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Developmental Toxicity Evaluation of Sodium Fluoride Administered to Rats and Rabbits in Drinking Water

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Sodium fluoride (NaF; Cas No. 7681-49-4) is used in fluoridating municipal water supplies, resulting in chronic exposure of millions of people worldwide. Because of a lack of pertinent developmental toxicity studies in the literature, sodium fluoride was administered *ad libitum* in deionized/filtered drinking water (to mimic human exposure) to Sprague–Dawley-derived rats (26/group) on Gestation Days (GD) 6 through 15 at levels of 0, 50, 150, or 300 ppm and New Zealand White rabbits (26/group) on GD 6 through 19 at levels of 0, 100, 200, or 400 ppm. Higher concentrations via drinking water were not practicable due to the poor palatability of sodium fluoride. Drinking water (vehicle) contained less than 0.6 ppm sodium fluoride (limit of detection) and sodium fluoride content of the feed was 12.4 ppm fluoride (rats) and 15.6 ppm fluoride (rabbits). Maternal food, water, body weights, and clinical signs were recorded at regular intervals throughout these studies. Animals were killed on GD 20 (rats) or 30 (rabbits) and examined for implant status, fetal weight, sex, and morphological development. In the high-dose group of both studies there was an initial decreased maternal body weight gain which recovered over time and a decreased water consumption—attributed to decreased palatability. No clear clinical signs of toxicity were observed. Maternal exposure to sodium fluoride during organogenesis did not significantly affect the frequency of postimplantation loss, mean fetal body weight/litter, or external, visceral or skeletal malformations in either the rat or the rabbit. The NOAEL for maternal toxicity was 150 ppm sodium fluoride in drinking water (~18 mg/kg/day) for rats, and 200 ppm (~18 mg/kg/day) for rabbits. The NOAEL for developmental toxicity was ≥300 ppm sodium fluoride (~27 mg/kg/day) for rats and ≥400 ppm (~29 mg/kg/day) for rabbits administered during organogenesis in drinking water. The total exposure to fluoride (mg F/kg body weight/day from food and drinking water combined) in the mid- and high-dose groups for both species was >100-fold higher than the range at 0.014–0.08 mg F/kg/day estimated for a 70-kg person from food and fluoridated (1 ppm) drinking water.

Since the 1800s, fluoride has been suspected to reduce dental caries (tooth decay) (Sognnaes, 1979). Research during the first half of the 20th century led to the first water fluoridation trials with sodium fluoride initiated in 1945 (Sognnaes, 1979). In the late 1970s it was estimated that over 200 million people worldwide (over 106 million in the United States) lived in areas where water supplies were fluoridated with hydrofluosilicic acid, sodium silicofluoride, or sodium fluoride. An additional 100 million people lived in areas that had nearly equivalent or higher natural fluoride concentrations in their drinking water (Sognnaes, 1979; Murray, 1986). Hydrofluosilicic acid is the chemical of choice for fluoridating large municipal water supplies because it does not present an occupational hazard from dust exposure and it is cheaper than sodium fluoride (Clayton and Clayton, 1981). Nevertheless, many smaller municipalities still use sodium fluoride to fluoridate their water supply because of the simplicity of the mixing equipment (Murray, 1986). In addition to its use in fluoridating water supplies, sodium fluoride is used as a pesticide for roaches and ants, as a component in glass and vitreous enamel processing, in frosting glass, in removing HF from exhaust gases to reduce pollution, as a wood preservative, as a steel degassing agent, and in electroplating (Budavari, 1989).

Animal studies of the potential effects of sodium fluoride on reproduction and development are limited in number. Abnormal spermatozoa were produced by male Swiss mice administered 8 mg/kg of fluoride by intraperitoneal injection for five consecutive days (Pati and Bhunya, 1987). On the other hand, two reports of oral administration of sodium fluoride in mice showed no effects on spermatogenesis. In the first study, B6C3F$_1$ mice were given 70 mg/kg sodium fluoride in an oral dose for five consecutive days with no effect on spermatogenesis (Li *et al.*, 1987). In the second study, B6C3F$_1$ mice were maintained on 75 ppm of sodium fluoride in drinking water for 21 weeks with no effects on

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spermatogenesis (Dunipace *et al.*, 1989). In contrast, another drinking water study conducted in mice reported that exposure to 500 or 1000 ppm of sodium fluoride for up to 3 months resulted in clear damage to spermatogenesis (reported in DHHS, 1991).

Sodium fluoride has been tested in fertility studies in several species. A 25-week two-generation fertility study was conducted in Swiss Webster mice at concentration levels of 50, 100, and 200 ppm of sodium fluoride in drinking water. Lethality occurred at the 200 ppm level, maternal toxicity was evident at 100 ppm, and 50 ppm was a no observable adverse effect level (NOAEL) (Messer *et al.*, 1973). The control animals were fed a low fluoride diet and exhibited an apparent decrease in fecundity compared to their fluoride-treated cohorts. A follow-up study by Tao and Suttie (1976) confirmed this effect and attributed it to low iron levels in the low fluoride diet. No effects were noted on reproductive performance in male and female mink maintained on diets containing from 33 to 350 ppm of sodium fluoride (Aulerich *et al.*, 1987). Hoffman *et al.* (1985) conducted a study on the reproductive effects of sodium fluoride in screech owls. The birds were fed 0, 40, or 200 ppm sodium fluoride and allowed to breed. Eggs and hatchlings were examined for effects. Egg volume was decreased at 40 and 200 ppm. At 200 ppm egg weight and length were also reduced and hatchling weight was decreased approximately 10% from control, but no gross abnormalities were observed.

