UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

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MEMORANDUM

SUBJECT: **Sodium Fluoride Toxicology Chapter** for the Reregistration Eligibility Decision (RED) Document. PC Code: 075202 (active). Case No. 3132

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Attached is the Toxicology chapter for the Sodium Fluoride RED document.

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1.0 HAZARD CHARACTERIZATION

Sodium fluoride is registered for commercial use only as a wood preservative for utility poles and railroad ties. Sodium fluoride products are used as supplemental wood treatments and are not intended for primary wood preservative or pressure treated wood preservation.

Sodium Fluoride is an inorganic substance which does not undergo hydrolysis typically like an organic compound. Sodium fluoride is water soluble and dissociates in water.

The acute toxicity database for sodium fluoride is considered complete. Sodium fluoride has a high order of toxicity via the oral route of exposure (Toxicity Category II) and a moderate order of toxicity via the dermal and inhalation routes of exposure (Toxicity Category III). Primary eye irritation studies classify sodium fluoride as corrosive (Toxicity Category I) whereas dermal irritation studies classify sodium fluoride as a mild or slight irritant (Toxicity Category IV). Sodium fluoride is not a dermal sensitizer.

For subchronic toxicity two National Toxicology Program (NTP) studies and five open literature studies (Bohatyrewicz, A. (1999); Paul et al. (1998); Pillai et al. (1988); Chinoy and Patel. (2001) and Shahshi et al. (1994)) were evaluated and considered acceptable. In one NTP study 10 F344/N rats/sex/dose were administered sodium fluoride in drinking water at doses of 0, 10, 30, 100, or 300 ppm for 6 months. **The NOAEL was 30 ppm and the LOAEL was 100 ppm**, based on the presence of hyperplasia in the glandular stomach. There were no treatment-related effects on mortality. In another NTP study, sodium fluoride was administered to 8-12 B6C3F1 mice/sex/dose in deionized water at doses of 0, 10, 50, 100, 200, 300 or 600 ppm for 6 months. The **NOAEL was determined to be 50 ppm in female mice, and could not be determined in males** based on the observation of increased osteoid of the tibia in 5/10 males dosed at 50 ppm. The **LOAEL was established to be 50 ppm in male mice and 100 ppm in female mice**, based on histopathology observed in bone. In one non-guideline study (Bohatyrewicz, A. 1999) 10 female 6-week-old Wistar rats/group were administered NaF at levels of 0, 8, 30, and 60 mg of fluoride/L in drinking water for 6 weeks. High fluoride intake (30 and 60 mg/L) was found to significantly decrease bone quality of the femoral shaft and neck of young rats while lower concentrations (8 mg/L) significantly increased the strength of the femoral neck from the control. In another study (Paul et al. 1998) 10 colony-bred adult Wistar female rats were treated orally (intubation) with daily, non-lethal doses of sodium fluoride at concentrations of 20 or 40 mg/kg for 60 days. The **subchronic toxicity NOAEL was less than 20 mg/kg/day (lowest dose tested). The LOAEL was ≤ 20 mg/kg/day**, based on significant reductions in body weight gain and suppressed spontaneous motor activity. A non-guideline toxicity study (Pillai et al. 1988) administered sodium fluoride daily to 5 Swiss albino mice (Haffkine strain) at a concentration of 5.2 mg/kg/day for 35 days. The **subchronic toxicity NOAEL was < 5.2 mg/kg/day (lowest dose tested). The LOAEL was ≤ 5.2 mg/kg/day**, based on significant decreases in body weight gain, and food and water consumption. In another study (Chinoy and Patel. 2001) 20 Adult female albino mice were administered 10

mg/kg/day sodium fluoride (in 0.2 mL water) for 30 days. A significant decline of ovarian protein, 3-beta-and 17-beta-hydroxysteroid dehydrogenase activities was observed. This could be related to increased cholesterol levels in the ovary suggesting altered steroidogenesis.

The database for developmental toxicity is considered complete with five studies, four in the rat and one in the rabbit. In one study (Bates et al. 1994), sodium fluoride (purity >99%) was administered *ad libitum* in drinking water to groups of 26 Sprague-Dawley rats/dose at dose levels of 0, 7, 18, or 27 mg NaF/kg/day (0, 50, 150, and 300 ppm, respectively) from gestation days (GD) 6 to 15. The **Maternal NOAEL was determined to be 18 mg/kg/day and maternal LOAEL 27 mg/kg/day**, based on reduced maternal body weight. There were no treatment-related clinical signs, increases in mortality or decreases in body weight in rats dosed with sodium fluoride. There were no treatment-related effects on mean live fetal body weight /litter, and the number of live fetuses. **The Developmental toxicity NOAEL is greater than or equal to 27 mg/kg/day (highest dose tested). The Developmental toxicity LOAEL is greater than 27 mg/kg/day (not established).**

In another study (Collins et al. 1995), female (CD:CRL: CD-BR, VAF+) rats were given drinking water containing 0, 10, 25, 100, 175, or 250 ppm fluoride (0, 1.4, 3.9, 15.6, 24.7, or 25.1 mg/kg bw). **A maternal Toxicity NOAEL of 175 ppm (24.7 mg/kg/day) and LOAEL of 250 ppm (25.1 mg/kg/day)** were determined, based on significant reductions in body weight gain, and food and water consumption. There were no incidences of maternal mortality, changes in behavior, clinical signs, or mottled teeth in dams treated with sodium fluoride. **A developmental toxicity NOAEL of ≥ 250 ppm (25.1 mg/kg/day; highest dose tested) and LOAEL > 250 ppm (25.1 mg/kg/day; not established)** were determined. There were no treatment-related effects in fetal body weight, litter sizes, or viable fetuses.

In a developmental toxicity study in the rat (Heindel et al 1996), Sprague-Dawley rats (26/group) were administered sodium fluoride ad libitum in deionized/filtered drinking water on gestation days 6-15 at levels 0, 50, 150, or 300 ppm (0, 6.6, 18.3, or 27.1 mg/kg/day, respectively). The feed contained 15.6 ppm of sodium fluoride (purity >99%). There were no treatment-related clinical signs, increases in mortality (100% survival), or decreases in body weights in rabbits dosed with sodium fluoride at the low- and mid-dose. A **maternal toxicity NOAEL was 18.3 mg/kg/day and LOAEL was 27.1 mg/kg/day**, based on reduced maternal body weight gain. The **developmental NOAEL was ≥ 27.1 mg/kg/day (highest dose tested) and LOAEL > 27.1 mg/kg/day (not established).** There were no treatment-related effects on mean live fetal body weight/litter, live fetal number, and prevalence of malformations.
In a study in rabbits (Heindel et al. 1996), New Zealand White rabbits (26/group) were administered sodium fluoride feed (15.6 ppm, purity >99%) ad libitum in deionized/filtered drinking water on gestation days (6-19 at levels of 0, 50, 150, or 300 ppm. The **maternal toxicity NOAEL was NOAEL 18 mg/kg/day and LOAEL was 29 mg/kg/day**, based on reduced maternal body weight gain. There were no treatment-related clinical signs, increases in mortality (100% survival), or decreases in body

weights in rabbits dosed with sodium fluoride at the low- and mid-dose. The **developmental toxicity NOAEL was established to be ≥ 29 mg/kg/day (highest dose tested) and LOAEL at > 29 mg/kg/day (not established).** There were no treatment-related effects in mean live fetal body weight/litter, live fetal number, and prevalence of malformations.

The database for reproductive toxicity is considered complete In one study (Collins et al. 2001), sodium fluoride was administered in drinking water at doses of 0, 25, 100, 175, and 250 ppm to (CD CRL: CD-BR) rats continuously for three generations. A NOAEL ≥ 250 ppm and LOAEL > 250 ppm were determined for maternal toxicity. There were no treatment-related effects on maternal mortality. A significant decrease from control in fluid consumption (30%) was observed at the 250 ppm dose level. The reproductive toxicity NOAEL and LOAEL were determined to be ≥ 250 ppm (highest dose tested) and > 250 ppm, respectively. There were no treatment-related effects in the mean number of corpora lutea, mean number of implantation sites, implantation efficiency, mean number of viable fetuses, and average percentage of early and late deaths per litter of dams. The developmental toxicity NOAEL was 175 ppm and the LOAEL was 250 ppm, based on decreased ossification of the hyoid bone. Fetal body weight was not affected by treatment with sodium fluoride. There was no evidence of toxicity in fetuses or pups of the F1 generation. The F2 generation fetuses and pups were unaffected by treatment with sodium fluoride with the exception of decreased ossification of the hyoid bone in the F2 fetuses at the 175 (not significant) and 250 ppm (significant) dose groups.
In another reproductive study (Collins et al. 2001); rats were administered 0, 25, 100, 175, or 250 ppm NaF in drinking water throughout three generations. The maternal toxicity NOAEL is ≥ 250 ppm (highest dose tested) and the maternal toxicity LOAEL > 250 ppm (not established). The reproductive toxicity NOAEL was ≥ 250 ppm and the corresponding LOAEL > 250 ppm (not established). There were no dose-related clinical effects observed and no significant differences were observed in F0 female food consumption while there was a 5% decrease (significant) reduction in F0 males at 250 ppm (in the first 7 weeks, and week 9 of the 10 week growth period). Fluid consumption was significantly reduced from control levels in the 175 and 250 ppm dose groups with decreases of 11 and 20% for F0 females, 9 and 20% for F0 males, 19 and 29% for F1 females, and 15 and 25% for F1 males, respectively. F1 males in the 100 ppm dose group drank significantly less (9%) than control animals. There were no significant or dose-related effects observed in implantation and reproductive parameters of any generation.
In a third study (Messer et al), 0, 50, 100, and 200 ppm sodium fluoride was administered via drinking water to 58, 55, 50, and 50 weaning female albino mice, respectively. The females mated and litters were normalized to 6 pups. Second generation mice from control and 50 ppm groups (38 and 44 animals, respectively) were mated and followed the same parameters as the parental group. Retardation of growth was observed in the 100 and 200 ppm F1 groups, with death in 50% of animals in the 200 ppm groups by 8 weeks of age. No litter production was seen at the 200 ppm group and only 9 litters at the 100 ppm were observed over a ten-week period.

The database for chronic toxicity is considered incomplete. In one study (Varner et al. 1998), adult male Long-Evans rats (7/group) received double deionized water (ddw) and

0.5 ppm aluminum fluoride, or ddw and 2.1 ppm sodium fluoride for 52 weeks. No differences were found between the body weights of rats in the different treatment groups although more rats died in the aluminum fluoride (5) ad the sodium fluoride group (3) than the control group (1). All levels in samples of brain and kidney were higher in both the aluminum fluoride and sodium fluoride groups relative to controls. The effects of the two treatments on cerebrovascular and neuronal integrity were qualitatively and quantitatively different. These alterations were greater in animals in the aluminum fluoride group than in the sodium fluoride group and greater in the sodium fluoride group than in controls.

The database for carcinogenicity consists of three studies; one open literature study in the rat and two National Toxicology Program studies. In one study (Maurer et al), Sprague-Dawley rats (70/group) were fed a diet containing 0, 4, 10, or 25 mg/kg/day sodium fluoride added to a low-fluoride diet for up to 99 weeks. There was no evidence of treatment-related incidence of carcinogenicity in Sprague-Dawley rats administered dietary sodium fluoride in concentrations up to 25 mg/kg/day for 2 years. All bone neoplasms observed were considered to be incidental and spontaneous and not related to sodium fluoride treatment, because of their low incidence and random distribution.

In one NTP study, 100, 70, 70, or 100 B6C3F1 mice/sex were administered sodium fluoride (purity >99%) in the drinking water at doses of 0, 25, 100, or 175 ppm (mice/sex) for 103 weeks (Male: 0, 2.4, 9.6, or 16.7 mg/kg/day; female: 0, 2.8, 11.3, or 18.8 mg/kg/day). There were no compound-related effects on mortality, body weight, food consumption, water consumption, hematology, or organ weights. Treatment-related clinical findings included a dose-dependent increase in white discoloration of the teeth (27%, 39%, 80%, and 100% in males and 19%, 43%, 84%, and 100% in females, from control to high dose, respectively) which occurred as early as Day 74 in the high-dose animals compared to Day 508 in the control animals. Serum alkaline phosphatase was significantly increased in high-dose females at 24 (29%) and 66 weeks (88%) and in high dose-males at 66 weeks (11%). Serum phosphorus levels were significantly decreased (13%) in high-dose males at 66 weeks. There was a significant increase in incisor dentine dysplasia in high-dose males (78% in controls versus 91% at the high dose). There was an increase in the incidence of myelofibrosis (femoral, humerus, maxilla, and thoracic) in female mice at all doses. **The NOAEL in male mice was 9.6 mg/kg/day and the corresponding LOAEL 16.7 mg/kg/day**, based on the clinical chemistry changes in alkaline phosphatase and serum phosphorus (males) at 66 weeks and bone lesions (dentine dysplasia). The **NOAEL for female mice was 11.3 mg/kg/day and the LOAEL was18.8 mg/kg/day**, based on the clinical chemistry changes in alkaline phosphatase and bone lesions (myelofibrosis).

In a combined chronic toxicity/oncogenicity study (NTP), 100, 70, 70, or 100 F344/N rats/sex were administered sodium fluoride (purity >99%) in the drinking water at doses of 0, 25, 100, or 175 ppm (mice/sex) for 103 weeks (male: 0, 1.3, 5.2, or 8.6 mg/kg/day; female: 0, 1.3, 5.5, or 9.5 mg/kg/day). **The NOAEL was determined to be < 1.3 mg/kg/day (lowest dose tested) and the LOAEL was determined to be 1.3 mg/kg/day,** based on dentine dysplasia in males and females, and ameloblast

degeneration in males. Mortality, body weight, body weight gain, food consumption, water consumption, hematology, and organ weights were not affected by exposure to sodium fluoride. Histopathology of the incisors noted dentine dysplasia (all dosed animals), degeneration of the ameloblasts (mid- and high-dose animals), and, to a lesser extent, degeneration of the odontoblasts (principally dosed males). Increases in the incidence and severity of osteosclerosis of the long bones were noted in the high-dose females (6/80 control; 18/81 high- dose, P=0.04). Three bone osteosarcomas were noted in high-dose males and one in a mid-dose male, with none in controls. A fourth osteosarcoma, not originating in the bone, was observed in an additional high-dose male

The database for mutagenicity is considered complete with five National Toxicology Program studies and numerous open literature studies. The results can be summarized as follows: (1) all studies conducting the bacterial gene mutation test concluded that sodium fluoride was not mutagenic to any of the 5 Salmonella typhimurium bacterial strains in the presence or absence of metabolic activation; (2) of the four studies that performed the in vitro mammalian cell gene mutation test, two concluded that sodium fluoride was not mutagenic at the HGPRT locus of Chinese hamster ovary cells and the rat epithelial cells (ARL 1), respectively. Two studies (Caspary et al., 1987; NTP) observed positive results in L5179Y and L5178Y mouse lymphoma cells in the absence and presence of metabolic activation; (3) six studies performed the in vitro mammalian chromosome aberration test and observed a dose- and time-dependent increase in chromosomal aberrations following administration of sodium fluoride. Hence, all of them found sodium fluoride to be positively mutagenic (4) three studies conducted the mammalian spermatogonial chromosomal aberration test, two of which reported positive results for mutagenicity; (5) of four studies that performed the mammalian bone marrow chromosomal aberration test, two reported positive results; (6) of four studies that conducted the mammalian erythrocyte micronucleus test, three reported sodium fluoride to be non-mutagenic; (7) only one study (Tong et al. 1988) conducted the bacterial DNA damage or repair test with negative results reported (8) two studies reported sodium fluoride to positively induce unscheduled DNA synthesis in mammalian cell culture (9) six studies conducted the in vitro sister chromatid exchange assay, only two of which reported sodium fluoride positively inducing sister chromatid exchanges (10) there was only one study that conducted an in vivo sister chromatid exchange assay (Li Y, et al. 1987). In this study, Male Chinese hamsters were administered sodium fluoride at concentrations of 0.1, 1, 10, 60 or 130 mg/kg. Sodium fluoride did not induce a SCE increase in CHBM cells; there was no evidence of mutagenicity. Death occurred in three out of the eight hamsters in the 130 mg/kg/day group. Although toxic effects were seen in the high dose group, there were no treatment-related increases in SCE.

In 1996, the EPA's Office of Prevention, Pesticides, and Toxic Substances classified sodium aluminofluoride (cryolite) as a "Group D" carcinogen (not classifiable as to carcinogenicity), citing the National Toxicology Program's carcinogenicity study of sodium fluoride (NTP, 1990). More recently, the National Acedemy of Sciences (NAS, 2006) at the request of the EPA, conducted a review of the toxicologic, epidemiologic, and clinical data on fluoride since the 1993 NAS report. With respect to carcinogenicity, the 2006 NAS report concluded that " on the basis of the committee's collective

consideration of data from humans, genotoxicity assays, and studies of mechanism of action in cell systems…the evidence on the potential of fluoride to initiate or promote cancers, particularly of the bone, is tentative and mixed." This recent conclusion is consistent with the past conclusion of OPPTS regarding carcinogenic potential of fluoride.

The database for metabolism consists of one study from the open literature. In a study by Hall et al. 1977, 6 adult male New Zealand rabbits were administered sodium fluoride in the diet (15 ppm), water (1 ppm), and in a single oral dose injected (0.5 mg/kg) directly into stomach through nasal catheter. Urine excretion following oral administration of sodium fluoride was 5 and 13% for 60 and 600 minutes, respectively. Under steady state conditions approximately 15% of fluoride ingested in food and water was absorbed by the animals. 15% was excreted in urine and 85% of ingested fluoride was removed via fecal excretion.

2.0 TOXICOLOGY DATA

The available toxicology data for Sodium Fluoride is listed below.

Table 1. Toxicology data for sodium fluoride

Test	MRID	Acceptable
870.1100 Acute Oral Toxicity	162945, 40928201, 43778501, 40932003	Yes
870.1200 Acute Dermal Toxicity	43778502, 40928202, 162946, 40932002	Yes
870.1300 Acute Inhalation Toxicity	43778503	Yes
870.2400 Primary Eye Irritation	162948, 40928204, 40932001, 41204001 43778504	Yes
870.2500 Primary Dermal Irritation	43778505, 162947, 40928203, 40932004	Yes
870.2600 Dermal Sensitization	43778506, 40866801 40866901	Yes
870.3100 Oral Subchronic (rodent)	NTP, 1990	yes
870.3700a Developmental Toxicity (rodent)	Open literature	yes
870.3700b Developmental Toxicity (nonrodent)	open literature	yes
870.3800 Reproduction	open literature	yes
870.4200a Oncogenicity (rat)	NTP study	yes
870.4200b Oncogenicity (mouse)	NTP study	yes
870.4300 Chronic/Oncogenicity	NTP study	yes

Test		MRID	Acceptable
870.5100	Mutagenicity-Gene Mutation-bacterial	Open literature	yes
870.5300	Mutagenicity-Gene Mutation mammalian	Open literature	yes
870.5375	Mutagenicity-In Vitro mammalian chromosome aberration test	Open literature	yes
870.5380	Mutagenicity-Spermatogonial chromosomal test	Open literature	yes
870.5385	Mutagenicity-Mammalian bone marrow chromosome aberration test	Open literature	yes
870.5395	Mutagenicity-Mammalian erythrocyte micronucleus Test	Open literature	yes
870.5550	Mutagenicity-UDS in mammalian cells in culture	Open literature	yes
870.5900	Mutagenicity-In Vitro sister chromatid exchange assay	Open literature	Yes
870.5915	Mutagencity-In Vivo sister chromatid exchange Assay	Open literature	yes
870.7485	General Metabolism	Hall, 1977	no
870.7600	Dermal Penetration	No study	--

* Open Literature studies

3.0 DATA GAPS

Intermediate- and long-term dermal risks that are of concern from occupational exposures involving pre-drilled hole spray applications using the mechanical pressure pumps and ground-line brush-on treatments could be refined if a dermal absorption study is conducted to determine actual dermal absorption of sodium fluoride and the result shows a low potential for dermal absorption, or from conduct of a repeated dose dermal toxicity study (90-day) to determine the route-specific NOAEL.

