264

CASWELL FILE

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

009682

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

AUG 18 1992

SUBJECT: CRYOLITE - Submission of a NTP Bioassay on Sodium Fluoride in Rats and Mice for Review with Respect to Satisfying the Chronic Toxicity/Carcinogenicity Data Required for Reregistration.

Tox. Chem. Nos: 264/769
PC No.: 075101
Project No.: 1-1732
Submission No.: 398926
DP Barcode: D166171

FROM: William B. Greear, M.P.H. William B. Greear 7/28/92
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: Larry Schnaubelt/Brigid Lowery, PM Team # 72
Reregistration Branch
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THRU: Marion P. Copley, D.V.M., Section Head Marion P. Copley 7/28/92
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)

KB 7/29/92

I. CONCLUSIONS:

The NTP Bioassays satisfy the requirements for Guideline Series 83-2 Carcinogenicity Studies in 2 species and the rat study satisfies the requirement for a Guideline Series 83-1 Chronic Toxicity Study.

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II. REQUESTED ACTION

SRRD has requested that Toxicology Branch I (TB-I) review the National Toxicology Program's Bioassay of Sodium Fluoride (NaF) in rats and mice and determine whether or not the NaF studies can be used in lieu of chronic toxicity/carcinogenicity studies on cryolite in rodents to support reregistration.

III. PRODUCT INFORMATION

Cryolite No. 264
Updated July, 1992

Cryolite is a common name for sodium fluoaluminate or sodium aluminofluoride (given variously as Na_3AlF_6, $3NaF.AlF_3$ or AlF_6Na_3). Its proprietary name is Kryocide and it is used as an insecticide on various crops such as apples, beans, broccoli, carrots, corn, cucumber, grapes, lettuce, peaches, tomatoes, etc., under 40 CFR 180.145. Tolerances have been established for insecticidal fluorine compounds, cryolite and synthetic cryolite at 7 ppm, except for kiwi fruit which was established at 15 ppm. Its molecular weight is 209.95. Its Chemical Abstract Registry Number is 1344-75-8 and its PC number is 075101.

IV. DATA REQUIREMENTS (40 CFR 158.340)		Cryolite Updated Required	No. 264 July, 1992 Satisfied
	Technical		
81-1	Acute Oral Toxicity	Y	Y
81-2	Acute Dermal Toxicity	Y	Y
81-3	Acute Inhalation Toxicity	Y	Y
81-4	Primary Eye Irritation	Y	Y
81-5	Primary Dermal Irritation	Y	Y
81-6	Dermal Sensitization	Y	Y
81-7	Acute Delayed Neurotoxicity (Hen)	Y	-
81-8	Acute Neurotoxicity (rat)	R	-
82-1	Subchronic Oral (Rodent)	Y	N [1]
82-1	Subchronic Oral (Nonrodent)	Y	N
82-2	21-Day Dermal	Y	N
82-3	90-Day Dermal	N	N
82-4	90-Day Inhalation	N	-
82-5	90-Day Neurotoxicity (Hen)	N	-
82-5	90-Day Neurotoxicity (Mammal)	R	-
83-1	Chronic Toxicity (Rodent)	Y	Y
83-1	Chronic Toxicity (Nonrodent)	Y	N
83-2	Carcinogenicity (rat)	Y	Y
83-2	Carcinogenicity (mouse)	Y	Y
83-3	Developmental Toxicity (rabbit)	W	-
83-3	Developmental Toxicity (Rat)	Y	Y
83-4	Reproduction	Y	N
83-5	Chronic/Carcinogenicity	-	-
84-2	Mutagenicity - Gene Mutation	Y	Y
84-2	Mutagenicity - Structural Chromosomal Aberration	Y	Y
84-2	Mutagenicity - Other Genotoxic Effects	Y	Y
85-1	General Metabolism	W	-
85-2	Domestic Animal Safety	N	-
85-3	Dermal Penetration	N	-
85-4	Visual System Studies	N	-

Y = Yes; N = No; W = Waived; R = Reserved

[1] Requirement satisfied by the chronic rat study

V. TOXICOLOGY PROFILE

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

CASWELL#: 264
CAS-REG#: 15096-52-3

TOXCHEM NO. 075101- Cryolite FILE LAST PRINTED: 06/16/92

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-3(a) Developmental Toxicity Study Species: rat Science Applications 1182008; 8/15/83	Kyrocide Lot #86-11-9	071392 250968	Levels tested by gavage - 0, 750, 1500, and 3000 mg/kg/day. Developmental NOEL > 3000 mg/kg/day (HDT), Maternal NOEL > 3000mg/kg/day (HDT).		Supplementary 002612 Supplementary 003775 Minimum 004552 007009
83-3(b) Developmental Toxicity Study Species: rabbit (2nd species) 12/21/87	Cryolite (Sodium fluo-aluminate)		The requirement for a teratology in a second species is waived based on lack of toxicity displayed in the rat study and other studies available.		007009
82-1(a) Feeding-3 month Species: rat Hazleton 6120-100; 11/27/85	Kryocide 96% pure	262372 00015800	Levels tested in Charles River 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, 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
82-1(b) Feeding-3 month Species: dog Wil Research Lab 75007; 1/86	Kryocide 96W 97.3% a.i. Batch #8401	262371 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 007009
82-2 Oral-3 week Species: rabbit Battelle Inst. W. Germany N4900-2001; 8/25/89	Crylolite (96% a.i.)	412248-01	NOEL = 25 mg/kg. LEL = 250 mg/kg (based on decreased body weight gain). Doses tested: 0, 25, 250 & 1000 mg/kg/day in NZW (SPF) str. In addition at 1000 mg/kg/day deaths. Clinical signs of toxicity (thin appearance, hypoactivity, anemia and changes in several clinical chemistry parameters occurred.		Guideline 007944
Feeding-28 day Species: rat Elars Bioresearch Lab Inc. 1821; 6/29/82	Kryocide 96.0%	071392	(A range finding study.) The only compound-related effect was a change in coloration and physical property (soft and granular enamel). Dietary levels: Group I - 0, 250, 500, 1000, 2000, 4000 ppm; Group II- 0, 10,000, 25,000, 50,000 ppm.		Minimum 002612 003775

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Feeding-28 day Species: dog Wil Research Lab WIL-75010; 7/25/85	Kryocide 96W 97.3% a.i. Batch #8401	262370	This study was not reviewed because doses were nearly identical to 90 day dog study.		Supplementary 005771
84-4 Mutagenic-DNA (POL) repair Species: E. coli Microbiological Associates T1693.104; 9/21/81	Kryocide	071392 250968 00128115	No mutagenic potential demonstrated. CDFA - EPA Review, 1/17/89: Agreement that this study's status be changed from acceptable to unacceptable.		Unacceptable 003775 Acceptable 004552 Unacceptable 007744
84-2(b) Mutagenic-in vivo cytogenetic Species: rat Microbiological Associates T1693.112; 10/2/81	Kryocide	071392 250968 00128115	No demonstrated toxic effects or mutagenic potential at HDT. CDFA - EPA Review, 1/17/89: Agreement that this study's status be changed from acceptable to unacceptable.		Unacceptable 003775 Acceptable 004552 007009 Unacceptable 007744
84-2(a) Mutagenic-Ames Species: salmonella Microbiological Associates T-1693.102; 9/29/81	Kryocide 96%	071392 250968 00129113	No mutagenic potential demonstrated. CDFA - EPA Review, 1/17/89: Agreement that this study's status be changed from acceptable to unacceptable.		Unacceptable 003775 Acceptable 004552 007009 Unacceptable 007744
84-2(a) Mutagenic-Ames Species: salmonella Pharmakon Res. Inst. Inc. PH-301-ANA00190; 03/19/91	Cryolite tech.	418384-01	Negative for gene mutation in Salmonella TA (Ames) strains, exposed to up to 10,000 ug/plate with/without activation.		Acceptable 009404
84-2(b) Mut- Chrom. aberr. in vitro Species: human lymphocytes Pharmakon Res. Inst. Inc. PH-324-ANA00190; 03/18/91	Cryolite tech.	418384-02	Negative for chromosome aberrations in human lymphocytes, cultured with/without activation up to 1000 ug/mL.		Acceptable 009404