In response to the finding of reproductive toxicity in multiple animal models following exposure to excess fluoride, a large-scale epidemiology study involving 1,513,022 women (ages 10–49 years) was conducted (Freni, 1994). Data were collected from index counties (i.e., counties having at least one drinking water source of >3 ppm) and compared to adjacent counties with water no fluoride water source >3 ppm. Based upon an analysis of population means, the total fertility rates were negatively associated with measures of fluoride exposure; that is, the higher the fluoride exposure, the lower the birth rate (Freni, 1994). In contrast, a retrospective study showed no correlation between sodium fluoride consumption and the prevalence of birth defects in Atlanta, Georgia or the prevalence of birth defects found in the National Cleft Lip and Palate Intelligence Database (DHHS, 1991).

Because of the lack of published studies designed to assess the developmental toxicity potential of sodium fluoride, the National Toxicology Program designed and performed developmental toxicity studies of sodium fluoride in rats and rabbits.

METHODS[3]

Chemical. Sodium fluoride (CAS No. 7681-49-4), obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI) was determined to be greater than 99% pure by elemental analysis and ion chromatography. Specific identities of the impurities were not determined.

Animals and husbandry. Cesarean-originated, barrier-sustained Crl:CD (BR) VAF/Plus outbred albino rats (CD rats)[4] or New Zealand White (NZW) SPF rabbits[5] were used in these studies. Rats were individually identified by tattoo; rabbits were eartagged.

After a 7-day quarantine period, individual breeding pairs of rats were cohabited overnight. The morning on which sperm were found in a vaginal lavage was designated as Gestational Day (GD) 0. The study was designed such that 26 rats per group were used for the developmental toxicity study and an additional 10 rats per group were used for blood collection. Animals were assigned to exposure groups by stratified randomization so that mean GD 0 body weights did not differ significantly among exposure groups. Individual maternal body weights for the animals used in the developmental toxicity study ranged from 221 to 274 g on GD 0 while mean body weights/group ranged from 248 to 253 g. The study was performed in two replicates with three consecutive breeding dates in the first replicate and five consecutive breeding dates in the second replicate. The last breeding date for the first replicate and the first breeding date of the second replicate were 47 days apart. Sperm-positive females were individually housed in solid-bottom polycarbonate cages with stainless-steel wire lids[6] and Ab-Sorb-Dri cage litter.[7] NIH-07 Rodent Chow[8] and either deionized/filtered water or the prepared sodium fluoride-spiked drinking water were available *ad libitum* throughout gestation.

Rabbits were approximately 5–5.5 months of age (females) and 6 months of age (males) on receipt. Females were in quarantine for approximately 2 weeks and were artificially inseminated approximately 3–6 weeks after arrival. The females received an intravenous injection of chorionic gonadotropin[9] (0.1 ml/kg). Semen was collected from the untreated males using an artificial vagina (Bredderman *et al.*, 1964) in conjunction with a teaser female. Prior to insemination, ejaculate collected from the males was evaluated for number of motile sperm. Females were inseminated between 1:00 and 5:00 PM with undiluted ejaculate on a day designated as GD O. The study was performed in two replicates with 34 days between replicates and 26 animals per group. Inseminated females were assigned to exposure groups by stratified randomization so that mean GD 0 body weights did not differ significantly among exposure groups. Individual maternal body weights ranged from 2770 to 4410 g on the day of insemination (GD 0) while mean body weights/group ranged from 3359 to 3442 g. Inseminated females were individually housed in stainless steel cages with mesh flooring.[10] Purina Certified Rabbit chow[11] and either deionized/filtered water or sodium fluoride-spiked drinking water were available *ad libitum* throughout gestation.

Environmental conditions (12:12 hr light: dark cycle; average temperature and average relative humidity 67°F and 52% for rabbit study and 72°F and 55% for rat study) were monitored and controlled by computer.[12]

Treatment. Timed-mated rats were allowed *ad libitum* access to deionized/filtered drinking water containing 0, 50, 150, or 300 ppm sodium fluoride from GD 6 through 15 (i.e., treated water was removed on the morning of GD 16). Preexposure analysis of drinking water solutions dem-

[3] The developmental toxicity studies reported here (except for the serum fluoride levels) were conducted in accordance with Food and Drug Administration Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies (FDA, 1988).

[4] Charles River Laboratories, Inc., Raleigh, NC.

[5] Hazelton Research Products Inc., Denver, PA.

[6] Laboratory Products, Rochelle Park, NJ.

[7] Laboratory Products, Garfield, NJ.

[8] Zeigler Brothers, Gardners, PA.

[9] Pregnyl, Organon, Inc., West Orange, NJ.

[10] Hoeltge, Inc., Cincinnati, OH.

[11] Ralston Purina Co., St. Louis, MO.

