CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

CRYOLITE

Chemical Code # 000173, Tolerance # 00145
SB 950 # 173

November 17, 1986
Revised: 9/3/87; 7/6/88; 10/27/89; 7/17/91; 3/01/95; 11/15/95

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effects
Chronic toxicity, dog:	No data gap, no adverse effects
Oncogenicity, rat:	No data gap, possible adverse effect[1]
Oncogenicity, mouse:	No data gap, no adverse effects[1]
Reproduction, rat:	No data gap, no adverse effects
Teratology, rat:	No data gap, no adverse effect
Teratology, Mouse	No data gap, no adverse effects
Gene mutation:	No data gap, no adverse effect

Chromosome effects: No data gap, no adverse effect2

DNA damage: No data gap, no adverse effect

Neurotoxicity: Not required at this time

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1 Based on a NTP study using sodium fluoride (CC: 537) as the test material.
2 Studies with sodium fluoride gave positive results for chromosomal effects.

All record numbers through 141548 (Document No. 145-047) submitted for SB-950 to support cryolite were examined. This includes all SB-950 studies indexed as of 11/08/95.

In the 1-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T951115

Summary of Toxicology Data revised by Kishiyama & Davis, 7/6/88; M. Silva, 10/89; T. Kellner, 7/17/91; Aldous and Gee, 3/01/95, and Aldous, 11/15/95.

These pages contain summaries only. Individual worksheets may contain additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

CHRONIC TOXICITY, RAT

The chronic rodent toxicity study requirement has been addressed by considering the subchronic study (below) along with sodium fluoride rat and mouse oncogenicity studies, and cryolite dog subchronic and chronic studies. Aldous, 11/15/95.

SUBCHRONIC, RAT

**145-043 139149 [U.S. EPA data review (DER) completed on 12/16/87, W.S. Woodrow (Primary reviewer), submitted as supporting information for Document No. 145-022, Record No. 043859, entitled "Subchronic Toxicity Study with Kryocide Insecticide in Rats"]. Dr. Woodrow's review classified the report as "Supplementary Data". Evidently the only reason that the study was not classified as "core minimum" data was the lack of a NOEL for accumulation of fluoride in bone (see p. 46 of this volume = p. 1 of the DER, also p. 17 of the DER). The essential data from the original report plus supplements are presented in this 1995 DPR review. CD rats were fed diets containing 0, 50, 5000, or 50000 ppm cryolite (96%) for at least 13 wk: about 10/sex/group were used for bone fluoride analyses, and about 30/sex/group were killed at termination for histopathology. An additional 10/sex were dosed for at least 13 wk, then maintained off treatment for 4 wk before sacrifice (recovery groups). The NOEL for toxicity is 50 ppm, based on (1) stomach lesions in both sexes, including epidermal hyperplasia and hyperkeratosis/acanthosis in the nonglandular portion, and submucosal lymphoid foci, mucosal atrophy, and chronic submucosal inflammation in the glandular portion, (2) basophilic granules in incisors (males), (3) hematology changes in both sexes (reduced HCT and Hb), and (4) clinical chemistry changes: reduced total protein (both sexes), and reduced albumin and inorganic phosphate levels (males). High dose-recovery groups indicated substantial recovery from the stomach lesions, and there was no residual hematology or clinical chemistry effect. Incisors were not examined microscopically in recovery rats. Absorption of fluoride from cryolite reached a plateau over the dose range of 5000 to 50000 ppm. NOTE: since the LEL is 100-fold higher than the NOEL, rodent oncogenicity studies on sodium fluoride (NTP Technical

Report No. 393) and the dog chronic study on cryolite (Study No. WIL-75033, Record No. 119207) are better studies for margin of safety evaluation. Data gap for rodent chronic study is filled, noting that U.S. EPA has determined that there are sufficient rodent chronic data available, and that this study, in conjunction with the above studies, adequately characterizes subchronic and chronic toxicity (see Discussion in this 1995 review). Study is reclassified as **not** indicative of a "possible adverse effect", considering the overall low toxicity, reversibility of major endpoints, apparent non-applicability of main focus of lesions (junction of forestomach and glandular stomach) to human anatomy, and availability of extensive human data on fluoride regarding effects on teeth and bones. **Acceptable (also completing rat chronic study requirements), with no adverse effects**. McGee, 9/26/86; Kishiyama and Davis, 7/5/88; and Aldous, 11/08/95.

145-022 043859 This is the original report of the Hazleton rat subchronic study. Please see updated 1-liner under Record No. 139149, above.

145-028 065012 Preliminary histopathology data on stomachs and incisors from 145-022 043859, above.

145-029 067215 Final Report Amendment No. 1 (histopathology data on stomachs and incisors from 145-022 043859, above.

145-019 043856 "The Registrant’s Justification for Exemption from Tolerance Submitted in Conjunction with Data Required under the Cryolite Registration Standard of June 28, 1983", dated March 1986.

CHRONIC, DOG

**145-041 119207 Tompkins, E.C., "One year dietary toxicity study in dogs with Kryocide", WIL Research Laboratories, Inc., Ashland, Ohio, 10/29/92. Study No. WIL-75033. Cryolite (designated by trade name Kryocide in report), purity 97.3-97.4%, was administered in diet at

concentrations of 0, 3000, 10000 or 30000 ppm to 4 beagles/sex/group for 52 weeks. Emesis shortly after eating was the only common clinical sign. Body weight decrements at 30000 ppm were transient in high dose females, and persistent in high dose males. There were no mortalities. No NOEL was established. Kidney changes (renal tubular regeneration, interstitial fibrosis, and tubular dilatation) were seen in most 10000 to 30000 ppm dogs. One or several of these changes were found in one male and one female at 3000 ppm, evidently a treatment effect. The kidney microscopic data are suitable for dose-response evaluations despite lack of a definitive NOEL. Signs of anemia were evident at 10000 to 30000 ppm, including a progressive decline over time in the standard RBC parameters, and a general increase in the numbers of immature or irregular RBC's. A related extramedullary hematopoiesis was found in liver and spleen at these dose levels. **Acceptable, with no adverse effects**. Kishiyama and Aldous, 2/6/95.

145-043 139150. EPA data review by W. Greear for Record No. 119207, above. Study was classified as "Core Minimum" (acceptable). As in the DPR review, the study was acknowledged not to have a NOEL, nevertheless meaningful dose-response relationships exist for all notable effects. There is no need for a DPR worksheet for this review. Aldous, 11/07/95.

SUBCHRONIC, DOG

021 043858 "90-Day Dietary Study in Dogs with Kryocide" (WIL, Report # WIL-75007, 1/18/86) Cryolite (97.3%) at 0, 500, 10,000 or 50,000 ppm fed to 6 (8 high dose)/sex/group, interim sac @ 0, 45 days and 28 day recovery period (high dose only). Approximate mean achieved dose levels were 17, 368, and 1692 mg/kg/day. No adverse effect-Decreased RBC, Hb, Hct, and RBC morphology changes at 50,000 ppm (extremely high-5% of diet). Plasma, urine and bone fluoride concentrations were dose related. Partial recovery during recovery period, except for sternal fluoride. NOEL = 10,000 ppm (368 mg/kg/day). Reviewed as ACCEPTABLE (McGee 9/26/86); Supplemental Study (Kishiyama & Davis 7/6/88)

020 043857 "28 Day Dietary Study in Dogs with Kryocide" (WIL, Report # WIL-75010, 7/25/85) Cryolite, (97.3%) fed at 0, 500, 10,000, 50,000 or 60,000 ppm for 28 days, 1 dog/sex/dose. Effect: 50,000 ppm female lost weight, had a decrease in food and water consumption, 60,000 ppm female was comparable to control. Urine and plasma fluoride levels increased in a dose-related manner though 50,000 ppm female value exceeded those of the 60,000 ppm female. McGee 9/24/86.

