exposed to T.W.A. 2.06 mg/L survived to the experiment termination.

Body Weight: The one female survivor in group one, and all of the test animals in group 2 lost body weight until approximately 7 days postexposure, and thereafter began to gain weight.

Toxic Signs: Group 1 animals (5.03 mg/L) displayed labored breathing, abnormal respiratory sounds, slowed righting reflex, slow movement, and lacrimation in two of the group animals.

One male animal in group 2 (2.06 mg/L) exhibited abnormal respiratory sounds, and one other group 2 male displayed a slowed righting reflex.

Necropsy: All of the rats that died exhibited red lungs with pale mottling; the animals that survived to termination at 14 days did not show these signs. The survivors did show varying degrees of mottling.

LC_{50} < 5.03 mg/L, > 2.06 mg/L (T.W.A)

Toxicity Category: III

Classification: Core Minimum Data

Guideline Reference No. 81-4

Confirmation of Conclusions in the DER

Study Type: Primary Eye Irritation Evaluation of Kryocide, Rabbit

Primary Reviewer: William S. Woodrow

Secondary Reviewer: William J. Burnam

Accession No.): 071392

Test Material: Kryocide, 96.0% pure

Study No.: 880531

Testing Facility: Raltech Scientific Services

Report Issued: September 21, 1981

Conclusion:

Moderate conjunctival redness and chemosis irritation that disappeared within 7 days.

Toxicity Category: III

Classification: Core Minimum Data

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William S. Woodrow 12/16/87

Supervisors Signature: Albin B. Kocialski 12/16/87

Primary Eye Irritation Evaluation of Kryocide, Rabbit. Sponsor: Pennwalt Corp. Tester: Raltech Scientific Services. #880531, September 21, 1981

Test Material: Finely ground kryocide (cryolite - Na_3AlF_6), 96% pure.

Nine young New Zealand White rabbits were acclimated to laboratory conditions for a period of 7 days before testing. The animals eyes were examined using fluorescein dye at least 24 hours before administering the test material, to screen for prior corneal injury. One-tenth gram of test material was placed on the everted lower lid of one eye of each of the nine rabbits. Upper and lower lids of treated eyes were briefly closed by hand for 1 second and released. Untreated eyes of each animal served as controls. The treated eyes of the remaining 6 rabbits were not flushed with water.

The treated eyes of all test rabbits were observed for ocular lesions at 24, 48, 72, and 96 hours and at 7 days posttreatment. At the 72 hour and again at the 7-day eye examinations, sodium fluorescein and ultraviolet light were employed to detect possible corneal injury. Ocular examination scoring was done according to Draize.

Results:

Irritation Scores	Unwashed Eyes	Washed Eyes
24 hours	5.3	3.7
48 hours	2.7	2.0
72 hours	0.7	1.3
96 hours	0.5	0.0
7 days	0.0	0.0

No corneal involvement; moderate conjunctival redness and chemosis irritation that disappeared within 7 days posttreatment.

Toxicity Category: III

Classification: Core-Minimum Data

Confirmation of Conclusions in the DER

Study Type: Primary Dermal Irritation Evaluation of Kryocide, Rabbit

Primary Reviewer: William S. Woodrow

Secondary Reviewer: William J. Burnam

Accession No. (MRID No.): 071392

Test Material: Kryocide, 96.0% pure

Study No.: 880531

Testing Facility: Raltech Scientific Services

Report Issued: September 21, 1981

Conclusion:

Primary irritation score 0.0; kryocide did not demonstrate any irritation potential.

Toxicity Category: IV

Classification: Core Minimum Data

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William S. Woodrow 12/16/87

Supervisors Signature: Allen B. Rowden 12/16/87

Primary Dermal Irritation Evaluation of Kryocide, Rabbit. Sponsor: Pennwalt Corp. Tester: Raltech Scientific Services. #880531, September 21, 1981.

Test Material:

Kryocide (Synthetic Cryolite) Na_3AlF_6, 95% pure. The test material was moistened and applied as received by the testing lab.

One-half (0.5 g) gram of moistened test material was applied to two abraded and two intact skin sites on each of three male and three female New Zealand White rabbits. The animals had been acclimated to laboratory conditions for a period of 7 days prior to testing. The backs and flanks of the animals were clipped to remove hair prior to test application. Five x5 cm gauze patches

were used to maintain the test material; etastoplast tape was then applied to the protected test sites. Collars were fitted to the animals during the 24-hour treatment period.