[12] Barber-Coleman Network Supervisor System, Diversified Environmental Control, Inc., Greensboro, NC.

onstrated the concentrations of sodium fluoride to be within a range of 104–107% of target concentrations. The drinking water provided to the control animals did not contain sodium fluoride above the limit of detection (0.6 ppm). Analysis of animal feed determined fluoride concentrations to be from 11.6 to 13.4 ppm with an average of 12.4 ppm (equivalent to 22.0 ppm sodium fluoride).

Artificially inseminated rabbits were allowed *ad libitum* access to drinking water containing 0, 100, 200, or 400 ppm sodium fluoride from GD 6 through 19 (i.e., treated water was removed on the morning of GD 20). Preexposure analysis of drinking water solutions demonstrated the concentrations of sodium fluoride to be within a range of 98–107% of target concentrations. In the drinking water provided to the control animals, fluoride was not found above the limit of detection, 0.6 ppm. Fluoride concentration in the feed ranged from 14.6 to 16.6 ppm of fluoride with an average of 15.6 ppm (equivalent to 27.7 ppm sodium fluoride).

Treatment and examination of animals in both studies was performed without knowledge of exposure levels.

Evaluations—Development toxicity study. Sperm-positive female rats were weighed, and food and water weights were recorded on the mornings of GD 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20. Animals were observed daily beginning on GD 6 for clinical signs of toxicity. All sperm-positive rats were killed on GD 20 by asphyxiation with CO2 followed by cervical dislocation. Ten sperm-positive females per dose level were added to the second replicate of this study. On GD 16, these 10 rats per dose level were killed by asphyxiation with CO_2, their pregnancy status was recorded, and 3–4 ml of blood was drawn into a collection tube[13] from the abdominal vena cava for measurement of serum fluoride levels.

Inseminated rabbits were weighed, and food and water weights were recorded on the mornings of GD 0 and every 2 days thereafter through GD 30. Animals were observed twice daily beginning on GD 6 for clinical signs of toxicity. On GD 20, 5 animals per group per replicate were randomly selected for determination of serum fluoride levels. Blood was drawn into a collection tube (see footnote 13) from an ear artery (3–4 ml) and the rabbits were returned to the study. All inseminated rabbits were killed on GD 30 by an iv lethal injection of sodium pentobarbital.

The maternal body, liver, right kidney, and intact uterus of the rats and rabbits were weighed and corpora lutea were counted. Uteri which had no visible implantation sites were stained with ammonium sulfide (10%) to detect very early resorptions (Salewski, 1964). Live fetuses were dissected from the uterus and anesthetized on ice (rats) or administered a lethal injection of sodium pentobarbital (rabbits). They were weighed, examined for external morphological abnormalities, and dissected for visceral examination by a fresh tissue dissection technique (Staples, 1974; Stuckhardt and Poppe, 1984). Half of the fetuses were decapitated prior to dissection; the heads were fixed in Bouin's solution and then examined by a free-hand sectioning technique (Wilson, 1965). All fetal carcasses (one-half without heads) were stained with Alcian blue/alizarin red S and examined for skeletal malformations (Marr *et al.*, 1988).

Analysis of serum fluoride. Serum fluoride analysis was performed as previously described (Bawden *et al.*, 1989).

Statistics. General linear models (GLM) procedures were applied for the analyses of variance (ANOVA) of maternal and fetal parameters (SAS Institute, 1989a,b, 1990a,b,c). Prior to GLM–ANOVA analysis, an arcsine–square root transformation was performed on all litter-derived percentage data to normalize the means (Snedecor and Cochran, 1967) and Bartlett's test for homogeneity of variance was performed on all data to be analyzed by ANOVA (Winer, 1962). GLM–ANOVA analysis determined the significance of dose–response relationships and the significance of dose effects, replicate effects, and dose × replicate interactions When ANOVA revealed a significant ($p < 0.05$) dose effect, Dunnett's test (Dunnett, 1955, 1964) and Williams' test (Williams, 1971, 1972) were used to compare treated to control groups. One-tailed tests were used for all pairwise comparisons except maternal body and organ weights, food and water consumption, fetal body weight, and percentage of males per litter. If a significant ($p < 0.05$) dose × replicate interaction occurred, then the data for that endpoint were analyzed separately for dose effects within each replicate in the study, as well as for all replicates combined. Nominal scale measures were analyzed by a χ^2 test for independence and by a test for linear trend on proportions (Snedecor and Cochran, 1967).

When a χ^2 test showed significant experimentwise differences, a one-tailed Fisher's exact test was used for pairwise comparisons of treatment and control groups.

Justification of exposure levels. The sodium fluoride concentrations tested in the rat study were 0, 50, 150, and 300 ppm. This corresponds to approximately 7.5, 22.5, and 45 mg/kg/day based on an estimated water consumption of 150 ml/kg body weight/day. The LD50 for sodium fluoride in rats is at least 52 mg/kg/day or approximately 350 ppm if administered in drinking water (RTECS, 1991). The concentrations were chosen after consideration of the data from the NTP 14-day study since data relative to a 10-day exposure regimen were lacking in other published studies (NTP, 1990). In that study, female rats given 400 ppm sodium fluoride in drinking water showed decreased body weight and water consumption and clinical signs of toxicity (dehydration, lethargy, and hunched posture) while 800 ppm killed all the animals. The high dose in this study (300 ppm) was chosen in an effort to induce some maternal toxicity while avoiding the potentially confounding effects of dehydration seen at the 400 ppm level in the 14-day study. The 300 ppm level was anticipated to be a nonlethal exposure based on the lack of lethality among animals exposed to 300 ppm sodium fluoride in the NTP 6-month study. The low exposure of 50 ppm was expected to produce no maternal toxicity based on the lack of effects related to sodium fluoride consumption in the NTP 2-year study at exposures up to 100 ppm.