ONCOGENICITY, RAT

DPR has determined to consider the following **sodium fluoride** oncogenicity study (Record No. 111528) in support of rat and mouse oncogenicity data requirements for cryolite (consistent with U.S. EPA position), based on dispositional studies in humans and experimental animals. These studies showed a high level of metabolism of cryolite to free fluoride (see the Cryolite Summary of Toxicology Data). See memorandum of J. Gee to O. Melnicoe dated 6/21/94 for details. Aldous, 2/28/95.

****145-039 111528** [RAT, see also separate review of mouse data under same record number] "NTP technical report on the toxicology and carcinogenesis studies of sodium fluoride (CAS No. 7681-49-4) in F344/N rats and B6C3F1 mice (Drinking Water Studies)", Battelle Columbus Laboratories, Dec., 1990. NTP Technical Report No 393. Sodium fluoride, purity 99%, was administered in the drinking water at concentrations of 0, 25, 100, or 175 ppm to 70-100 F344/N rats/sex/group for up to 2 years. This study did not seek, nor did it achieve, a NOEL for the most commonly recognized chronic effect of excess fluoride (dental fluorosis). Mottling and occasional frank discoloration of teeth occurred at all levels tested. Treatment-induced dysplasia of incisor dentine was observed microscopically at all dose levels in males and females. A general increase in osteosclerosis was observed in 175 ppm females, providing a "NOEL" for non-neoplastic effects (other than on dentition) of 100 ppm. Several bone osteosarcomas were noted in 100 ppm males (1/50) and 175 ppm males (3/80), which are considered "equivocal evidence" of oncogenic activity. The study is **acceptable** as an

oncogenicity study, with minor deviations from FIFRA guidelines. The bone-derived osteosarcomas indicate a **"possible adverse effect"**. Kishiyama and Aldous, 2/8/95.

145-039 111527 J.K. Maurer, M.C. Chang, B.G. Boysen, and R.L. Anderson, "Two-year carcinogenicity study of sodium fluoride in rats" [report submitted in support of cryolite data requirements]. Conducting laboratory not stated: author affiliations were Proctor and Gamble Co., Cincinnati, OH and Hazleton Laboratories America, Inc., Madison, WI. Report is from J. Nat. Cancer Inst. 82 (13), 1118-1126 (1990). Sodium Fluoride, >99% purity, was mixed with low-fluoride control diet at 0, 4, 10, or 25 mg/kg and fed to 70 Sprague-Dawley rats/sex/group for up to 95 weeks (males) or 99 weeks (females). Additional rats, fed untreated commercial rodent chow, served as a second control for this study. Interim sacrifices of up to 10/sex/group were scheduled at 26 and 53 weeks. Dose-related changes without NOELs were found for dental fluorosis or for bone changes (based on subperiosteal hyperostosis in cranial bone). No oncogenicity was indicated. Study is **unacceptable and not upgradeable, with no adverse effects indicated**. Kishiyama and Aldous, 2/16/95.

ONCOGENICITY, MOUSE

See paragraph under "Oncogenicity, Rat" regarding use of sodium fluoride NTP drinking water studies in support of cryolite oncogenicity data requirements. Aldous, 2/28/95.

**145-039 111528 [MOUSE, see also separate review of rat data under same record number] "NTP technical report on the toxicology and carcinogenesis studies of sodium fluoride (CAS No. 7681-49-4) in F344/N rats and B6C3F1 mice (Drinking Water Studies)", Battelle Columbus Laboratories, December 1990. NTP Technical Report No 393, December 1990. Sodium fluoride, purity 99%, was administered in the drinking water to B6C3F1 mice at 0, 25, 100 or 175 ppm. Eighty mice/sex were assigned for a 2-year term at 0 and 175 ppm, as were 50/sex of 25 and 100 ppm groups. An additional 10/sex/group/interval were assigned to interim sacrifice groups at weeks 24 or 66. No NOEL was found for the expected response, dental fluorosis (increases in mottled or discolored teeth were found at all dose levels). The "NOEL" for other findings was

100 ppm: elevated serum alkaline phosphatase activities were found in high dose males and females. The study is **acceptable** as an oncogenicity study, with minor deviations from FIFRA guidelines. No adverse effects are indicated. Kishiyama and Aldous, 2/14/95.

REPRODUCTION, RAT

145-042 129131 Schroeder, R.E., "A two-generation dietary reproduction study in rats with Kryocide (cryolite)", Pharmaco LSR, Inc., Toxicology Services North America, East Millstone, NJ., Project # 90-3633. Report date of final revision: 9 March 1994. Cryolite, 96% purity, was administered in diets of 30 CD*-Crl: CD* (SD) BR rats per sex per group at 0, 200, 600, or 1800 ppm through two generations with 1 mating per generation. Overall mean achieved dose during the 14-week pre-mating periods was estimated to be 15, 45, and 138 mg/kg/day for the 200, 600, and 1800 ppm groups, respectively. There was no NOEL for parental rats (discoloration of incisors was dose-related at all levels). Excluding apparent dental fluorosis, a conservative parental NOEL = 600 ppm, based on minor body weight gain decrements in high dose F1 males. Reproductive NOEL = 600 ppm (reduced pup body weight gain at 1800 ppm during lactation, gross changes in weanlings such as pale kidneys, pale liver, and enlarged heart). Study was not accepted as originally submitted, however supplemental data in Record No. 141548 (below) led to an upgrade to **acceptable status. **No adverse effects are indicated.** H. Green and C. Aldous, 1/27/95; Aldous, 11/08/95.

145-047 141548 Schroeder, R., Addendum II to Record No. 129131, above. Date of present addendum: Oct. 9, 1995. The newly submitted data clarify methods for selection of F1 parental rats, and provide individual pup necropsy data for all pups culled at day 4 in both generations. The latter did not identify treatment effects. The reproduction study is **acceptable** as amended. No change in reported findings. Aldous, 11/08/95.

145-043 139151. U.S. EPA review of Record No. 129131, above. Study was classified as "Core Guideline Data", and identified the same NOEL's as the DPR review. Aldous, 11/08/95 (no worksheet).

TERATOLOGY, RAT

**025 059221 "Final Report for a Teratology Study of Kryocide Insecticide in Albino Rats" (Science Applications Inc., Study No. 1182008, 3/17/83) Kryocide (lot 86-11-9, purity of 97.6% from record # 59217 in 025) given by oral gavage to 30 per group at 0, 750, 1500, or 3000 mg/kg, days 6 - 19 of gestation, (SD) fBR rats; only clinical observation was whitening of the dams' teeth starting around day 16 or 17 of gestation; NO ADVERSE EFFECT no developmental toxicity or maternal toxicity reported; maternal and developmental NOELs $\geq$ 3000 mg/kg/day - FIFRA states that 1000 mg/kg/day is an acceptable high dose for relatively non-toxic chemicals. ACCEPTABLE. Gee 8/31/87
EPA adequate.

TERATOLOGY, MOUSE

145-040 112070 Nemec, M.D., "A developmental toxicity study of Kryocide* in mice", WIL Research Laboratories, Inc., 12/17/91. Cryolite, 97.3%, was administered by gavage in 0.5% aq. methylcellulose suspension to Crl:CD-1* (ICR)BR mice (25 pregnant dams/group) at dose levels of 0, 30, 100, or 300 mg/kg/day over days 6 to 15 of gestation. The apparent maternal NOEL = 30 mg/kg/day, based on dose-related mortality and indications of treatment-related lesions of the glandular stomach. The developmental NOEL = 100 mg/kg/day, based on bent ribs and bent scapulae. The study was classified as not acceptable, but upgradeable in the 2/1/95 DPR review. Cited deficiencies were that earlier studies (subsequently submitted as Record Nos. 139642 and 139628) had not been provided, yet would have bearing on interpretation of this study; and further that it was not clear that dosing suspensions were refrigerated between treatments. The latter was confirmed (see 1-liner below). Study is now classified as **acceptable. **No adverse effects are indicated**. Kishiyama and Aldous, 2/1/95; and Aldous, 11/01/95.