TOXCHEM NO. 075101- Cryolite FILE LAST PRINTED: 06/16/92

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
84-4 Mutagenic-unscheduled DNA synt Species: rat hepatocytes Pharmakon Res. Inst. Inc. PH-311-ANA00190; 03/18/91	Cryolite tech.	418384-03	Negative for unscheduled DNA synthesis ("repair") in rat hepatocytes cultured up to toxic doses (50+ ug/mL).		Acceptable 009404
85 Metabolism Species: rat Pennwalt WT-12-82; 2/1/83	Kryocide	071392	The study was inadequate in measuring absorption & or excretion in the rat. 80% of the dose was excreted in the feces and less than 1% in the urine. Fluoride levels in the blood were very low. Total fluoride levels in the bone were 3 times higher (3000 vs. 1000 ppm) than controls. Approximately less than 10% of the daily dose is absorbed from the G.I. tract and excreted in 24 hrs in the urine and feces. The rest of the dose most probably is not absorbed & is excreted in the feces.		Acceptable 002612 003775 Supplementary 006479
Registration standard	Kryocide				007009
85-1 Metabolism Species:	Cryolite		Data requirement is waived because cryolite behaves like the free fluoride ion.		007500
81-1 Acute oral LD50 Species: rat Hazleton 814515; 11/10/83	Kryocide 96.0%	252071	LD50 > 5 gm/kg (only level tested).	4	Minimum 001696 003775 007009 Guideline 008987
81 Acute oral LD50 Species: rat Raltech Sci. Services Lab 880531; 9/21/81	Na3Al6 (Kryolide)	071392	LD50 > 1.5 gm/kg.	3	Supplementary 002612 003775
81-2 Acute Dermal LD50 Species: rabbit Elars Bioresearch Lab Inc. 1685-6; 7/20/81	Na3Al6 (Kryolide) 96.0%	071392	LD50 > 2.1 gm/kg.	3	Minimum 002621 003775 007009 Guideline 008987

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

TOXCHEM NO. 075101- Cryolite FILE LAST PRINTED: 06/16/92

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-3 Acute inhalation LC50 Species: rat Litton Bionetics Inc. 22098; 5/19/81	Na3Al6 (Kryolide) 96.0%	071392	LC50 > 2.06 mg/L and < 5.03 mg/L.	3	Minimum 002612 003775 007009 008987
81-4 Primary eye irritation Species: rabbit Raltech Sci. Services Lab 880531; 9/21/81	Na3Al6 (Kryolide) 96.0%	071392	Moderate conjunctival redness and irritation - disappeared within 7 days.	3	Minimum 002612 003775 007009 008987
81-5 Primary dermal irritation Species: rabbit Raltech Sci. Services Lab 880531; 9/21/81	Na3Al6 (Kryolide) 96.0%	071392	PIS = 0.0	4	Minimum 002612 003775 007009 008987
81-6 Dermal sensitization Species: guinea pig Hazleton 814516; 12/13/83	Kryocide 96.0%	252071	Negative results.		Minimum 001696 007009

Guideline Series 83-2, Carcinogenicity Study - mouse

009682

MRID No.: N/A

Test Material: Sodium Fluoride

Study Number: NTP TR 393; NIH Publ. 91-2848

Testing Facility: Battelle Columbus Laboratories, Columbus, OH

Author: National Toxicology Program

Report Issued: December 1990

Conclusions

NOEL < 25 ppm (M=2.4 mg/kg/day;F=2.8 mg/kg/day)
LEL = 25 ppm (based on attrition of teeth - M; discoloration and mottling of teeth - M,F; increased bone fluoride - M,F)

In addition, at 175 ppm (M=16.7 mg/kg/day;F=18.8 mg/kg/day) males had dentine dysplasia, females had increased attrition of teeth and increased alkaline phosphatase levels)

No evidence of carcinogenic activity in male or female B6C3F1 mice (according to NTP).

Classification: Core-Minimum Data

Guideline Series 83-5, Combined Chronic/Carcinogenicity Study-rat

MRID No.: N/A

Test Material: Sodium Fluoride

Study Number: NTP TR 393; NIH Publ. 91-2848

Testing Facility: Battelle Columbus Laboratories, Columbus, Ohio

Report Issued: December 1990

Conclusions:

NOEL < 25 ppm (1.3 mg/kg/day)
LEL = 25 ppm (based on mottling of teeth, dentine incisor dysplasia, increased serum, urine and bone fluoride levels in M&F; incisor odontoblast and incisor ameloblast degeneration in M)

In addition, at 100 ppm males had attrition, deformity, and discoloration of the teeth, females had incisor ameloblast degeneration; at 175 ppm males had malocclusion, females had attrition, deformity and discoloration of the teeth, incisor odontoblast and ameloblast degeneration of the teeth and osteosclerosis. Equivocal evidence of carcinogenicity in males. No evidence of carcinogenicity in females.

Classification: 83-1 Chronic Toxicity: Core-Minimum Data
83-2 Carcinogenicity: Core-Minimum Data

VI. DATA GAPS

82-1 Subchronic Oral (Nonrodent)
(may be satisfied by 83-1)

83-1 Chronic Toxicity (Nonrodent)
83-4 Reproduction

VII. ACTION TAKEN TO REMOVE DATA GAPS AND OBTAIN ADDITIONAL INFORMATION

The sponsor is herein informed of the data gaps on cryolite.

VIII. REFERENCE DOSE (RfD)

At present, a RfD has not been established.

IX. PENDING REGULATORY ACTIONS

There are no pending regulatory actions against this pesticide at this time that TB-I is aware of.

X. TOXICOLOGICAL ISSUES

A. Data Requirement

In a memorandum dated 9-27-89, TB-I responded to a request of the sponsor to waive the requirement for certain data. It was determined that the data requirement for a metabolism study should be waived because of the amount of data readily available on sodium fluoride. Data waivers were requested for chronic toxicity, carcinogenicity and reproduction studies. TB-I recommended that the data waiver for the chronic dog and the reproduction studies be denied. However, it was decided that the request to waive the requirement for a chronic toxicity study in rodents and for carcinogenicity studies in two species be held in abeyance pending submission and evaluation of the NTP Bioassay on Sodium Fluoride. The rat and mouse bioassays have been evaluated in this action (see DERs attached). Both rat and mouse studies are acceptable as Guideline Series 83-2 Carcinogenicity Studies and the rat study is acceptable as a chronic toxicity study (83-1).