4.0 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of database for Acute Toxicity: The acute toxicity database for sodium fluoride is considered complete. For the technical grade active ingredient, sodium fluoride has a high order of toxicity via the oral route of exposure (Toxicity Category II) and a moderate order of toxicity via the dermal and inhalation routes of exposure (Toxicity Category III). Primary eye irritation studies classify sodium fluoride as corrosive (Toxicity Category I) whereas dermal irritation studies classify sodium fluoride as a mild or slight irritant (Toxicity Category IV). Sodium fluoride is not a dermal sensitizer.

The acute toxicity data for sodium fluoride is summarized below in Table 2.

Table 2. Acute toxicity data for sodium fluoride technical a.i.				
Guideline Number	**Study Type/Test substance (% a.i.)**	**MRID Number/ Citation**	**Results**	**Toxicity Category**
870.1100 (§81-1)	Acute Oral – Rat Purity 95.6% - Sodium Fluoride	43778501	LD_{50} (combined) = 105 (93-119 CL) Male LD_{50} = 120 mg/kg Female LD_{50} = 89 mg/kg	II
870.1200 (§81-2)	Acute Dermal – Rat Purity 95.6% - Sodium Fluoride	43778502	LD_{50} > 2000 mg/kg	III
870.1300 (§81-3)	Acute Inhalation - Rat Purity 95.6% - Sodium Fluoride	43778503	LC_{50} = 1.00 mg/L	III
870.2400 (§81-4)	Primary Eye Irritation - Rabbit Purity 95.6% - Sodium Fluoride	43778504	Severely irritating to unwashed eyes	II
870.2500 (§81-5)	Primary Dermal Irritation- Rabbit purity 95.6% – Sodium Fluoride	43778505	Slightly Irritating	IV
870.2600 (§81-6)	Dermal Sensitization - Guinea pig purity 95.6 % - Sodium Fluoride	43778506	Buehler: Not a skin sensitizer	No
870.2600 (§81-6)	Dermal Sensitization - Guinea pig purity not reported	40866801	Not a dermal sensitizer	No

4.2 Subchronic Toxicity

Adequacy of database for Subchronic Toxicity: Two National Toxicology Program studies and five open literature studies were evaluated and considered acceptable.

870.3100 90-Day Oral Toxicity study in rodents – Rats

A 6-month oral toxicity study (NIH Publication No. 91/2848, 1990) was designed to determine the subchronic oral toxicity of sodium fluoride (>99% purity) administered to 10 F344/N rats/sex/dose in deionized drinking water at dose levels of 0, 10, 30, 100, or 300 ppm (approximately 0, 0.05, 1.5, 5.0, and 15.0 mg/kg/day).

There were no treatment-related effects on mortality. Food and water consumption was decreased compared to the control at the 300 ppm dose level with reductions of 13 and 8% for males and 18 and 19% for females, respectively. There were significant decreases in mean body weight in both males (84% of controls) and females (90% of controls) of the 300 ppm dose group. Mean body weight changes were also decreased in the 300 ppm-treated rats; however, this reduction was only significant in the males (21%). Additional treatment-related effects were noted in 300-ppm treated rats including clinical observations of dental fluorosis (chalky white appearance of teeth from week 6 to 26, overgrowth of upper incisors from week 6 to 17, unusual wear pattern of incisors, occlusal surface of the lower incisor worn to the gumline) and rough hair coats observed from week 18 to 26.

The principle pathological effects associated with the administration of sodium fluoride were observed in the incisor teeth and stomach. Five male rats (of 6 total) treated with 300 ppm sodium fluoride effects had focal or multifocal degeneration of the enamel organ (degeneration was termed dysplasia for this lesion, small aggregation of enamel-like material trapped within cell layers collectively termed dysplasia), columnar ameloblasts flattened or lost (atrophy), disorganized cells of the stratum intermedium that contained less cytoplasm and fewer secretory vacuoles. Effects of the glandular stomach were observed with sodium fluoride treatment. Acute inflammation (7/10 rats), hyperplasia (10/10 rats), necrosis (10/10 rats), and inflammatory, infiltrate, lymphocytic effects (7/10 rats) were observed at the 300 ppm dose level. Inflammatory, infiltrate, lymphocytic effects were also observed at 30 ppm in 2 or 10 male rats while hyperplasia was found in 5 or 10 male rats at 100 ppm. Mucosa of glandular stomach of most male rats in the 300 ppm dose groups appeared thickened and focal or multifocal punctate hemorrhages were observed in 4/10 males. Multiple, small non-perforated ulcers were observed in 1 male rat of the 300 ppm dose group.

Glandular stomach inflammatory, infiltrate, lymphocytic effects were observed in 1 and 4 of 10 female rats at 30 and 300 ppm, respectively. Additional effects were observed at 100 ppm (2/10, hyperplasia) and 300 ppm (9/10, hyperplasia; 9/10, necrosis). Focal or multifocal punctate hemorrhages of the glandular stomach were observed in 1/10 females. Perforated ulcers of the glandular stomach were found in the 300 ppm-treated females.

Measurement of fluoride content revealed a dose-dependent increase in fluoride concentration in bone and urine, while elevated levels of fluoride in plasma were only observed in 300-ppm treated rats.

Histologically, the hyperplasia of the mucosal epithelium of the glandular stomach, which ranged from subtly focal to diffuse, was found in 100 and 90% of the males and females, respectively, at 300 ppm. This effect was accompanied by minimal individual cell necrosis (apoptosis) in the pyloric region as evidenced by acute inflammation in several males at 300 ppm. Compared to the control, mucous cells in the epithelium was slightly decreased and the number of mitotic figures at the base of the gastric pits was increased. Columnar cells were stained more basophilic and epithelium lining the gastric pits contained 1 or several cells with pyknotic nuclei, fragments of nuclear debris, or residual bodies. Focal basal cell hyperplasia of the stratified squamous epithelium was located adjacent to the lining ridge (junction of the glandular stomach and forestomach) in nearly all 300-ppm treated rats. Hyperplasia of the mucosal epithelium of the glandular stomach was also observed in males (5/10) and females (2/10) of the 100 ppm dose group, but individual necrosis was not. Microscopic evidence of the effects of the test article on the incisors included focal or multifocal degeneration of the enamel organ in 300-ppm males (5/10), localized in the maturation zone near the apical end of the tooth.

The Subchronic Toxicity NOAEL is 100 ppm. The Subchronic Toxicity LOAEL is 300 ppm, based on decreased body weight and food and water consumption, increased body weight changes, and macroscopic effects (dental fluorosis and glandular stomach effects including acute inflammation, apoptosis in the pyloric region, focal or multifocal punctate hemorrhages, perforated ulcer of the glandular stomach, and multiple, small, nonperforated ulcers).

This 6-month oral (drinking) toxicity study in F344/N rats is **ACCEPTABLE-NONGUIDELINE** as a range-finding study for the 2-year chronic toxicity in rats, and does not satisfy the guideline requirement for a subchronic oral toxicity study (OPPTS 870.3100; OECD 408) in rats. The study is classified as nonguideline based on the omission of hematology and clinical chemistry analyses, urinalysis, organ weights, ophthalmoscopic examinations, and various organs/tissues for microscopic examination. In addition, without test article intake data, it is difficult to draw definitive conclusions regarding the dose level and treatment-related effects.

870.3100 90-Day Oral Toxicity study in rodents – Mouse

A 6-month oral toxicity study (NIH Publication No. 91/2848, 1990) was designed to examine the subchronic toxic effects of sodium fluoride (>99% purity) administered to 8-12 $B6C3F_1$ mice/sex/dose in deionized water at dose levels of 0, 10, 50, 100, 200, 300 or 600 ppm (approximately 0, 1.5, 7.5, 15.0, 30.0, 45.0, and 90 mg/kg bw/day)
There were premature deaths, including sacrifice due to moribundity, observed at 300 ppm (male, 12.5%) and 600 ppm (males, 44%; females, 82%) dose levels. Clinical signs of thin appearance, hunched posture and weakness were observed in several of the decedents prior to premature sacrifice. Clinical signs in surviving animals included chalky white incisors (≥100 ppm) and chipped teeth (≥300 ppm). The effects on the

incisors correlated with microscopic findings, which included focal or multifocal degeneration of the enamel organ.

Food consumption in 600 ppm males was approximately 77% of controls. Food consumption in the other treatment groups, and water consumption in all treatment groups were within 20% of control values. Mean body weight was significantly decreased in 600 ppm treated males (20%) and in 200 and 300 ppm treated females (16 and 13%, respectively). Mean body weight gain was significantly decreased form control in 200, 300, and 600 ppm males (9, 5, and 20%, respectively) and in 200 and 300 ppm females (16 and 13%, respectively). These parameters were also decreased in the 600 ppm females, but did not reach statistical significance, likely due to the reduced number of animals in this group as a result of premature deaths.

There was a dose-dependent increase in fluoride content in bone and urine. Due to the pooling of plasma samples for sufficient volume for analysis, meaningful statistical analyses in this fluid could not be performed. The data indicate that there was generally a dose-dependent increase in fluoride concentration in the plasma.

Animals that died prior to the termination of the study had noted histopathology in the kidney, liver, testes, and/or myocardium. Nephrosis in the kidneys and/or myocardial degeneration and mineral accumulation in degenerate myofibers were the likely causes of death in some of the decedents. Multifocal megalocytosis and syncytial alteration were observed primarily in the livers of 600-ppm treated mice. Necrosis, tubule degeneration, and the presence of multinucleated giant cells in the seminiferous tubules were also noted in the males that died prior to terminal sacrifice; however, it could not be determined if these effects were due to treatment with sodium fluoride or secondary to the mice dying from other unrelated causes. Bone lesions, in the form of increased osteoid, were observed in the femurs and tibias of treated mice at concentrations of ≥50 ppm for males and ≥100 ppm for females. This histopathology is indicative of altered rates of bone deposition and remodeling as a result of treatment. Further, treatment with sodium fluoride induced degeneration of the incisors as evidenced by degeneration (dysplasia) in ≥300-ppm treated mice.

The Subchronic Toxicity NOAEL is 10 ppm in male mice and 50 ppm in female mice. The LOAEL is 50 ppm for males and 100 ppm for females, based on histopathology observed in bone with degeneration in tibias and femurs of animals.

This 6-month oral toxicity study in the $B6C3F_1$ mice is **ACCEPTABLE-NONGUIDELINE** as a range-finding study for the 2-year chronic toxicity in mice, and does not satisfy the guideline requirement for a subchronic oral toxicity study (OPPTS 870.3100; OECD 408) in mice. The study is classified as nonguideline based on the omission of hematology and clinical chemistry analyses, urinalysis, organ weights, ophthalmoscopic examination, and various organs/tissues for microscopic examination. In addition, without test article intake data, it is difficult to draw definitive conclusions regarding the dose level and treatment-related effects.

Non-guideline Oral Subchronic Toxicity - Rat

A non-guideline toxicity study (Paul, Ekambaram, et al. 1998) was designed to determine spontaneous motor activity and motor coordination in 10 colony-bred adult Wistar female rats that were treated orally (intubation) with daily, non-lethal doses of sodium fluoride at concentrations of 20 or 40 mg/kg/day for 60 days.

There were significant dose-dependent decreases of 17 and 30% for food intake and 14 and 37% for body weight gain at the 20 and 40 mg/kg/day dose levels, respectively. Total protein concentrations in serum (low-dose, 13%; high-dose, 38%), liver (low-dose, 22%; high-dose, 42%), and skeletal muscle (low-dose, 15%; high-dose, 31%) were also significantly reduced in a dose-related manner in animals treated with sodium fluoride.

Spontaneous motor activity was suppressed in a dose-dependent manner with decreases of 15 and 29% at the 20 and 40 mg/kg/day dose levels, respectively. However, motor coordination was not altered in treated animals. Total blood cholinesterase activity was reduced at the low- and high-dose, although there was no evidence of change in acetyl-cholinesterase activity of the cerebral cortex, brain stem, or cerebellum.

Food intake reductions may account for the decrease in protein concentration or a direct deleterious action of fluoride on protein metabolism can also play a role in depleting protein in sensitive tissues. Thus, a decreased food intake together with a depletion of protein in soft tissues accounted for an inhibition of body growth in sodium fluoride-treated animals. Sodium fluoride deprived skeletal muscle of total protein and suppressed blood cholinesterase activity; although, these effects are unlikely to have a deteriorating action on neuromuscular function. However, similar sodium fluoride doses can produce neurobehavioral deficit resulting in an inhibition of spontaneously occurring locomotor activity.

The Subchronic Toxicity NOAEL is less than 20 mg/kg/day (lowest dose tested). The LOAEL is less than or equal to 20 mg/kg/day, based on significant reductions in body weight gain and suppressed spontaneous motor activity.

Non-guideline Oral Subchronic Toxicity - Mouse

A non-guideline subchronic toxicity study (Pillai, Mathai, et al. 1988) was designed to assess the toxicity effects of sodium fluoride (purity not reported) on the male mouse. Sodium fluoride was administered daily to 5 Swiss albino mice of a laboratory colony Haffkine strain at a concentration of 5.2 mg/kg/day for 35 days. These mice were also provided with a low-fluoride diet and water supply (< 1ppm fluoride).

There were significant changes in hematological analyses with decreases in red blood cells, lymphocytes, hemoglobin, albumin, total protein, cholesterol, glucose, and alkaline phosphatase. Statistically significant increases were observed in white blood cells, monocytes, basophils, and eosinophils. Food and water consumption was significantly decreased in treated animals compared to controls. There were significant, treatment-related decreases from control in body weight gain of sodium fluoride-treated mice after

day 19 of the treatment period. A significant relationship between food and water consumption and the body weight was observed in the controls, but not in the treated animals.

Significant increases in fluoride content were measured in the kidneys, stomach, brain, liver, and intestines of the sodium fluoride-treated animals when compared to the controls. The increases were 3.5- and 1.5-fold greater than control in the kidneys and stomach, respectively, while the brain, intestines, and liver exhibited 2-fold increases over control. There was no evidence of sperm abnormalities following treatment with sodium fluoride.

The Subchronic toxicity NOAEL is less than 5.2 mg/kg/day (lowest dose tested). The LOAEL is less than or equal to 5.2 mg/kg/day, based on significant decreases in body weight gain, and food and water consumption.

4.3 Prenatal Developmental Toxicity

Adequacy of database for Prenatal Developmental Toxicity: The database for developmental toxicity is considered complete.

870.3700a Developmental Toxicity – Rat

A prenatal developmental toxicity study (Bates, et al.) was designed to evaluate postnatal growth and viability of 104 prenatally exposed mated female CD rats. Sodium fluoride (purity >99%) was administered *ad libitum* in drinking water to groups of 26 rats/dose at dose levels of 0, 7, 18, or 27 mg NaF/kg/day (0, 50, 150, and 300 ppm, respectively) from gestation days (GD) 6 to 15. Females were weighed,and observed for clinical signs of toxicity daily starting on GD 6. Food and water weights were recorded every other day during GD 0 to 20. The maternal body, liver, right kidney, and intact uterus were weighed and corpora lutea were counted. Each live fetus was weighed and examined for external, visceral, and skeletal malformations.

There were no treatment-related clinical signs, increases in mortality (100% survival), or decreases in body weight in rats dosed with sodium fluoride. The maternal body weight gain during the first two days of exposure (GD 6 to 8) was significantly reduced (55%) at 300 ppm (27 mg/kg/day) relative to controls. The mean maternal body weight gain and water consumption during the treatment period was also significantly reduced, possibly due to a decrease in palatability. **The Maternal toxicity NOAEL is 18 mg/kg/day. The Maternal toxicity LOAEL is 27 mg/kg/day, based on reduced maternal body weight gain.**

There were no changes in reproductive parameters in treated animals compared to controls. **The Reproductive toxicity NOAEL is greater than or equal to 27 mg/kg/day (highest dose tested). The Reproductive toxicity LOAEL is greater than 27 mg/kg/day (not established).**

There were no treatment-related effects observed in mean live fetal body weight/litter or the number of live fetuses. A dose-related increase in the percent of litters with one or more externally malformed fetuses, externally malformed fetuses/litter, and skeletally malformed fetuses/litter occurred, however these increases were not statistically significant. **The Developmental toxicity NOAEL is greater than or equal to 27 mg/kg/day (highest dose tested). The Developmental toxicity LOAEL is greater than 27 mg/kg/day (not established).**

870.3700a Developmental Toxicity – Rat

A prenatal developmental toxicity study (Collins, Sprando, et al. 1995) was designed to evaluate the developmental toxicity effects of sodium fluoride (100% purity) administered to caesarean-derived, viral antibody-free CD-CRL:CD-BR, VAF + female rats, 33-37 animals/dose, in drinking water at concentrations of 10, 25, 100, 175, or 250 ppm (1.4, 3.9, 15.6, 24.7, or 25.1 mg/kg/day, respectively). Animals were also fed a low-fluoride diet (7.95 ppm fluoride). Prior to treatment with sodium fluoride, 1 male for every 2 female rats were mated and once females were pregnant treated drinking water was administered. On gestation day (GD) 20, a caesarean section was performed and the uterus was opened and examined for resorption sites, implantation sites and live or dead fetuses.

There were no incidences of maternal mortality, changes in behavior, clinical signs, or mottled teeth in dams treated with sodium fluoride. In the 100 ppm dose group, there was 1 female rat that exhibited multiple, apparently random, clinical findings (exudate from the eye and nose, and overgrown teeth) that were not associated with treatment. The 250 ppm dose group experienced significant decreases in food and water consumption, and body weight gain that were 7, 30, and 11% less than controls, respectively. A significant reduction (10.7%), from control, in fluid consumption was observed in animals treated with 175 ppm sodium fluoride; however, there were no other treatment-related changes found at this dose level. **The Maternal toxicity NOAEL is 175 ppm (24.7 mg/kg/day). The Maternal toxicity LOAEL is 250 ppm (25.1 mg/kg/day), based on significant reductions in food and water consumption and body weight gain. (The similarity in the mg/kg/day concentrations can be attributed to decreases in water consumption, which was greater at 250 ppm. At 175 ppm, there was a decrease in water consumption; however, there were no significant changes in feed consumption and body weight gain.)**

The pregnancy rate was greater than 90% for all groups. There was a significant decrease in the mean number of corpora lutea/female in dams of the 250 ppm dose group; however, because the number of corpora lutea is determined at birth, this decrease is considered to be random. There were no significant changes in reproductive parameters in treated animals when compared to controls. **The Reproductive toxicity NOAEL is greater than or equal to 250 ppm (25.1 mg/kg/day; highest dose tested). The Reproductive toxicity LOAEL is greater than 250 ppm (25.1 mg/kg/day; not established).**

There were no treatment-related effects on fetal body weight, litter sizes, or viable fetuses. Several external variations were observed in control and treated animals; however, there were no significant increases in the number of fetuses with at least 1, 2, or 3 variations, or in the number of litters with fetal sternebral variations. There was no evidence of teratogenicity observed in the rats following administration of phenol. **The Developmental toxicity NOAEL is greater than or equal to 250 ppm (25.1 mg/kg/day; highest dose tested). The Developmental toxicity LOAEL is greater than 250 ppm (25.1 mg/kg/day; not established).**

870.3700a Developmental Toxicity – Rat

A prenatal developmental toxicity study (Heindel et al. 1996) was designed to evaluate postnatal growth and viability of 104 prenatally exposed mated female Sprague-Dawley rats. Sodium Fluoride was administered *ad libitum* in drinking water to groups of 26 rats/dose at dose levels of 0, 6.6, 18.3, or 27.1 mg NaF/kg/day (0, 50, 150, and 300 ppm, respectively) from gestation days (GD) 6 to 15. Females were weighed, and food and water weights were recorded every other day during GD 0 to 20 and observed for clinical signs of toxicity daily starting on GD 6. The maternal body, liver, right kidney, and intact uterus were weighed and corpora lutea were counted. Each live fetus was weighed and examined for external, visceral, and skeletal malformations.