145-045 139642 Nemec, M.D., "Developmental toxicity study of Kryocide* in mice", WIL Research Laboratories, Inc. Study No. WIL-160004, Jan. 6, 1992. Kryocide*, purity of 97.3%, was administered via gavage at concentrations of 0 (0.5% Methylcellulose), 100, 300 or 1000

mg/kg/day to 30 mated Crl:CD-1* (ICR) BR mice/group during gestation days 6 through 15. Maternal toxicity NOEL = 100 mg/kg/day. Mortality was 40% and 10% for high and mid dose groups, respectively, with occasional necropsy reporting of "red stomach contents" or "reddened adrenals". Food consumption and body weight gains were reduced at 1000 mg/kg/day. Survival was too low at 1000 mg/kg/day to meaningfully assess treatment effects on fetuses, however a small increase in incidences of cleft palate and a single incident of open eyelid contributed toward a general increase in malformations in this group. There were no definitive developmental effects at or below 300 mg/kg/day, however a single incident of the variation "bent ribs" was considered an equivocal indication of a treatment effect, so that 100 mg/kg/day is the developmental NOEL. This study is not independently acceptable, however it provides useful data, and justifies dose levels used in the teratology study which followed (Record No. 112070). Kishiyama and Aldous, Nov. 1, 1995.

145-044 139628 Nemec, M.D., "A dose range-finding developmental toxicity study of Kryocide* in mice", WIL Research Laboratories, Inc. Study No. WIL-160003, 12/16/91. Dose levels of 0, 10, 30, 100, 300, and 1000 mg/kg/day were administered on p.c. days 6-15 to 8 Crl:CD-1* (ICR)BR mice/group. No clear developmental nor maternal toxicity was identified at any dose. Pregnancy rates were very low (2 groups with as few as 3 pregnant dams/group: not treatment-related), hence this pilot study was of limited utility for rangefinding. Dose levels used in Record No. 139642, above, were based on this study. This pilot study does not indicate "possible adverse effects", and has no apparent utility for hazard assessment. No DPR worksheet is needed. Aldous, 11/1/95.

[No record number, material has been inserted at the front of Document No. 145-044]: A 1-page FAX memo from M.D. Nemec of WIL Research Laboratories, Inc. to Gail Arce of Elf Atochem, dated July 12, 1995 confirms from study records that the developmental study (Record No. 112070) used dosing suspensions which were stored refrigerated between treatments. This addresses one of the two issues relating to acceptability of that study. Aldous, 11/1/95 (no worksheet is appropriate for this clarification).

TERATOLOGY, RABBIT

No study on file. Not required by EPA for Cryolite (1/17/89).

037 88779 Protocol: "Range-finding Developmental Toxicity Study of Kryocide in Rabbits" WIL Research Laboratories, Inc. Not reviewed. T. Kellner, 7/17/91.

037 88780 Protocol: "Developmental Toxicity Study of Kryocide in Rabbits" WIL Research Laboratories, Inc. Not reviewed. T. Kellner, 7/17/91.

GENE MUTATION

********** NOTE: FILENAMES FOR THE FOUR REVIEWS OF MUTAGENICITY STUDIES UNDER RECORD NO. 111528 HAVE BEEN SLIGHTLY CHANGED TO CONFORM TO DOS FILENAME CHARACTER LIMITATIONS. ********* ALDOUS, 10/22/96

** 025 059217, 059218 "Activity of Kryocide in the Salmonella/Microsomal Assay for Bacterial Mutagenicity," (Microbiological Associates, 9/29/81). Kryocide, 97.6% pure was tested in Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100, both with and without rat liver microsomal activation (Aroclor 1254-induced), at 0 (vehicle = DMSO), 0.05, 0.15, 0.50, and 5.0 mg/plate (triplicate). No adverse effect. No mutagenicity was observed at any dose level. It has been shown, however, that Salmonella is insensitive to metals such as fluorine. This study was previously reviewed as unacceptable (J. Gee, 8/17/87) due to the fact that only one trial was performed. In light of new Federal Regulations, in a negative study if sufficient toxicity is demonstrated and a limit test is utilized, there is no need to perform a repeat trial. Currently, this test is complete and ACCEPTABLE. M. Silva, 8/24/89.
EPA: inadequate (1/17/89).

CHROMOSOME EFFECTS

025 059220 "Activity of T1693 in the in vivo Cytogenetics Assay in Rodents" (Microbiological Associates, Study Number T1693.112, 10/2/81) Kryocide (96%) given by oral gavage to 5 males per group at 0, 0.6, 1.8, or 6.0 g/kg body weight/day for five consecutive days; sacrificed 4 hours after the last dosing; TEM as positive control; 50 metaphase cells scored per animal and the mitotic index determined for each animal; NO ADVERSE EFFECT on chromosomes is reported. Study is UNACCEPTABLE but possibly upgradeable with justification of using only males and submission of historical control data. Gee 8/28/87
EPA: inadequate (1/17/89).

** 145-038 92498 SanSebastian, J. "In Vitro Chromosome Aberration Analysis of Kryocide in Human Lymphocytes" (Pharmakon Research International, Inc., Report # PH 324-ANA-001-90, 3/18/91). Cryolite (Kryocide*, Sodium aluminofluoride), lot # 86-12, 97.3% purity was tested for chromosome aberration in mitogen (PHA) stimulated human donor lymphocytes in whole blood during the G_0/G_1, S and G_2 stages of the cell cycle with and without metabolic activation by Aroclor-1254 stimulated rat liver S-9 fraction with 2 cultures/dose/test condition/stage of cell cycle in 1 experiment (positive controls were evaluated at the S phase only); dose levels were 0 (untreated), 0 (solvent control), 100, 500 and 1000 µg/ml. No adverse effects were noted (no increase in the proportion of aberrant metaphases or total aberrations/cell at any dose level or at time interval tested); ACCEPTABLE. (Kellner and Gee, 7/11/91).

DNA DAMAGE

025 059219 "Activity of T1693 in a DNA Repair Test using Escherichia coli - Final Report" (Microbiological Associates, Study Number T1693.104, 9/21/81) Kryocide (96%) disk assay in Escherichia coli strains W3100/polA$^+$ and p3478/polA$^-$, with and without rat liver activation at 0, 0.1, 0.3, or 1.0 mg/disk in 20 ul DMSO, in triplicate, single trial. UNACCEPTABLE (no cytotoxicity in either strain = no test.) Gee 8/27/87
EPA: inadequate (1/17/89).

** 145-038 92499 SanSebastian, J. "Rat Hepatocyte Primary Culture/DNA Repair Test on Kryocide" (Pharmakon Research International, Inc., Study # PH 311-ANA-011-90, 3/18/91). Cryolite (Kryocide*, Sodium aluminofluoride), lot # 86-12, 97.3% purity was tested for unscheduled DNA synthesis (UDS) potential in vitro using cultured cells (10^5 cells/culture) from a male Fischer 344 rat liver with 3 cultures/dose at 0 (control), 1, 5, 10, 50, 100, 125, 250, 500 and 1000 µg/ml (the top five doses were not scored because of cytotoxicity). No adverse effects were noted (i.e. cryolite did not induce DNA damage in cultured rat liver cells at doses up to 50 µg/ml); ACCEPTABLE. (Kellner and Gee, 7/12/91).

NEUROTOXICITY

Not required at this time.