B. 6 (a)(2) Data

1. Developmental Toxicity

The sponsor submitted preliminary information from a

range-finding study in rabbits in which unexpected toxicity was observed and simultaneously requested that the study, instead, be conducted in mice. The range-finding study in rabbits produced mortality at 30-1000 mg/kg/day whereas in a rat study the NOEL was 3000 mg/kg/day. The difference in toxicity was attributed to the very sensitive nature of the rabbit stomach as demonstrated by studies conducted with antibiotics, and the knowledge that cryolite is a stomach poison. TB-I had no objection to conducting a developmental toxicity study in mice instead of rabbits, but requested that the sponsor also submit the results of the range-finding study in rabbits. (See memorandum of W. Greear dated 3-4-91). [The range-finding study in rabbits and the mouse developmental study have recently been submitted and are under review.]

2. Reproduction

The sponsor submitted information from a pilot reproduction study in rats in which toxicity and mortality occurred at all dose levels (5000 - 20000 ppm). A second pilot study was proposed and TB-I will evaluate the main study when it is completed. It was determined that no imminent hazard could be identified for cryolite at this time (see memorandum of M. Copley dated 10-22-91).

XI. OTHER

The following study has been evaluated herein:
National Toxicology Program (1990) NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Fluoride Cas No. 7681-49-4 in F344/N Rats and $B_6C_3F_1$ Mice (Drinking Water Studies), NIH Public. No. 91-2848 (NTP TR 393), December 1990, Research Triangle Park, NC 27709.

Results

Rat:

NOEL < 25 ppm (1.3 mg/kg/day)
LEL = 25 ppm (based on mottling of teeth, dentine incisor dysplasia, increased serum, urine and bone fluoride levels in M&F; incisor odontoblast and incisor ameloblast degeneration in M).

In addition, at 100 ppm males had attrition, deformity, and discoloration of the teeth, females had incisor ameloblast degeneration; at 175 ppm males had malocclusion, females had attrition, deformity and discoloration of the teeth, incisor odontoblast and ameloblast degeneration of the teeth and osteosclerosis. Equivocal evidence of carcinogenicity in males . No evidence of carcinogenicity in

females.

Classification: 83-1 Chronic Toxicity: Core-Minimum Data

83-2 Carcinogenicity: Core-Minimum Data

Study Acceptability:

The study satisfies the requirement for a Guideline Series 83-2 Carcinogenicity Study and for a Guideline Series 83-1 Chronic Toxicity Study.

Mouse:

NOEL < 25 ppm (M=2.4 mg/kg/day; F=2.8 mg/kg/day)
LEL = 25 ppm (based on attrition of teeth - M, F; increased bone fluoride - M,F)

In addition, at 175 ppm (M=16.7 mg/kg/day;F=18.8 mg/kg/day) males had dentine dysplasia, females had increased attrition of teeth and increased alkaline phosphatase levels). No evidence of carcinogenic activity in male or female B6C3F1 mice.

Classification: Core-Minimum Data

Study Acceptability:

The study satisfies the requirement for a Guideline Series 83-2 Carcinogenicity Study in mice.

Guideline Series 83-2

Reviewed By: William B. Greear, M.P.H. William B. Greear 6/10/92
Review Section IV, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. Marion Copley 6/29/92
Review Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

Study Type: NTP Carcinogenesis Bioassay - Mouse

PC No.: 075202
TOX Chem. No.: 769
MRID No.: N/A
CAS No.: 7681-49-4

Test Material: Sodium Fluoride

Study Number: NTP TR 393; NIH Publ. 91-2848

Sponsor: National Toxicology Program
Research Triangle Park, NC 27709

Testing Facility: Battelle Columbus Laboratories
Columbus, OH

Title of Report: Toxicology and Carcinogenesis Studies of Sodium Fluoride in F344/N Rats and B6C3F1 Mice (Drinking Water Studies)

Author: National Toxicology Program

Report Issued: December 1990

Conclusions

NOEL < 25 ppm (M=2.4 mg/kg/day;F=2.8 mg/kg/day)
LEL = 25 ppm (based on attrition of teeth - M; discoloration and mottling of teeth - M,F; increased bone fluoride - M,F)

In addition, at 175 ppm (M=16.7 mg/kg/day;F=18.8 mg/kg/day) males had dentine dysplasia, females had increased attrition of teeth and increased alkaline phosphatase levels)

No evidence of carcinogenic activity in male or female B6C3F1 mice (according to NTP).

Classification: Core-Minimum Data

Study Acceptability:

The study satisfies the requirement for a Guideline Series 83-2 Carcinogenicity Study in mice.

A. Materials:

1. Test compound - sodium fluoride; Description: white, crystalline water-soluble powder; Batch No.: A022085; Purity 99%.

2. Test Animals - Species: mouse; Strain: B6C3F1; Age: 4 weeks on arrival; Weight: male - 22.7 to 23.4 g (mean), female - 19.0 to 19.5 g (mean); Source: NCI Frederick Cancer Research, Frederick, MD.

B. Study Design:

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

Test Group	Dose in Water (ppm)	Main Study 103 Weeks Male	Main Study 103 Weeks Female	Interim Sac. 24 Weeks Male	Interim Sac. 24 Weeks Female	Interim Sac. 66 Weeks Male	Interim Sac. 66 Weeks Female
1. Control	0	80	80	10	10	10	10
2. Paired Cont.*	0	50	50	--	--	--	--
3. Low (LDT)	25	50	50	10	10	10	10
4. Mid (MDT)	100	50	50	10	10	10	10
5. High (HDT)	175	80	80	10	10	10	10

*During each week that one or more animals from any treated group were found dead or sacrificed in a moribund condition, one mouse of the same sex from this group was killed.

The mice were individually housed in polycarbonate cages in an animal room with temperature 19.4 to 26.1 °C, relative humidity 22 to 76 percent, a light-on/light-off cycle of 12 hours, and there were 10 room air changes per hour. After a 12 to 13 day quarantine period, a complete necropsy was conducted on 3 male and 2 female mice.

2. Dose Preparation - Dose formulations were made weekly by mixing appropriate amounts of the test material with deionized water. Stability studies were conducted and the concentration of the test material in each dose formulation was analyzed at 1- to 2-month intervals.

Results - The test material at a concentration of 25 ppm was stable in deionized water at 25 °C for 3 weeks in sealed polypropylene containers that were protected from light. All dose formulations were within ± 10% of the target concentrations throughout the study.

3. Animals received food (NIH-07 diet) and deionized water with or without the test material ad libitum.

4. Statistics - The probability of survival was estimated by the product-limit procedures of Kaplan-Meier. Tests for possible dose-related effects on survival used Cox's method for testing two groups for equality and Tarone's life table test for dose-related trends. The primary method used for the analysis of tumor incidence was logistic regression analysis. Prevalence analysis was used to adjust for intercurrent mortality. Life table tests, appropriate for rapidly lethal tumors, the Fisher exact test, and the Cochran-Armitage trend tests were used to analyze the data. Tests of significance included pairwise comparison of each treated group with the controls. Continuity-corrected tests were used in the analysis of tumor incidence and reported p values were one-sided. Treated groups were compared to the concurrent controls and the NTP historical control data base.

5. Quality assurance was conducted by an independent quality assurance contractor, Integrated Laboratory Systems.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for mortality and morbidity. Clinical observations were made weekly through Week 13 and monthly thereafter.