Twenty-four hours following application, treated skin sites were uncovered, wiped, and the degree of edema and erythema was recorded according to the Draize system of scoring. A second reading was made at 72 hours.

Results:

Primary irritation score = 0.0; Kryocide did not demonstrate any irritation potential in this experiment.

Toxicity Category: IV

Classification: Core Minimum Data

Guideline Reference No. 81-6

Confirmation of Conclusions in the DER

Study Type: Dermal Sensitization Evaluation of Kryocide in Guinea Pigs

Primary Reviewer: William S. Woodrow

Secondary Reviewer: Edwin Budd

Accession No. (MRID No.): 252071

Test Material: Kryocide formulation, 96.0% pure

Study No.: 814516

Testing Facility: Hazleton Laboratories

Report Issued: December 13, 1983

Conclusion:

Kryocide was not found to be a sensitizing agent.

Classification: Core Minimum Data

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William S. Woodrow 12/16/87

Supervisors Signature: Alan B. Rowitz 12/16/87

Dermal Sensitization Evaluation of Kryocide in Guinea Pigs. Sponsor: Pennwalt Corp. Tester: Hazleton Laboratories. Report No. 814516, December 13, 1983.

Test Material: Kryocide formulation.

Twenty-four male Hartley strain guinea pigs were divided into: a) one group of 10 test animals, b) one group of 10 non-sensitized (but challenged animals), and c) one group of 4 positive control animals.

A dosing range study was conducted prior to the sensitization study to determine a threshold level for Kryocide; the test material for the actual study was administered at 50% w/v in 0.9% saline for both the sensitizing and challenge doses.

For the positive control with DNCB (dinitrochlorobenzene), a concentration of 0.3% DNCB w/v in 80% ETOH for the sensitizing doses, and 0.1% w/v in acetone for a challenge dose was used.

Guideline Reference No. 81-6

The delayed hypersensitivity test was actually a delayed contact hypersensitivity potential test. All of the challenge and sensitizing applications were 0.4 mL.

Hair was removed from the back of each animal prior to testing. Four-tenths mL of appropriate test material was placed on an adhesive pad, and the pad placed on a skin test site and maintained in place with occlusive dressing for a period of 6 hours, after which the dressing application pad was removed.

The test chemical and positive applications were similarly applied.

All animals (excepting untreated controls) received one application per week, for a total of three applications. Two weeks following the third sensitizing application a challenge dose of 90% w/v test material in 0.9% saline was administered to the flank opposite to the sensitizing doses of all test animals, and at the same time, all of the untreated control animals received the same challenge dose.

The positive control animals were similarly challenged with a 0.1% w/v suspension of DNBC in acetone.

All application and challenge sites were examined for dermal irritation at 24 and 48 hours posttreatment according to the Buehler method[1].

The Buehler sensitization scoring scale:

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without erythema	3.0

Results:

1. Test animals (Kryocide). One animal showed very faint erythema during sensitizing phase; 0.5 score. No reactions to challenge applications.

2. Positive controls (DNCB). All animals received a score of 2.0 (moderate erythema), which was considered positive.

3. Untreated controls (challenged). No reactions.

1/ Buehler, E.V.; Ritz, H.L. (1980) Planning, conduct, and interpretation of guinea pig sensitizing patch tests. Current concepts in cutaneous toxicity. Page 28.

Note: All animals on test appeared normal throughout the study. Body weights throughout the study were normal, except one of the untreated control animals, "which showed a 7 gram weight loss during the last 3 days of the study."

Conclusions: Kryocide was not found to be a sensitizing agent.

Classification: Core Minimum Data

007009

Guideline Reference No. 84-2

Confirmation of Conclusions in the DER

Study Type: Salmonella/Microsomal Mutagenicity Assay of Kryocide Technical.

Primary Reviewer: Dr. Irving Mauer

Secondary Reviewer: W.J. Burnam; Accession No. 071392
Dr. Albin Kocialski; Accession No. 250968

Accession Nos.: 071392, 250968, MRID No. 129113

Test Material: Kryocide, 96.0% pure

Study No.: T-1693-102

Testing Facility: Science Applications, inc.

Report Issued: August 1, 1983

Conclusion:

Additional data and final report remendations satisfy the deficiencies listed in the original review (March 14, 1983) and the study is now acceptable.