LITERATURE REVIEW (SUPPLEMENTAL)

028 065013 "Updated Literature Review. Fluoride Metabolism and Toxicity" This review is of minimal utility because it is unsigned and undocumented by references. No worksheet done. Davis 7/5/88

013 The document dated June, 1983, contains the EPA Registration Standard for this compound and identifies data gaps in all required toxicology study categories i.e., chronic toxicity, oncogenicity, teratogenicity (partial for rat), reproduction, and all mutagenicity study types.

Gaps in the data base precluded completion of the Agency’s risk assessment on this chemical. EPA had deferred its reassessment of this compound until essential data on residues and toxicology tests are available.

Reference to the wealth of available toxicological literature on fluoride is made including "other toxicity data previously submitted on cryolite, such as a teratology study in rats,...

and ...mutagenicity tests". CDFA now has these studies on file (September, 1987). Davis, 7/6/88.

031 This document, dated October 28, 1988 contains the results of the EPA assessment from the Reregistration Standard (May 1988) regarding mutagenicity studies (no further testing required) and rabbit teratology studies (based on negative findings in the rat studies, no further testing is required).

In addition, "Responses to Toxicological Issues From the Guidance For the Reregistration of Pesticide Products Containing Cryolite as the Active Ingredient (EPA Case Number 0087)" was included. EPA requires:

1. A new rat metabolism (pharmacodynamic) study must be performed in order to accurately quantify the amount of fluoride available from Cryolite so the reference dose (RfD) can be established.

   Pennwalt contended that Cryolite metabolism in animals and humans has been thoroughly described. Studies performed by Pennwalt (humans and rats) and studies in the literature indicated that fluoride retention from Cryolite should not exceed 80%. EPA agreed with Pennwalt's position that performing a new metabolism study would yield no new information. Currently, Pennwalt is submitting a summary of in-house and literature studies to fulfill the requirement for a metabolism study, in support of a waiver request. EPA responded positively to the proposal (Attachments A, 031 071324 & Attachment B, 032 070550).
2. The Agency also requires new chronic feeding, oncogenicity and reproduction studies. This decision was based upon the results of the subchronic rat feeding study (022 043859) which demonstrated stomach lesions at $\geq$ 5,000 ppm (NOEL).

   Pennwalt presents the argument that the stomach lesions were due to irritation of the stomach from the acidity of HF formed in the stomach. In support of this proposal, a literature summary was provided to demonstrate that the stomach lesions were not specific to cryolite, but were typical to fluoride (Attachment C, 032 070618). The Toxicology Branch of the EPA agreed that a chronic study using sodium fluoride would

be sufficient to meet chronic studies on cryolite. Currently, the National Testing Program is performing a chronic rat and mouse study with sodium fluoride, data from which could be used by Pennwalt to support their views. The data, however, are not completed and therefore are not available for review. Meanwhile, Pennwalt submitted a literature study performed by Tao and Suttie (University of Wisconsin, Madison), which demonstrates that sodium fluoride had no effect on reproduction in mice at 2 or 100 ppm (Attachment D, 032 070622).

METABOLISM

031 071324 "Cryolite Animal Metabolism," (Pennwalt Corporation, 10/28/88), Summary of in-house and literature studies on cryolite. Cryolite serves as a source of fluoride and is essentially metabolized as free fluoride. Released in aqueous solution, the fluoride is deposited primarily in teeth and bone. Teeth striations have been observed in rat (3 to 13 ppm fluorine in diet) and cryolite in new bone of rabbit mandible (12 to 50 mg fluorine/day). Osteomalacia was produced in sheep with cryolite (60 mg fluorine/day). These effects are in response to fluoride's interference with normal calcium metabolism. Cryolite hydrolyzes in vitro to produce fluoride anion instantaneously under acidic (pH 5: 15.5%), neutral (pH 7: 36.8%) or basic (pH 9: 43.3%) conditions. The same effect probably also occurs in vivo, based upon the rapid assimilation into the bone as well as it's efficient membrane permeability. Hydrolysis products of cryolite may re-associate to form similar salts. Differences in acute toxicity between cryolite and other fluoride salts is potentially due to solubility. Inefficient absorption occurs at levels necessary to cause acute fluoride toxicity symptoms. Chronic effects, however, are similar to those produced by simple fluoride salts. M. Silva, 8/22/89.

031 071325 "The Comparative Toxicity of Fluorine in Calcium Fluoride and in Cryolite," (University of Illinois, 3/29,39). Cryolite (synthetic product), marketed as an insecticide, consisted of 47% fluorine and calcium fluoride were fed in diet and drinking water to albino rats (10 females and 14 males/treatment group) at 0.58 mg/kg for 14 weeks. Several of the

rats, irrespective of treatment groups showed hematuria lasting 1 or 2 days in the first week. Striations in tooth enamel began to appear during the 8th week of treatment and were visible in all rats by the end of the 10th week. Data demonstrate that the action of fluorine from cryolite is indistinguishable from that of calcium fluoride when both are administered in aqueous solutions at the rate of 0.58 mg/kg daily. Approximately 96% of the fluorine retained (13 ppm in food) is deposited in the skeleton, while the rest is equally divided between teeth and soft tissues. M. Silva, 8/10/89.

031 071326 "A Comparison of the Toxicity of Fluorine in the Form of Cryolite Administered in Water and in Food," (University of Illinois, Urbana, 3/29/39). Albino rats (10 females and 2 males/group) were fed cryolite in diet (10 ppm fluorine) or administered in solution (9.1 mg cryolite/l) for 19 or 20 weeks. Hematuria was observed in animals receiving cryolite in drinking water. Growth was slowed due to frequent food refusal. Refusal was twice as frequent for rats receiving cryolite solution than for those with cryolite in diet. However there was no effect upon the method of fluorine administration upon body growth or appearance of tooth striations. A greater concentration of fluorine was found in bone, teeth and soft tissues of all rats receiving cryolite in water. Retention of fluorine was 18.6% less for rats fed cryolite in diet versus solution. This is apparently due to decreased absorption in the alimentary tract. When low doses of fluorine are continually ingested, there is a smaller percentage retention in the body. The authors considered 1 ppm fluorine in drinking water (upper limit of safety) to be the equivalent of 2.4 to 4.8 ppm of fluorine in total food (depending upon the amount of water intake with the critical fluorine concentration). M. Silva, 8/10/89.

031 071327 "Adaptation of the Growing Rat to the Ingestion of a Constant Concentration of Fluorine in the Diet," (University of Illinois, 1/8/40). Albino rats were fed fluorine (as cryolite) in diet at 4, 6.5 and 12.5 ppm (1 male and 2 females/group) for 22-32 weeks, depending upon respiratory impairment. Samples of urine and feces were collected after 27, 56 and 70 days of feeding. Collection periods lasted from 6 to 14 days. At termination, body length, empty weight (of carcass) and fluorine content of skeleton, teeth and soft tissue were determined. Results showed that growing rats adapt themselves to continuous ingestion of low

levels of fluorine by increasing their excretion of fluorine in feces and urine. Rate of adaptation decreases with time but maximum adaptation is 60 to 100% in proportion of ingested fluorine eliminated by kidney and intestine. The greater the proportion of fluorine in the consumed food, the lesser the efficiency of adaptation. As fluorine consumption increases, the concentration deposited in teeth becomes greater than in bone. M. Silva, 8/11/89.

031 071328 "The Comparative Assimilation of Fluorine by Growing Rats During Continuous and Intermittent Dosage," (University of Illinois, 7/2/40). Twelve pairs of littermate rats (sex/group not specified) were fed 1.4 ppm fluorine in diet for approximately 20 weeks. Additionally, one rat/pair was administered 1 ml of aqueous solution containing cryolite (18 ppm fluorine)/3 grams of food. Every 3rd day the other rat was provided, in diet, with as much cryolite as its pair mate received from aqueous dosage, so that the total intakes of fluorine by pair mates was equalized every three days for the total 3 day period. When fluorine is continuously administered as synthetic cryolite to growing rats, there is a greater retention of fluorine in bones and possibly teeth than when cryolite is administered intermittently (in the same quantity). Regarding fluorine effects in humans, where variable amounts are sprayed on foods which are consumed intermittently, the effects may be less than predicted from experiments where fluorine administration is continuous. M. Silva, 8/11/89.