   Results - Survival was comparable among control and treated groups (see Table 1). Males in the control and 175 ppm groups had 73 and 81 percent survival, respectively. Females in the control and 175 ppm groups had 66 and 65 percent survival, respectively.

Table 1: Animal Survival

	Control	25 ppm	100 ppm	175 ppm
Male				
No. of Mice	99	70	70	100
No. Surviving 2 years	58	39	37	65
Interim Kill	20	20	19	20
Percent Survival	73	78	73	81
Mean Survival (days)	707	704	678	705

Table 1: Animal Survival (Cont'd)

	Control	25 ppm	100 ppm	175 ppm
Female				
No. of Mice	100	70	70	99
No. Surviving 2 years	53	38	34	52
Interim Kill	20	18	20	19
Percent Survival	66	73	68	65
Mean Survival (days)	693	688	681	655

Males in the 25, 100, and 175 ppm groups had increases in the incidence of attrition of the teeth, 31, 24, and 28 percent, when compared with controls, 19%. The incidence of attrition was increased in only females in the 175 ppm group of 8 percent compared with 0% for the control and remaining treated groups. Discoloration of the teeth was also increased in males in the 25, 100 and 175 ppm groups (incidence: 43, 84 and 100%, respectively) when compared to controls, 27%. Discoloration of the teeth was similarly increased in females in the 25, 100, and 175 ppm groups (incidence: 43, 84, and 100%, respectively) when compared to controls, 19 percent. Mottling of the teeth was increased in males in the 25, 100, and 175 ppm groups (incidence: 64, 86, and 96%) when compared to controls, 26%. Mottling of the teeth was increased in females in the 25, 100, and 175 ppm groups (incidence: 45, 94, and 98%, respectively) when compared to controls, 15% (see Table 2).

Table 2: Incidence of Tooth Abnormalities in Mice at Week 104

	Dose Level (ppm)			
Lesion	0	25	100	175
	Males			
Attrition	11 (19)[1]	12 (31)	9 (24)	18 (28)
Discoloration	27 (27)	27 (39)	56 (80)	100 (100)
Mottling	16 (26)	25 (64)	32 (86)	62 (95)

[1]Percent incidence

Table 2: Incidence of Tooth Abnormalities in Mice at Week 104 (Cont'd)

Lesion	Dose Level (ppm) 0	25	100	175
	Females			
Attrition	0	0	0	4 (8)
Discoloration	19 (19)	30 (43)	59 (84)	99 (100)
Mottling	8 (15)	17 (45)	32 (94)	51 (78)

2. Body Weight - The animals were weighed initially, weekly through Week 13, and monthly thereafter.

   Results - Unremarkable. The body weight of males in the 0 and 175 ppm groups at Week 13 were 37.7 and 36.2 g, respectively. The body weight of females in the 0 and 175 ppm groups were 31.7 and 30.7 g, respectively. This represents a decrease of 4.0 percent in males and 3.2 percent in females in the 175 ppm group when compared to controls.

3. Food and Water Consumption and Compound Intake - Every 4 weeks food and water consumption were recorded for a 1-week period.

   Results - Food and water consumption were comparable among the control and treated groups. Male and female average daily food consumption ranged from 4.9 to 5.2 g for males and 5.4 to 5.8 g for females. Average daily water consumption ranged from 4.1 to 4.2 g for males and 4.4 to 4.6 g for females. Compound intake was estimated to be 2.4, 9.6, and 16.7 mg/kg/day for males in the 25, 100, and 175 ppm groups, respectively. Compound intake was estimated to be 2.8, 11.3, and 18.8 mg/kg/day for females in the 25, 100, and 175 ppm groups, respectively.

4. Blood was collected at 24 and 66 weeks for hematology and clinical analysis from all animals (10/sex/group) sacrificed at that time. The CHECKED (X) parameters were examined.

   a. Hematology

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

Results - Unremarkable.

b. Clinical Chemistry

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

Results - Alkaline phosphatase was increased in females in the 175 ppm group at 24 weeks by 29 percent and by 11 percent at 66 weeks when compared to controls (see Table 3).

Table 3: Serum Alkaline Phosphatase in Females (IU/L) and Percent (%) Increase When Compared to Controls

	Dose Level (ppm)			
Week	0	25	100	175
24	200.1	179.4 (-10)	214.4 (7)	258.5** (29)
66	167.3	164.5 (-2)	171.7 (4)	185.7* (11)

*Significantly different from controls at $p \leq 0.05$.
**Significantly different from controls at $p \leq 0.01$.

Fluoride levels in bone (humerus) were increased in treated groups in a dose-response relationship. Males and females in the 25, 100, and 175 ppm groups were increased by 85 to 102 percent, 376 to 423 percent and 618 to 677 percent, respectively, when compared to controls at 24 weeks. At 66 weeks, bone fluoride levels were increased in animals in the 25, 100, and 175 ppm groups by 108 to 170 percent, 385 to 414 percent, and 675 to 693 percent, respectively, when compared to controls. At 105 weeks, bone fluoride levels were increased in animals in the 25, 100, and 175 ppm groups by 66 to 123 percent, 377 to 399 percent and 532 to 691 percent, respectively, when compared to controls (see Table 4).

Table 4: Bone Fluoride Levels in Mice (ug/mg ash) and Percent (%) Increase Compared to Controls

Time	Control	25 ppm	100 ppm	175 ppm
24 Weeks				
Male	0.360	0.728 (102)*	1.884 (423)*	2.796 (677)*
Female	0.395	0.731 (85.1)*	1.880 (376)*	2.837 (618)*
66 Weeks				
Male	0.558	1.163 (108)*	2.868 (414)*	4.324 (675)*
Female	0.595	1.606 (170)*	2.883 (385)*	4.716 (693)*
105 Weeks				
Male	0.719	1.606 (123)*	3.585 (399)*	5.690 (691)*
Female	0.917	1.523 (664)*	4.370 (377)*	6.241 (532)*

*Significantly different from controls at $p \leq 0.015$.

5. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. Only gross lesions were examined in the 25 and 100 ppm groups at the 24-week sacrifice. The (XX) organs, in addition, were weighed.

X	Digestive system
	Tongue
X	Salivary glands
X	Esophagus
X	Stomach
X	Duodenum
X	Jejunum
X	Ileum
X	Cecum
X	Colon
X	Rectum
XX	Liver
X	Gallbladder
X	Pancreas
	Respiratory
X	Trachea

X	Cardiovasc./Hemat.
	Aorta
X	Heart
X	Bone marrow
X	Lymph nodes
X	Spleen
	Thymus
	Urogenital
XX	Kidneys
X	Urinary bladder
X	Testes
X	Epididymides
X	Seminal vesicle
X	Ovaries
X	Uterus
X	Clitoral gland

X	Neurologic
XX	Brain
X	Periph. nerve (when neurolog. signs present)
X	Spinal cord (3 levels) (when neurolog. signs present)
X	Pituitary
X	Eyes (optic nerve)

X	Respiratory
X	Lung
X	Nasal cavity and turbinates
X	Pharynx (when grossly abnormal)

X	Urogenital
X	Preputial gland

X	Glandular
X	Adrenals
X	Lacrimal gland
X	Mammary gland
X	Parathyroids
X	Thyroids
	Other
X	Bone
X	Skeletal muscle
X	Skin
X	All gross lesions and masses
X	Teeth

Results

a. Organ Weight - Unremarkable (includes organ/body weight ratios).

b. Gross pathology - not reported.

c. Microscopic pathology

1) Non-neoplastic - The incidence of dentine dysplasia was increased in males in the 175 ppm group when compared to the controls; the incidences were 62/79 (78%), 44/50 (88%), 43/51 (84%), and 73/80 (91%) in the 0, 25, 100, and 175 ppm groups, respectively.