Selection of the high concentration for rabbits was based, in part, on a limited palatability study in which nonpregnant rabbits (3/group) were given 0, 50, 150, or 300 ppm sodium fluoride in drinking water for 14 days (NTP, unpublished data). Sodium fluoride at 300 ppm caused no specific indications of toxicity, but the overall pattern of water consumption suggested a mild taste aversion. Therefore a somewhat higher concentration of sodium fluoride (400 ppm) was selected as the highest concentration for the developmental toxicity study, and the mid and low concentrations were set at $\frac{1}{2}$ and $\frac{1}{4}$ of the high concentration, respectively Excessive maternal toxicity was not anticipated at this level of intake because the anticipated daily dose (25 mg/kg/day) was only 1/8 of the reported oral LD50 for sodium fluoride in rabbits (e.g. 200 mg/kg according to RTECS, 1991).

RESULTS

Rats

Maternal effects. No animals died during the course of this study (Table 1). No treatment-related clinical signs were observed in confirmed-pregnant animals during or after administration of sodium fluoride. Maternal body weight gain during the first 2 days of exposure (GD 6 to 8) was significantly reduced at 300 ppm relative to controls (14.3 ± 0.9 g for control vs 6.3 ± 2.1 g for treated). The mean maternal body weight gain for the treatment period as a whole exhibited a decreasing trend, and the comparison among groups for an effect of dose approached statistical significance ($p = 0.0671$, main effect of dose by ANOVA). There was no indication of an effect on posttreatment weight gain (data

[13] Vacutainer SST tubes, Becton–Dickinson Vacutainer Systems U.S.A., Rutherford, NJ.

TABLE 1
Maternal Toxicity in CD Rats Given Sodium Fluoride in Drinking Water on Gestational Days 6 through 15

	Sodium fluoride (ppm, in water)			
	0	50	150	300
Maternal pregnancy status				
No. treated	26	26	26	26
No. removed	0	0	0	0
No. dead or euthanized	0	0	0	0
No. (%) pregnant at euthanization	26 (100)	25 (96)	23 (89)	25 (96)
Maternal body weight changes (g)[a,b]				
Gestation wt gain (GD 0–20)	166.2 ± 3.9	156.4 ± 5.1	163.6 ± 4.2	159.0 ± 5.2
Treatment wt gain (GD 6–16)§	72.3 ± 2.1	68.7 ± 2.4	69.2 ± 1.6	63.6 ± 2.6
Corrected wt gain[c]	75.3 ± 2.8	74.4 ± 3.3	71.1 ± 2.7	72.6 ± 2.7
Gravid uterine wt	90.9 ± 2.5	81.9 ± 4.9	92.5 ± 3.3	86.4 ± 4.3
Maternal organ weights[a]				
Liver (g)	18.22 ± 0.42	17.89 ± 0.39	17.82 ± 0.34	18.70 ± 0.48
Right kidney (g)	1.27 ± 0.03	1.23 ± 0.04	1.26 ± 0.04	1.31 ± 0.04
Maternal food consumption[a,d]				
Treatment period (GD 6–16)				
Absolute (g/day)	26.0 ± 0.5	25.1 ± 0.5	25.7 ± 0.5	24.6 ± 0.4
Relative (g/kg/day)§	80.3 ± 0.9	79.2 ± 1.0	80.0 ± 0.8	77.1 ± 0.9
Posttreatment period (GD 16–20)				
Absolute (g/day)	27.5 ± 0.5	26.9 ± 0.5	27.1 ± 0.3	27.7 ± 0.5
Relative (g/kg/day)	70.3 ± 0.7	70.6 ± 0.9	70.0 ± 0.7	71.7 ± 0.9
Maternal water consumption[a,d]				
Treatment period (GD 6–16)				
Absolute (g/day)§	41.4 ± 1.0	41.9 ± 1.3	39.0 ± 1.4	28.8 ± 0.8*
Relative (g/kg/day)§	128.1 ± 2.5	132.1 ± 3.9	122.3 ± 4.1	90.3 ± 2.1*
Posttreatment period (GD 16–20)				
Absolute (g/day)	48.8 ± 1.1	52.4 ± 1.7	47.9 ± 1.6	46.9 ± 1.6
Relative (g/kg/day)	124.7 ± 2.6	137.4 ± 4.3*	123.5 ± 4.0	121.2 ± 3.2
Average sodium fluoride intake (mg NaF/kg/day)				
From drinking water (GD 6 to 16)	—	6.6 ± 0.2	18.3 ± 0.6	27.1 ± 0.6
% Fluoride from water (GD 6 to 16)	≥20%	75.4%	89.2%	92.7%
% Fluoride from feed (GD 6 to 16)	80–100%	24.6%	10.8%	7.3%
Average fluoride intake (mg F/kg/day)				
From water (GD 6 to 16)	—	2.99 ± 0.09	8.30 ± 0.28	12.26 ± 0.28
From feed (GD 6 to 16)	1.00 ± 0.01	0.98 ± 0.01	0.99 ± 0.01	0.96 ± 0.01
Total (GD 6 to 16)	1.00 ± 0.01	3.97 ± 0.09	9.29 ± 0.28	13.21 ± 0.29