There were no treatment-related clinical signs, increases in mortality (100% survival), or decreases in body weights in rabbits dosed with sodium fluoride at the low- and mid-dose. The maternal body weight gain of the high dose group on GD 6-8 was 56% less than the control. During the treatment period, as a whole, there was not a significant difference in mean body weight gain; however, a decreasing trend that approached statistical significance was observed. The water consumption during the treatment period was significantly reduced at the high-dose. The food consumption was decreased at the high-dose during GD 8-10, but was normal thereafter. **The Maternal toxicity NOAEL is 18.3 mg/kg/day. The LOAEL is 27.1 mg/kg/day, based on reduced maternal body weight gain.**

There were no treatment-related effects on mean live fetal body weight/litter, live fetal number, and prevalence of malformations. **The Developmental toxicity NOAEL is greater than or equal to 29 mg/kg/day (highest dose tested). The LOAEL is greater than 29 mg/kg/day (not established)**.

870.3700b Developmental Toxicity – Rabbit

A prenatal developmental toxicity study (Heindel et al. 1996) was designed to evaluate postnatal growth and viability of 104 prenatally exposed mated female New Zealand White Rabbits. Sodium Fluoride was administered *ad libitum* in drinking water to groups of 26 rabbits/dose at dose levels of 0, 10, 18, or 29 mg NaF/kg/day (0, 100, 200, and 400 ppm, respectively) from gestation days (GD) 6 to 19. Females were weighed, and food and water weights were recorded every other day during GD 0 to 30 and observed for

clinical signs of toxicity daily starting on GD 6. Pregnancy rates were 84, 87, 78, and 83% in the control to high exposure groups, respectively. The maternal body, liver, right kidney, and intact uterus were weighed and corpora lutea were counted. Each live fetus was weighed and examined for external, visceral, and skeletal malformations.

There were no treatment-related clinical signs, increases in mortality (100% survival), or decreases in body weights in rabbits dosed with sodium fluoride at the low- and mid-dose. During the treatment period, as a whole, there was not a significant difference in mean body weight gain. The high-dose (400 ppm) group, during GD 6 to 8, experienced a mean weight loss of 112 grams versus an mean weight gain of 14 grams for the control. During GD 10 to 12, the 400 ppm group recovered with a mean weight gain of 71 grams versus 22 grams for the control. The water consumption during the treatment period was significantly reduced, possibly due to a decrease in palatability. Overal the maternal food consumption was decreased at the high-dose with a significant decrease from GD 6 to 8. **The Maternal toxicity NOAEL is 18 mg/kg/day. The LOAEL is 29 mg/kg/day, based on reduced maternal body weight gain.**

There were no treatment-related effects on mean live fetal body weight/litter, live fetal number, and prevalence of malformations. **The Developmental toxicity NOAEL is greater than or equal to 29 mg/kg/day (highest dose tested). The LOAEL is greater than 29 mg/kg/day (not established)**.

4.4 Reproductive Toxicity

Adequacy of database for Reproductive Toxicity: The database for reproductive toxicity is considered complete.

870.3800 Reproduction – Rat

A reproduction and fertility effects study (Collins, Sprando, et al. 2001) was designed to determine any generational, possibly cumulative, effects on the development of offspring when sodium fluoride (purity not reported) was ingested continuously through multiple generations of caesarean-derived CD CRL:CB-BR, viral antibody-free rats. Sodium fluoride was administered to 48 rats/sex/dose at concentrations of 25, 100, 175, or 250 ppm in drinking water for 10 weeks. Animals were also fed a low-fluoride (7.95 ppm) NIH-07 diet. Following the treatment period, female rats were mated 1:1 with males. On gestation day (GD) 20 a caesarean section was performed on 8 mated F0 females to measure reproductive and developmental parameters. The uterus was opened and examined for presence and position of resorption sites, implantation sites, and live or dead fetuses. The remaining F0 females were allowed to litter and wean their pups. On postnatal day (PND) 21, 36/sex/dose group, F1 rats were randomly selected to provide the F2 generation. Both fluoridated drinking water and the low-fluoride diet were provided as before for 10 weeks, and animals were mated as previously described. F1 dams were euthanized and caesarean sections were performed on GD 20.
There were no treatment-related effects on maternal mortality. A significant decrease from control in fluid consumption (30%) was observed at the 250 ppm dose level. There

were no other changes in F0 maternal generation. Significant decreases from control were observed in fluid consumption, with reductions of 28 and 31% in the F1 dams at the 175 and 250 ppm dose levels, respectively. The decreases in fluid consumption corresponded with decreased palatability of the solution. Food consumption was significantly reduced (11%) in F1 dams when compared to control in the 175 ppm dose group. There was a 14% decrease from control in the body weight gain of F1 females (dams) treated with 175 ppm. These reductions at 175 ppm were considered random because of the lack of effect in 250 ppm group. Gravid uterine weight measurements showed no dose-related differences. **The Maternal toxicity NOAEL is greater than or equal to 250 ppm (highest dose tested). The Maternal toxicity LOAEL is greater than 250 ppm (not established).**

There were no treatment-related effects in the mean number of corpora lutea, mean number of implantation sites, implantation efficiency, mean number of viable fetuses, or average percentage of early and late deaths per litter of dams. **The Reproductive toxicity NOAEL is greater than or equal to 250 ppm (highest dose tested). The Reproductive toxicity LOAEL is greater than 250 ppm (not established).**

Fetal body weight was not affected by treatment with sodium fluoride. There was no evidence of toxicity in fetuses or pups of the F1 generation. Similarly, the F2 generation fetuses and pups were unaffected by treatment with sodium fluoride with the exception of decreased ossification of the hyoid bone in the F2 fetuses at the 175 (not significant) and 250 ppm (significant) dose groups. **The Offspring toxicity NOAEL is 175 ppm. The Offspring toxicity LOAEL is 250 ppm, based on decreased ossification of the hyoid bone.**

Non-guideline Reproduction – Rat

A special reproductive and fertility effects toxicity study (Aráibi, et al. 1989) was designed to assess the effects of sodium fluoride (purity not reported) on the reproductive performance of the adult male albino rat. Sodium fluoride was administered to 15 rats/dose at concentrations of 100 or 200 ppm in the diet for 60 days. Blood samples from cardiac puncture were collected from 5 animals of each dose group and the control group. These animals were sacrificed (cervical dislocation) and testes were removed. The remaining animals were mated with normal females (1 male to 2 females) to examine fertility.

Lesions on the teeth (mottling and erosion of enamel), a characteristic commonly associated with sodium fluoride exposure, were observed in animals at the end of the experiment. Males treated with sodium fluoride seemed to show less interest toward females when compared to those animals of the control group. The number of pregnant females were decreased 10 and 40% from controls in groups treated with 100 and 200 ppm, respectively. High-dose animals exhibited significant reductions in the number of pregnant females. The number of newborns produced by the 100 and 200 ppm dose groups were significantly less than controls, with decreases of 30 and 57%, respectively. There was a decrease in average litter size for both dose levels, although neither

reduction was significantly different from controls.

Mean tubular diameters were significantly less than controls with 3 and 7% decreases in diameter for the 100 and 200 ppm dose levels, respectively. There were 94 and 93% (significant) increases in peritubular membrane thickness in the low- and high-dose groups, respectively. Treatment of animals with 200 ppm sodium fluoride resulted in significant decreases from control in percentage of seminiferous tubules containing spermatozoa. There were decreases in mean testosterone levels in the serum of treated animals with 29 (nonsignificant) and 71% (significant) reductions from controls observed in the 100 and 200 ppm dose groups, respectively.

There was a decrease in reproductive performance of male rats exposed to a high intake of sodium fluoride in spite of the absence (until the end of the experiment) of clinical signs in the teeth that are characteristic features of fluorosis. The testes of 200 ppm sodium fluoride-treated rats exhibited impairments of spermatogenesis and steroidogenesis based on changes in mean diameter of seminiferous tubules, the thickness of peritubular membranes, percentage of seminiferous tubules containing spermatozoa, and serum testosterone levels. The researchers suggested that sodium fluoride appears to be antispermatogenic and the decrease in testosterone may account for the decrease of mated females in sodium fluoride-treated groups. However, the results of this study could not be verified.

4.5 Chronic Toxicity

Adequacy of database for Chronic Toxicity: There is only one chronic toxicity study available for sodium fluoride. This study is considered inadequate for assessment of chronic toxicity. However, chronic toxicity/carcinogenicity data are available for sodium fluoride that address the chronic toxicity of this chemical.

4.6 Carcinogenicity

Adequacy of database for Carcinogenicity: The database for carcinogenicity consists of three studies; one open literature study in the rat and two National Toxicology Program studies.

870. 4200a Oncogenicity – Rat

A 24-month carcinogenicity study (Maurer, et al. 1990) was designed to assess the oncogenic potential of sodium fluoride (>99% purity) when administered to groups of 70 male and 70 female Albino Sprague-Dawley rats. The treatment groups were fed a low-fluoride diet that was supplemented with sodium fluoride at concentrations of 4, 10, or 25 mg/kg/day (groups 3-5, respectively) and the control groups were fed either the low-fluoride diet (group 1) or the normal laboratory chow (group 2) (Purina Certified Rodent Chow; fluoride content not determined) for up to 99 weeks. The study was designed to continue for 24 months or until survival for a single group of males or a single group of females was 20% or less. The survival was 20% or less at week 95 for males receiving 4

mg/kg/day and at week 99 for females in the low-fluoride control group. At 26 weeks, sufficient rats were killed to retain 60 animals/sex/group. Ten rats/sex were killed after 52 weeks to assess fluoride toxicity.

There was no evidence of treatment-related incidence of carcinogenicity in Sprague-Dawley rats administered dietary sodium fluoride in concentrations up to 25 mg/kg/day for 2 years. All bone neoplasms observed were considered to be incidental and spontaneous and, not related to sodium fluoride treatment because of their low incidence and random distribution. **The incidence of preneoplastic and neoplastic lesions at any site in rats of either sex was not altered by the adminstration of sodium fluoride. Sodium fluoride was not carcinogenic to rats within the confines of this study.**

There were no treatment-related increases in mortality in rats receiving sodium fluoride in the feed for 95 or 99 weeks. Survival rates for groups 1-5 were approximately 35, 44, 20, 43, and 48% for males (95 weeks) and 20, 50, 35, 42, and 22% for females (99 weeks), respectively. There was no evidence of a significant, positive dose-related trend in mortality for either sex; however, females in the 4 and 10 mg/kg/day dose groups exhibited significantly greater survival rates than that of the low-fluoride control.

At study termination, diet consumption for the 25 mg/kg/day (group 5) was significantly reduced when compared to the control (group 1), with decreases of approximately 20 and 18% for males and females, respectively. Body weight gain was significantly less than the control for the 25 mg/kg/day dose group. Both male and female rats administered the high-dose of sodium fluoride experienced decreases of roughly 25% in mean body weight gain.

Clear evidence of fluoride toxicity was seen in the teeth, bones, and stomach, the severity of which was related to dose and duration of treatment. At sodium fluoride concentrations of 4 mg/kg/day or greater, dental changes occurred including incisors malformations and fractures, and enamel hypoplasia. Treatment-related bone effects, mostly skull, were observed at concentrations of 10 mg/kg/day and greater. Affected bones were white, thick, and found to have roughened surfaces and subperiosteal hyperostosis. There was a lack of bone marrow cavities in the new bone. There was an increase in incidence and severity of chronic inflammation of the gastric glandular mucosa in rats treated with sodium fluoride doses at or above 10 mg/kg/day.

At the 25 mg/kg/day dose level, animals experienced decreased plasma glucose levels and specific gravity of urine. Males exhibited a decrease in plasma globulins that was probably related to the reduced diet consumption. The lack of survival effect indicates that these small parameters did not significantly affect the general health of the rats.

Levels of fluoride in the urine and bone of animals increased as the level of sodium fluoride in the diet increased. The fluoride concentration of the urine was a linear function of the ingested dose, regardless of whether the fluoride was endogenous to the diet or added as sodium fluoride. The low-fluoride diet yielded a proportionately lower level of fluoride in bone than the added sodium fluoride.

The diet consumption for males in group 2 (lab chow control) was significantly less (18%) than that of the control (group 1, low-fluoride diet). However, this same group (2, lab chow) exhibited a 10% decrease in mean body weight gain compared to control. Females of the lab chow control group exhibited a survival rate that was overall greater than the sodium fluoride-treated females and significantly greater than the low-fluoride control. The results associated with the animals maintained on laboratory chow were equivocal and prevented comparisons with the treated and control groups fed the low-fluoride diet.

870.4200 Oncogenicity – Mouse

In a carcinogenicity study (NTP TR 393*),* male and female B6C3F1 mice on a low fluoride diet were administered sodium fluoride (NaF; 99% a.i., Lot A022085) at concentrations in deionized drinking water of 0 (100 mice/sex), 25 (70 mice/sex), 100 (70 mice/sex), or 175 ppm (100 mice/sex) for 103 weeks. The average daily doses of NaF corresponding to these drinking water concentrations were estimated to be 2.4, 9.6, or 16.7 mg/kg in males, and 2.8, 11.3, or 18.8 mg/kg in females. Including the fluorine available from the low-fluoride diet, the estimated total daily fluoride ion intakes for control, low-, mid-, and high-dose groups were 0.6, 1.7, 4.9, and 8.2 mg/kg in males and 0.6, 1.9, 5.7, and 9.1 mg/kg in females. Ten mice/sex/dose group were sacrificed at 24 and 66 weeks. Fluoride concentrations in bone (humerus) were determined from samples taken from all animals at interim sacrifice and 10 randomly selected mice/sex at terminal sacrifice. Complete hematology and select clinical chemistry analyses were conducted on all interim sacrifice animals. Organ weights were taken only at interim sacrifice and only of the liver, both kidneys, and the brain.

There were no compound-related effects on mortality, body weight, food consumption, water consumption, hematology, or organ weights. Treatment-related clinical findings included a dose-dependent increase in white discoloration of the teeth (27%, 39%, 80%, and 100% in males and 19%, 43%, 84%, and 100% in females, from control to high dose, respectively) which occurred as early as Day 74 in the high-dose animals compared to Day 508 in the control animals. Serum alkaline phosphatase was significantly increased in high-dose females at 24 (29%) and 66 weeks (88%) and in high dose-males at 66 weeks (11%). Serum phosphorus levels were significantly decreased (13%) in high-dose males at 66 weeks. There was a significant increase in incisor dentine dysplasia in high-dose males (78% in controls versus 91% at the high dose). There was an increase in the incidence of myelofibrosis (femoral, humerus, maxilla, and thoracic) in female mice at all doses. **Based on the clinical chemistry changes in alkaline phosphatase (males and females) and serum phosphorus (males) at 66 weeks and bone lesions (dentine dysplasia, males; myelofibrosis, females), the LOAELs in males and females were 16.7 and 18.8 mg/kg/day, respectively and the NOAELs in males and females were 9.6 and 11.3 mg/kg/day, respectively.**

At the doses tested, a treatment-related increase in any tumor incidence was not noted when compared to controls. Dosing was considered adequate based on the overall

changes noted in the high-dose animals. However, it should be noted that the mice may have been able to tolerate a higher dose.

This carcinogenicity study in mice is **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for a carcinogenicity study [OPPTS 870.4200; OECD 451] in mice.

870.4300 Combined Chronic Toxicity/ Oncogenicity – Rodent

A 24-month study (NIH Publication No. 91/2848, 1990) was
designed to examine the chronic and carcinogenic effects of sodium fluoride (99% purity) on male and female F344/N rats fed a low fluoride diet. Sodium fluoride (NaF) was administered in deionized drinking water to animals at concentrations of 0 (100 rats/sex), 25 (70 rats/sex), 100 (70 rats/sex), or 175 ppm (100 rats/sex) for 103 weeks. These concentrations were equivalent to average daily doses of NaF from drinking water of 0, 1.3, 5.2, or 8.6 mg/kg for male rats and 0, 1.3, 5.5, or 9.5 mg/kg for females. Including the fluoride content in the diet, the estimated average daily doses of fluoride ion at these concentrations were 0.2, 0.8, 2.5, and 4.1 mg fluoride/kg for males and 0.2, 0.8, 2.7, and 4.5 mg fluoride/kg for females. An additional 50 age-matched control rats served as concurrent controls for animals dying prior to terminal sacrifice in the exposed groups.

Mortality, body weight, body weight gain, food consumption, water consumption, hematology, and organ weights were not affected by exposure to NaF. Fluoride concentration increased with dose in blood (serum) at Weeks 27 and 66, and bone and urine at Weeks 27, 66, and 105. Analysis of bone fluoride revealed an increase with dose and age. Urinary calcium was observed to be significantly increased in high-dose females.

Tooth discoloration (whitening and mottling) was noted in all treated animals with attrition, deformity, and occasional malocclusions noted in the high- and/or mid-dose males. Histopathology of the incisors noted dentine dysplasia (all dosed animals), degeneration of the ameloblasts (mid- and high-dose animals), and, to a lesser extent, degeneration of the odontoblasts (principally dosed males). These effects were dose-dependent in males and showed similar results in females, although without the strong dose-dependence noted in the male rats. Increases in the incidence and severity of osteosclerosis of the long bones were noted in the high-dose females (7.5% control; 22% high-dose, P=0.04).

The Chronic Toxicity NOAEL is less than 1.3 mg/kg/day. The Chronic Toxicity LOAEL is 1.3 mg NaF/kg/day in both sexes, based on dentine dysplasia in males and females, and ameloblast degeneration in males.

Three bone osteosarcomas were noted in high-dose males and one in a mid-dose male, with none in controls. A fourth osteosarcoma, not originating in the bone, was observed

in an additional high-dose male. Dosing was considered adequate based on tooth deformities and discoloration; dentine dysplasia and degeneration in the ameloblasts and odontoblasts, bone osteosarcomas in males and osteosclerosis in females. Trend analyses revealed that, at the doses tested, there was a significant treatment-related increase in the incidence of bone osteosarcomas in males but the incidence was not significantly increased in the high-dose males as compared to controls when comparisons were made either within the animals scheduled for terminal sacrifice or all animals (including the interim sacrifice and concurrent control animals). In those animals scheduled for terminal sacrifice, statistical analysis of all organ osteosarcoma in dosed animals as compared to controls also failed to show significance. The study authors failed to perform the statistical analysis all osteosarcoma analysis among all animals. That analysis, done by the contractor, did reveal a significant difference between the high dose and control groups. Due to the fact that bone osteosarcoma incidence of the high-dose as compared to the control group was not significant, but displayed a significant positive trend, the occurrence of these rare tumors was considered equivocal evidence of carcinogenicity in male rats by the study authors. Such a conclusion was bolstered by the fact that bone osteosarcomas were not observed in treated females or in the parallel study in $B6C3F_1$ mice (TR393, NIH Publication No. 91/2848). However, with the significant difference between high dose animals and controls in the all organ osteosarcoma incidence analysis when all animals are considered, the reviewer believes that the occurrence of osteosarcomas in the male rats should have been considered some evidence, if not clear evidence, of the carcinogenic activity of sodium fluoride.

This chronic/carcinogenicity study in the rat is **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for a chronic/carcinogenicity study [OPPTS 870.4300; OECD 453] in rats.

4.7 Mutagenicity

Adequacy of database for Mutagenicity Toxicity: The database for mutagenicity is considered complete. Several open literature studies are available as well as data from the National Toxicology Program.

The mutagenicity data for sodium fluoride is summarized below in Table 3.