2) Neoplastic - The incidence of malignant lymphoma (24%) and malignant lymphoma and histiocytic sarcoma combined (30%) were marginally increased in females in the 175 ppm group when compared to control (malignant lymphoma - 14%; combined - 20%; see Table 5). (The term histiocytic sarcoma is synonymous with malignant lymphoma according to NTP.) The incidence of malignant lymphoma in females in the 175 ppm group (30%) is similar to the mean incidence in historical controls (34.6%) and within the range of historical controls at the testing laboratory, 18 to 48 percent. There was an increase in the incidence of hepatoblastoma in males in the 25 ppm (1/50; 2%), 100 ppm (1/51; 2%), and 175 ppm (3/80; 4%) groups when compared to controls (0/79; 0%). The incidence of hepatoblastoma was increased in females in the 25 ppm (1/52; 2%) and 175 ppm (2/80; 3%) when compared to controls (0/80; 0%). Hepatocellular neoplasms with the embryonal cell

TABLE 5
Malignant Lymphomas and Histiocytic Sarcomas in Female Mice in the 2-Year Drinking Water Studies of Sodium Fluoride

	Control	25 ppm	100 ppm	175 ppm
Malignant Lymphoma (Lymphocytic)				
Overall rates[a]	2/80 (3%)	0/52 (0%)	1/50 (2%)	5/80 (6%)
Malignant Lymphoma (Mixed)				
Overall rates	4/80 (5%)	5/52 (10%)	7/50 (14%)	8/80 (10%)
Malignant Lymphoma (Undifferentiated Cell Type)				
Overall rates	5/80 (6%)	0/52 (0%)	3/50 (6%)	6/80 (8%)
All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or Undifferentiated Cell Type)[b]				
Overall rates	11/80 (14%)	5/52 (10%)	11/50 (22%)	19/80 (24%)
Adjusted rates[c]	19.3%	12.4%	30.0%	32.6%
Terminal rates[d]	8/53 (15%)	4/38 (11%)	9/34 (26%)	14/52 (27%)
First incidence (days)	652	587	379	241
Life table tests[e]	P=0.012	P=0.282N	P=0.181	P=0.069
Logistic regression tests[e]	P=0.010	P=0.333N	P=0.145	P=0.051
All Organs: Histiocytic Sarcoma				
Overall rates	5/80 (6%)	3/52 (6%)	2/50 (4%)	5/80 (6%)
Adjusted rates	7.9%	7.2%	4.9%	8.0%
Terminal rates	2/53 (4%)	2/38 (5%)	0/34 (0%)	1/52 (2%)
First incidence (days)	562	584	587	584
Life table tests	P=0.515	P=0.577N	P=0.452N	P=0.577
Logistic regression tests	P=0.481N	P=0.590N	P=0.405N	P=0.580N
All Organs: Malignant Lymphoma or Histiocytic Sarcoma				
Overall rates	16/80 (20%)	8/52 (15%)	13/50 (26%)	24/80 (30%)
Adjusted rates	26.2%	19.3%	33.4%	38.4%
Terminal rates	10/53 (19%)	6/38 (16%)	9/34 (26%)	15/52 (29%)
First incidence (days)	562	584	379	241
Life table tests	P=0.022	P=0.282N	P=0.301	P=0.089
Logistic regression tests	P=0.023	P=0.335N	P=0.267	P=0.077

[a] Number of tumor-bearing animals/number of animals necropsied
[b] 2-year historical incidence for untreated control groups at study laboratory (mean): 145/419 (34.6%); historical incidence for untreated control groups in NTP studies (mean ± SD): 693/2209 (31.4% ± 14.0%)
[c] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.

TABLE 6
Hepatocellular Neoplasms in Male and Female Mice in the 2-Year Drinking Water Studies of Sodium Fluoride

	Control	25 ppm	100 ppm	175 ppm
Male				
Hepatocellular Adenoma[a]				
Overall rates[b]	50/79 (63%)	34/50 (68%)	30/51 (59%)	53/80 (66%)
Hepatocellular Carcinoma[c]				
Overall rates	25/79 (32%)	15/50 (30%)	13/51 (25%)	15/80 (19%)
Hepatoblastoma				
Overall rates	0/79 (0%)	1/50 (2%)	1/51 (2%)	3/80 (4%)
Hepatocellular Neoplasms (Adenoma, Carcinoma, Hepatoblastoma)				
Overall rates	62/79 (78%)	39/50 (78%)	37/51 (73%)	61/80 (76%)
Adjusted rates[d]	86.0%	82.9%	84.0%	82.4%
Terminal rates[e]	48/58 (83%)	31/39 (79%)	30/37 (81%)	52/65 (80%)
First incidence (days)	420	619	579	529
Logistic regression tests[f]	P=0.410N	P=0.581N	P=0.496N	P=0.470N
Female				
Hepatocellular Adenoma[g]				
Overall rates	49/80 (61%)	28/52 (54%)	23/50 (46%)	34/80 (43%)
Hepatocellular Carcinoma[h]				
Overall rates	14/80 (18%)	11/52 (21%)	8/50 (16%)	12/80 (15%)
Hepatoblastoma				
Overall rates	0/80 (0%)	1/52 (2%)	0/50 (0%)	2/80 (3%)
Hepatocholangiocarcinoma				
Overall rates	0/80 (0%)[i]	0/52 (0%)	0/50 (0%)	1/80 (1%)
Hepatocellular Neoplasms (Adenoma, Carcinoma, Hepatoblastoma, Hepatocholangiocarcinoma)				
Overall rates	55/80 (69%)	33/52 (63%)	26/50 (52%)	43/80 (54%)
Adjusted rates	84.4%	78.5%	64.8%	72.6%
Terminal rates	43/53 (81%)	29/38 (76%)	20/34 (59%)	36/52 (69%)
First incidence (days)	358	587	527	361
Logistic regression tests	P=0.077N	P=0.339N	P=0.056N	P=0.116N

[a] 2-year historical incidence for untreated control groups at study laboratory (mean): 70/414 (16.9%); historical incidence for untreated control groups in NTP studies (mean ± SD): 323/2197 (14.7% ± 7.9%)
[b] Incidence expressed as number of animals with lesion/total number of animals necropsied
[c] 2-year historical incidence for untreated control groups at study laboratory (mean): 72/414 (17.4%); historical incidence for untreated control groups in NTP studies (mean ± SD): 358/2197 (16.3% ± 6.9%)
[d] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal.
[g] 2-year historical incidence for untreated control groups at study laboratory (mean): 23/417 (5.5%); historical incidence for untreated control groups in NTP studies (mean ± SD): 131/2202 (5.9% ± 3.7%)
[h] 2-year historical incidence for untreated control groups at study laboratory (mean): 11/417 (2.6%); historical incidence for untreated control groups in NTP studies (mean ± SD): 78/2202 (3.5% ± 2.4%)
[i] A hepatocholangiocarcinoma occurred in 1/42 paired control female mice.

type (hepatoblastoma) is rare with an incidence of 0/2197 and 1/2202 historical control males and females. The incidences of primary hepatocellular neoplasms were comparable among the treated and control groups (see Table 6).