031 071329 "The Effect of Dietary Calcium and Phosphorus on the Assimilation of Dietary Fluorine," (University of Illinois, 1/3/41). Experiment I: Growing albino rats, 12 pairs of littermates (unspecified sex; average weight = 120 g) and 8 pairs of younger littermates (44 g) were fed in diet cryolite (9.4 ppm fluorine, 0.23% calcium). One rat/pair received this low calcium ration (Ca:P = 0.44 to 1), while the other rat received a diet containing 0.73% calcium (Ca:P = 1.40 to 1). Feeding was terminated when each rat had consumed 1 kg of food. Results: A heavier dry fat-free skeleton was obtained as dietary calcium increased. Fluorine retention was decreased 10-13%, especially in teeth and soft tissues. Data suggested that lower assimilation of fluorine with increased dietary calcium was greater in the younger group. Experiment II: Four trios of rats (48 grams) were fed 500 grams of food in one of three diets where calcium (0.71%) and fluorine (32 ppm) were constant, but phosphorous was varied (0.14, 0.54 and 0.71% of diet). Results: Appetite increased as dietary phosphorus

increased. Growth rate was higher and dry, fat-free skeleton was heavier with increased dietary phosphorous. Fluorine retention and distribution were not effected (although the ratio of fluorine/bone weight was decreased.) Experiment III: The same protocol as the second experiment, except 0.18, 0.51 and 0.63% phosphorous, 0.74% calcium and 13 ppm fluorine was administered. Animals were fed 600 grams of food. Results: Similar to those of Experiment II. Calcium appears to protect against dietary fluorine by impairing assimilation in tissues (teeth and soft tissues) where the most deleterious effects would be exerted. M. Silva, 8/11/89.

031 071330 "The Assimilation of Fluorine by Rats From Natural and Synthetic Cryolite and From Cryolite-Sprayed Fruits," (University of Illinois, 6/30/41). Twelve pairs or trios of rats, depending upon the number on rations to be compared, were selected on the basis of sex, litter membership and body weight and fed equal amounts within the pairs or trios. Initially, 1-4 animals/litter were sacrificed for base level of fluorine. Experiment I: Domestic synthetic cryolite (particle size $\leq$ 1 µm, 44.9% fluorine) and natural Greenland cryolite (commercial form $\leq$ 5 µm, 46.2% fluorine; specially ground form $\leq$ 1 µm, 50.5% fluorine) was fed (9.4 ppm fluorine). Results showed that fluorine of synthetic cryolite is retained significantly more than fluorine from natural cryolite, probably due to solubility. Experiment II: Peaches sprayed with coarsely ground natural cryolite or finely ground special natural cryolite and un-sprayed peaches (dried and ground to fine powder) were found to contain 100 ppm, 94 ppm and 1.5 ppm respectively. The 2 sprayed-peach powders were put into diet (fluorine = 9.6 ppm). Results showed the degree of fineness to which cryolite is ground may modify the assimilation of the contained fluorine. Weathering of natural Greenland cryolite on sprayed fruit does not affect it’s assimilation in rats. Experiment III: Weathered synthetic cryolite was sprayed on apples in one group and the other group had unweathered synthetic cryolite mixed with unsprayed apple powder. Sprayed apples were dried and powdered then both diets were fed to rats (6.8 ppm fluorine). Results showed weathering on fruit of synthetic cryolite lowers the assimilation of it’s fluorine. Possibly, the more soluble compounds of fluorine contained in cryolite are leached out by weathering. Experiment IV: Freshly picked sprayed apples (using synthetic cryolite) were dried and ground to a powder. Sprayed apples allowed to age at a warm temperature and "wax up" for several weeks were then dried and ground. Rats were fed the

two diets containing 11 ppm fluorine. Results showed the development of a wax coat on apples sprayed with cryolite may decrease the assimilation of the fluorine. M. Silva, 8/14/89.

031 071331 "The Absorption and Excretion of Fluoride," (University of Cincinnati, 10/12/42). I: Pure fluoride was administered to adult humans for 20 weeks after each meal (2 mg/dose) either in small gelatin capsules as a dry salt or in solution in distilled water. II: Sodium fluoride (2 mg/dose) was fed in solution after each meal to adult humans for 14 weeks. III: Calcium fluoride (2 mg/dose after each meal) was administered for 4 weeks. IV: Solid calcium fluoride was given for 3 weeks in previously described doses. V: Bone meal was fed (4 mg/meal) for 5 weeks. VI: Cryolite was fed in diet (2 mg fluoride/dose at 0.4887 g fluoride/gram cryolite) for 3 weeks. Feces and urine were sampled for fluoride content in order to determine absorption. Fluid and food intake were also monitored. Conclusions: At normal levels of fluoride intake the urinary plus fecal output equals the intake. Excretion and absorption increase with higher levels of fluoride intake. The absorption of fluorine is dependent upon the aqueous solubility of the fluoride salt and its state at the time of ingestion. With cryolite, the amount excreted in urine was comparable to that of sodium fluoride while the amount in feces was intermediate between bone meal or solid calcium fluoride and that found when solutions of fluoride were administered. M. Silva, 8/16/89.

031 071332 "The Absorption and Excretion of Fluorides. III. Further Observations on Metabolism of Fluorides at High Levels of Intake," (University of Cincinnati, 12/20/48). Human subjects were fed 36 mg of fluoride as cryolite every third day for 3 weeks, cryolite solution for 5 weeks and sodium fluoride for 2 weeks in a program where several fluoride-containing salts and solids were administered sequentially for 102 weeks. Fluoride intake and output were monitored. At 12-25 mg/day of aqueous salt, absorption was 93-97%. When solid salts were ingested at 6, 12, 18 or 36 mg, absorbed fluoride was 62-77%. As the amount of fluoride absorbed daily was increased the urine output increased. Spillage in urine increased when subjects ingested fluoride in daily quantities well in excess of those found normally in diet. This spillage continued in progressively decreasing rate for at least 2 years. M. Silva, 8/16/89.

031 071333 "Non Dental Physiological Effects of Trace Quantities of Fluoride," (NIH, 1945). Fluoride is highly retained in teeth and bone, but there is no evidence that it is required in these tissues. Ingested quantities of fluorine up to 3.0-4.0 mg/day is 90% eliminated by human adults. There is no effect of bone or kidney dysfunction when human adults are exposed to 5.0 ppm/day of fluorine in water. Children may show mottled enamel (hypocalcified enamel in teeth) when exposed to fluorine in excess of 1.0 ppm daily in drinking water. M. Silva, 8/17/89.

031 071334 "Topical Application of Fluoride and Fluoride Absorption," (University of Cincinnati, 8/1/43). Aqueous sodium fluoride (2 mg total) was applied to the teeth a human subject (1 subject studied). Application took 5 days. Teeth were examined 7 days prior to treatment, the 5 days of treatment and 23 days post treatment. Results showed the therapeutic application of a solution of sodium fluoride to the teeth can be done without appreciable systemic absorption of the material. M. Silva, 8/17/89.