Table 3. Genotoxicity Profile for Sodium Fluoride

Guideline No./Study type/Citation	Results	Positive or Negative for Mutagenicity
Gene Mutation		
870.5100 **Bacterial reverse mutation test** Gocke et al. (1981). Mutagenicity of Cosmetics Ingredients Licensed by	**5 Tester strains of Salmonella typhimurium** There was no evidence of induced mutant colonies over background following administration of sodium	**NEGATIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
the European Communities. Mutation Research 90.2:91-109. **Open Literature**	fluoride in the presence or absence of metabolic activation. The numbers of his+ revertants observed with treatment were not significantly different from control with any of the study concentrations of sodium fluoride. Sodium fluoride was not mutagenic to any of the 5 Salmonella typhimurium bacterial strains in the presence or absence of metabolic activation.	
870.5100 **Bacterial reverse mutation test** Haworth et al. (1983). Salmonella Mutagenicity Test Results for 250 Chemicals. Env. Mutagenesis Supplement 1:3-142. **Open Literature**	**Incubation concentration up to 10 mg/plate** There was no evidence of induced mutant colonies over background. Positive controls produced appropriate responses in corresponding strains of the bacterial reverse mutagenesis test. S. typhimurium did not show mutagenic activity in the presence or absence of metabolic activation following administration of sodium fluoride.	**NEGATIVE**
870.5100 **Bacterial reverse mutation test** Li, Y., Dunipace, A., Stookey, G. (1987). Absence of Mutagenic and Antimutagenic Activities of Fluoride in Ames Salmonella Assays. Mutation Res 190:229-236. **Open Literature**	**Bacterial Tester Strains TA97a, TA98, TA100, TA102, and TA1535** **0.44, 4.42, 44.2, 88.4m 221.1, 442.1, 1105.3, 2210.5, or 4421.0 µg/plate NaF** Sodium fluoride was not mutagenic in the Salmonella typhimurium bacterial strains in the presence or absence of metabolic activation. Toxic effects were first observed at concentrations ≥ 1100 µg/plate in various strains. The strains ranged from the most sensitive to least sensitive; 97a, 102, 100, 1535 and 98. The incorporation of metabolic activation increased the number of revertants, but did not significantly influence the toxic effects of sodium fluoride on the bacteria. There was no evidence of induced mutant colonies over background following administration of sodium fluoride in the presence or absence of metabolic activation. The numbers of his+ revertants observed with the treatment were not significantly different from control with any of the study concentrations of sodium fluoride.	**NEGATIVE**
870.5100 **Bacterial reverse mutation test** Martin, G. et al. (1979). Lack of Cytogenic Effects in Mice or	**Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, and TA 100** **0.1-500 µg/plate NaF**	**NEGATIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
Mutations in Salmonella Receiving Sodium Fluoride. Mutation Res 66:159-167. **Open Literature**	There was no evidence of induce mutant colonies over background. Positive controls produced appropriate responses in corresponding strains of the bacterial reverse mutagenesis test. S. typhimurium did not show mutagenic activity in the presence or absence of metabolic activation following administration of sodium fluoride.	
870.5100 **Bacterial reverse mutation test** Tong et al. (1988). The Lack of Genotoxicity of Sodium Fluoride in a Battery of Cellular Tests. Cell Biology and Toxicology 4.2:173-186. **Open Literature**	**Bacterial Tester Strains, Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, and TA 100** **10, 20, 40, 80, 160, or 320 µg/plate** Sodium fluoride was not mutagenic to any of the 5 Salmonella typhimurium bacterial strains in the presence or absence of metabolic activation. The higher doses (80-320 µg/plate) were slightly cytotoxic. However at all doses there was no evidence of induced mutant colonies over background following administration of sodium fluoride in the presence or absence of metabolic activation. The numbers of his+ revertants observed with treatment were not significantly different from control with any of the study concentrations of sodium fluoride.	**NEGATIVE**
870.5100 **Bacterial reverse mutation test** Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, Technical Report Series No. 393, NIH Publication No. 91-2848, December 1990, Pgs. 1-477. **Acceptable** Guideline	**Strains TA98, TA100, TA1535, and TA1537 of S. typhimurium** **0, 100, 333, 1000, 3333, and 10000 µg/plate** There was no clear evidence, or a concentration related positive response, of induced mutant colonies over background.	**NEGATIVE**
870.5300 **In Vitro mammalian cell gene mutation test**	**-S9 Trial 1-200, 300, 400, 500, 600, 0r 800 µg/mL** **Trial 2-50, 100, 200, 300, 400, 500, or 600 µg/mL**	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
Caspary, W. et al. (1987). Mutagenic Activity of Fluorides in Mouse Lymphoma Cells. Mutation Res 187:165-180 **Open Literature**	**+S9: Trial 1-100, 200, 300, 400, 500, or 600 µg/mL Trial 2-50, 100, 200, 300, 400, 500 or 600 400, 500, or 600 µg/mL** Sodium fluoride was mutagenic in L5179Y mouse lymphoma cells in the presence and absence of metabolic activation. There was evidence of general toxicity in the 300-500 µg/mL sodium fluoride concentration range and lethality usually occurred at higher concentrations (600-800 µg/mL). In the absence of metabolic activation, cytotoxicity was apparent at the 800 µg/mL sodium fluoride dose level in Trial 1. Cytotoxicity was observed in the presence of metabolic activation at 600 µg/mL. There were significant increases in mutation frequency following administration of sodium fluoride. Trial 1 (-S9) experienced 1.8-, 1.6-, 1.9-, and 2.9-fold increases at sodium fluoride concentrations of 300, 400, 500 and 600 µg/mL, respectively, while Trial 2 (-S9) exhibited a 1.6-fold increase at the 500 µg/mL dose. In the presence of metabolic activation, 1.5-, 3.1-, and 3.6- fold increases in Trial 1 and 1.6-, 1.9-, and 2.3-fold increases in Trial 2 occurred at sodium fluoride 400, 500, and 600 µg/mL and 300, 400, and 500 µg/mL, respectively. Sodium fluoride had a significant effect in gene mutations at the TK locus, although the addition of metabolic activation ha no apparent effect on either the toxicity or mutagenic activities of sodium fluoride. The measured mutant colony size was predominantly small.	
870.5300 **In Vitro mammalian cell gene mutation test** Oberly et al. (1990). An Evaluation of the CHO/HGPRT Mutation Assay Involving Suspension Cultures and Soft Agar Cloning: Results for 33 Chemicals. Environmental and Molecular Mutagenesis 16:260-271.	**Chinese Hamster Ovary (CHO)/HGPRT+ cells, Strain K1-BH4** **250, 500, 600, 700, or 800 µg/mL in –S9** **200, 400, 450, 500, 550, 600, or 700 µg/mL in +S9** All doses greater than 450 µg/mLwith and without activation were toxic, as is evident by the relative total growth of 38% or less. Sodium fluoride was not mutagenic at the HGPRT locus of Chinese hamster ovary cells.	**NEGATIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
Open Literature		
870.5300 **In Vitro mammalian cell gene mutation test** Tong et al. (1988). The Lack of Genotoxicity of Sodium Fluoride in a Battery of Cellular Tests. Cell Biology and Toxicology 4.2:173-186. **Open Literature**	**ARL 1 (rat liver epithelial cell line)** **2, 10, 20, 40, 80, or 160 µg/mL** The higher doses (80 and 160 µg/mL) were toxic and were not analyzed for gene mutatins. Sodium fluoride at doses up to 40 µg/mL did not result in any significant increase in TGR mutants above the control. Sodium fluoride was not mutagenic at the HGPRT locus.	**NEGATIVE**
870.5300 **In Vitro mammalian cell gene mutation test** Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, Technical Report Series No. 393, NIH Publication No. 91-2848, December 1990, Pgs. 1-477. **Acceptable** Guideline	**Trifluorothymidine (TFT)-resistant cells at the thymidine kinase (TK) locus exposed to sodium fluoride in lymphoma L5178Y cells cultured in vitro in either distilled water or culture medium** There was evidence of a concentration related positive response of induced mutant colonies over background. Sodium fluoride was tested up to cytotoxic concentrations (the maximum dose tested was 1000 µg/mL in one laboratory and 800 µg/mL in another). Mutant frequencies increased in a dose-related manner. Statistically significant ($p<0.05$) responses were observed in all trials, ±S9, at the high doses. Mutant fractions (vs. the solvent control response) at the highest doses tested in each trial were reported to be 83.0x10-6 vs. 29.5x10-6, 41.3x10-6 vs. 24.3x10-6, 134.0x10-6 vs. 58.0x10-6, and 195.5x10-6 vs. 51.0x10-6 in cultures tested in the absence of metabolic activation, and 94.0x10-6 vs. 25.8x10-6 and 75.7x10-6 vs. 33.0x10-6 in cells tested in the presence of metabolic activation.	**POSITIVE**
Chromosome Aberration		
870.5375 **In Vitro mammalian chromosome aberration test** Aardema MJ, et al. (1989). Sodium Fluoride-Induced Chromosome Aberrations in Different Stages of the Cell Cycle: A Proposed	**Cells exposed to 465, 650, 911, 1276, or 1786 ug/mL NaF** **In separate experiments, cells exposed to 100 ug/mL of sodium fluoride for 1 or 2 hours or concentrations of 0.1, 1.0, 10, 25, 50, 75, and 100 ug/mL for 3 hours**	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
Mechanism. Mutation Research 223:191-203. **Open Literature**	A high level of toxicity was observed at 1786 go/mL (high-dose) that limited chromosome aberration analysis to the lower dose groups. There was an increase in average cell generation time (AGT) as the concentration of sodium fluoride increased; indicating a treatment-related cell-cycle delay. At 20 hours after treatment, greater than 50% of the cells were in their first mitosis at 911 and 1276 go/mL +/- S9 and at 465 and 650 go/mL +S9. Overall, there was a significant increase in the percentage of aberrant cells in sodium fluoride treated groups at the 8 and 20 hour post-treatment harvesting of cells. There were 4.5- and 3.5-fold increases in the percentage of aberrant cells, at the 8 hour harvest time, in the 465 and 911 go/mL dose group, respectively, in the absence of metabolic activation. In the presence of S9, the 465, 650, and 1276 go/mL dose groups exhibited 6.3-, 3.7-, and 5.0-fold increases, respectively, in the number of aberrant cells. In the 20 hour harvesting assay there was only one significant increase in the number aberrant cells; a 6.5-fold increase at the 1276 go/mL sodium fluoride in the presence of metabolic activation. In both harvest (8 or 20 hours) the aberrations were almost exclusively chromatid-type deletions and gaps. There was evidence of endoreduplicated cells observed in the chromosome aberration screening assay. At the 465, 650, 911, and 1276 go/mL dose levels, the percentages of endoreduplicated cells were 2, 3, 5, and 11% for -S9 and 4, 12, 15, and 14% for +S9, respectively. These cells all had an M0/M1 staining pattern indicating they had gone through 0 or 1 round of DNA synthesis. Endoreduplicated cells were also observed in the CHO 8 and 20 hour harvest time assays. At the 8 hour timepoint in the absence of metabolic activation there was a 6% increase in endoreduplicated cells with the 1276 go/mL dose of sodium fluoride. There were endoreduplicated cell increases of 6, 16, and 22% in -S9 and 28, 26, and 34% in +S9 at the 20 hour harvest time point for the sodium fluoride concentrations of 650, 911, and 1276 go/mL, respectively. In separate experiments cells were exposed to 100 go/mL of sodium fluoride for 1 or 2 hours or	