D. Discussion

Attrition of the teeth was increased in females in the 175 ppm group and in all treated male groups. The incidence of discoloration and mottling of the teeth were increased in all male and female treated groups. Alkaline phosphatase was increased in females in the 175 ppm group when compared to controls. Bone fluoride levels were increased in males and females in all NaF treated groups. The incidence of dentine dysplasia was increased in males in the 175 ppm group. It was concluded by NTP that there was no evidence of carcinogenic activity in male or female mice administered sodium fluoride in drinking water for 2 years.

Guideline Series 83-5

Reviewed By: William B. Greear, M.P.H. William B. Greear 7/23/92
Review Section IV, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M.
Review Section IV, Toxicology Branch I (H7509C) Marion Copley 7/28/92

DATA EVALUATION REPORT

Study Type: NTP Carcinogenesis Bioassay - Rat

PC No.: 075202
TOX Chem. No.: 769
CAS No.: 7681-49-4

Test Material: Sodium Fluoride

Study Number: NTP TR 393; NIH Publ. 91-2848

Sponsor: National Toxicology Program
Research Triangle Park, NC 27709

Testing Facility: Battelle Columbus Laboratories
Columbus, OH

Title of Report: Toxicology and Carcinogenesis Studies of Sodium Fluoride in F344/N Rats and B6C3F1 Mice (Drinking Water Studies)

Author: National Toxicology Program

Report Issued: December 1990

Conclusions:

NOEL < 25 ppm (1.3 mg/kg/day)
LEL = 25 ppm (based on mottling of teeth, dentine incisor dysplasia, increased serum, urine and bone fluoride levels in M&F; incisor odontoblast and incisor ameloblast degeneration in M)

In addition, at 100 ppm males had attrition, deformity, and discoloration of the teeth, females had incisor ameloblast degeneration; at 175 ppm males had malocclusion, females had attrition, deformity and discoloration of the teeth, incisor odontoblast and ameloblast degeneration of the teeth and osteosclerosis. Equivocal evidence of carcinogenicity in males. No evidence of carcinogenicity in females.

Classification: 83-1 Chronic Toxicity: Core-Minimum Data
83-2 Carcinogenicity: Core-Minimum Data

Study Acceptability: The study satisfies the requirement for a Guideline Series 83-2 Carcinogenicity Study, and for a Guideline Series 83-1 Chronic Toxicity Study.

A. Materials:

1. Test Compound: Sodium fluoride; Description: white, crystalline water-soluble powder; Batch No.: A022085; Purity: 99%.

2. Test Animals: Species: rat; Strain: F344/N; Age: 4 weeks on arrival; Weight: male - 133 to 136 (mean), female - 99 to 105 (mean); Source: NCI Frederick Cancer Research, Frederick, MD.

B. Study Design:

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

Test Group	Dose in Water (ppm)	Main Study 103 Weeks Male	Main Study 103 Weeks Female	Interim Sac. 27 Weeks Male	Interim Sac. 27 Weeks Female	Interim Sac. 66 Weeks Male	Interim Sac. 66 Weeks Female
1. Control	0	80	80	10	10	10	10
2. Paired Cont.*	0	50	50	--	--	--	--
3. Low (LDT)	25	50	50	10	10	10	10
4. Mid (MDT)	100	50	50	10	10	10	10
5. High (HDT)	175	80	80	10	10	10	10

*During each week that one or more animals from any treated group were found dead or sacrificed in a moribund condition, one rat of the same sex from this group was killed.

The rats were housed five per cage in polycarbonate cages in an animal room with temperature 19.4 to 26.1 °C, relative humidity 22 to 76 percent, a light-on/light-off cycle of 12 hours, and there were 10 room air changes per hour. After a 12 to 13 day quarantine period, a complete necropsy was conducted on 20 rats/sex.

2. Dose Preparation - Dose formulations were made weekly by mixing appropriate amounts of the test material with deionized water. Stability studies were conducted and the concentration of the test material in each dose formulation was analyzed at 1- to 2-month intervals.

Results - The test material at a concentration of 25 ppm was stable in deionized water at 25 °C for 3 weeks in sealed polypropylene containers that were protected from light. All dose formulations were within ± 10% of the target concentrations throughout the study.

3. Animals received food (NIH-07 diet) and deionized water with or without the test material ad libitum.

4. Statistics - The probability of survival was estimated by the product-limit procedures of Kaplan and Meier. Tests for possible dose-related effects on survival used Cox's method for testing two groups for equality and Tarone's life table test for dose-related trends. The primary method used for the analysis of tumor incidence was logistic regression analysis. Prevalence analysis was used to adjust for intercurrent mortality. Life table tests, appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend tests were used to analyze the data. Tests of significance included pairwise comparison of each treated group with the controls. Continuity-corrected tests were used in the analysis of tumor incidence and reported p values were one-sided. Treated groups were compared to the concurrent controls and the NTP historical control data base.

5. Quality assurance was conducted by an independent quality assurance contractor, Integrated Laboratory Systems.

C. Method and Results:

1. Observations - Animals were inspected twice daily for mortality and morbidity. Clinical observations were made weekly through Week 13 and monthly thereafter.

   Results - There were no treatment-related effects on mortality (see Table 1). Males in the control and 175 ppm groups had 53 percent survival. Females in the control and 175 ppm groups had 74 and 67 percent survival, respectively. The incidence of attrition of the teeth was increased in males in the 100 ppm (30%) and 175 ppm (50%) when compared to controls (0%), increased in females in the 175 ppm. Deformity of the teeth was increased in males in the 100 ppm (17%) and 175 ppm (27%) groups when compared to controls (1%), and in females in the 175 ppm group (8%) when compared to controls (0%). Discoloration of the teeth was increased in males in the 100 ppm (21%) and 175 ppm (31%) groups when compared to controls (1%). Discoloration of the teeth was increased in females in the 175 ppm group (13%) when compared to controls (1%). Mottling of the teeth was increased in males in the 25 ppm (55%), 100 ppm (96%) and 175 ppm (100%) groups when compared to controls (5%). Mottling of the teeth was increased in females in the 25 ppm (85%), 100 ppm (94%), and 175 ppm (98%) group when compared to controls (see Table 2).

Table 1: Animal Survival

	Control	25 ppm	100 ppm	175 ppm
Male				
No. of Rats	100	70	70	100
No. Surviving 2 years	42	25	23	42
Interim Kill	19	19	20	20
Percent Survival	53	49	47	53
Mean Survival (days)	668	687	671	675
Female				
No. of Rats	100	70	70	100
No. Surviving 2 years	59	31	34	54
Interim Kill	20	20	20	19
Percent Survival	74	62	68	67
Mean Survival (days)	697	702	703	697

Table 2: Tooth Abnormalities (Percent) in Survivors at Week 104

Observation[a]	Control	25 ppm	100 ppm	175 ppm
Male				
Attrition[b]	0	0	7 (30)	22 (50)
Deformity[c]	1 (1)	0	12 (17)	27 (27)
Discoloration[c]	1 (1)	2 (3)	15 (21)	31 (31)
Malocclusion[c]	1 (1)	1 (1)	2 (3)	13 (13
Mottling[b]	2 (5)	22 (85)	22 (96)	44 (100)
Female				
Attrition	0	0	1	2 (4)
Deformity	0	0	1	8 (8)
Discoloration	0	2 (3)	2 (3)	8 (8)
Malocclusion	1 (1)	0	0	1 (1)
Mottling	0	8 (26)	32 (94)	53 (98)

[a]Discoloration designates an overall effect, while mottling indicates variegated discoloration. The terms are not mutually exclusive.
[b]The incidence for this observation are for the lower incisors of animals observed at Week 104 only (males: N = 43, 26, 23, 44; females: N = 59, 31, 34, 54).
[c]The incidences for this observation include interim and terminal sacrifice animals (males and females: N = 100, 70, 70, 100).