Classification: Acceptable study

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William S. Woodrow 12/16/87

Supervisors Signature: Albin B. Kocialski 12/16/87

March 23, 1983, Accession No. 071392, Salmonella/Microsomal Mutagenicity (Ames) Assay of Kryocide Technical.

Classification: Unacceptable (a repeat test was not performed, no toxicity testing was reported, and chemical characterization of the test material was not included).

July 12, 1985, Accession No. 250968

Salmonella/Microsomal Mutagenicity Assay (Ames) of Kryocide Technical.

"Additional data and final report emendations satisfy the deficiencies listed in original review (March 14, 1983) and study is now acceptable."

Guideline Reference No. 84-2

Confirmation of Conclusions in the DER

Study Type: In Vivo Cytogenetic Evaluation of Kryocide Technical

Primary Reviewer: Irving Mauer

Secondary Reviewer: W. J. Burnam; Accession No. 071392
Albin Kocialski; Acession No. 250968

Accession No.: 071392 (March 23, 1983) MRID No. 128115
250968 (July 12, 1985)

Test Material: Kryocide, 96.0% pure

Study No.: T-1693-112

Testing Facility: Microbiological Associates

Report Issued: October 12, 1981

Conclusion:

Additional data and reevaluation of original review (March 15, 1983) satisfy requirements of an adequate test, and thus this study is considered acceptable.

Classification: Acceptable study

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William A. Woodside 12/16/87

Supervisors Signature: Albin B. Kocialski 12/16/87

March 23, 1983, Accession No. 071392; In Vivo Cytogenetic Evaluation of Kryocide Technical

Classification:

Unacceptable: Test chemical not characterized; solubility, nature and conc. of impurities not stated; it is possible that insufficient dosage may have been administered.

July 12, 1985; Accession No. 250968; In Vivo Cytogenetic Evaluation of Kryocide Technical

"Additional data and re-evaluation of original review (3/15/83) satisfy requirements of an adequate test, and thus this study is considered acceptable."

007009

Guideline Reference No. 84-2

Confirmation of Conclusions in the DER

Study Type: DNA Repair Evaluation of Kryocide Technical

Primary Reviewer: Irving Mauer

Secondary Reviewer: W. J. Burnam; Accession No. 071392
Albin Kocialski; Acession No. 250968

Accession No.: 071392 (March 23, 1983) MRID No. 128115
250968 (July 12, 1985)

Test Material: Kryocide, 96.0% pure

Study No.: T-1693-104

Testing Facility: Microbiological Associates

Report Issued: September 21, 1981

Conclusion:

Additional data and revisions in amended final report satisfy deficiencies listed in original review (March 15, 1983) satisfy requirements of an adequate test, and this study is now considered acceptable.

Classification: Acceptable study

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William J. Woodrow 12/16/87

Supervisors Signature: Albin B. Kocialski 12/16/87

March 23, 1983, Accession No. 071392; DNA Repair Evaluation of Kryocide Technical

Classification:

Inconclusive (the highest dose may not have been used, solubility data were not provided, and chemical characterization of the test material was not included).

July 12, 1985; Accession No. 250968; DNA Repair Evaluation of Kryocide Technical

"Additional data and revisions in amended final report satisfy deficiencies listed in original review (March 15, 1983) and the study is now acceptable."

Guideline Reference No. 82-1

Confirmation of Conclusions in the DER

Study Type: 90-Day Dog Feeding

Primary Reviewer: William S. Woodrow

Secondary Reviewer: Albin Kocialski

Accession No. (MRID No.): 262371

Test Material: Cryolite Batch No. 8401, 97.3% pure

Study No.: WIL-75007

Testing Facility: WIL Research Laboratories

Report Issued: January 1986

Conclusion:

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 (HDT), based on decreased body weight, body weight gain, food consumption, and hematology. No NOEL for fluoride accumulation in bone. LEL for fluoride accumulation in bone was 500 ppm (LDT).

Classification: Supplementary Data

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William S. Woodrow 12/16/87

Supervisors Signature: Albin B. Kocialski 12/16/87

007009

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

DATA EVALUATION REPORT

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

Accession Number: 262371 MRID NO.: 157999

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	Nitrate
	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 subjected 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.

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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.