031 071335 "A Study of the Comparative Toxicity of Cryolite Fluorine and Sodium Fluoride for the Rat," (University of Wisconsin). I. Weanling albino rats (35-45 grams, 5/dose) were fed 0.1105, 0.0554 or 0.0276% cryolite; 0.132, 0.066, 0.033 or 0.0154% NaF; 0.259, 0.1295 or 0.0648% $AlCl_3$ + 6NaF for 12 weeks. II. Three groups of weanling rats (5/group) were given distilled water or 4 ppm of fluorine as aqueous cryolite or sodium fluoride for 8 weeks. At the end of each experiment femurs and tibiae were removed for fluorine analysis. Results showed at 4 ppm in drinking water, sodium fluoride or cryolite resulted in identical storage of fluorine in the bones of growing rats. At higher dosage, less fluorine was found in the skeleton when fed as cryolite or as the sodium fluoride + aluminum chloride, than when fed as sodium fluoride. When fed at a level of 0.06% in diet, NaF was twice as toxic as cryolite or NaF + $AlCl_3$. Cryolite and NaF + $AlCl_3$ were equally toxic. It appears that the NaF portion of the $AlF_3.3NaF$ molecule is responsible for its toxic properties. M. Silva, 8/17/89.

031 071336 "A Feeding Study With Dairy Cows Using Kryocide Insecticide (Synthetic Cryolite)," (Pennwalt Corporation, 1/10/86). Lactating Holstein cows (3/group) were fed 0, 50, 150 and 500 ppm Kryocide (97.3% synthetic cryolite, lot 8401, 52.8% fluoride) in grain

(10.35 grams Kryocide/kg premix) for 28 days. Results showed no major changes in fluoride levels due to treatment with Kryocide. Treatment with 500 ppm Kryocide produced an increase in liver fluoride from 0.91 ppm to 1.21 ppm and kidney fluoride increased from 1.99 ppm to 2.32 ppm. M. Silva, 8/17/89.

031 071339 "A Feeding Study With Chickens Using Kryocide Insecticide (Synthetic Cryolite)," (Wil Research Laboratories, Inc., 10/24/85). Leghorn laying hens (8 groups with 5/group) were given Kryocide (97.3% synthetic cryolite, Lot 8401, 52.8% fluoride) in feed at 0, 8.6, 26 or 86 ppm (duplicate groups) for 35 days. Kryocide in diet did not affect egg production. Levels of fluoride in eggs was <0.01 ppm (no increase over normal levels). No increase was found in tissue levels of fluoride after treatment (control = 0.27-1.01 ppm; high dose group = 0.32-1.07 ppm). M. Silva, 8/17/89.

031 071341 "Fluorine in Foods," (NIH, 8/26/49). Most foods contain from 0.2-0.3 ppm fluorine. Exceptions to this are tea (75 to 100 ppm) and seafoods (5-15 ppm). Cow’s milk contains 0.1-0.2 ppm fluorine. Fluorine in soil and water has little or no influence on the fluorine content of edible plant produce. Fluorine in foods is available for assimilation in humans. Daily human diets (exclusive of drinking water) contains 0.2-0.3 mg fluorine. Additional intake of fluorine during formative tooth life, via drinking water containing 1.0 ppm is advantageous. M. Silva, 8/17/89.

031 071342 "Metabolism of Inorganic Fluoride," (Review article in: Fluorine Chemistry, J.H. Simmons, ed., Vol. IV, Academic Press, N.Y. & London, 137-191, 1965). Fluoride in cryolite is as available (93%) as other soluble fluoride. Fluoride crosses the placental barrier. Data (with study references) for cryolite metabolism and disposition in adult males (solid and solution form) is given. This review article illustrates the extensive research performed on fluoride metabolism in in vivo studies. M. Silva, 8/21/89.

031 071343 "Fluoride in the Body," (in Fluoridation: The Great Dilemma, Coronado Press Inc., Lawrence, Kansas, 47-54, 1978). Normally, fluoride is taken into the blood within 10 minutes after ingestion and reaches a maximum concentration 50 minutes later. By diffusion,

approximately 47% is absorbed through the upper bowels and 27% through the stomach wall. Free fluoride ion penetrates capillaries and reaches cells of body organs, especially the bones. Fluoride can be stored in virtually any tissue in the body and is eliminated primarily through kidneys, but also through feces, sweat, saliva, tears and milk. The percentage eliminated in each compartment is variable. M. Silva, 8/21/89.

031 071344 "Fluoride in Soft Tissues," (in Fluoridation: The Great Dilemma, Coronado Press Inc., Lawrence, Kansas, 148-174, 1978). Fluoride inhibits glycolysis and other enzyme systems. This article discusses the effects of fluoride toxicity on enzymes, kidneys, heart, arteries, central nervous system, gastrointestinal tract, thyroid, parathyroid, ears, eyes and skin in humans and other animals. M. Silva, 8/21/89.

031 071345 "Distribution of Fluoride Among Body Compartments," in Continuing Evaluation of the Use of Fluorides, E. Johansen, ed., Western Press, Boulder, Colorado, 159-185, 1979. This review considers the compartmental analysis of fluoride. It discusses data showing relationships between serum fluoride and fluoride concentrations in other compartments. In addition, evidence for a relationship of serum fluoride to age and toxicity is considered. Sodium fluoride is the primary compound under consideration. In general, serum fluoride is a good steady-state predictor of the concentrations in other compartments of the body. Daily fluctuations of fluoride in blood are usually held to a narrow range of approximately 30% due to dampening by the large bone compartment. The effectiveness of a simple compartment model for fluoride means there are probably no complex regulating systems. M. Silva, 8/21/89.

031 071346 "Absorption of Various Fluorine Compounds From the Gastrointestinal Tract of the Rat," Am. J. Physiol., 191:549-550, 1957, (NIH, 1957). Various fluorine compounds (cryolite was not among them, however NaF, which supposedly behaves like cryolite was), were compared for their absorption from the gastrointestinal tract of rat. Groups of 10 male Holtzman rats (110-120 grams) were fasted for 72 hours, then intubated with 2.0 ml of each of 7 compounds (NaF, Na2SiF6, Na2PO3F, SnF2, KPF6, Et4NPF6 and KBF4) containing 100 ug F/ml solution. Results showed that covalently bound fluorine compounds, such as DPF6, Et4NPF6 and KBF4, were absorbed at a greater rate than the fluorine ion. M. Silva, 8/21/89.

031 071347 "Metabolism of Fluorine 18 in Domestic Animals," (Am. J. Physiol. 182:383-389, 1955). In this paper, a description of a) the removal of F18 from the blood of sheep and cattle, b) the appearance of ingested F18 in sheep blood, c) excretion into the digestive tract of sheep, d) tissue distribution in sheep, e) incorporation in the developing hen's egg and f) secretion into the milk of dairy cow was given. Results showed fluorine absorption occurred rapidly in sheep and cows, probably from the rumen. Peak blood value was 1% of ingested dose (at 2-5 hours in sheep and cow respectively). Selective localization of F18 was like that of calcium. Ingested F18 was deposited in various parts of hen's egg and cow's milk. M. Silva, 8/21/89.

031 071348 "Halide Transport in Red Blood Cells," (Acta. Physiol. Scand., 46:19-41, 1959). In this study, a flow tube is described for the measurement of rapid transport processes (half-times between 0.05 and 1 sec in suspensions of cells which have an average diameter greater than 1u) in cells. Ratios of cell to medium concentrations of various halides at equilibrium were measured by isotope dilution (F = 0.50) in beef red cells. Similar ratios were measured in human cells. M. Silva, 8/21/89.

031 071349 "Rates of Elimination of Fluoride Stored in the Tissues of Man," (University of Cincinnati College of Medicine, 1952). Human subjects (3 men & 1 woman, ages 25-45), who had lived at least 15 years in Chicago or Cincinnati where fluoride in water $\leq$ 0.2 ppm were treated with additional fluoride (3 to 36 mg/day) for 2 to 130 weeks. One of the subjects had lived in a region where fluoride in water was 2.4 to 4.4 ppm. Every item of food and drink ingested by the subjects during the experimental period was analyzed for fluoride content. The amount of fluoride ingested by each subject was subtracted from the amount found in the urine and feces and the differences were recorded daily or weekly. Results showed that fluoride is stored in human tissues for months or years during which as little as 3 mg of NaF was ingested/day. Demonstrable storage of fluoride had occurred in the subject who lived in Amarillo, Texas where fluoride in water was high. M. Silva, 8/22/89.