2. Body Weight - The animals were weighed initially, weekly through Week 13, and monthly thereafter.

   Results - Unremarkable.

3. Food and Water Consumption and Compound Intake - Every 4 weeks food and water consumption were recorded for a 1-week period.

   Results - Food and water consumption were comparable among control and treated groups. Male and female average daily food consumption ranged from 17.2 to 17.4 g and 11.2 to 11.3 g, respectively. Average daily water consumption ranged from 19.8 to 21.2 g for males and 13.1 to 13.6 g for females. Compound intake was estimated to be 1.3, 5.2, and 8.6 mg/kg/day for males in the 25, 100, and 175 ppm groups and 1.3, 5.5, and 9.5 mg/kg/day for females in the 25, 100, and 175 ppm groups.

4. Blood was collected at 27 and 66 weeks for hematology and clinical analysis from all animals (10/sex/group) sacrificed at that time. The CHECKED (X) parameters were examined.

   a. Hematology

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

   Results - Unremarkable.

   b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium		Albumin
	Chloride		Blood creatinine
	Magnesium		Blood urea nitrogen
X	Phosphorus		Cholesterol
	Potassium		Globulins
	Sodium		Glucose

X	Enzymes	X	Other (cont'd)
X	Alkaline phosphatase		Total bilirubin
	Cholinesterase		Total protein
	Creatinine phosphokinase		Triglycerides
	Lactic dehydrogenase	X	Serum fluoride
	Serum alanine aminotransferase (also SGPT)	X	Humerus fluoride
	Serum aspartate aminotransferase (also SGOT)	X	Urine fluoride

Results - Serum fluoride concentrations were significantly increased in females treated at 100 ppm (94%) and 175 ppm (118%) at 27 weeks and in all treated males (control to high: 238, 955, 1909%) and females (control to high: 146, 633, 997%) at 66 weeks (see Table 3). Urine fluoride concentrations were increased in all treated groups (males greater than 232%; females greater than 340%) at 27 and 66 weeks when compared to controls. Bone fluoride levels were increased in all male treated groups (> 120%) and all female treated grups (> 143%) at 27, 66, and 105 weeks.

Serum fluoride levels were increased in males in the 175 ppm group (62%) at 27 weeks and in all male treated groups (low to high - 34, 66, 133%) at 66 weeks. Serum fluoride levels were increased in all female treated groups at 27 week (low to high - 69, 94, 118%) and at 66 weeks (low to high 20, 56, 169%).

Table 3: Bone, Serum, and Urine Fluoride Concentration (ug/mg ash) and Percent (%) Increase Compared to Controls

Time	Control	25 ppm	100 ppm	175 ppm
27 Weeks				
Male				
Bone	0.253	0.617** (144)	1.685** (566)	2.936** (1060)
Serum	0.063	0.041 (-34.9)	0.065 (3.2)	0.102 (61.9)
Urine	0.919	3.055** (232)	10.473** (1040)	17.110 (1762)
Female				
Bone	0.320	0.805** (152)	2.081** (550)	3.236** (911)
Serum	0.051	0.086 (68.6)	0.099* (94.1)	0.112** (118)
Urine	1.09	5.00** (391)	18.51** (1598)	32.97** (2925)

*Significantly different from controls at $p \leq 0.05$.
**Significantly different from controls at $p \leq 0.01$.

Table 3: Bone, Serum, and Urine Fluoride Concentration (ug/mg ash) and Percent (%) Increase Compared to Controls (Cont'd)

66 Weeks				
Male				
Bone	0.357	0.871** (144)	2.563** (618)	4.020** (1026)
Serum	0.067	0.090* (34.3)	0.111** (65.7)	0.156** (133)
Urine	1.01	3.41** (238)	10.66** (955)	20.29** (1909)
Female				
Bone	0.425	1.045** (146)	3.115** (633)	4.622** (997)
Serum	0.071	0.085* (19.7)	0.107** (50.7)	0.191** (169)
Urine	0.851	3.748** (340)	13.500** (1486)	26.778** (3047)
105 Weeks				
Male				
Bone	0.445	0.978** (120)	3.648** (720)	5.263** (1083)
Female				
Bone	0.554	1.348** (143)	3.726** (573)	5.554** (903)

*Significantly different from controls at $p \leq 0.05$.
**Significantly different from controls at $p \leq 0.01$.

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

X	Digestive system
	Tongue
X	Salivary glands
X	Esophagus
X	Stomach
X	Duodenum
X	Jejunum
X	Ileum
X	Cecum
X	Colon
X	Rectum
XX	Liver[1]
X	Gallbladder

X	Cardiovasc./Hemat.
	Aorta
X	Heart
X	Bone marrow
X	Lymph nodes
X	Spleen
X	Thymus
	Urogenital
XX	Kidneys[1]
X	Urinary bladder
X	Testes
X	Epididymides
X	Prostate

X	Neurologic
XX	Brain[1]
	Periph. nerve (when neurolog. signs present)
X	Spinal cord (3 levels) (when neurolog. signs present)

X	Pancreas	X	Seminal vesicle	X	Pituitary
	Respiratory	X	Ovaries	X	Eyes (optic nerve)
X	Trachea	X	Uterus		Glandular
X	Lung	X	Clitoral gland	X	Adrenals
X	Nasal cavity and turbinates	X	Preputial gland		Lacrimal gland
X	Pharynx (when grossly abnormal)			X	Mammary gland
				X	Parathyroids
				X	Thyroids
					Other
					Bone
				X	Skeletal muscle
				X	Skin
				X	All gross lesions and masses
				X	Teeth

*At the 27-week sacrifice, only gross lesions were examined in the 25 and 100 ppm groups.
[1]Weights only determined at the 27- and 66-week sacrifices.

Results

a. Organ Weight - unremarkable (includes organ/body weight ratios).

b. Gross pathology - not reported.

c. Microscopic pathology
1) Non-neoplastic - The incidence of osteosclerosis was increased in females in the 175 ppm group when compared to controls (control - 6/80; 175 ppm - 18/81). Alterations of the teeth were associated with administration of the test material (see Table 4). Dentine incisor dysplasia was increased in males and females in all treated groups. Incisor odontoblast degeneration was increased in males in all treated groups and in females in the 175 ppm group. Incisor ameloblast degeneration was increased in males in all treated groups and in females in the 100 and 175 ppm groups.

Table 4: Incidence of Lesions of the Tooth

Lesion	Control	Paired Control	25 ppm	100 ppm	175 ppm
Male (N)	(80)	(45)	(51)	(50)	(80)
Dentine incisor dysplasia	4 (5%)		7 (14%)	14 (28%)	30 (38%)
Incisor odontoblast degeneration			1 (2%)	2 (4%)	4 (5%)
Incisor ameloblast degeneration			1 (2%)	7 (14%)	23 (29%)
Female					
Dentine incisor dysplasia		2 (6%)	8 (16%)	4 (8%)	10 (12%)
Incisor odontoblast degeneration					
Incisor ameloblast degeneration				1 (2%)	7 (9%)

2) Neoplastic - One male in the 100 ppm group and 3 males in the 175 ppm group had osteosarcoma of the bone. This lesion was not observed in the control or in the 25 ppm male rats or in female rats (see Table 5).