Guideline Reference No. 82-1

Confirmation of Conclusions in the DER

Study Type: 90-Day Subchronic Rat Study

Primary Reviewer: William S. Woodrow

Secondary Reviewer: Albin B. Kocialski

Accession No. (MRID No.): 262372

Test Material: Kryocide, 96.0% pure

Study No.: 6120-100

Testing Facility: Hazleton Laboratories American, Inc.

Report Issued: November 27, 1983

Conclusion:

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: Supplementary Data

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William S. Woodrow 12/16/87

Supervisors Signature: Albin B. Kocialski 12/16/87

007009

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 MRID NO.: 0015800

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)	50,000	50	50
including 128-day recovery: 10/sex (basal diet after 100 days treatment)	0	30	30
5. Sentinel*			

*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 Stablity	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)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count	X	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		Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase		Total protein
	Cholinesterase	X	Triglycerides
	Creatinine phosphokinase		Fluoride
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		

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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	-	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	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		X		X	
	Digestive system		Cardiovasc./Hemat.		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		Grandular
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
		X	Cervix		and masses

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	Male				Female			
Group	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 rate 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 leumens were not 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, micro-abscesses, 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 acinar atrophy of the pancreas.

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

	Males				Females			
Groups	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, regenerate 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 livers 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 Data	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	-	2.5	33.8	69.7	
	SD	-	-	13.75	34.83	
	N	-	1	10	10	
90	Mean	3.1	3.7	22.6	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 Values

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	-	-	-	-	190.92
	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	5352.88	
	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	1157.83	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		Fluoride (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	-
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	-

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

Day of Study		Fluoride (ppm) 0	50	5000	50,000	Recovery
Males						
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

Day of Study		Fluoride (ppm) 0	50	5000	50,000	Recovery
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 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 quantative 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/

Guideline Reference No. 83-3

Confirmation of Conclusions in the DER

Study Type: Teratology Evaluation of Kryocide, Rats

Primary Reviewer: William S. Woodrow

Secondary Reviewer: Albin B. Kocialski

Accession No. (MRID No.): 250968

Test Material: Kryocide, Lot NB No. 86-11-9

Study No.: 1182008

Testing Facility: Science Applications, Inc.
Division of Toxicology

Report Issued: August 1, 1983

Conclusion:

Kryocide insecticide did not demonstrate a teratogenic potential, when tsted at 750, 1500, or 3000 mg/kg in rats. Whitening of teeth in treated dams was the only change in either dams or fetuses.

NOEL for maternal and fetotoxicity is 3000 mg/kg body weight (HDT).

Classification: Core Minimum Data

This reviewer is in general agreement with the conclusions of the DER.

Reviewer Signature: William S. Woodrow 12/16/87

Supervisors Signature: Albin B. Kocialski 12/16/87

Review of a Final Kryocide Teratology Study by Woodrow. The Preliminary Report was Reviewed (as mentioned above) by Gary Burin, March 22, 1983.

Final Report for a Teratology Study of Kryocide Insecticide in Albino Rats; Sponsor: Pennwalt Corp. Tester: Science Applications, Inc., Division of Toxicology. Study No. 1182008, August 1, 1983

Test Material:

Kryocide Insecticide, Lot NB No. 86-11-9; sodium fluoaluminate.

Control material - carboxymethyl-cellulose (CMC) - Lot No. 8M04, dissolved in distilled water to form a 0.2% solution. The CMC was refrigerated between use periods.

Dose levels of Kryocide were suspended in 0.2% CMC on a weight/weight basis, and were prepared for 2 and 4 day periods before new batches were made ready. Prepared suspensions were stirred at least 5 minutes prior to dosing, and were stirred continuously throughout dosing using a magnetic stirrer. The formulated doses were refrigerated between use periods.

Samples of the dose levels were taken on the first and last days of formulation both for determination of homogeneiety and test material concentration.

Thirty female rats, 73 days of age per each of 0, 750, 1500, and 3000 mg/kg dose levels were bred to sexually mature male rats following a 7-day acclimation period, and were dosed by oral gavage, following vaginal smear for sperm and/or vaginal plug evidence of breeding. The day that breeding evidence was established was considered day 0 of gestation.

The pregnant female rats were dosed as described above at 10 mL/kg body weight on days 6 through 19 of gestation.

Food and water were available ad libitum. The dose levels chosen were based upon the results of a prior dose range-finding study. All study dams were observed for mortality 2 x daily, and were observed for feces and urine output and condition or activity at least once daily. Females showing signs of premature birth were sacrificed on the day of observation.