031 071350 "The Effect of the Level of Calcium Intake on the Calcification of Bones and Teeth During Fluorine Toxicosis," (University of Wisconsin, 1932). Young rats were fed various

stock (no added fluorine or calcium), high or low fluoride and/or calcium-containing diets (diets were described in a referenced paper in the same journal, same volume) for 6-40 weeks (2-10 animals/group). Parathyroids, tibias, femurs and incisor teeth were removed and analyzed for fluoride at termination of the experiment. Results showed that at 0.15% sodium fluoride in the diet, there was a variable effect on the ash of bone. Animals on diets low in calcium and on a stock diet of moderate calcium content, showed a decreased ash weight. On a high calcium diet, ash weight was increased. Total ash weight of teeth in all diets containing calcium was decreased when compared to the stock diet or the stock diet + fluorine. M. Silva, 8/22/89.

031 071351 "Studies of the Effects of Dietary Sodium Fluoride on Dairy Cows," (University of Wisconsin, 12/5/57). Holstein heifers (2 years old) which were bred were fed NaF at 3-5 ppm (basal diet), 20, 30, 40, 50 or 60 ppm of fluorine (3-4 cows/group) for 5.5 years (6 lactations). At termination, heart, liver, kidney, pancreas, adrenal, thyroid and a blood sample were obtained for fluorine analysis, gross pathology and histology. In addition, the metacarpal, metatarsal, mandible, maxilla and frontal bones and the 11th and 12th ribs were analyzed for fluorine. Results showed that cows on the basal ration stored less than 1000 ppm fluorine in skeleton. Stored fluorine increased with increase in diet. Bone fluorine varied with bone type. Fluorine toxicosis was associated with fluorine in compact bone and of cancellous bone in excess of 5500 and 7000 ppm respectively. Concentrations below 4500 ppm showed no toxicosis. Fluorine in soft tissues increased with increases in diet. Due to a narrow margin in concentration between normal and fluorosed tissues, the use of soft tissue analysis as a criterion of fluorine toxicosis is unreliable. M. Silva, 8/22/89.

031 071352 "Balances of Fluorine Ingested From Various Sources in Food and Water by Five Young Men," (University of Maryland, 3/30/45). Five men (19-27 years) spent 8 hours/day for 5 days in an experimental chamber which controls environmental temperature and humidity (temperatures were 84-84 ^{o}F, relative humidity = 49-52% or 100-101 ^{o}F, r.h. = 66-70%) which varied during the day. Their diets were supplemented with 3.0 mg fluorine daily (taken in different forms, including cryolite). Dermal excretion of fluorine, feces and urine were assayed. Food aliquots were also tested. Results showed the absorption of fluorine from

cryolite is significantly less than from Galesburg, Illinois drinking water or from NaF in food or water. Intake, therefore, is dependent upon solubility of fluorine compound and whether it is solid or in solution. Fluorine ingested in food (as cryolite) up to 3.0 mg/day was largely eliminated. Fluorine (as cryolite) as residues on apples and pears, when ingested up to 3.0 mg/day may not be expected to induce fluoride toxicosis. M. Silva, 8/22/89.

031 071354 "The Surface Chemistry of Bone," (University of Rochester, 8/3/50). Bone from shafts of long bones of adult rabbits was freed of soft tissue and ashed by boiling in alkaline glycol. Experiments were performed to determine fluorine impregnation on Ca, P, HCO_3 and OH exchange. Results showed fluoride replaces either OH or HCO_3 ions in the surfaces of the mineral phase. M. Silva, 8/22/89.

032 070550 "Cryolite: Factor For Fluoride Retention," (Pennwalt Corporation, 10/28/88). This document summarizes several literature studies and argues that fluoride retention from cryolite should not exceed 80%. However, since fluoride retention varies a great deal among individuals, on average, assimilation probably ranges from 40-60%. Fluoride storage from cryolite varies depending upon whether it is ingested in solid verses liquid (more efficient). Retention of fluoride from cryolite ingested in food varies from 27.5% to 70.7% (similar results were obtained with NaF). In rat studies, fluoride retained from cryolite was 20% less than that in drinking water. NaF studies showed 20.4% and 21.5% less fluoride retained when obtained from food than drinking water. In humans, fluoride retained from cryolite ranged from 34.1% to 48%, based upon studies where fluoride was quantified from feces and urine. Another study showed fluoride retention from cryolite was -10.8%

to 5%, based upon quantification of fluoride in urine, feces and perspiration. Fluoride loss in perspiration was 19.1 to 30%. M. Silva, 8/23/89.

032 070617 "The Relative Assimilation of Fluorine From Fluorine-Bearing Minerals and Food (Tea) and From Water and Food," (University of Illinois, 7/28/89). Experiment I: CaF_2 (7.8 ppm) and NaF (9.0 ppm) were administered in water and NaF was administered in food (9.0 ppm) to rats (10/group) for 14 weeks. At termination, bones, teeth and soft tissues were examined for fluorine content. Control rats were sacrificed and their fluorine was assessed. Experiment II. Green tea (130 ppm F), rock phosphate (3.9% F & 34.96% Ca) and NaF were used in this experiment. Rats (12/group) were fed either, 644.3 ppm $CaHPO_4$ + 21.7 ppm NaF, 5% ground green tea leaves or rock phosphate (228 ppm) and $CaHPO_4$ (375 ppm). The experiments were designed to have equal percentages of Ca and F and 9-12 ppm fluorine. Results showed that at low levels if intake, NaF is no more assimilable by the rat body than CaF_2, but is more assimilable than fluorine in green tea (5%), which is more assimilable than fluorine in rock phosphate. Fluorine of NaF in drinking water is 21% more completely assimilated than the same compound consumed in the same amounts in the food. In a similar experiment previously carried out with cryolite, the decrease in assimilation when cryolite was administered in food was 20%. M. Silva, 8/24/89.

032 070618 "Cryolite: Stomach Irritation Associated With Hydrogen Fluoride Formation," (Summary of scientific studies by Pennwalt). Recent studies by Pennwalt have demonstrated stomach irritation in rats fed cryolite. Pennwalt contends these responses are due to fluorine rather than cryolite per se. According to Pennwalt, clinical investigators have demonstrated that small amounts of fluoride salts which are capable of releasing free fluoride will produce high enough levels of HF in the stomach to result in gastric distress (several references and examples are cited), ranging from stomach upset and gastric ulcers in adults to stomach hemorrhages in young children and infants. It is known that cryolite produces free fluoride and that HF would be produced in an acidic medium such as the stomach. Therefore, Pennwalt requests that the stomach irritation noted in the toxicological studies performed with cryolite be considered as relating to data for NaF (and other salts capable of dissociation) rather than be a basis for special concern. Pennwalt requests their cryolite

studies be considered an extension of the vast amount of fluoride toxicology data already available. M. Silva, 8/23/89.

032 070619 "Prenatal and Postnatal Ingestion of Fluorides: Fourteen Years of Investigation: Final Report," (Department of HEW, 10/61). CaF_2 NaF, and Na_2PO_8F (all with approximately the same amount of F ion) were administered to gravid women and children through their 8th year of life, during the formation of dental enamel. One tablet/day was prescribed and could be chewed, or taken by liquid. Placental tissues and cord blood were analyzed and clinical data were summarized. Results showed that fluoride ingested by gravid women is stored in the placenta and passes to the fetal blood stream. Fluoride in fetal blood supply makes developing teeth more resistant to caries. 1% of patients had side effects in the form of dental fluorosis which is reversed by removing the supplements. M. Silva, 8/23/89.