[a] NaF-treated drinking water was removed on the morning of GD 16; includes all dams pregnant at euthanization; mean ± SEM; GD, gestational day.
[b] Body weights were recorded in the morning of each designated gestational day.
[c] Weight change during gestation minus gravid uterine weight.
[d] Body weights were recorded in the morning of each designated gestational day. Relative food and water consumption (g/kg/day) was calculated using these weights.
§ $p < 0.05$, linear trend test.
* $p < 0.05$, Dunnett's test.

not shown). Maternal water consumption of the animals given 300 ppm sodium fluoride was significantly decreased for each period of observation from GD 6 to 16 (Fig. 1). This was also reflected in the absolute and relative water consumptions during the administration period as a whole (GD 6–16) which were significantly decreased in the animals at 300 ppm sodium fluoride (Table 1). Maternal food consumption was also decreased in the animals given 300 ppm sodium fluoride from GD 8 to 10 (Fig. 2), but did not differ from controls for any other period of measurement. Analysis of total fluoride consumption indicated that an average of 24.6, 10.8, and 7.3% of the total fluoride intake came from the feed for the animals in the low- to high-dose group, respectively (Table 1). Average fluoride intake from food and water was 1.00, 3.97, 9.29, and 13.21 mg F/kg/day for control through high-dose groups.

Maternal liver and kidney weights on GD 20 were not different from control (Table 1). Examination of uteri dem-

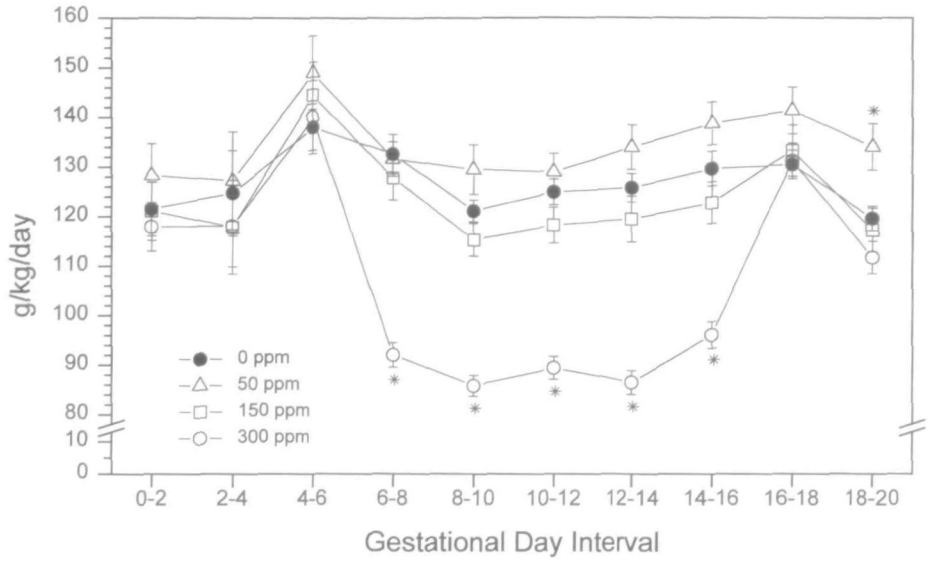

FIG. 1. Maternal relative water consumption of rats given sodium fluoride in drinking water on GD 6 through 15. Data are presented as means ± SEM for each measurement period. *$p < 0.05$, pairwise comparison to the concurrent control group.

onstrated that 100% (26/26), 96% (25/26), 89% (23/26), and 96% (25/26) of the mated animals in the control through high-dose groups were pregnant.

Embryo–fetal effects. All of the pregnant animals had one or more live fetuses on GD 20. There were no significant differences between the sodium fluoride groups and the control group in the average number of corpora lutea, implantations, live fetuses, or in the percentage of early deaths (resorptions), or late fetal deaths per litter (Table 2). The percentages of pre- and postimplantation losses per litter and mean pup weights were not significantly different from control values (Table 2). Statistical examination of the prevalence of morphological abnormalities from the fetuses in this study showed no significant effects for pairwise comparisons of sodium fluoride-treated groups with the control group (Tables 2 and 3). Although there were no significant pairwise effects from sodium fluoride administration, the percentage of litters with one or more externally malformed fetuses, the percentage of externally mal-