Body weights were recorded on days 0, 6 through 19 of gestation and prior to laparohysterectomy on day 20 of gestation.

Dams were sacrificed on day 20 of gestation by CO_2 asphyxiation.

At necropsy, the thoracic and abdominal cavities were examined for gross lesions. The ovaries were examined and the number of corpora lutea were recorded. The uterus was opened and the number and distribution of live and dead fetuses in each uterine horn was recorded. Each fetus was examined for external anomalies; individual fetal body weights and sex were recorded. Approximately one-half of the fetuses from each litter were randomly selected for decapitation and later head examination for internal changes following fixing and cross-sectioning in 1 mm sections.

Fetuses selected for head examinations and the remaining fetuses from each litter were subject to fresh tissue visceral examinations. The umbilical blood vessels, stomach, spleen, pancreas, liver, and disphragm were observed for size, shape and location. The fetal sex was verified and the kidneys, adrenal glands, uretus and urinary bladder were examined for size, shape and location. All abnormalities were recorded.

The fetal thoracic cavity contents examination included heart blood vessels, heart valves, lungs, trachea, esophagus. Abnormalities were recorded and atypical tissues were removed for further examination.

Each fetus was fixed in 70% ETOH and stained with alizarin prior to skeletal examinations. Bones of the neck region, the sternum, pectoral girdle, ribs, extremities, pelvic girdle, and the vertebral column were examined. Bones not fully ossified, absent, misshapen, fused, bifurcated, malaligned, or of unusual size were described and recorded.

Statistical tests were conducted on a DEC-10 computer using standardized statistical programs from the Statistical Package for the Social Sciences[1] or a Fortran Applications Program for the Gladen[2], and Modified Jonckheere[3] Analyses.

The dam weight gain was analyzed using a two-way analysis of variance. The number of implantations, number of corpora lutea and litter size were analyzed using one-way ANOVA. Live fetal body weights were analyzed using an Analysis of Covariance with litter size as the Co-variant. Embryo-lethality (no. dead + no. resorbed no. implantations) was analyzed using the modified Jonckheere test and Kruskal-Wallis[4] test; malformations and

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1 Nie, N. H., Jenkin, C. H., Steinbrenner, K. and Bert, D. H. Statistical Package for the Social Sciences, 2nd Ed., McGraw Hill Book Co., 1975.

2 Gladen B. The Use of the Jackknife to Estimate Proportions from Toxicological Data in the Presence of Litter Effects, J. Am. Stat. Assoc., 74, 278-283, 1979.

3 Lin, F. O. and J. K. Hasseman. A Modified Jonckheere Test Against Ordered Alternatives when Ties are Present at a Single Extreme Value, Biomed. Z Bd, 18, 623-631, 1976.

4 Conover, W. J., Practical Nonparametric Statistics, John Wiley and Sons, Inc., p. 256, 1971.

variations were analyzed using the Kruskal-Wallis and Gladen tests.

Results

A. Maternal Effects

Clinical observations - The only clinical observations were confined to three dams: No. 48 dosed at 750 mg Kryocide/kg delivered easily on day 20 of gestation. No. 74 dosed at 1500 mg/kg exhibited respiratory congestion on day 16-20 of gestation. No. 102 dosed at 3000 mg/kg displayed a porphorin discharge from both eyes, unthrifty fur, was hypoactive and moribund; this animal was removed from the study on day 11 of gestation and was subjected to necropsy.

The only clinical sign attributable to Kryocide treatment was whitening of dam tooth enamel for all of the treated animals (750, 1500, or 3000 mg/kg) beginning on day 16 or 17 of gestation. None of the described clinical signs (excepting teeth whitening) appeared to be treatment-related. Control dam teeth were unaffected.

A variety of lesions were observed in control and treated dams during laparohysterectomy, following CO_2 sacrifice:

Two control animals showed right or left hydronephrosis, and one control animal displayed purulent material in the right lung lobe.

One dam dosed at 750 mg/kg displayed a whitish-green nodule in a diaphragmatic lung lobe.

Two dams dosed at 1500 mg/kg showed hydronephrosis of right or left kidneys, and a third animal showed mottling throughout the lungs, the left lung was absent, the right lobe was small and contained white purulent material.