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
	concentrations of 0.1, 1.0, 10, 25, 50, 75, and 100 go/mL for 3 hours. There were no significant changes from control in chromosome aberrations until the 3 hour incubation assay. Sodium fluoride at doses greater than 10 go/mL induced increases in the percentage of aberrant cells that were significant at concentrations greater than or equal to 50 go/mL. The types of aberrations were chromatid deletions, isochromatid deletions, and a large number of gaps but not chromatid exchanges.In this CHO assay, cell-cycle kinetic studies indicated that aberrations were induced in cells exposed to sodium fluoride at the 20 hour harvest time (G1/S phase) but the increases in aberrant cells were greater at the 8 hour time point where most of the metaphases were from cells exposed to sodium fluoride in the G2 stage of the cell cycle. This sensitivity of the G2 cells was evident in the 3 hour exposure assay with increases in aberrant cells at concentrations greaterthan 10 go/mL; concentrations that are relatively much greater than levels present in water or dentifrices. The researchers suggest that the level of sodium fluoride-induced mutagenicity is dependent on both the cell-cycle stage that cells are in during exposure and the length of time until harvest. Sodium fluoride induced positive mutagenic results in CHO cells at concentrations greater than or equal 50 go/mL when exposed for 3 hours in the presence and absence of metabolic activation. However, longer exposure times (8 or 20 hours) required greater concentrations of sodium fluoride (greater than or equal to 465 go/mL) to achieve mutagenic results.	
870.5375 **In Vitro mammalian chromosome aberration test** Albanese. (1987). Sodium Fluoride and Chromosome Damage (In Vitro Human Lymphocyte and In Vivo Micronucleus Assays). Mutagenesis 2:497-499. **Open Literature**	**Cells exposed to 20 or 40 ug/mL NaF for 28 or 2 hours, +/-S9** Sodium fluoride was mutagenic in human peripheral blood lymphocytes in both the presence and absence of metabolic activation. However, mutagenicity appeared to be dependent on exposure time and concentration. There were significant dose-dependent increases in chromosome aberrations in the experiment without metabolic activation; with 6- and 18-fold increases in total number of damaged cells at the 20 and 40 go/mL dose levels, respectively, after 28 hours of incubation. In the presence of metabolic activation, there was a significant 2.5-fold increase in total number of	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
	damaged cells over control at the 40 go/mL dose level, after 2 hours of incubation. The chromosome aberrations observed following the administration of sodium fluoride were predominantly gaps, breaks, and fragments. No exchange-type aberrations (the type thought to correlate better with the carcinogenic potential of chemicals) were found at any dose level or exposure period.	
870.5375 **In Vitro mammalian chromosome aberration test** Khalil. (1995). Chromosome Aberrations in Cultured Rat Bone Marrow Cells Treated with Inorganic Fluorides. Mutation Research 343:67-74. **Open Literature**	**Bone marrow cells of Sprague-Dawley rats** **0.1, 1.0, 10 or 100 uM** Chromosomal aberrations in bone marrow cells increased following the administration of sodium fluoride in a dose- and time-dependent manner. Sodium fluoride was mutagenic in Sprague-Dawley rat bone marrow cells within the confines of this study. Overall, there was a significant increase in the percentage of aberrant cells in sodium fluoride treated groups for the 12-, 24-, and 36-hour exposures. Only the 0.1 uM treatment, 12-hour exposure cells did not have a significant increase in breaks/cell or in the percent of aberrant cells compared to controls. The increased aberrations at the other treatment levels mainly consisted of simple aberrations, such as breaks and fragments. Small number of complex aberrations, such as chromatid exchanges and rings, occurred sporadically, at doses 1.0, 10.0, and 100.0 uM. The number and percentage of aberrations increased with the increasing concentrations of sodium fluoride and with the prolongation of treatment. The only significant exposure time-related effects observed were between the 12- and 36-hour exposures at concentrations of 1.0, 10.0, and 100.0 uM.	**POSITIVE**
870.5375 **In Vitro mammalian chromosome aberration test** Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water	**Chinese hamster ovary cells exposed to sodium fluoride concentrations between 50 and 1600 µg/mL with metabolic activation or between 16 and 800 µg/mL without metabolic activation** There was evidence of a concentration related positive response of chromosome aberrations	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
Studies). National Toxicology Program, Technical Report Series No. 393, NIH Publication No. 91-2848, December 1990, Pgs. 1-477. **Acceptable** Guideline	induced over background in one laboratory testing without metabolic activation; no other evidence of a positive response.	
870.5375 **In Vitro mammalian chromosome aberration test** Tsutsui T, et al. (1984). Sodium Fluoride-Induced Morphological and Neoplastic Transformation, Chromosome Aberrations, Sister Chromatid Exchanges, and Unscheduled DNA Synthesis in Cultured Syrian Hamster Embryo Cells. Cancer Research 44.3:938-941 **Open Literature**	**Syrian hamster embryo (SHE) cells exposed to sodium fluoride at 50 or 100 ug/mL for 16 hours or 100 or 200 ug/mL for 28 hours** There was a dose- and time-dependent increase in chromosomal aberrations following administration of sodium fluoride in Syrian hamster embryo cells. Sodium fluoride was mutagenic in SHE cells within the confines of this study. Treatment-related effects on mortality were observed in SHE cells administered sodium fluoride. At sodium fluoride concentrations of 75, 100, and 125 go/mL,/mL, there were decreases in cell survival that were 10, 47, and 61%, respectively, less than control. There were significant dose-dependent increases in chromosome aberrations in the sodium fluoride-treated SHE cells. Sodium fluoride incubation of 16 hours exhibited increases in aberrant metaphases that were 9- and 24-fold greater than the control at the 50 and 100 go/mL/mL dose levels, respectively. After 28 hours of exposure, the 100 and 200 go/mL/mL dose levels of sodium fluoride induced aberrant metaphases that were 19- and 29.5-fold greater than control, respectively. The chromosomal aberrations were predominantly gaps and some breaks.	**POSITIVE**
870.5375 **In Vitro mammalian chromosome aberration test** Tsutsui, T., N. Suzuki, et al. (1984). Cytotoxicity, Chromosome Aberrations and Unscheduled DNA Synthesis in Cultured Human Diploid Fibroblasts Induced by Sodium Fluoride. Mutation Research 139:193-198.	**Cytotoxicity test: sodium fluoride administered to JHU-1 cells at concentrations of 50, 100, or 150 go/mL/mL for 1, 2, 6, 12, or 24 hours.** **Mutagenicity assay: Sodium fluoride was administered to JHU-1 cells at concentrations of 25, 50 or 75 go/mL/mL for 12 hours of exposure and 20 or 40 ug/mL for 24 hours exposure** There was a dose- and time-dependent increase in	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
Open Literature	chromosomal aberrations following administration of sodium fluoride in cultured human diploid fibroblasts. Sodium fluoride was mutagenic in JHU-1 cells within the confines of this study. Treatment-related cytotoxic effects were observed in JHU-1 cells administered sodium fluoride. Cell survival decreased as the sodium fluoride concentration and duration of exposure increased. At sodium fluoride concentrations of 50, 100, and 150 go/mL, there were decreases in cell survival that were 100, 98, and 90% after 1 hour; 100, 75, and 65% after 2hours; 70, 48, and 40% after 6 hours; 55, 22, and 15% after 12 hours; and 17, 7, and 1% after 24 hours of exposure, respectively. Sodium fluoride was cytotoxic to JHU-1 cells and cell survival decreased linearly with increasing dose or exposure time. There were significant dose- and time-dependent increases in chromosome aberrations in the sodium fluoride-treated JHU-1 cells. The 12 hour sodium fluoride incubation exhibited increases in aberrant metaphases that were 3.5- and 22.4-fold greater than the control at the 25 and 50 go/mL dose levels, respectively. Sodium fluoride at 75 go/mL provided few metaphases to analyze for chromosomal aberrations. After 24 hours of exposure, the 20 and 40 go/mL dose levels of sodium fluoride induced aberrant metaphases that were 7- and 47-fold greater than control, respectively. The chromosomal aberrations were predominantly gaps and some breaks.	
870.5380 **Mammalian Spermatogonial Chromosomal aberration test** Li, Dunipace, and Stookey. (1987). Effect of Fluoride on the Mouse Sperm Morphology Test. J. Dent. Res. 66:1509-1511. **Open Literature**	**B6C3F1 male mice fed a low fluoride diet (<0.2 ppm) via stomach intubation at concentrations of 0.1, 1.0, 10, 20, 35, or 70 mg/kg. Treated daily for 5 days.** The frequency of abnormal sperm in NaF-treated groups was not significantly different from controls. NaF did not cause spermatogenic damage as determined by the frequency of sperm abnormalities and weights of testes. There was an increase in bone fluoride content with increasing dosage; concentrations less than or equal to 10 mg/kg exhibited significantly lower bone	**NEGATIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
	fluoride content than concentrations greater than or equal to 20 mg/kg. The increase in bone fluoride demonstrated that fluoride was adequately absorbed following intubation, and therefore, the route of administration of NaF used was justified. NaF was nonspermatogenic in male mice and supports the view point that Fl has no adverse mutagenic effects.	
870.5380 Mammalian Spermatogonial Chromosomal aberration test Mohamed and Chandler. (1982). Cytological Effects of Sodium Fluoride on Mice. Dept. of Biology and School of Medicine, University of Kansas City, Missouri. Presented at the 12th I.S.F.R. Conference. **Open Literature**	**Male BALB/c mice administered a low fluoride diet (0.263 ppm) for 1 week and sodium fluoride in drinking water (1, 5, 10, 50, 100, or 200 ppm) for 3 or 6 weeks** There were significant treatment-related increases from controls in aberration rates among spermatocytes. There was evidence of cytogenetic damage found in animals administered sodium fluoride. Sodium fluoride within the parameters of this study was found to be mutagenic. In the three week study, all of the treatments demonstrated a significantly higher frequency of chromosomal aberrations compared to controls. A dose-related response occurred at doses 5 to 200 ppm, but the frequency of aberrations in the low dose (1 ppm) compared to the 5 ppm group did not express the same increasing trend. In the three week study, all of the treatments demonstrated a significantly higher frequency of chromosomal aberrations compared to controls. A dose-related response occurred at doses 5 to 100 ppm, but the frequency of aberrations in the low dose (1 ppm) compared to the 5 ppm group and the 100 ppm group compared to the 200 ppm group did not express the same increasing trend.	**POSITIVE**
870.5380 Mammalian Spermatogonial Chromosomal aberration test Pati and Bhunya. (1987). Genotoxic effect of an environmental pollutant, sodium fluoride, in mammalian in vivo test system. Carylogia 40:79-87.	**Male Swiss mice administered sodium fluoride as intraperitoneal injections** **10, 20, or 40 mg/kg** Sodium fluoride was mutagenic to spermatogonial cells in Swiss mice within the confines of this study. There was a dose-related increased in the number of abnormal sperm that increased with increasing dose.	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
Open Literature	The mean percentages of sperm abnormalities were 6.4, 6.8 and 7.6% for the 10, 20, and 40 mg/kg dose levels, respectively, of sodium fluoride. There were significant dose-dependent increases in the frequency of spermatogonial aberrations that were 3.1-, 3.3-, and 3.7-fold greater than the control at the sodium fluoride doses of 10, 20, and 40 mg/kg, respectively. The higher incidence of sperm abnormalities induced by sodium fluoride may be a measure of the genetic damage caused in the germline cells. There were significant increases over control in the number of spermatogonial aberrations in animals receiving sodium fluoride.	
870.5385 **Mammalian bone marrow chromosomal aberration test** Martin G, et al. (1979). Lack of Cytogenetic Effects in mice or mutations in salmonella receiving sodium fluoride. Mutation Res 66:159-167. **Open Literature**	**Male BALB/c mice administered sodium fluoride in the diet (0.5 ppm) and in drinking water (1,5, 10, 50, or 100 ppm) for six weeks** There were no significant treatment-related differences observed in aberration rates among bone marrow cells. There was no evidence of cytogenetic damage found in animals administered sodium fluoride.	**NEGATIVE**
870.5385 **Mammalian bone marrow chromosomal aberration test** Mohamed and Chandler. (1982). Cytological Effects of Sodium Fluoride on Mice. Dept. of Biology and School of Medicine, University of Kansas City, Missouri. Presented at the 12th I.S.F.R. Conference. **Open Literature**	**Male BALB/c mice administered a low fluoride diet (0.263 ppm) for 1 week and sodium fluoride in drinking water (1, 5, 10, 50, 100, or 200 ppm) for 3 or 6 weeks** There were significant treatment-related differences observed in aberration rates among bone marrow cells. There was evidence of cytogenetic damage found in animals administered sodium fluoride. Sodium fluoride within the parameters of this study was found to be mutagenic. In the three week study, all of the treatments demonstrated a significantly higher frequency of chromosomal aberrations compared to controls. A dose-related response occurred at doses 1 to 50 ppm, but the aberration frequencies in the higher doses were not significantly different from each other. In the six week study, all of the treatments demonstrated a significantly higher frequency of chromosomal aberrations compared to controls. A	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
	dose-related response occurred at doses 1 to 10 ppm, but the frequency of aberrations in the higher doses were not significantly different from each other.	
870.5385 **Mammalian bone marrow chromosomal aberration test** Pati and Bhunya. (1987). Genotoxic Effect of an Environmental Pollutant, Sodium Fluoride, in Mammalian In Vivo Test System. Carylogia 40:79-87. **Open Literature**	**Swiss mice administered sodium fluoride orally (40 mg/kg); ip (10 or 20 mg/kg for 24 hours; 40 mg/kg for 6, 24, or 48 hours or 8 injections of 5 mg/kg intraperitoneally for 120 hours); subcutaneously (40 mg/kg) for 6, 24, or 48 hours** Sodium fluoride was found to be mutagenic in Swiss mice bone marrow cells within the confines of this study. There were significant dose-related increases in chromosomal aberrations in the 24 hour experiment. Intraperitoneal injections of sodium fluoride at concentrations of 10, 20, and 40 mg/kg were 3.3-, 4.3-, and 5.2-fold greater than control, respectively. Similar and significant results were observed in the oral and subcutaneous experiments with 5.5- and 5.0-fold increases, respectively, over controls at the 40 mg/kg dose level. There were 1.9 and 3.3-fold increases over control at the 40 mg/kg dose levels of sodium fluoride administered intraperitoneally at 6 (not significant) and 48 hours (significant), respectively. The multiple, 5-time dosing of 8 mg/kg resulted in a significant 2.9-fold increase over control in mouse chromosomal aberrations. There were treatment-related aberrations, including chromatid gaps and breaks, isochromatid gaps, fragments and exchanges in mouse bone marrow cells. Gaps were observed more frequently than breaks. Sodium fluoride induced dose- and time-dependent increases in the number of chromosomal aberrations, but there was no evidence of route-sensitivity. There was no practical difference observed at the same dose level in the 3 administration routes employed. Additionally, the chronic, repeated exposure of fractionated doses induced less aberrations than that of an equivalent dose treated once. The increases in chromosome aberrations were significantly greater than control in all experiments with one exception. Intraperitoneal injection of 40 mg/kg sodium fluoride over 6 hours failed to induce a significant number of chromosomal aberrations.	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
870.5385 **Mammalian bone marrow chromosomal aberration test** Zeiger et al. (1994). Cytogenetic Studies of Sodium Fluoride in Mice. Mutagenesis 9:467-471. **Open Literature**	**Male B6C3F1 mice administered fluoride in the diet for 1 week and sodium fluoride in drinking water (100, 200 or 400 ppm) for 7 days/week for 6 weeks** There were no significant treatment-related differences observed in aberration rates among metaphase and anaphase bone marrow cells. There was no evidence of cytogenetic damage found in animals administered sodium fluoride. Three of sixteen mice from the 400 ppm group died during the sixteen-week treatment period. A decrease in body weight gain and water consumption occurred at the 200 and 400 ppm group. There were no other treatment-related signs.	**NEGATIVE**
Other Genotoxicity		
870.5395 **Mammalian erythrocyte micronucleus test** Albanese. (1987). Sodium Fluoride and Chromosome Damage (In Vitro Human Lymphocyte and In Vivo Micronucleus Assays). Mutagenesis 2:497-499. **Open Literature**	**Oral gavage of 500 or 1000 mg/kg NaF to Male Alpk:APF Sprague-Dawley rats** There were no significant increases in the frequency of micronucleated polychromatic erythrocytes in rat bone marrow cells at the concentrations of sodium fluoride used in this study. Sodium fluoride was not mutagenic in Alpk:APFSD rat bone marrow cells. There were no treatment-related effects on mortality in any of the low-dose (500 mg/kg) group; however, 4 of the 5 rats in the 1000 mg/kg group died prior to the 48 hour sampling period. No other abnormal signs were observed in the remaining animals in other dose/time groups. The ratio of normochromatic erythrocytes (NCEs) to PCEs was 1:1; which indicated that at the doses used sodium fluoride was not cytotoxic to the bone marrow cells.	**NEGATIVE**
870.5395 **Mammalian erythrocyte micronucleus test** Gocke et al. (1981). Mutagenicity of Cosmetics Ingredients Licensed by the European Communities. Mutation Research 90.2:91-109 **Open Literature**	**Male and female NMRI mice and Sprague-Dawley rats** There were no a significant increases in the frequency of micronucleated polychromatic erythrocytes in mouse bone marrow cells at the concentrations of sodium fluoride used in this study. Sodium fluoride was not mutagenic in NMRI mouse bone marrow cells.	**NEGATIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
	There were no treatment-related effects on mortality (100% survival) or MNPCEs in mice receiving administrations of phenol.	
870.5395 **Mammalian erythrocyte micronucleus test** Pati and Bhunya. (1987). Genotoxic effect of an environmental pollutant, sodium fluoride, in mammalian in vivo test system. Carylogia 40:79-87. **Open Literature**	**Swiss mice administered intraperitoneal NaF (10, 20, or 40 mg/kg) 2 times over 24 hours** There were treatment-related increases in MNPCEs in animals administered sodium fluoride, with 3-, 3.5-, and 5.15-fold increases over control at the 10, 20, and 40 mg/kg dose levels, respectively. These increases were significantly greater than the control with the exception of the low-dose (10 mg/kg). The induction of MN in bone marrow cells increased with sodium fluoride dose. MN frequency was at its highest in PCEs and least in immature white cells. There was evidence of mutagenicity in mouse bone marrow cells administered sodium fluoride within the confines of this study.	**POSITIVE**
870.5395 **Mammalian erythrocyte micronucleus test** Zeiger et al. (1994). Cytogenetic Studies of Sodium Fluoride in Mice. Mutagenesis 9:467-471. **Open Literature**	**Male B6C3F1 mice administered fluoride in the diet for 1 week and sodium fluoride in drinking water for 7 days/week for 6 weeks.** **100, 200, or 400 ppm** There were no significant increases in the frequency of micronucleated polychromatic erythrocytes and normochromatic erythrocytes in B6C3F1 mice peripheral blood cells in the parameters of this study. Sodium fluoride was not mutagenic in B6C3F1 mice peripheral blood cells. Three of sixteen mice from the 400 ppm group died during the sixteen-week treatment period. A decrease in body weight gain and water consumption occurred at the 200 and 400 ppm group. There were no other treatment-related signs.	**NEGATIVE**
870.5500 **Bacterial DNA damage or repair tests** Tong et al. (1988). The Lack of Genotoxicity of Sodium Fluoride in a Battery of Cellular Tests. Cell Biology and Toxicology 4.2:173-	**Male Fischer F-344 rat hepatocyte primary cultures (HPC) administered sodium fluoride (2, 10, 20, 40, 80, or 160 ug/mL) for 18 hours** There was no significant increase in net nuclear grain counts at sodium fluoride concentrations up to 160 go/mL. Sodium fluoride did not elicit DNA repair	**NEGATIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
186. **Open Literature**	synthesis in the rat hepatocytes.	
870.5550 **Unscheduled DNA synthesis in mammalian cell culture** Tsutsui T, et al. (1984). Sodium Fluoride-Induced Morphological and Neoplastic Transformation, Chromosome Aberrations, Sister Chromatid Exchanges, and Unscheduled DNA Synthesis in Cultured Syrian Hamster Embryo Cells. Cancer Research 44.3:938-941. **Open Literature**	**Syrian hamster embryo (SHE) cells exposed to sodium fluoride (10, 20, or 40 ug/mL) for 4, 8, 12, 24, or 33 hours.** A dose- and time-dependent increase in unscheduled DNA synthesis was observed following administration of sodium fluoride in Syrian hamster embryo cells. Sodium fluoride was mutagenic in SHE cells within the confines of this study. Treatment-related effects on mortality were observed in SHE cells administered sodium fluoride. At sodium fluoride concentrations of 75, 100, and 125 go/mL, there were decreases in cell survival that were 10, 47, and 61%, respectively, less than control. There was no evidence of UDS at any dose of sodium fluoride in the 4 or 8 hour exposure time period. However, significant dose- and time-dependent increases in UDS were observed in the SHE cells treated with all three doses of sodium fluoride for 12 hours or greater. After 12 hours of exposure, the UDS (as measured by [3H]dThd cpm/culture well (x 10-2)) was at a level of 0.55 and 1.30 [3H]dThd cpm/culture well (x 10-2) for the 20 and 40 go/mL, respectively, dose groups. Sodium fluoride induced UDS levels of 0.75, 1.45, and 2.30 after 24 hours of exposure and 0.45, 2.25, and 5.55 after 33 hours of exposure at concentrations of 10, 20, and 40 go/mL, respectively.	**POSITIVE**
870.5550 **Unscheduled DNA synthesis in mammalian cell culture** Tsutsui, T., N. Suzuki, et al. (1984). Cytotoxicity, Chromosome Aberrations and Unscheduled DNA Synthesis in Cultured Human Diploid Fibroblasts Induced by Sodium Fluoride. Mutation Research 139:193-198.	**Cytotoxicity Test: cytotoxicity test sodium fluoride administered to JHU-1 cells at concentrations of 50, 100, or 150 go/mL for 1, 2, 6, 12, or 24 hours.** **Mutagenicity Assay: Sodium fluoride was administered to JHU-1 cells at concentrations of 50, 70, 100, 150, 200, 300, or 400 go/mL for 4, 8, 12, 16, or 24 hours** There was a significant dose-dependent increase in	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
Open Literature	unscheduled DNA synthesis following administration of sodium fluoride in cultured human diploid fibroblasts. Sodium fluoride was mutagenic in JHU-1 cells within the confines of this study. Treatment-related cytotoxic effects were observed in JHU-1 cells administered sodium fluoride. Cell survival decreased as the sodium fluoride concentration and duration of exposure increased. At sodium fluoride concentrations of 50, 100, and 150 go/mL, there were decreases in cell survival that were 100, 98, and 90% after 1 hour; 100, 75, and 65% after 2hours; 70, 48, and 40% after 6 hours; 55, 22, and 15% after 12 hours; and 17, 7, and 1% after 24 hours of exposure, respectively. Sodium fluoride was cytotoxic to JHU-1 cells and cell survival decreased linearly with increasing dose or exposure time. UDS was not induced by sodium fluoride treatment over the dose range of 50-5000 go/mL for 1 hour. There were increases in the level of UDS after 4 hours of exposure; however, none exceeded 7 [3H]TdR cpm/culture well (x 10-2) and were not significantly different from untreated cells. No significant UDS was detected until the cells were treated for longer than 4 hours. The UDS levels were 9, 22, 29, and 41 for 8 hours and 3, 22, 44, and 52 for 12 hours of exposure at the sodium fluoride concentrations of 100, 150, 200, and 300 go/mL, respectively. The UDS levels increased with dose after 16 hours of exposure, with 15, 39, and 47 [3H]TdR cpm/culture well (x 10-2) at 150, 200, and 300 go/mL sodium fluoride, respectively. The inducibility was markedly decreased in cells treated for 24 hours; most likely a result of cytotoxicity.	
870.5900 **In Vitro sister chromatid exchange assay** Khalil A, Da'Dara A. (1994). The Genotoxic and Cytotoxic Activities of Inorganic Fluoride in Cultured Rat Bone Marrow Cells. Arch Environ Contam Toxicol 26:60-63. **Open Literature**	**Bone marrow cells of Sprague-Dawley rats from tibia and femurs** **0.1, 1, 10, 100, 1,000 and 10,000 uM for 12, 24, and 36 hours** Cell survival and cell division was significantly reduced at the high-doses (1,000 and 10,000 uM). However, Sodium fluoride did not induce a SCE increase in bone marrow cells; there was no evidence of mutagenicity.	**NEGATIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
870.5900 **In Vitro sister chromatid exchange assay** Li Y, et al. (1987). Genotoxic Effects of Fluoride Evaluated by Sister-Chromatid Exchange. Mutation Res 192:191-201. **Open Literature**	**Male CHO cells** **0.05, 0.5, 1.0, 2.10, 4.20, 5.30, or 6.30 mM** The 5.30 and 6.30 mM dose levels of sodium fluoride were toxic and were not evaluated. There were no significant increases from controls in SCEs in CHO cells exposed to 0.05 to 4.20 mM sodium fluoride. Sodium fluoride did not induce a SCE increase in CHO cells; there was no evidence of mutagenicity.	**NEGATIVE**
870.5900 **In Vitro sister chromatid exchange assay** Tong et al. (1988). The Lack of Genotoxicity of Sodium Fluoride in a Battery of Cellular Tests. Cell Biology and Toxicology 4.2:173-186. **Open Literature**	**Human peripheral blood lymphocytes (HPBL) exposed to sodium fluoride (2, 10, 20, 40, 80, or 160 ug/mL) for 72 hours** The 160 go/mL dose of sodium fluoride was toxic as indicated by total lack of cell entering the mitotic cycle. SCEs of cells exposed to sodium fluoride concentrations of 80 go/mL or lower did not differ significantly from the control. Sodium fluoride did not induce an increase in SCEs in HPBL cells; there was no evidence of mutagenicity.	**NEGATIVE**
870.5900 **In Vitro sister chromatid exchange assay** Tong et al. (1988). The Lack of Genotoxicity of Sodium Fluoride in a Battery of Cellular Tests. Cell Biology and Toxicology 4.2:173-186. **Open Literature**	**Chinese hamster cells (CHO) exposed to sodium fluoride (2, 10, 20, 40, 80, or 160 ug/mL) for 24-27 hours** The 80 and 160 go/mL dose levels of sodium fluoride were toxic. There were no significant increases from control in SCEs in CHO cells exposed to 2-40 go/mL sodium fluoride. Sodium fluoride did not induce a SCE increase in CHO cells; there was no evidence of mutagenicity.	**NEGATIVE**
870.5900 **In Vitro sister chromatid exchange assay** Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, Technical Report Series	There was evidence of a concentration related positive response in SCEs induced over background in one of two studies performed, while the second did not find any evidence of a positive response.	**POSITIVE**

Table 3. Genotoxicity Profile for Sodium Fluoride		
Guideline No./Study type/Citation	**Results**	**Positive or Negative for Mutagenicity**
No. 393, NIH Publication No. 91-2848, December 1990, Pgs. 1-477. **Acceptable** Guideline		
870.5900 **In Vitro sister chromatid exchange assay** Tsutsui T, et al. (1984). Sodium Fluoride-Induced Morphological and Neoplastic Transformation, Chromosome Aberrations, Sister Chromatid Exchanges, and Unscheduled DNA Synthesis in Cultured Syrian Hamster Embryo Cells. Cancer Research 44.3:938-941 **Open Literature**	**Syrian hamster embryo (SHE) cells exposed to sodium fluoride (20, 40, or 80 ug/mL) for 24 hours** A dose-dependent increase in sister chromatid exchanges was observed following administration of sodium fluoride in Syrian hamster embryo cells. Sodium fluoride was mutagenic in SHE cells within the confines of this study. Treatment-related effects on mortality were observed in SHE cells administered sodium fluoride. At sodium fluoride concentrations of 75, 100, and 125 go/mL, there were decreases in cell survival that were 10, 47, and 61%, respectively, less than control. There were significant dose-dependent increases in SCE frequency in the sodium fluoride-treated SHE cells. After 24 hours of exposure, the frequency of SCEs increased 1.4-, 1.6-, and 2.1-fold over control at the 20, 40, and 80 go/mL dose levels of sodium fluoride, respectively.	**POSITIVE**
870.5915 **In Vivo sister chromatid exchange assay** Li Y, et al. (1987). Genotoxic Effects of Fluoride Evaluated by Sister-Chromatid Exchange. Mutation Res 192:191-201. **Open Literature**	**Male Chinese hamsters** **0.1, 1, 10, 60 or 130 mg/kg** Sodium fluoride did not induce a SCE increase in CHBM cells; there was no evidence of mutagenicity. Death occurred in three out of the eight hamsters in the 130 mg/kg/day group. Although toxic effects were seen in the high dose group, there were no treatment-related increases in SCE.	**NEGATIVE**

4.8 Neurotoxicity

Adequacy of database for Neurotoxicity: The database for neurotoxicity is considered incomplete.