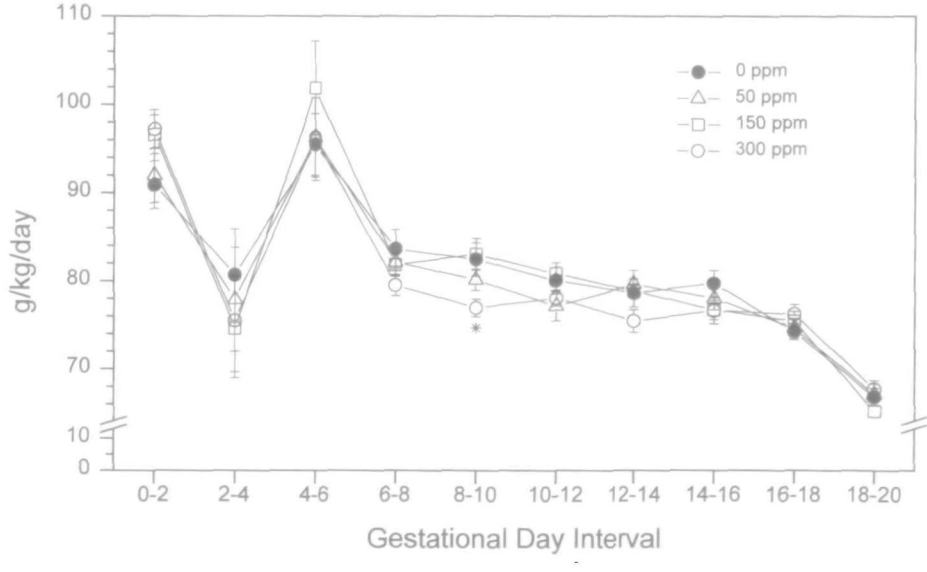

FIG. 2. Maternal relative food consumption of rats given sodium fluoride in drinking water GD 6 through 15. Data are presented as means ± SEM for each measurement period. *$p < 0.05$, pairwise comparison to the concurrent control group.

TABLE 2
Developmental Toxicity in CD Rat Fetuses Following Maternal Inbibition of Sodium Fluoride on Gestational Days 6 through 15

	Sodium fluoride (ppm, in water)			
	0	50	150	300
All litters[a,b]	26	25	23	25
No. corpora lutea/dam	16.8 ± 0.5	15.8 ± 0.4	16.3 ± 0.3	16.0 ± 0.6
No. implantation sites/litter	16.2 ± 0.5	14.1 ± 0 9	15.9 ± 0.3	15.3 ± 0.8
% Preimplantation loss/litter	4.3 ± 1.6	8.1 ± 3.1	2.7 ± 0.8	8.4 ± 3.4
% Resorptions/litter	2.3 ± 0.7	1.8 ± 0.7	1.1 ± 0.5	2.7 ± 0.9
% Litters with resorptions	9	6	4	8
% Late deaths/litter[c]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
% Litters with late deaths[c]	0	0	0	0
Live litters[b,d]	26	25	23	25
No. live fetuses/litter	15.8 ± 0.5	13.9 ± 0.9	15.7 ± 0.3	14.9 ± 0.8
Avg male fetal body wt/litter (g)	3.8 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.1
Avg female fetal body wt/litter (g)	3.6 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.5 ± 0.1
% Male fetuses/litter	50.4 ± 2.2	52.4 ± 3.2	50.6 ± 1.9	54.0 ± 3.2
% Externally malformed fetuses/litter[e]§	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.8 ± 0.6
% Viscerally malformed fetuses/litter[e]	3.7 ± 1.9	8.4 ± 4.1	3.3 ± 2.1	5.7 ± 1.6
% Skeletally malformed fetuses/litter[e]§	0.0 ± 0.0	0.5 ± 0.5	1.5 ± 0.8	1.4 ± 0.7
% Malformed fetuses/litter[e]	3.7 ± 1.9	8.9 ± 4.1	4.9 ± 2.1	6.9 ± 1 9

[a] Includes all dams pregnant at euthanization; litter size, no. implantation sites per dam.
[b] Reported as the mean ± SEM
[c] There were no late fetal deaths in any dose group, therefore statistical analyses for this parameter were not conducted
[d] Includes only dams with live fetuses; litter size equals number of live fetuses per dam.
[e] Fetuses with one or more malformations.
§ $p < 0.05$, linear trend test.

formed fetuses per litter and the percentage of skeletally malformed fetuses per litter, exhibited increasing trends with increasing dose of sodium fluoride (Table 2 and 3). No significant effects were noted in the prevalence of variations in the examined fetuses (Table 3).

Serum fluoride levels. Fluoride levels in maternal serum samples (10/group) collected on GD 16 were (mean ± SD) 0.007 ± 0.002, 0.035 ± 0.040, 0.039 ± 0.039, and 0.187 ± 0.076 ppm fluoride for the control through high-dose groups, respectively.