Three dams dosed at 3000 mg/kg displayed right or left kidney hydronephrosis. One dam dosed at this rate was necropsied on day 11 of gestation (in a moribund state), and showed stomach-scattered pinpoint hemorrhages; the cause of this animal's illness could not be determined. None of the lesions observed at necropsy or terminal laparohysterectomy appeared to be treatment related.

Quoted from the tester's report:

Table 1
SUMMARY OF MATERNAL DATA IN RATS
ADMINISTERED KRYOCIDE® INSECTICIDE

Dose Level (mg/kg)	Control	750	1500	3000
Number of Dams Pregnant/ Number Sperm Positive (%)	29/30 (96.7)	28/30 (93.3)	26/30 (86.7)	30/30 (100)
Mortalities (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Actual Mean Body Weight Change (g)[a]	89.1 ±17.84*	92.1 ±22.69	90.8 ±21.18	90.3 ±15.71
Corrected Mean Body Weight Change (g)[b]	30.7 ±13.51	28.5 ±17.11	26.9 ±12.26	25.3 ±13.95
Mean Number of Corpora Lutea	10.7 ±1.87	11.2 ±1.39	11.3 ±2.36	11.0 ±1.52

* Data accompanied by plus or minus one standard deviation.
a Day 20 weight (grams) - Day 6 weight (grams).
b Day 20 weight (grams) - Day 6 weight (grams) - gravid uterus weight (grams).

End of quotation.

An analysis of variance applied to the dam weights at day 6 of gestation and at cesarean section (day 20 of gestation) showed no significant effects were observed for weight gain during pregnancy between the control and treated groups (p = .894). No significant differences in numbers of corpora lutea were determined when analyzed statistically (p=.381). Only one dam was removed from the study due to moribund condition; all other control and test animals survived to experimental term.

B. Prenatal Effects

A summary of prenatal effects prepared by the tester is shown below:

TABLE 2

SUMMARY OF PRENATAL DATA IN RATS ADMINISTERED KRYOCIDE INSECTICIDE

Dose Level (mg/kg)	Control	750	1500	3000
Number of Dams Pregnant	29	28	26	30
Number of Litters Examined (Fetuses)	28[a] (278)	27[b] (285)	26 (275)	29[c] (311)
Number of Implantations	300	300	286	325
Mean Number of Implantations/Litter	10.3 ± 2.42*	11.1 ±1.69	11.0 ± 2.95	11.2 ±1.47
Percent Live Fetuses[d]	92.7	95.0	96.0	95.7
Mean Number of Live Fetuses/Litter	9.6 ±3.24	10.6 ±2.12	10.6 ±2.89	10.7 ±1.49
Mean Fetal Weight (g) All:	3.8 ±0.58	3.8 ±0.30	3.8 ±0.28	3.8 ±0.31
Males:	3.9 ±0.58	3.9 ±0.31	3.9 ±0.29	3.9 ±0.32
Females:	3.8 ±0.59	3.6 ±0.31	3.7 ±0.26	3.7 ±0.28
Number of Resorbed Fetuses	22	15	11	14
Number of Dead Fetuses	0	0	0	0
Percent Dead and Resorbed Fetuses	7.3	5.0	3.8	4.3
Mean Sex Ratio (M:F)	1.1:1.0	1.1:1.0	1.0:1.0	1.1:1.0

* Data accompanied by plus or minus one standard deviation.
a One dam had entire litter resorbed (number 15).
b One dam delivered early (number 43).
c One dam was removed from study on day 11 of gestation due to moribund condition (number 102).
d Percent of live fetuses calculated as the number of live fetuses divided by the number of implantations.

End of quotation.

No differences between control and test animals were found when various prenatal parameters were analyzed statistically:

a. The number of implantations in control and test groups were not significantly different; p=.521.

b. Litter sizes were not significantly different between control and test groups; p=.691.

c. Live fetal weights for control and test animals were not significantly different, at a p value of .370.

d. A statistical trend analysis to determine the possibility of a trend in embryolethality difference between control and test animals showed no significant differences; p=.354.

e. A test for embryolethality between control and test groups of animals, also showed no significant differences; p=.496 (embryolethality was defined as the number of dead fetuses plus the number or resorptions divided by the number of implantations).