Special Developmental Neurotoxicity Study

A developmental neurotoxicity study (Mullenix, et al. 1995) was designed to determine critical periods of CNS susceptibility at various stages of development and to evaluate the effects of sodium fluoride (purity not reported) on the developing brain of Sprague-Dawley rats. Seven and nine pregnant dams were administered 0.13 mg/kg sodium fluoride via subcutaneous injection two to three times daily on gestation days (GD) 14-18 and 17-19, respectively, (a total of nine injections per group, at least four hours apart). At birth, litters were culled to ten pups/dam (5 male and 5 female, where possible). At 21 days of age, 19-27 pups/sex/dose were administered 75, 100, 125, or 175 ppm fluoride in the drinking water for 6 or 20 weeks. Behavior tests were performed on the rats at 9-, 14-, or 19-weeks of age and plasma fluoride levels were measured thereafter. The behavior was tested in a computer recognition system that classified acts in a novel environment and quantified act initiations, total times, and time structures. In an additional, adult exposure study, 21-24 adult (12-week old) rats/sex/dose were administered 0 or 100 ppm sodium fluoride in deionized water for 5 to 6 weeks. Body weights were recorded weekly for all test groups.
No maternal or offspring toxicity was indicated by reduced body weight in dams during prenatal treatment or in their pups soon after birth. However, prenatal exposure to sodium fluoride altered the behavioral outcome in male offspring when exposure occurred on GD 17-19 and consisted of time structure changes in eleven behaviors and behavioral sequences. The behavioral differences did not coincide with the plasma fluoride levels.

Body-weight was significantly reduced from the control group in 3-week old rats administered 125 ppm fluoride. Concentrations below 125 ppm did not affect body weight gain during 6-week exposures. Plasma fluoride levels were significantly increased in all test groups compared to control groups. The same direction of behavioral change (initiation and total time) occurred in treated animals when compared to controls. This change was independent and unrelated to sex of the animal, exposure time (6 or 16 weeks), or dose level (100 or 125 ppm). The act of standing and the related attention cluster tended to increase in total time, while the other acts consistently decreased in initiations and total times.

The adult exposure to 100 ppm sodium fluoride had a significant effect on female behavior consistent with the behavioral change in the 3-week old rats. Similar behavioral time structure effects occurred when adult and weanling exposed rats approached 5 months of age.

The effect on behavior varied with the timing of exposure during CNS development. There were differences between behavioral changes in weanling and adult exposure when compared to prenatal exposures. Prenatally induced behavioral effects were unaccompanied by changes in body weight or elevated plasma fluoride levels. The behavioral effects induced by weanling and adult exposures were accompanied often by weight reduction and always by elevated plasma fluoride levels.

Rats were exposed to sodium fluoride at concentrations ranging from 75-125 ppm for 6 or 20 weeks. Plasma fluoride levels reached 0.059-0.640 ppm and after 6 weeks of consuming 75 and 100 ppm of sodium fluoride animals exhibited greater plasma fluoride levels than animals treated with 125 ppm. The researchers suggest that there was a taste aversion that limited the water consumption at the 125 ppm level; prolonging the period needed to attain plasma levels that were achieved in 6 weeks by the two lower exposure levels. The levels of fluoride in plasma, best predicted effects on behavior.

4.9 Metabolism and Pharmacokinetics

The database for metabolism consists of one study from the open literature. In a study by Hall et al. 1977, 6 adult male New Zealand rabbits were administered sodium fluoride in the diet (15 ppm), water (1 ppm), and in a single oral dose injected (0.5 mg/kg) directly into stomach through nasal catheter. Urine excretion following oral administration of sodium fluoride was 5 and 13% for 60 and 600 minutes, respectively. Under steady state conditions approximately 15% of fluoride ingested in food and water was absorbed by the animals. 15% was excreted in urine and 85% of ingested fluoride was removed via fecal excretion.

5.0 TOXICITY ENDPOINT SELECTION

5.1 See Section 7.1, Summary of Toxicological Doses and Endpoint Selection, Table 2.

6.0 FQPA CONSIDERATIONS

FQPA considerations are not applicable to sodium fluoride. There are no food use tolerances for this chemical and indirect food contact is not expected from the current uses of this chemical.

7.0 SUMMARY OF TOXICOLOGICAL DOSES AND ENDPOINTS FOR Sodium Fluoride FOR USE IN HUMAN RISK ASSESSMENT

7.1 Summary Table of Toxicological Dose and Endpoint Selection (Table 4)

Table 4. Sodium Fluroide for Use in Human Risk Assessment

Exposure Scenario	Dose (mg/kg/day) used in risk assessment UF	Special FQPA SF and Level of Concern for Risk Assessment	Study and Toxicological Effects
Dietary Risk Assessments			
Acute Dietary (general population and females 13-49)	No appropriate endpoints were identified that represent a single dose effect. Therefore, this risk assessment is not required.		

Table 4. Sodium Fluroide for Use in Human Risk Assessment			
Exposure Scenario	**Dose (mg/kg/day) used in risk assessment UF**	**Special FQPA SF and Level of Concern for Risk Assessment**	**Study and Toxicological Effects**
Chronic Dietary	No appropriate endpoints were identified that represent a single dose effect. Therefore, this risk assessment is not required.		
Non-Dietary Risk Assessments			
Short -Term Dermal (1 - 30 Days)	LOAEL = 20 mg/kg/day	**Target MOE**=300 (10x inter-species extrapolation, 10x intra-species variation, 3x for use of LOAEL)	Oral Subchronic Toxicity – Rat (Sodium Fluoride) LOAEL = 20 mg/kg/day, based on significant reductions in body weight gain and suppressed spontaneous motor activity.
Intermediate -Term Dermal (30 Days- 6 months)	NOAEL = 1.5 mg/kg/day	**Target MOE**=100 (10x inter-species extrapolation, 10x intra-species variation)	6-month NTP oral toxicity study-mouse LOAEL = 7.5 mg/kg/day based on histopathology observed in bone with degeneration in tibias and femurs of animals
Long-Term Dermal (> 6 months)	LOAEL = 1.3 mg/kg/day	**TARGET MOE =** 100 (10x inter-species extrapolation, 10x intra-species variation)	2-year NTP chronic toxicity/carcinogenicity study in rats LOAEL = 1.3 mg/kg/day, based on dentine dysplasia in males and females, and ameloblast degeneration in males
Short-term Inhalation (1-30 days)	LOAEL = 20 mg/kg/day	**Target MOE**=300 (10x inter-species extrapolation, 10x intra-species variation, 3x for use of LOAEL) Note: 10x route extrapolation for confirmatory inhalation study.	Oral Subchronic Toxicity – Rat (Sodium Fluoride) LOAEL = 20 mg/kg/day, based on significant reductions in body weight gain and suppressed spontaneous motor activity.

Table 4. Sodium Fluroide for Use in Human Risk Assessment			
Exposure Scenario	**Dose (mg/kg/day) used in risk assessment UF**	**Special FQPA SF and Level of Concern for Risk Assessment**	**Study and Toxicological Effects**
Intermediate-term Inhalation	NOAEL = 1.5 mg/kg/day	**Target MOE**=100 (10x inter-species extrapolation, 10x intra-species variation) Note: 10x route extrapolation for confirmatory inhalation study.	6-month NTP oral toxicity study-mouse LOAEL = 7.5 mg/kg/day based on histopathology observed in bone with degeneration in tibias and femurs of animals
Long-term Inhalation	LOAEL = 1.3 mg/kg/day	**TARGET MOE =**300 (10x inter-species extrapolation, 10x intra-species variation, 3x for use of LOAEL) Note: 10x route extrapolation for confirmatory inhalation study.	2-year NTP chronic toxicity/carcinogenicity study in rats LOAEL = 1.3 mg/kg/day, based on dentine dysplasia in males and females, and ameloblast degeneration in males
Cancer	Sodium fluoride has been classified as a "Group D" (not classifiable as to carcinogenicity). This conclusion is consistent with the recent report by the National Academy of Sciences which concluded that 'the evidence on the potential of fluoride to initiate or promote cancers, particularly of the bone, is tentative and mixed.'		

8.0 TOXICITY PROFILE TABLES

8.1 Acute Toxicity Profile Table - (See Section 4.1, Acute Toxicity, Table 2).

8.2 Subchronic, Chronic and Other Toxicity Profiles Table (Tables 5).

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
870.3100 (§ 82-1) 90-Day oral toxicity in rodents Purity: 99%	Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, Technical Report Series No. 393, NIH Publication No. 91-2848, December 1990, Pgs. 1-477. **Acceptable** Guideline 10 F344/N rats/sex/dose administered sodium fluoride at doses of 0, 10, 30, 100, or 300 ppm for 6 months	**NOAEL = 30 ppm** **LOAEL = 100 ppm**, based on the presence of hyperplasia in the glandular stomach There were no treatment-related effects on mortality. Treatment-related effects were noted in 300-ppm treated rats including clinical observations of dental fluorosis (chalk white appearance of teeth, overgrowth of upper incisors, occlusal surface of the lower incisor worn to the gum, unusual wear pattern of incisors) and rough hair coat, decreased food and water consumption, and reductions in mean body weight and body weight change. Measurement of fluoride content revealed a dose-dependent increase in fluoride concentration in bone and urine, while elevated levels of fluoride in plasma were only observed in 300-ppm treated rats. Treatment-related macroscopic effects were observed in the 300-ppm treated rats. A majority of the high-dose males exhibited thickened stomachs. Focal or multifocal punctate hemorrhages, perforated ulcer of the glandular stomach, and multiple, small, nonperforated ulcers were also observed in several 300-ppm males and/or females. These macroscopic changes were supported by microscopic evidence of pathology. Dose-dependent increases in incidence and severity of hyperplasia and necrosis of the glandular stomach was observed in rats treated with ≥100 ppm. Diffuse hyperplasia

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		of the mucosal epithelium of the glandular stomach was noted in 5/10 males and 2/10 females treated with 100 ppm, and in 10/10 males and 9/10 females treated with 300 ppm. This effect was accompanied by minimal individual cell necrosis (apoptosis) in the pyloric region in 300-ppm treated rats, and by evidence of acute inflammation in several males at 300 ppm. Focal basal cell hyperplasia of the stratifiedsquamous epithelium was located adjacent to the limiting ridge in nearly all 300-ppm treated rats. Microscopic evidence of the effects of the test article on the incisors included focal or multifocal degeneration of the enamel organ in 300-ppm males (5/10), localized in the maturation zone near the apical end of the tooth.
870.3100 (§ 82-1) 90-Day oral toxicity in rodents Purity: 99%	Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, Technical Report Series No. 393, NIH Publication No. 91-2848, December 1990, Pgs. 1-477. **Acceptable** Guideline 8-12 B6C3F1 mice/sex/dose administered deionized water at doses of 0, 10, 50, 100, 200, 300 or 600 ppm for 6 months	**NOAEL = 50 ppm in female mice, and could not be determined in males** based on the observation of increased osteoid of the tibia in 5/10 males dosed at 50 ppm. **LOAEL = 50 ppm in male mice and 100 ppm in female mice** based on histopathology observed in bone. Premature deaths, including sacrifice due to moribundity, occurred at 300 ppm (1 male) and 600 ppm (4 males and 9 females) dose levels. Clinical signs of thin appearance, hunched posture and weakness were observed in several of the decedents prior to premature sacrifice. Clinical signs in surviving animals included chalky white incisors (≥100 ppm) and chipped teeth

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		(≥300 ppm). The effects on the incisors correlated with microscopic findings, which included focal or multifocal degeneration of the enamel organ. Mean body weight was significantly decreased in 600-ppm treated males and in 200- and 300-ppm treated females. Mean body weight gain was significantly decreased in ≥200-ppm males and in 200- and 300-ppm females. These parameters were also decreased in the 600-ppm females, but did not reach statistical significance, likely due to the reduced number of animals in this group as a result of premature deaths. Food consumption in 600-ppm males was approximately 77% of controls. Food consumption in the other treatment groups, and water consumption in all treatment groups were within 20% of control values. There was a dose-dependent increase in fluoride content in bone and urine. Due to the pooling of plasma samples for sufficient volume for analysis, meaningful statistical analyses in this fluid could not be performed. The data indicate that there was generally a dose-dependent increase in fluoride concentration in the plasma. Animals that died prior to the termination of the study had noted histopathology in the kidney, liver, testes, and/or myocardium. Nephrosis in the kidneys and/or myocardial degenerationand mineral accumulation in degenerate myofibers were the likely causes of death in some of the decedents. Multifocal megalocytosis and syncytial alteration were

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		observed primarily in the livers of 600-ppm treated mice. Necrosis, tubule degeneration, and the presence of multinucleated giant cells in the seminiferous tubules were also noted in the males that died prior to terminal sacrifice; however, it could not be determined if these effects were due to treatment with sodium fluoride or secondary to the mice dying from other unrelated causes. Bone lesions, in the form of increased osteoid, were observed in the femurs of treated mice at ≥100 ppm, and in the tibias at 50 ppm. This histopathology is indicative of altered rates of bone deposition and remodeling as a result of treatment. Further, treatment with sodium fluoride induced degeneration of the incisors as evidenced by degeneration (dysplasia) in ≥300-ppm treated mice.
Non-Guideline Oral Subchronic (Rodent) Purity not reported	Bohatyrewicz, A. (1999). Effects of Fluoride on Mechanical Properties of Femoral Bone in Growing Rats. Fluoride 32:47-54. **Open Literature** 10 female 6-week-old Wistar rats/group administered NaF at levels of 0, 8, 30, and 60 mg of fluoride/L in drinking water for 6 weeks. Femoral bones from each rat were assayed for bending strength	High fluoride intake (30 and 60 mg/L) significantly decrease bone quality of the femoral shaft and neck of young rats. NaF administered in lower concentrations (8 mg/L) significantly increases the strength of the femoral neck from the control.
Non-Guideline Oral Subchronic (Rodent) Purity not reported	Paul, V. et al. (1998). Effects of Sodium Fluoride on Locomotor Behavior and a Few Biochemical Parameters in rats. Environmental Toxicol and Pharmacol 6:187-	Subchronic Toxicity: **NOAEL < 20 mg/kg/day (lowest dose tested)** **LOAEL ≤ 20 mg/kg/day** based on significant reductions in body

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	191. **Open literature** 10 female Wistar rats/dose administered Sodium Fluoride via oral intubation at dose levels of 20 or 40 mg/kg/day for 60 days.	weight gain and suppressed spontaneous motor activity. There were significant dose-dependant decreases of 17 and 30% for food intake and 14 and 37% for body weight gain at the 20 and 40 mg/kg/day dose levels, respectively. Total protein concentrations in serum (low-dose, 13%; high-dose, 38%), liver (low-dose, 22%; high-dose, 42%), and skeletal muscle (low-dose, 15%; high-dose, 31%) were also significantly reduced in a dose-related manner in animals treated with sodium fluoride. Spontaneous motor activity was suppressed in a dose-dependant manner with decreases of 15 and 29% at the 20 and 40 mg/kg/day dose levels, respectively. However , motor co-ordination was not altered in treated animals. Total blood cholinesterase activity was reduced at the low- and high-dose, although there was no evidence of change in acetyl-cholinesterase activity of the cerebral cortex, brain stem, or cerebellum. Food intake reductions may account for the decrease in protein concentration of a direct deleterious action of fluoride on protein metabolism can also play a role in depleting protein in sensitive tissues. Thus, a decreased food intake together with a depletion of protein in soft tissues accounted for an inhibition of body growth in sodium fluoride-treated animals. Sodium fluoride deprived skeletal muscle of total protein and suppressed blood cholinesterase activity; although, these effects are unlikely to have a

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		deteriorating action on neuromuscular function. However, similar sodium fluoride doses can produce neurobehavioral deficit resulting in an inhibition of spontaneously occurring locomotor activity.
Non-Guideline Oral Subchronic (Rodent) Purity not reported	Pillai et al. (1988). Effect of Subacute Dosage of Fluoride on Male Mice. Toxicology Letters 44:21-29. **Open Literature** 5 Male Swiss albino mice administered 5.2 mg F/kg/day for 35 days.	**NOAEL ≤ 5.2 mg/kg/day (lowest dose tested)** **LOAEL ≤ 5.2 mg/kg/day**, based on significant decreases in body weight gain, and food and water consumption. There were significant changes in hematological analyses with decreases in red blood cells, lymphocytes, hemoglobin, albumin, total protein, cholesterol, glucose, and alkaline phosphatase. Statistically significant increases were observed in white blood cells, monocytes, basophils, and eosinophils. Food and water consumption was significantly decreased in treated animals compared to controls. There were significant treatment-related decreases from controls in body weight gain of sodium fluoride-treated mice after day 19 of the treatment period. A significant relationship between food and water consumption and the body weight was observed in the controls, but not in the treated animals. Significant increases in fluoride content were measured in the kidneys, stomach, brain, liver, and intestines of the sodium-fluoride-treated animals when compared to the controls. The increases were 3.5- and 1.5 fold greater than control in the kidneys and stomach, respectively, while the brain,

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		intestines, and liver exhibited 2-fold increases over control. There was no evidence of sperm abnormalities following treatment with sodium fluoride.
Non-Guideline Oral Subchronic (Rodent) Purity: 99%	Chinoy and Patel. (2001). Effects of Sodium Fluoride and Aluminum Chloride on Ovary and Uterus of Mice and Their Reversal by Some Antidotes. Fluoride 1:9-20. **Open Literature** 20 Adult female albino mice administered 10 mg/kg/day NaF for 30 days.	Significant decline of ovarian protein and 3-beta- and 17-beta-hydroxysteroid dehydrogenase activities, which could be related to increased cholesterol levels in the ovary suggesting altered steroidogenesis.
Special Study Subchronic (subcutaneous injection) Toxicity Purity not reported	Shahshi et al. (1994). Effect of Long-term Administration of Fluroide on Levels of Protein, Free Amino Acids and RNA in Rabbit Brain. Fluoride 27.3:155-159. **Open Literature** Albino rabbits administered sodium fluoride via subcutaneous injection for 100 days at 0, 5, 10, 20, and 50 mg/kg/day. 12 animals/group	Fluoride treated rabbits showed a significant decline in soluble, basic, and total protein and free amino acid levels. RNA content rapidly decreased, except in male rabbits treated with 5 and 10 mg/kg/day sodium fluoride. Decreased body weight gain in the 20 and 50 mg/kg/day groups. Some animals in the 10, 20, and 50 mg/kg/day groups showed paralysis by day 35. No rabbits in the 50 mg/kg/day group survived the experiment.
870.3700a Developmental Toxicity (Rodent) Purity > 99%	Bates et al. (1994). Final report on the developmental toxicity of sodium fluoride (Cas No. 7681-49-4) in Sprague-dawley rats. RTI, RTP NC, for NTP (PB95-110193). **Open Literature** Administered ad libitum in deionized/filtered drinking water	Maternal toxicity: NOAEL = 18 mg/kg/day LOAEL = 27 mg/kg/day, based on reduced maternal body weight. There were no treatment-related clinical signs, increases in mortality (100% survival), or decreases in body weight in rats dosed with sodium fluoride. The maternal body weight gain during