032 070622 "Evidence for a Lack of an Effect of Dietary Fluoride Level on Reproduction in Mice," (University of Wisconsin, 12/22/75. Weanling Webster mice (10 grams) were fed a basal low-fluoride diet or the same diet supplemented with 2 ppm NaF (25-35 mice/group) and bred for 1-4 litters in a 25 week period. Second and 3rd generation females were selected from 4th litter pups of the previous generation. They were raised to weaning, then fed the same diets (2 ppm fluorine) as their dams with the same protocol as that of the first generation. In this second generation, however a third group was added and fed a diet containing 100 ppm fluoride when mice were 8 weeks old. This experiment was carried through 3 generations. At termination, blood samples were taken as well as liver, kidneys and femur for fluoride analysis. No significant effects were observed on growth of pups, reproductive response, litter size, weight of pups or incidence of stillbirth. Plasma and femur fluoride was higher in the animals fed 100 ppm, but there was no difference between the animals fed the basal diet and 2 ppm fluoride. The results failed to confirm earlier reports that fluoride in the maternal diet is essential for reproduction in the mouse. M. Silva, 8/23/89.

034 071031 "The Measurement of Fluoride, With Special Reference to Milk, Using a Fluoride Ion-Selective Electrode, and an Investigation of the Interferences Caused by Certain Ionic Species," (Polytechnic of the South Bank, 7/78). A fluoride ion-selective electrode was used

to test the effects of Na, Ca and Mg on fluoride concentrations in aqueous solutions. Results were inconclusive when examining Ca and Mg exchanges when fluoride is added to milk. Proteins in the synthetic milk interfered with instrument readings. M. Silva, 8/22/89.

Conclusion: Based upon the data and literature presented by Pennwalt, it appears that the metabolism of cryolite has been thoroughly characterized even though a specific Guideline study has not been performed. Many of the studies cited were performed on sodium fluoride. However, the availability of fluorine in cryolite is similar to that of sodium fluoride (as shown in the literature) and availability of fluorine is the primary concern for possible adverse effects. Although CDFA agreed with Pennwalt that there was no need to perform further metabolism studies with cryolite, it is necessary to abide by the decisions of the EPA. CDFA [subsequently DPR] requests that Pennwalt forward any correspondence with EPA, so that we remain informed on their final decision.

SODIUM FLUORIDE STUDIES IN DATA CATEGORIES FOR WHICH ACCEPTABLE CRYOLITE STUDIES EXIST

GENE MUTATION

145 - 039 111528 "Mutagenicity of Sodium Fluoride in Σαλμονελλα τψπηιμυριυμ", (SRI, A part of National Toxicology Program's Technical Report No. 393, December 1990). Sodium fluoride, purity not stated, at concentrations of 0 (dimethylsulfoxide), 100, 333, 1000, 3333, or 10000 µg/plate, was tested on Σαλμονελλα τψπηιμυριυμ tester strains TA98, TA100, TA1535 and TA1537 with and without metabolic activation with Aroclor 1254-induced S9 from male Syrian hamster or Sprague-Dawley rat. Exposure time was 20 minutes preincubation followed by 48 hours after plating. No evidence of an increase in reverse mutations. **No adverse effect indicated.** UNACCEPTABLE for sodium fluoride but upgradeable with submission of details of methods and missing individual data. **Supplemental** for cryolite. (Kishiyama and Gee, 3/01/95)

145- 039 111528 "Induction of Trifluorothymidine Resistance in Mouse L5178Y Cells by Sodium Fluoride", (Litton Bionetics and Inveresk Research International, A part of National

Toxicology Program's Technical Report No. 393, December 1990). Sodium fluoride was tested for induction of trifluorothymidine resistance in Mouse L5178Y lymphoma cells. Litton Bionetics tested sodium fluoride at concentrations of 50, 100, 200, 300, 400, 500, 600, 700 and 800 µg/mL with and without Aroclor 1254-induced Fischer 344/N rat liver (S9) in triplicate, two trials. Inveresk Research Laboratory tested at 62.5, 125, 250, 500, 600, 700. 800, 900 and 1000 µg/mL in duplicate, two trials, without S9 activation. Exposure time was 4 hours for both laboratories. Sodium fluoride tested positive at doses ranging from 300 to 600 µg/mL with and without S9 activation in Litton's studies and at 62.5, 125 and 1000 µg/mL and also at 800 and 900 µg/mL in Inveresk's first and second trials, respectively. **Possible adverse effect indicated: Elevated chromosomal abnormalities** (increased mutant fraction reported to be primarily small colonies). UNACCEPTABLE (Need details of methods used, protocol, and individual data). Upgradeable for sodium fluoride. **Supplemental for cryolite.** (Kishiyama and Gee, 2/28/95)

CHROMOSOME EFFECTS

145 - 039 111528 "Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Sodium Fluoride", (studies by two independent labs: Litton Bionetics Inc. and Environmental Health Research and Testing, Inc. [EHRT]. Studies were considered together as part of National Toxicology Program's Technical Report No. 393, December 1990). Sodium fluoride was tested in Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges in both studies.

Litton Bionetics, Inc.: sodium fluoride (NaF) concentrations at 6.7, 20.0, 66.7 and 200 µg/mL for trial 1 and 50.0, 75.0, 100.5 and 125 µg/mL for trial 2 without S9. NaF concentrations of 330, 350 and 400 µg/mL for trial 1 and 1200, 1400 and 1600 µg/mL for trial 3 with S9 (from the livers of Aroclor 1254-induced male Sprague-Dawley rats). Harvest time was extended 4.5 to 6.5 hours for high dose groups. **Possible adverse effect indicated: Sodium fluoride at doses of 66.7 and 75.0 µg/mL without S9 and 1200 - 1400 µg/mL with S9 induced SCEs.**

EHRT: sodium fluoride concentrations were 1.6, 5.0, 16.0, 50.0 and 160 µg/mL for trial 1 and 5.0, 25.0, 50.0, 75.0 and 100 µg/mL for trial 2 without S9. NaF concentrations of 16,

50, 160.0, 500.0 and 1600.0 µg/mL with S9 for trial 1 [the only trial with S9]. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats. There was no evidence of induction of SCEs in this study.

Studies from both laboratories are UNACCEPTABLE for sodium fluoride (insufficient information on methods). Possibly upgradeable. **Supplemental for cryolite.** (Kishiyama and Gee, 3/01/95).

145 - 039 111528 "Induction of Chromosomal; Aberrations in Chinese Hamster Ovary Cells by Sodium Fluoride", (Two independent studies, by Environmental Health Research and Testing, Inc. (EHRT), and Litton Bionetics Inc. (LBI). Studies were presented as supplementary data to the NTP rat and mouse oncogenicity study report under this same record number. Sodium fluoride was tested in Chinese hamster ovary (CHO) cells for induction of chromosomal aberrations in both studies. One hundred cells were scored per concentration.

EHRT: Sodium fluoride (NaF) concentrations were 16, 50, 160, and 500 µg/mL, or 300, 400, 500 and 600 µg/mL, or 300, 400, 500, 600 and 800 µg/mL without S9 for trials 1, 2 and 3, respectively. NaF concentrations were 50, 160, 500 and 1000 µg/mL, or 400, 600, 800 and 1000 µg/ml with S9 from the livers of Aroclor 1254-induced male Sprague-Dawley rats for the respective trials 1 and 2. **Possible adverse effect:** Sodium fluoride at doses 400 µg/mL and above (without S9) increased chromosomal aberrations in trials two and three.

LBI studies: NaF concentrations were 150, 176 and 200 µg/mL without S9 and 1200, 1400 and 1600 µg/mL with S9 (from the livers of Aroclor 1254-induced male Sprague-Dawley rats). Results were negative.

Studies from both laboratories are UNACCEPTABLE (insufficient information on methods, no individual data) for NaF. **Supplemental** for cryolite. Possibly upgradeable for NaF. (Kishiyama and Gee, 3/01/95)