C. Fetal Malformations and Variations

Table 3 below quoted from the tester's report, presents data as mean proportions to facilitate comparison of statistical tests calculated as proportions, versus the raw data:

Guideline Reference No. 83-3

Quoted from the tester's report:

Table 3

SUMMARY OF MEAN LITTER PROPORTIONS[a] OF MALFORMATIONS AND VARIATIONS IN RATS ADMINISTERED KRYOCIDE INSECTICIDE

Dose Level (mg/kg)	Control	750	1500	3000
Total Number of Litters Examined	28	27	26	29
External[b] Malformations	0.036 ±0.189[c]	0.016 ±0.060	0.005 ±0.025	0.007 ±0.027
Visceral[d] Variations	0.000 ±0.000	0.000 ±0.000	0.003 ±0.018	0.000 ±0.000
Skeletal Malformationa	0.006 ±0.023	0.027 ±0.091	0.000 ±0.000	0.009 ±0.046
Variations	0.177 ±0.210	0.110 ±0.192	0.152 ±0.166	0.130 ±0.185
Head Examinations Malformations	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000
Variations	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000
Other[e]	0.007 ±0.038	0.034 ±0.161	0.000 ±0.000	0.003 ±0.017

Table 3 (cont'd)

SUMMARY OF MEAN LITTER PROPORTIONS[a] OF MALFORMATIONS AND VARIATIONS IN RATS ADMINISTERED KRYOCIDE INSECTICIDE

Dose Level (mg/kg)	Control	750	1500	3000
Total Number of Litters Examined	28	27	26	29
Abnormal Fetuses[f]	0.042 ±0.189	0.044 ±0.143	0.005 ±0.025	0.016 ±0.065

[a] Mean litter proportions were derived by obtaining the proportion of viable fetuses per litter which had at least one malformation or variation, and then calculating the grand mean of these proportions for the entire group. Individual fetuses may have more than one type of malformation and/or variation.
[b] No external variations were observed in any dose level.
[c] All data accompanied by plus or minus one standard deviation.
[d] No visceral malformations were observed in any dose level.
[e] Specimens which were inadvertently damaged during dissection or processing and, therefore, were considered unsuitable for evaluation.
[f] All fetuses with one or more malformations.

End of quotation.

Table 3 footnotes state that no external variations or visceral malformations were seen at any dose level.

Table 3 indicates that two types of external fetal malformations were found in control and all treated group fetuses; the litter from control dam no. 7 contained all runt animals, and one fetus from a dam treated with 750 mg/ml displayed an umbilical cord hernia.

Visceral variations were confined to one fetus from a dam treated with 1500 mg/kg (left ureter - slight hydroureter).

One dam treated with 750 mg/kg contained a number of fetuses that displayed a number of skeletal malformations, and a number of fetuses from one dam treated with 3000 mg/kg showed absent sacral vertebrae. The thoracic centrum was absent in each of two fetuses from two untreated dams, and was also absent from two fetuses from one dam and in two fetuses from two different dams treated with 750 mg/kg. A classification of all malformations combined showed no significant differences existed between control and treated groups ($p = .331$).

Various kinds of skeletal variation were observed in both control and fetuses from treated dams, as indicated in Table 3; the most common variations included absent or incompletely ossified sternbrae. An examination of Table 3 shows that the occurrence of skeletal variations was greater in the control fetuses; statistical analyses indicated that no significant differences in skeletal variations existed between fetuses from control or treated dams ($p = .261$).

Conclusions:

Kryocide insecticide tested at 750, 1500, or 3000 mg/kg in rats did not demonstrate a teratogenic potential, and was not shown to be fetotoxic. A whitening of the dam teeth in treated animals was the only change in either dams or fetuses that was attributable to Kryocide treatment.

No significant differences between control and treated dam body weight gains, or numbers of corpora lutea were found.

Parameters of prenatal comparisons between control and treated dams showed no statistically significant differences, including: number of implantations, mean number of implantations per litter, the percent of live fetuses, the mean number of live fetuses per litter, all mean fetal weights, the number of resorbed fetuses, the number of dead fetuses, the percent of dead and resorbed fetuses, and the mean male and female sex ratio.

No statistically significant differences were found to exist between litters from control or treated dams for external, visceral, skeletal or head examinations for malformations or variations.

The NOEL for maternal and fetotoxicity is 3000 mg/kg body weight (HDT).

Classification: Core Minimum Data

94845:R:I:Draft:Woodrow:C.Disk:KENCO:11/27/87:SG
R:88939:Kocialski:C.Disk:KENCO:12/07/87:EE:LF:CB

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