Table 5: Osteosarcoma of the Bone in Male Rats

Bone Osteosarcoma	Control	25 ppm	100 ppm	175 ppm
Overall rate[1]	0/80	0/51	1/50 (2%)	3/80 (4%)[3]
Adjusted rate[2]	0%	0%	4.3%	5.3%
First Incidence	--	--	729	388
Logistic regression tests	P = 0.027[4]	--	P = 0.380[5]	P = 0.099[5]

[1]No. tumor-bearing animals/No. animals necropsied
[2]Kaplan-Meier estimated tumor incidence at the end of study after adjustment for intercurrent mortality
[3]One extraskeletal osteosarcoma in a high-dose male
[4]Trend test
[5]Pairwise comparisons

[The incidence of osteosarcoma at any site was 10/2106 (0.5%) in male historical control rats. The greatest incidence observed was 6 percent in any control group.]

The incidence of squamous cell papilloma or carcinoma combined arising from the epithelium of the oral mucosa was marginally increased in males and females in the 175 ppm group (see Table 6).

[The incidence of squamous cell neoplasms of the oral mucosa was 14/2106 (0.7%) in historical male rats and 12/2153 (0.6%) in historical control female rats. The highest incidence observed in any control group was 4 percent.] Follicular cell adenomas of the thyroid were observed in 1/49 males in the 100 ppm group and in 3/80 males in the 175 ppm group. Follicular cell carcinomas of the thyroid occurred in 1/80 control males and in 1/51 males in the 25 ppm group and in 1/80 males in the 175 ppm group. The follicular cell carcinoma in the male in the 175 ppm group was observed at 66 weeks. However, the incidence of the follicular cell tumors in the treated groups was not significantly different from the controls. In addition, the incidence of follicular cell neoplasms in males in the 175 ppm group was within the range of historical controls [26/2086 (1.2%), range 0-6%].

Keratoacanthomas were observed in three females in the 175 ppm group, whereas none occurred in the lower dose groups or in controls. However, other benign tumors arising from stratified squamous epithelium were observed in one control female (a trichoepithelioma) and in one paired control female (squamous papilloma). The incidence of squamous cell neoplasms in females in the 175 ppm group were not significantly greater than in controls.

D. Discussion:

Animals in the 100 and 175 ppm groups exhibited attrition, deformity, discoloration, and mottling of the teeth. Animals in the 25 ppm group exhibited only mottling of the teeth. Serum, urine, and bone fluoride levels were increased in all treated animals. The fluoride levels showed a dose-response relationship. Osteosclerosis was increased in females in the 175 ppm group. Sensitive

TABLE 6
Lesions of the Oral Cavity in Rats in the 2-Year Drinking Water Studies of Sodium Fluoride

	Control	25 ppm	100 ppm	175 ppm
Male				
Tongue: Squamous Hyperplasia				
Overall rates[a]	0/80 (0%)	1/51 (2%)	0/50 (0%)	0/80 (0%)
Oral Cavity (Oral Mucosa, Tongue, or Pharynx): Squamous Papilloma				
Overall rates	0/80 (0%)	1/51 (2%)	1/50 (2%)	2/80 (3%)
Oral Mucosa: Squamous Cell Carcinoma				
Overall rates	0/80 (0%)	0/51 (0%)	1/50 (2%)	1/80 (1%)
Oral Cavity (Oral Mucosa, Tongue, or Pharynx): Squamous Papilloma or Squamous Cell Carcinoma[b]				
Overall rates	0/80 (0%)[c]	1/51 (2%)	2/50 (4%)	3/80 (4%)
Adjusted rates[d]	0.0%	4.0%	6.0%	5.9%
Terminal rates[e]	0/42 (0%)	1/25 (4%)	0/23 (0%)	1/42 (2%)
First incidence (days)	—	729 (T)	681	620
Logistic regression tests[f]	P=0.082	P=0.397	P=0.142	P=0.123
Female				
Tongue: Squamous Hyperplasia				
Overall rates	1/80 (1%)	0/50 (0%)	1/50 (2%)	1/81 (1%)
Oral Cavity (Pharynx): Squamous Papilloma				
Overall rates	0/80 (0%)	1/50 (2%)	1/50 (2%)	1/81 (1%)
Oral Mucosa: Squamous Cell Carcinoma				
Overall rates	1/80 (1%)	0/50 (0%)	0/50 (0%)	2/81 (2%)
Oral Cavity (Oral Mucosa or Pharynx): Squamous Papilloma or Squamous Cell Carcinoma[g]				
Overall rates	1/80 (1%)	1/50 (2%)	1/50 (2%)	3/81 (4%)
Adjusted rates	1.5%	3.2%	2.9%	4.5%
Terminal rates	0/59 (0%)	1/31 (3%)	1/34 (3%)	0/54 (0%)
First incidence (days)	674	729 (T)	729 (T)	628
Logistic regression tests	P=0.211	P=0.654	P=0.654	P=0.303

(T)Terminal sacrifice

[a] Incidence expressed as number of animals with lesion/total number of animals necropsied

[b] 2-year historical incidence for untreated control groups at study laboratory (mean): 1/350 (0.3%); historical incidence for untreated control groups in NTP studies (mean ± SD): 14/2106 (0.7% ± 1.3%)

[c] One male rat in the paired control group had a squamous cell carcinoma of the oral mucosa (Table A1 and A3).

[d] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality

[e] Observed incidence at terminal kill

[f] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal.

[g] 2-year historical incidence for untreated control groups at study laboratory (mean): 2/348 (0.6%); historical incidence for untreated control groups in NTP studies (mean ± SD): 12/2153 (0.6% ± 1.0%)

incisor dysplasia was increased in males and females in all treated groups. Incisor odontoblast degeneration was increased in males in all treated groups and in females in the 175 ppm group. Incisor ameloblast degeneration was increased in males in all treated groups and in females in the 100 and 175 ppm groups. Osteosarcoma of the bone was only observed in one male in the 100 ppm group and in three males in the 175 ppm group. NTP considers this to be equivocal evidence of carcinogenicity in male F344/N rats. "Other" tumors present were squamous cell neoplasms of the epithelium of the oral mucosa, follicular cell adenomas and carcinomas and benign tumors arising from stratified squamous epithelium. The occurrence of these "other" tumors was associated with administration of the test material.

The study satisfies the requirement for a Guideline Series 83-2 Carcinogenicity Study, and for a Guideline Series 83-1 Chronic Toxicity Study. The deficiencies are that the following data were not provided: clinical chemistry (minimal data) and urinalysis. In addition, a NOEL was not determined. However, additional clinical chemistry data will be obtained in a forthcoming Chronic Feeding Study in dogs. It is currently known that in humans levels of 1-2 ppm in drinking water may produce slight dental fluorosis (discoloration). This effect is not been considered to be a toxic effect. Due to an abundance of data on the effects of fluoride in humans, additional data is not required to define a NOEL in rats for this endpoint. Therefore this study is acceptable as a Guideline Series 83-1 Chronic Toxicity Study.

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