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	to Sprague-Dawley-derived rats (26/group) on Gestation days 6-15 at levels 0, 50, 150, or 300 ppm. Rats killed on gestation by day 20 and examined. Feed contained 12.4 ppm.	the first two days of exposure (GD 6 to 8) was significantly reduced (55%) at 300 ppm (27 mg/kg/day) relative to controls. The mean maternal body weight gain and water consumption during the treatment period was, also, significantly reduced, possibly due to a decrease in palatability. Reproductive toxicity: NOAEL ≥ 27 mg/kg/day (highest dose tested) LOAEL > 27 mg/kg/day (not established) There were no treatment-related effects on mean live fetal body weight /litter, and the number of live fetuses. A dose-related increase in the percent of litters with one or more externally malformed fetuses, the percent of externally malformed fetuses/litter, and the percent of skeletally malformed fetuses/litter occurred however was not statistically significant.
870.3700a Developmental Toxicity (Rodent) Purity not reported	Collins, T et al. (1995). Developmental Toxicity of Sodium Fluoride in Rats. Fd Chem Toxicol 33:951-960. **Open Literature** Female (CD:CRL: CD-BR, VAF+) rats were given drinking water containing 0, 10, 25, 100, 175, or 250 ppm Fluoride (0, 1.4, 3.9, 15.6, 24.7, or 25.1 mg/kg bw) 34, 35, 33, 33, 33, 35 female rats for each dose Caesarean sections were performed on gestation day 20.	Maternal Toxicity: NOAEL = 175 ppm (24.7 mg/kg/day) LOAEL = 250 ppm (25.1 mg/kg/day), based on significant reductions in body weight gain, and food and water consumption. There were no incidences of maternal mortality, changes in behavior, clinical signs, or mottled teeth in dams treated with sodium fluoride. In the 100 ppm dose group, there was 1 female rat that exhibited multiple, apparently random, clinical findings (exudate from the eye and nose, and overgrown teeth) that was not associated with treatment. The 250 ppm dose group experienced significant

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		decreases in food and water consumption, and body weight gain that were 7, 30 and 11 % respectively, less than controls. A significant reduction (10.7%) from control, in fluid consumption was observed in animals treated with 175 ppm sodium fluoride; however, there were no other treatment-related changes found at this dose level. Reproductive toxicity: NOAEL ≥ 250 ppm (25.1 mg/kg/day; highest dose tested) LOAEL > 250 ppm (25.1 mg/kg/day; not established) The pregnancy rate was greater than 90% for all groups. There was a significant decrease in the mean number of corpora lutea/female in dams of the 250 ppm dose group; however, because number of corpora lutea is determined at birth, this decrease is considered to be random. There were no significant changes in reproductive parameters in treated animals when compared to controls. Developmental toxicity: NOAEL ≥ 250 ppm (25.1 mg/kg/day; highest dose tested) LOAEL > 250 ppm (25.1 mg/kg/day; not established) There were no treatment-related effects in fetal body weight, litter sizes, or viable fetuses. Several external variations were observed in control and treated animas; however, there were no significant increases in the number of fetuses with at least 1,2 or 3 variations, or in the number of litters with fetal sternebral variations. There was no evidence of teratogenicity

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		observed in the rats following administration of phenol.
870.3700a Developmental Toxicity (Rodent) Purity > 99%	Heindel, J. et al. (1996). Developmental Toxicity Evaluation of Sodium Fluoride Administered to Rats and Rabbits in Drinking Water. Fund Applied Toxicol 30:162-177. **Open Literature** Administered ad libitum in deionized/filtered drinking water to Sprague-Dawley rats (26/group) on gestation days 6-15 at levels 0, 50, 150, or 300 ppm (0, 6.6, 18.3, or 27.1 mg/kg/day, respectively). Rats killed on gestation day 20 and examined. Feed contained 15.6 ppm.	Maternal toxicity: NOAEL = 18.3 mg/kg/day LOAEL = 27.1 mg/kg/day, based on reduced maternal body weight gain There were no treatment-related clinical signs, increases in mortality (100% survival), or decreases in body weights in rabbits dosed with sodium fluoride at the low- and mid-dose. The maternal body weight gain of the high dose group on GD 6-8 was 56% less than the controld. During the treatment period, as a whole, there was not a significant difference in mean body weight gain; however, a decreasing trend that approached statistical significance was observed. The water consumption during the treatment period was significantly reduced at the high-dose. The food consumption was decreased at the high-dose during GD 8-10, but was normal thereafter. Reproductive toxicity: NOAEL >= 27.1 mg/kg/day (highest dose tested) LOAEL > 27.1 mg/kg/day (not established). There were no changes in reproductive parameters in treated animals when compared to controls. Developmental toxicity: NOAEL >= 27.1 mg/kg/day (highest dose tested) LOAEL > 27.1 mg/kg/day (not established) There were no treatment-related effects on mean live fetal body

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		weight/litter, live fetal number, and prevalence of malformations.
870.3700b Developmental Toxicity (Non- Rodent) Purity > 99%	Heindel, J. et al. (1996). Developmental Toxicity Evaluation of Sodium Fluoride Administered to Rats and Rabbits in Drinking Water. Fund Applied Toxicol 30:162-177. **Open Literature** Administered ad libitum in deionized/filtered drinking water to New Zealand White rabbits (26/group) on gestation days (6-19 at levels of 0, 50, 150, or 300 ppm. Rats killed on gestation day 30 and examined. Feed contained 15.6 ppm	Maternal toxicity: NOAEL = 18 mg/kg/day LOAEL = 29 mg/kg/day, based on reduced maternal body weight gain. There were no treatment-related clinical signs, increases in mortality (100% survival), or decreases in body weights in rabbits dosed with sodium fluoride at the low- and mid-dose. The high-dose (400 ppm) group, during GD 6 to 8, experienced a mean weight loss of 112 grams versus a mean weight gain of 14 grams for the control. During the GD 10 to 12, the 400 ppm group recovered with a mean weight gain of 71 grams versus 22 grams for the control. During the treatment period, as whole, there was not a significant difference in mean body weight gain. The water consumption during the treatment period was significantly reduced, possibly due to a decrease in palatability. The food consumption was decreased during the first four days of treatment, but was normal thereafter. Reproductive toxicity: NOAEL ≥ 29 mg/kg/day (highest dose tested) LOAEL > 29 mg/kg/day (not established) There were no changes in reproductive parameters in treated animals when compared to controls. Developmental toxicity: NOAEL ≥ 29 mg/kg/day (highest

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		dose tested) LOAEL > 29 mg/kg/day (not established) There were no treatment-related effects in mean live fetal body weight/litter, live fetal number, and prevalence of malformations.
Non-guideline Developmental Toxicity (Rodent) Purity not reported	Elbetieha, A et al. (2000). Fertility Effects of Sodium Fluoride in Male Mice. Fluoride 33:128-134. **Open Literature** 80 sexually mature Swiss mice exposed to 0, 100, 200, 300 ppm NaF via drinking water for 4 weeks (0, 12.35, 21.80, 39.19 mg/kg/day) and 10 weeks (0, 8.85, 15.64, 27.25 mg/kg/day) (10 mice/group/exposure period) Males mated after exposure periods to untreated female mice	2/10 and 3/10 mice died during 10 week exposure at 100 and 300 ppm, respectively. 200 and 300 ppm for 4 weeks caused significant increase in the relative weights of preputial glands. Mice tested for ten weeks showed no significant increase in any reproductive organ. Mice tested for 4 weeks ha d no effect on male fertility. 100, 200 and 300 ppm for 10 weeks caused a significant increase in resorptions, a decrease in implantations and pregnancies in untreated females mated with NaF treated males.
870.3800 Reproduction Purity not reported	Collins, T et al. (2001). Developmental Toxicity of Sodium Fluoride Measured During Multiple Generations. Fd Chem Toxicol 39:867-876. **Open Literature** Administered 0, 25, 100, 175, 250 mg of NaF in drinking water to (CD CRL: CD-BR) rats continuously for three generations. Parental generation (F0) was treated for ten weeks and mated within groups. On gestation day 20m caesarian sections were performed on 8 F0 females per group and their litters (F1) observed. The remaining F0 females were allowed to litter.	Maternal toxicity: NOAEL ≥ 250 ppm (highest dose tested) LOAEL > 250 ppm (not established) There were no treatment-related effects on maternal mortality. A significant decrease from control in fluid consumption (30%) was observed at the 250 ppm dose level. There were no other changes in F0 maternal generation. There were significant decreases from control of 28 and 31% in fluid consumption in the F1 dams at the 175 and 250 ppm dose levels, respectively. The decreases in fluid consumption corresponded

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Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Caesarian sections were performed on all of the F1 generation females (36/group) and were observed along with their litters.	with decreased palatability of the solution. Food consumption was significantly reduced (11%) in F1 dams when compared to control in the 175 ppm dose group. There was a 14% decrease from control in the body weight gain of F1 females (dams) treated with 175 ppm. These reductions at 175 ppm were considered random because of the lack of effect in the 150 pp, group. Gravid uterine weight measurements showed no doe-related differences. Reproductive toxicity: NOAEL ≥ 250 ppm (highest dose tested) LOAEL > 250 ppm (not established) There were no treatment-related effects in the mean number of corpora lutea, mean number of implantation sites, implantation efficiency, mean number of viable fetuses, and average percentage of early and late deaths per litter of dams. Offspring toxicity: NOAEL = 175 ppm LOAEL = 250 ppm, based on decreased ossification of the hyoid bone. Fetal body weight was not affected by treatment with sodium fluoride. There was no evidence of toxicity in fetuses or pups of the F1 generation. Similarly, the F2 generation fetuses and pups were unaffected by treatment with sodium fluoride with the exception of decreased ossification of the hyoid bone in the F2 fetuses at the 175 (not significant) and 250 ppm (significant) dose groups.

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
870.3800 Reproduction Purity not reported	Collins, T et al. (2001). Multigenerational Evaluation of Sodium Fluoride in Rats. Food and Chemical Toxicology 39.6:601-13. **Open Literature** Rats administered 0, 25, 100, 175, or 250 ppm NaF in drinking water throughout three generations.	The Maternal toxicity NOAEL is ≥ 250 ppm (highest dose tested). The Maternal toxicity LOAEL > 250 ppm (not established). Reproductive toxicity: NOAEL ≥ 250 ppm (highest dose tested). LOAEL > 250 ppm (not established). Rats were monitored daily during the 10 week growth period and only 2 animals died; 1 F0 male at 25 ppm and 1 F1 female of control dose groups. There were no dose-related clinical effects observed. No significant differences were observed in F0 female food consumption while there was a 5% decrease (significant) reduction in F0 males at 250 ppm (in the first 7 weeks, and week 9 of the 10 week growth period). F1 females exhibited an overall decrease in food consumption but never significantly different for control. Males of the F1 generation consumed less food than controls but in a dose-related or significant manner. Fluid consumption was significantly reduced from control levels in the 175 and 250 ppm dose groups with decreases of 11 and 20% for F0 females, 9 and 20% for F0 males, 19 and 29% for F1 females, and 15 and 25% for F1 males, respectively. F1 males in the 100 ppm dose group drank significantly less (9%) than control animals. The decrease in fluid consumption was attributed to a reduced palatability. Weight gain of F0 females and males showed a significant negative linear regression for the

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		10 weeks but only the individual weight gain of F0 males in 250 ppm dose group was statistically significantly less than controls. There was a 6% reduction from 367.0 to 345.9 g in first generation females treated with the high-dose (250 ppm) of sodium fluoride. F0 female mating indices (mating and fertility) were over 90% in all groups, although these were slightly (but not significantly) decreased at the 250 ppm sodium fluoride dose level. Similarly, F1 female mating indices exceeded 90% with slight but not significant decreases in the 25 and 250 ppm groups; indicating a lack of compound-related effects. There were no significant or dose-related effects observed in implantation and reproductive parameters of any generation. Survival indices of the F2 generation (implantation, live-births, days 4, 7, 14, and 21 survival and lactation indices) were calculated for both male and female offspring. Neither significant nor dose-related effects were observed (data not shown in this study).
870.3800 Reproduction Purity not reported	Messer et al. Influence of Fluoride Intake on Reproduction in Mice. J. Nutr. 103:1319-1326. **Open Literature** Weaning female albino mice administered 0, 50, 100, and 200 ppm NaF via drinking water to 58, 55, 50, and 50 animals, respectively. Females mated and litters were normalized to 6 pups and a	Maternal Toxicity: Offspring toxicity: Retardation of growth in the 100 and 200 ppm F1 groups, with death in 50% of animals in the 200 ppm groups by 8 weeks of age. Reproductive toxicity: No litter production at the 200 ppm group and only 9 litters at the 100 ppm over a ten-week period. 50 ppm group had progressive decrease in litter production in both

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	maximum of 4 litters were analyzed. Second generation mice from control and 50 ppm groups (38 and 44 animals, respectively) were mated and followed the same parameters as the parental group.	generations, but considered insignificant differences.
Non-guideline Reproduction Purity not reported	Araibi et al. (1989). The Effect of High Fluoride on the Reproductive Performance of the Male Rat. J. Biol. Sc. Res. 20:19-20. **Open Literature** Male albino rats administered sodium fluoride in the diet for 60 days 15 mice/dose 100 or 200 ppm	Lesions on the teeth (mottling and erosion of enamel), a characteristic commonly associated with sodium fluoride exposure, were observed in animals at the end of the experiment. Males treated with sodium fluoride seemed to show less interest toward females when compared to those animals of the control group. The number of pregnant females were decreased 10 and 40% from controls in groups treated with 100 and 200 ppm, respectively. High-dose animals exhibited significant reductions in the number of pregnant females. The number of newborns produced by the 100 and 200 ppm dose groups were 30 and 57% (significant), respectively, less than controls. There was a decrease in average litter size for both dose levels, although neither reduction was significantly different from controls. Mean tubular diameters were significantly less than controls with 3 and 7% decreases in diameter for the 100 and 200 ppm dose levels, respectively. There were 94 and 93% (significant) increases in peritubular membrane thickness in the low- and high-dose groups, respectively. Treatment of animals with 200 ppm sodium fluoride resulted in significant

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Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		decreases from control in percentage of seminiferous tubules containing spermatozoa. There were decreases in mean testosterone levels in the serum of treated animals with 29 (nonsignificant) and 71% (significant) reductions from controls observed in the 100 and 200 ppm dose groups, respectively There was a decrease in reproductive performance of male rats exposed to a high intake of sodium fluoride in spite of the absence (until the end of the experiment) of clinical signs in the teeth that are characteristic features of fluorosis. The testes of 200 ppm sodium fluoride-treated rates exhibited impairments of spermatogenesis based on changes in mean diameter of seminiferous tubules, the thickness of peritubular membranes, spermatozoa, and serum testosterone levels. The researchers suggested that sodium fluoride appears to be antispermatogenic and the decrease in testosterone may account for the decrease of mated females in sodium fluoride-treated groups.
Non-guideline Reproduction Purity not reported	Ream et al. (1983). Bone Morphology of Weaning Rats from Dams Subjected to Fluoride. Cell Tissue Res 233:689-691. **Open Literature** 0 or 150 ppm fluoride as NaF in drinking water administered to 12 female Sprague-Dawley rats for 10 weeks prior to breeding and during 3 successive pregnancy and lactation periods. Rebreeding periods commenced immediately following a 3 week	The amount of fluoride transferred to the offspring and incorporated into the skeleton is not sufficient to cause a visible structural alteration in the growth and development if the long bones.

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	lactation period and all litters were normalized to 8 pups were sacrificed and femur removed for analysis.	
Non-guideline Reproduction Purity not reported	Shivarajashankara et al. (2002). Histological Changes in the Brain of Young Fluoride-Intoxicated Rats. Fluoride 35:12-21. **Open Literature** 0.5 (control), 30 or 100 ppm fluoride (as NaF) in drinking water administered to female Wistar albino rats, respectively, during the last (3rd) week of pregnancy and throughout the lactation period. Litters exposed to same dose levels for up to ten weeks.	30 ppm fluoride did not show any notable alterations in brain histology, whereas rats exposed to 100 ppm fluoride showed significant neurodegenerative changes in the hippocampus, amygdale, motor cortex, and cerebellum. Changes included decrease in size and number of neurons in all regions, decrease in the number of Purkinje cells in the cerebellum, and signs of chromatolysis and gliosis in the motor cortex. These histological changes suggest a toxic effect of high-fluoride intake during the early developing stages of life on the growth, differentiation, and sub cellular organization of brain cells in rats.
Non-guideline Reproduction Purity not reported	Trabelsi, M et al. (2001). Effect of Fluoride on Thyroid Function and Cerebellar Development in Mice. Fluoride 34: 165-173. **Open Literature** 0 or 500 mg/L NaF in drinking water to pregnant and lactating mice, from the 15th day of pregnancy to the 14th day after delivery. Litter size was reduced to 8 pups for the control and tested group.	Tested group pups showed 35% decrease in body weight, a 75% decrease in the plasma free T4 level, a 27% decrease in cerbellar protein, and a 17% decrease in cerebral protein compared to the control. (Graphs missing in study).
870.4100a Chronic Toxicity (Rodent) Purity not reported	Varner, J.A. et al. (1998). Chronic Administration of Aluminum-Fluoride and Sodium Fluoride to Rats in Drinking Water: Alterations in Neuronal and Cerebrovascular Integrity. Brain Research 784:284-298.	No differences were found between the body weights of rats in the different treatment groups although more rats died in the aluminum fluoride (5) ad the NaF group (3) than the control group (1). All levels in samples of brain and kidney were higher in both

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Open Literature Adult male Long-Evans rats received double deionized water (ddw) and 0.5 ppm Aluminum Fluoride, or ddw and 2.1 ppm NaF for 52 weeks. 7 animals/group	the aluminum fluoride and NaF groups relative to controls. The effects of the two treatments on cerebrovascular and neuronal integrity were qualitatively and quantitatively different. These alterations were greater in animals in the aluminum fluoride group than in the NaF group and greater in the NaF group than in controls.
Non-guideline Chronic Toxicity (Rodent) Purity not reported	Turner et al. (1995). Fluoride Reduced Bone Strength in Older Rats. J. Dent Res. 74:1475-1481. **Open Literature** Four groups of 64 to 66 rats administered 0, 5, 15, or 50 ppm of fluoride via drinking water for exposure periods of 3, 6, 12, or 18 months.	Femoral failure load was not significantly decreased in rats treated for 3 to 6 months , but was decreased as much as 23% in rats treated 12 to 18 months at 50 ppm fluoride.
870.4200a Oncogenicity (Rat) Purity not reported	Maurer et al. (1990). Two-Year Carcinogenicity Study of Sodium Fluoride in Rats. J. Natl. Cancer Inst. 82:1118-1126. **Open Literature** Sprague-Dawley rats fed a diet containing 0, 4, 10, or 25 mg/kg/day NaF added to a low-fluoride diet for up to 99 weeks 70 rats/group	There was no evidence of treatment-related incidence of carcinogenicity in Sprague-Dawley rats administered dietary sodium fluoride in concentrations up to 25 mg/kg/day for 2 years. All bone neoplasms observed were considered to be incidental and spontaneous and not related to sodium fluoride treatment, because of their low incidence and random distribution. The incidence of preneoplastic and neoplastic lesions at any site in rats of either sex was not altered by the administration of sodium fluoride. Sodium fluoride was not carcinogenic to rats within the confines of this study. At study termination, diet consumption for the 25 mg/kg/day (group 5) was significantly reduced when compared to the control (group

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Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		1), with decreases of approximately 20 and 18% for males and females, respectively. Body weight gain was significantly less than the control for the 25 mg/kg/day dose group. Both male and female rats administered the high-dose of sodium fluoride experienced decreases of roughly 25% in mean body weight gain. Clear evidence of fluoride toxicity was seen in the teeth, bones, and stomach, the severity of which was related t dose and duration of treatment. At sodium fluoride concentrations of 4 mg/kg/day or greater, dental changes occurred including incisors malformations and fractures, and enamel hypoplasia. Treatment-related bone effects, mostly skull, were observed at concentrations of 10 mg/kg/day and greater, affected bones were white, thick, and found to have roughened surfaces and subperiostal hyperostosis. There was lack of bone marrow cavities in the new bone. There was an increase in incidence and severity of chronic inflammation of the gastric glandular mucosa in rats treated with sodium fluoride doses at or above 10 mg/kg/day.
870.4200b Oncogenicity (Mouse) Purity = 99%	Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, Technical Report Series No. 393, NIH Publication No. 91-2848, December 1990, Pgs. 1-477. **Acceptable** Guideline	Male: NOAEL = 9.6 mg/kg/day LOAEL = 16.7 mg/kg/day, based on the clinical chemistry changes in alkaline phosphatase and serum phosphorus (males) at 66 weeks and bone lesions (dentine dysplasia) Female: NOAEL = 11.3 mg/kg/day LOAEL = 18.8 mg/kg/day, based on the clinical chemistry changes