Rabbits

Maternal effects. No animals died during the course of this study (Table 4), and no clinical signs of fluoride toxicity occurred in the treated animals. Maternal water intake of the animals given 400 ppm sodium fluoride was significantly decreased for each period of observation from GD 6 to 20 (Fig. 3). This was also reflected in the absolute and relative water consumption during exposure (GD 6–20) which were significantly decreased in the animals exposed to 400 ppm sodium fluoride (Table 4). Postdosing water consumption was normal in these animals indicating the probability of decreased palatability of the 400 ppm solution. Maternal relative food consumption (g/kg/day) was decreased in the animals given 400 ppm sodium fluoride from GD 6 to 8 and increased in the animals given 100 ppm sodium fluoride from GD 12 to 14 (Fig. 4). Overall, the maternal relative food consumption was decreased at 400 ppm sodium fluoride during the treatment period (Table 4). No significant reduction of body weight occurred during administration of sodium fluoride (Table 4). However, maternal body weight change for the animals receiving 400 ppm sodium fluoride was significantly lower than controls for the period from GD 6 to 8 (average weight gain of 14 g for controls vs average weight loss of 112 g at 400 ppm), probably as a result of decreased food and water consumption. Maternal body weight change was significantly increased from GD 10 to 12 (average weight gain of 22 g for controls vs 71 g at 400 ppm). Maternal body weight change during treatment (GD 6–20) and maternal gestational weight change corrected for gravid uterine weight were unaffected by administration of sodium fluoride (Table 4). Analysis of total fluoride consumption in drinking water and feed (g/animal/day) indicated that an average of 15.6, 8.6, and 4.9% of the total fluoride intake came from the feed for the animals in the low- to high-dose groups, respectively (Table 4). Average fluoride intake from food and water was 0.78, 5.75, 8.79, and 13.72 mg F/kg/day, for control through high-dose groups.

Maternal liver and kidney weights on GD 30 were not different from control (Table 4). Examination of uteri dem-

TABLE 3
Morphological Abnormalities Observed in CD Rat Fetuses Following Maternal Inbibition of Sodium Fluoride on Gestational Days 6 through 15: Listing by Defect Type[a]

	Sodium fluoride (ppm, in water)			
	0	50	150	300
No. fetuses examined[b]	410	347	362	373
No. litters examined[c]	26	25	23	25
All malformations				
No. fetuses with any malformations[d]	13	19	17	26
% Fetuses with any malformations	3.2%	5.5%	4.7%	7 0%
No. litters with any malformations[e]	6	10	9	12
% Litters with any malformations	23 1%	40.0%	39.1%	48.0%
External malformations				
No. fetuses with external malformations[d]	0	0	1	3
% Fetuses with external malformations	0.0%	0.0%	0.3%	0 8%
No. litters with external malformations[e]	0	0	1	2
% Litters with external malformations†	0 0%	0.0%	4.3%	8.0%
Craniorachischisis				2 (2)
Anophthalmia. Bilateral				1 (1)
Microphthalmia: Bilateral				1 (1)
Ectocardia				1 (1)
Anasarca				1 (1)
Gastroschisis			1 (1)	1 (1)
Skeletal malformations				
No. fetuses with skeletal malformations[d]	0	1	6	5
% Fetuses with skeletal malformations	0.0%	0.3%	1.7%	1.3%
No. litters with skeletal malformations[e]	0	1	4	4
% Litters with skeletal malformations	0.0%	4.0%	17.4%	16.0%
Cleft sternum			1 (1)	
Fused ribs and fused rib cartilage				1 (1)
Bipartite cartilage, bipartite ossification center				
Thoracic centrum				5 (4)
Bipartite cartilage, dumbbell ossification center		1 (1)	1 (1)	
Thoracic centrum			4 (3)	
Visceral malformations				
No. fetuses with visceral malformations[d]	13	18	11	22
% Fetuses with visceral malformations	3.2%	5.2%	3.0%	5.9%
No. litters with visceral malformations[e]	6	9	5	12
% Litters with visceral malformations	23.1%	36.0%	21.7%	48.0%
Enlarged lateral ventricle	13 (5)	15 (7)	11 (5)	19 (10)
Renal agenesis: Right				1 (1)
Small kidney				1 (1)[f]
Grossly displaced kidney				1 (1)[f]
Hydronephrosis: Right			1 (1)	1 (1)
Hydroureter				
Bilateral		1 (1)		
Left		1 (1)		1 (1)
Right		1 (1)	1 (1)	
All variations				
No. fetuses with any variations[d]	59	45	43	40
% Fetuses with any variations	14 4%	13.0%	11.9%	10.7%
No. litters with any variations[e]	17	18	17	19
% Litters with any variations	65.4%	72.0%	73.9%	76.0%

onstrated that 84% (21/25), 87% (20/23), 78% (18/23), and 83% (20/24) of the mated animals in the control through high-dose groups were pregnant at euthanization (Table 4).

Embryo–fetal effects. There were no significant differences between the sodium fluoride-exposed groups and the control in the average number of corpora lutea, implants, or live fetuses, or in the percentage of early deaths (resorptions), or late fetal deaths per litter (Table 5). The percentages pre- and postimplantation losses (early and late deaths) per litter were not significantly different from