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	100, 70, 70, or 100 B6C3F1 mice/sex administered sodium fluoride in the drinking water at doses of 0, 25, 100, or 175 ppm (mice/sex) for 103 weeks. (male: 0, 2.4, 9.6, or 16.7 mg/kg/day) (female: 0, 2.8, 11.3, or 18.8 mg/kg/day)	in alkaline phosphatase and bone lesions (myelofibrosis) There were no compound-related effects on mortality, body weight, food consumption, water consumption, hematology, or organ weights. Treatment-related clinical findings included a dose-dependent increase in white discoloration of the teeth (27%, 39%, 80%, and 100% in males and 19%, 43%, 84%, and 100% in females, from control to high dose, respectively) which occurred as early as Day 74 in the high-dose animals compared to Day 508 in the control animals. Serum alkaline phosphatase was significantly increased in high-dose females at 24 (29%) and 66 weeks (88%) and in high dose-males at 66 weeks (11%). Serum phosphorus levels were significantly decreased (13%) in high-dose males at 66 weeks. There was a significant increase in incisor dentine dysplasia in high-dose males (78% in controls versus 91% at the high dose). There was an increase in the incidence of myelofibrosis (femoral, humerus, maxilla, and thoracic) in female mice at all doses.
870.4300 Chronic/Oncogenicity (Rodent) Purity = 99%	Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, Technical Report Series No. 393, NIH Publication No. 91-2848, December 1990, Pgs. 1-477. **Acceptable** Guideline 100, 70, 70, or 100 F344/N	Three bone osteosarcomas were noted in high-dose males and one in a mid-dose male, with none in controls. A fourth osteosarcoma, not originating in the bone, was observed in an additional high-dose male. Dosing was considered adequate based on tooth deformities and discoloration; dentine dysplasia and degeneration in the ameloblasts and odontoblasts, bone osteosarcomas in males and osteosclerosis in females. Trend

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	rats/sex administered sodium fluoride in the drinking water at doses of 0, 25, 100, or 175 ppm (mice/sex) for 103 weeks. (male: 0, 1.3, 5.2, or 8.6 mg/kg/day) (female: 0, 1.3, 5.5, or 9.5 mg/kg/day)	analyses revealed that, at the doses tested, there was a significant treatment-related increase in the incidence of bone osteosarcomas in males but the incidence was not significantly increased in the high-dose males as compared to controls when comparisons were made either within the animals scheduled for terminal sacrifice or all animals (including the interim sacrifice and concurrent control animals). In those animals scheduled for terminal sacrifice, statistical analysis of all organ osteosarcoma in dosed animals as compared to controls also failed to show significance. The study authors failed to perform the statistical analysis all osteosarcoma analysis among all animals. That analysis, done by the contractor, did reveal a significant difference between the high dose and control groups. Due to the fact that bone osteosarcoma incidence of the high-dose as compared to the control group was not significant, but displayed a significant positive trend, the occurrence of these rare tumors was considered equivocal evidence of carcinogenicity in male rats by the study authors. Such a conclusion was bolstered by the fact that bone osteosarcomas were not observed in treated females or in the parallel study in B6C3F1 mice (TR393). However, with the significant difference between high dose animals and controls in the all organ osteosarcoma incidence analysis when all animals are considered, the reviewer believes that the occurrence of osteosarcomas in the male rats should have been considered some evidence, if not clear

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		evidence, of the carcinogenic activity of sodium fluoride. NOAEL < 1.3 mg/kg/day (lowest dose tested) LOAEL = 1.3 mg/kg/day, based on dentine dysplasia in males and females, and ameloblast degeneration in males Mortality, body weight, body weight gain, food consumption, water consumption, hematology, and organ weights were not affected by exposure to NaF. Fluoride concentration increased with dose in blood (serum) at Weeks 27 and 66, and bone and urine at Weeks 27, 66, and 105. Analysis of bone fluoride revealed an increase with dose and age. Urinary calcium was observed to be significantly increased in high-dose females. Tooth discoloration (whitening and mottling) was noted in all treated animals with attrition, deformity, and occasional malocclusions noted in the high- and/or mid-dose males. Histopathology of the incisors noted dentine dysplasia (all dosed animals), degeneration of the ameloblasts (mid- and high-dose animals), and, to a lesser extent, degeneration of the odontoblasts (principally dosed males). Increases in the incidence and severity of osteosclerosis of the long bones were noted in the high-dose females (6/80 control; 18/81 high- dose, P=0.04).
Special Study Developmental Neurotoxicity Purity not reported	Mullenix et al. (1995). Neurotoxicity of Sodium Fluoride in Rats. Neurotoxicology and Teratology 17:169-177. **Open Literature**	No maternal or offspring toxicity was indicated by reduced body weight in dams during prenatal treatment or in their pups soon after birth. However, prenatal exposure to sodium fluoride

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Sprague-Dawley rats administered NaF via subcutaneous injection during the prenatal period on gestation periods 14-18 and 17-19. Weanlings received drinking water containing 0, 75, 100, or 125 ppm F for 6 to 20 weeks. 3-month old adults received 100 ppm for 6 weeks.	altered the behavioral outcome in male offspring when exposure occurred on GD 17-19 and consisted of time structure changes in eleven behaviors and behavioral sequences. The behavioral differences did not coincide with the plasma fluoride levels. Body-weight was significantly reduced from the control group in 3-week old rats administered 125 ppm fluoride. Concentrations below 125 ppm did not affect body weight gain during 6-week exposures. Plasma fluoride levels were significantly increased in all test groups compared to control groups. The same direction of behavioral change (initiation and total time) occurred in treated animals when compared to controls. This change was independent and unrelated to sex of the animal, exposure time (6 or 16 weeks), or dose level (100 or 125 ppm). The act of standing and the related attention cluster tended to increase in total time, while the other acts consistently decreased in initiations and total times. The adult exposure to 100 ppm sodium fluoride had a significant effect on female behavior consistent with the behavioral change in the 3-week old rats. Similar behavioral time structure effects occurred when adult and weanling exposed rats approached 5 months of age. The effect on behavior varied with the timing of exposure during CNS development. There were differences between behavioral changes in weanling and adult exposure when compared to prenatal exposures. Prenatally induced behavioral effects were unaccompanied by

Table 5. Subchronic, Chronic, and other Toxicity Profiles for Sodium Fluoride		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		changes in body weight or elevated plasma fluoride levels. The behavioral effects induced by weanling and adult exposures were accompanied often by weigh reduction and always by elevated plasma fluoride levels. Rats were exposed to sodium fluoride at concentrations ranging from 75-125 ppm for 6 or 20 weeks. Plasma fluoride levels reached 0.059-0.640 ppm and after 6 weeks of consuming 75 and 100 ppm of sodium fluoride animals exhibited greater plasma fluoride levels than animals treated with 125 ppm. The researchers suggest that there was a taste aversion that limited the water consumption at the 125 ppm level; prolonging the period needed to attain plasma levels that were achieved in 6 weeks by the two lower exposure levels. The levels of fluoride in plasma best predicted effects on behavior.
870.7485 General Metabolism	Hall et al. (1977). Kinetic Model of Fluoride Metabolism in the Rabbit. Environmental Research 13:285-302. **Open Literature** Adult male New Zealand rabbits were administered sodium fluoride in the diet, water, and in a single oral dose injected directly into stomach through nasal catheter 15 ppm in the diet 1 ppm in the water 0.5 mg/kg oral 6 rabbits	Urine excretion following oral administration of NaF was 5 and 13% for 60 and 600 minutes, respectively. Under steady state conditions approximately 15% of fluoride ingested in food and water was absorbed by animal. 15% was excreted in urine and 85% of ingested fluoride was involved in fecal excretion.

9.0 REFERENCES

MRID	CITATION
162945	Wingard, B. (1984) Acute Oral LD50 Study in Rats Using NG-84: Study No. 410-1844. Unpublished study prepared by Toxigenics, Inc. 26 p.
162946	Kreuger, J. (1984). Acute Dermal Toxicity Study in Rabbits Using NG-84at a Dose Level of 2 Grams per Kilogram of Body Weight: Study No. 410-1845. Unpublished study prepared by Toxigenics, Inc. 14p.
162947	Mellon, K. (1984). Primary Dermal Irritation Study in Rabbits Using NG-84: Study No. 410-1846. Unpublished study prepared by Toxigenics, Inc. 14 p.
162948	Doyle, G. (1984). Primary Eye Irritation Study in Rabbits Using NG-84: Study No. 410-1847. Unpublished study prepared by Toxigenics, Inc. 16 p.
40866801	Siglin, J. (1988). Delayed Contact Hypersensitivity Study in Guinea Pigs with Patox-Lite: Final Report: SLS Study No. 3191.8. Unpublished study prepared by Springborn Life Sciences, Inc. 24 p.
40866901	Siglin, J. (1988). Delayed Contact Hypersensitivity Study in Guinea Pigs with Adz-Pad (EPA): Final Report: SLS Study No. 3191.9. Unpublished study prepared by Springborn Life Sciences, Inc. 23 p.
40928201	Naas, D. (1988). Acute Oral Toxicity (LD50) Study in Albino Rats with Copper Naphthenate/Sodium Fluoride Grease: Final Report: Project No. WIL-127001. Unpublished study prepared by WIL Research Laboratories, Inc. 21 p.
40928202	Naas, D. (1988). Acute Dermal Toxicity (LD50) Study in Albino Rabbits with Copper Naphthenate/Sodium Fluoride Grease: Final Report: Project ID WIL-127002. Unpublished study prepared by WIL research Laboratories, Inc. 29p
40928203	Naas, D. (1988). Primary Dermal Irritation Study in Albino Rabbits with Copper Naphthenate/ Sodium Fluoride Grease: Final Report: Project IN WIL 127003. Unpublished study prepared by WIL Research Laboratories, Inc. 17 p.

40928204 Naas, D. (1988). Primary Irritation Study in Albino Rats with Copper Naphthenate/Sodium Fluoride Grease: Final Report: project ID WIL-127004. Unpublished study prepared by Bioassay Systems Corp. 19 p.

40932001 Goodband , J. (1982). Primary Eye Irritation Test Performed on Osmoplastic: Project No. 11005. Unpublished study prepared by Bioassay Laboratories, Inc. 21 p.

40932002 Goodband, J. (1982). Acute 14-Day Dermal Range Finding Determination Performed on Osmoplastic, Batch No. C059: Project No. 11005. Unpublished study prepared by Bioassay Systems Corp. 10p.

40932003 Goodband, J. (1982). Acute Oral LD_{50} Determination Performed on Osmoplastic: Project No. 11005. Unpublished study prepared by Bioaasay Systems Corp. 19p.

40932004 Goodband, J. (1982). Primary Dermal Irritation Test Performed on Osmoplastic: Project No. 11005. Unpublished study prepared by Bioassay Systems Cor. 12 p.

41204001 Naas, D. (1989). Primary Eye Irritation Study in Albino Rabbits with Patox II: Project ID WIL-127009. Unpublished

43778501 Wnorowski G. (1995). Acute Oral Toxicity Defined LD50 (in Rats): Composite NaF: Lab Project Number: 3719:P320. Unpublished study prepared by Product Safety Labs. 28 p.

43778502 Wnorowski G. (1995). Acute Dermal Toxicity Limit test (in Rats): Composite NaF: Lab Project Number: 3722:P322. Unpublished study prepared by Product Safety Labs. 15 p.

43778503 Wnorowski, G. (1995). Acute Inhalation Toxicity Defined LC50 (in Rats): Composite NaF: Lab project Number: 3724:P330. Unpublished study prepared by Product Safety Labs. 42p.

43778504 Wnorowski, G. (1995). Primary Eye Irritation (in Rabbits): Composite NaF: Lab project Number: 3720:P324. Unpublished study prepared by product Safety Labs. 26 p.

43778505 Wnorowski, G. (1995). Primary Skin Irritation (in Rabbits): Composite NaF: Lab Project Number: 3721: P326. Unpublished study prepared by Product Safety Labs. 16 p.

43778506 Wnorowski, G. (1995). Dermal Sensitization Test-Buehler Method (in Guinea Pigs): Composite NaF: Lab Project Number: 3723: P328. Unpublished study prepared by Product Safety Labs. 24 p.

Open Literature

Aardema MJ, et al. (1989). Sodium Fluoride-Induced Chromosome Aberrations in Different Stages of the Cell Cycle: A Proposed Mechanism. Mutation Research 223:191-203.

Albanese. (1987). Sodium Fluoride and Chromosome Damage (In Vitro Human Lymphocyte and In Vivo Micronucleus Assays). Mutagenesis 2:497-499.

Araibi et al. (1989). The Effect of High Fluoride on the Reproductive Performance of the Male Rat. J. Biol. Sc. Res. 20:19-20.

Bates et al. (1994). Final report on the developmental toxicity of sodium fluoride (Cas No. 7681-49-4) in Sprague-dawley rats. RTI, RTP NC, for NTP (PB95-110193).

Bohatyrewicz, A. (1999). Effects of Fluoride on Mechanical Properties of Femoral Bone in Growing Rats. Fluoride 32:47-54.

Caspary, W. et al. (1987). Mutagenic Activity of Fluorides in Mouse Lymphoma Cells. Mutation Res 187:165-180

Chinoy and Patel. (2001). Effects of Sodium Fluoride and Aluminum Chloride on Ovary and Uterus of Mice and Their Reversal by Some Antidotes. Fluoride 1:9-20.

Collins, T et al. (1995). Developmental Toxicity of Sodium Fluoride in Rats. Fd Chem Toxicol 33:951-960.

Collins, T et al. (2001). Developmental Toxicity of Sodium Fluoride Measured During Multiple Generations. Fd Chem Toxicol 39:867-876.

Collins, T et al. (2001). Multigenerational Evaluation of Sodium Fluoride in Rats. Food and Chemical Toxicology 39.6:601-13.

De Lopez O et al. (1976). Plasma Fluoride Concentrations in Rats Acutely Poisoned with Sodium Fluoride. Toxicology and Applied Pharmacology 37:75-83.

Elbetieha, A et al. (2000). Fertility Effects of Sodium Fluoride in Male Mice. Fluoride 33:128-134.

Essman et al. (1981). Histaminergic Mediation of the Response of Rat Skin to Topical Fluorides. Arch Dermatol Res 21:325-340

Gocke et al. (1981). Mutagenicity of Cosmetics Ingredients Licensed by the European Communities. Mutation Research 90.2:91-109.

Hall et al. (1977). Kinetic Model of Fluoride Metabolism in the Rabbit. Environmental Research 13:285-302.

Heindel, J. et al. (1996). Developmental Toxicity Evaluation of Sodium Fluoride Administered to Rats and Rabbits in Drinking Water. Fund Applied Toxicol 30:162-177.

Heindel, J. et al. (1996). Developmental Toxicity Evaluation of Sodium Fluoride Administered to Rats and Rabbits in Drinking Water. Fund Applied Toxicol 30:162-177.

Haworth et al. (1983). Salmonella Mutagenicity Test Results for 250 Chemicals. Env. Mutagenesis Supplement 1:3-142.

Khalil A, Da'Dara A. (1994). The Genotoxic and Cytotoxic Activities of Inorganic Fluoride in Cultured Rat Bone Marrow Cells. Arch Environ Contam Toxicol 26:60-63.

Khalil. (1995). Chromosome Aberrations in Cultured Rat Bone Marrow Cells Treated with Inorganic Fluorides. Mutation Research 343:67-74.

Li, Y., Dunipace, A., Stookey, G. (1987). Absence of Mutagenic and Antimutagenic Activities of Fluoride in Ames Salmonella Assays. Mutation Res 190:229-236.

Li, Dunipace, and Stookey. (1987). Effect of Fluoride on the Mouse Sperm Morphology Test. J. Dent. Res. 66:1509-1511.

Li Y, et al. (1987). Genotoxic Effects of Fluoride Evaluated by Sister-Chromatid Exchange. Mutation Res 192:191-201

Lim et al. (1978). LD50 of SnF_2, NaF, and Na_2PO_3 in the Mouse Compared to the Rat. Caries Res. 12:177-179.

Lim et al. (1978). LD50 of SnF_2, NaF, and Na_2PO_3 in the Mouse Compared to the Rat. Caries Res. 12:177-179.

Martin, G. et al. (1979). Lack of Cytogenic Effects in Mice or Mutations in Salmonella Receiving Sodium Fluoride. Mutation Res 66:159-167.

Maurer et al. (1990). Two-Year Carcinogenicity Study of Sodium Fluoride in Rats. J. Natl. Cancer Inst. 82:1118-1126

Messer et al. Influence of Fluoride Intake on Reproduction in Mice. J. Nutr. 103:1319-1326.

Mohamed and Chandler. (1982). Cytological Effects of Sodium Fluoride on Mice. Dept. of Biology and School of Medicine, University of Kansas City, Missouri. Presented at the 12th I.S.F.R. Conference.

Mullenix et al. (1995). Neurotoxicity of Sodium Fluoride in Rats. Neurotoxicology and Teratology 17:169-177.

Oberly et al. (1990). An Evaluation of the CHO/HGPRT Mutation Assay Involving Suspension Cultures and Soft Agar Cloning: Results for 33 Chemicals. Environmental and Molecular Mutagenesis 16:260-271.

Pati and Bhunya. (1987). Genotoxic effect of an environmental pollutant, sodium fluoride, in mammalian in vivo test system. Carylogia 40:79-87.

Paul, V. et al. (1998). Effects of Sodium Fluoride on Locomotor Behavior and a Few Biochemical Parameters in rats. Environmental Toxicol and Pharmacol 6:187-191.

Pillai et al. (1987). Acute Toxicity of Fluorife to Mice. Fluoride 20.2:68-70.

Pillai et al. (1988). Effect of Subacute Dosage of Fluoride on Male Mice. Toxicology Letters 44:21-29.

Ream et al. (1983). Bone Morphology of Weaning Rats from Dams Subjected to Fluoride. Cell Tissue Res 233:689-691.

Shahshi et al. (1994). Effect of Long-term Administration of Fluroide on Levels of Protein, Free Amino Acids and RNA in Rabbit Brain. Fluoride 27.3:155-159.

Shivarajashankara et al. (2002). Histological Changes in the Brain of Young Fluoride-Intoxicated Rats. Fluoride 35:12-21.

Skare J et al. (1986). Lack of DNA-Strand Breaks in rat Testicular Cells after In Vivo Treatment with Sodium Fluoride. Mutation Res 170:85-92.

Tong et al. (1988). The Lack of Genotoxicity of Sodium Fluoride in a Battery of Cellular Tests. Cell Biology and Toxicology 4.2:173-186.

Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, Technical Report Series No. 393, NIH Publication No. 91-2848, December 1990, Pgs. 1-477.

Tsutsui T, et al. (1984). Sodium Fluoride-Induced Morphological and Neoplastic Transformation, Chromosome Aberrations, Sister Chromatid Exchanges, and Unscheduled DNA Synthesis in Cultured Syrian Hamster Embryo Cells. Cancer Research 44.3:938-941

Tsutsui, T., N. Suzuki, et al. (1984). Cytotoxicity, Chromosome Aberrations and Unscheduled DNA Synthesis in Cultured Human Diploid Fibroblasts Induced by Sodium Fluoride. Mutation Research 139:193-198.

Trabelsi, M et al. (2001). Effect of Fluoride on Thyroid Function and Cerebellar Development in Mice. Fluoride 34: 165-173.

Turner et al. (1995). Fluoride Reduced Bone Strength in Older Rats. J. Dent Res. 74:1475-1481.

Varner, J.A. et al. (1998). Chronic Administration of Aluminum-Fluoride and Sodium Fluoride to Rats in Drinking Water: Alterations in Neuronal and Cerebrovascular Integrity. Brain Research 784:284-298.

Zeiger et al. (1994). Cytogenetic Studies of Sodium Fluoride in Mice. Mutagenesis 9:467-471.