TABLE 3—*Continued*

	Sodium fluoride (ppm, in water)			
	0	50	150	300
External variations				
No. fetuses with external variations[d]	1	0	0	3
% Fetuses with external variations	0.2%	0.0%	0.0%	0.8%
No. litters with external variations[e]	1	0	0	3
% Litters with external variations	3.8%	0.0%	0.0%	12.0%
Blood in amniotic sac				1 (1)
Clubbed limb without bone change				2 (2)
Hematoma: Head	1 (1)			
Skeletal variations				
No. fetuses with skeletal variations[d]	51	36	40	30
% Fetuses with skeletal variations	12.4%	10.4%	11.0%	8.0%
No. litters with skeletal variations[e]	16	16	16	15
% Litters with skeletal variations	61.5%	64.0%	69.6%	60.0%
Rib on lumbar I: Bilateral full			1 (1)	
Left full			2 (2)	
Bilateral rudimentary	27 (9)	6 (5)	8 (5)	9 (8)
Left rudimentary	10 (9)	9 (8)	19 (10)	9 (4)
Right rudimentary	5 (4)	3 (2)	5 (3)	3 (2)
Dumbbell cartilage, dumbbell ossification center	1 (1)		4 (2)	3 (3)
Thoracic centrum				
Normal cartilage, bipartite ossification center	2 (1)	14 (7)	3 (3)	4 (3)
Lumbar centrum	8 (4)	18 (10)	3 (3)	6 (5)
Thoracic centrum				
Visceral variations				
No. fetuses with visceral variations[d]	10	9	3	9
% Fetuses with visceral variations	2.4%	2.6%	0.8%	2.4%
No. litters with visceral variations[e]	7	6	3	8
% Litters with visceral variations	26.9%	24.0%	13.0%	32.0%
Distended ureter: Bilateral	3 (3)	3 (3)	2 (2)	4 (3)
Left	5 (4)	4 (4)	1 (1)	1 (1)
Right	2 (2)	2 (2)		3 (3)
Small renal papilla: Left		1 (1)		
Right				1 (1)
Displaced testis: Left				1 (1)[f]

Note. Parentheses indicate number of litters with affected fetuses.

[a] The incidence of individual defects is expressed as the number of individual fetuses exhibiting that defect. Thus, a single fetus may be represented more than once in listing individual defects. Approximately 50% of the fetuses were decapitated prior to skeletal staining; heads were fixed in Bouin's solution and examined using a free-hand section method. Number of litters is indicated in parentheses.

[b] Only live fetuses were examined.

[c] Includes only litters with live fetuses.

[d] Fetuses with one or more malformations or variations, as applicable.

[e] Litters with one or more malformed or variant fetuses.

[f] These defects occurred in the same fetus. The left kidney was small and lower than normal; the left kidney was also rotated so that the renal pelvis faced ventrally. The left testis was higher than normal and was attached to the left kidney.

† $p < 0.05$, test for linear trend on proportions.

control values (Table 5). Evaluation of male fetal body weight revealed no effects among the sodium fluoride groups; a decreasing trend was noted for female fetal body weight, but the groupwise comparison (ANOVA) for treatment effect was not significant (Table 5). Statistical examination of the prevalence of morphological abnormalities from the fetuses in this study showed no significant effects for pairwise comparisons of sodium fluoride-treated groups with the control group (Tables 5 and 6). No significant effects were noted in the prevalence of variations in the examined fetuses (Tables 5 and 6).

Serum fluoride levels. Serum fluoride levels reflected the increased fluoride intake of the animals given sodium fluoride (i.e. 0.06 ± 0.04, 0.24 ± 0.10, 0.39 ± 0.14, and 0.70 ± 0.33 ppm, mean ± SD, from the control to high-dose groups, respectively).

TABLE 4
Maternal Toxicity in NZW Rabbits Given Sodium Fluoride in Drinking Water on Gestational Days 6 through 19

	Sodium fluoride (ppm in water)			
	0	100	200	400
Maternal pregnancy status				
No. treated	26	26	26	26
No. removed	1[a]	3[b]	3[c]	2[d]
No. dead or euthanized	0	0	0	0
No. (%) pregnant at euthanization	21 (84.00)	20 (86.96)	18 (78.26)	20 (83.33)
Maternal body weight changes (g)[e,f]				
Gestation wt gain (GD 0 to 30)	610 ± 47	685 ± 58	690 ± 51	590 ± 47
Treatment wt gain (GD 6 to 20)	253 ± 22	297 ± 30	265 ± 25	222 ± 26
Corrected wt gain[g]	175 ± 68	241 ± 72	191 ± 53	125 ± 57
Gravid uterine wt	435 ± 50	443 ± 38	499 ± 41	464 ± 31
Maternal organ weights[e]				
Liver (g)	113.0 ± 6.3	111.9 ± 6.4	108.1 ± 4.3	105.4 ± 4.0
Right kidney (g)	9.2 ± 0.3	9.0 ± 0.3	9.1 ± 0.3	8 5 ± 0.3
Maternal food consumption[e,h]				
Treatment period (GD 6 to 20)				
Absolute (g/day)§	186.6 ± 7.3	208.9 ± 10.0	189.0 ± 10.6	161.5 ± 7.8
Relative (g/kg/day)§	49.7 ± 1.6	54.1 ± 1.9	51.5 ± 2.9	43.2 ± 2.2*
Posttreatment period (GD 20 to 30)				
Absolute (g/day)	150.4 ± 9.1	173.5 ± 11.6	141.3 ± 8.0	146.5 ± 10.5
Relative (g/kg/day)	38.0 ± 2.2	41.9 ± 2.7	36.8 ± 2.7	37.4 ± 2.6
Maternal water consumption[e,h]				
Treatment period (GD 6 to 20)				
Absolute (g/day)§	384.7 ± 19.4	389.7 ± 17.7	347.1 ± 13.2	271 0 ± 13.1*
Relative (g/kg/day)§	101.7 ± 5.3	103.2 ± 4.3	90.6 ± 2.5	73.0 ± 2.8*
Posttreatment period (GD 20 to 30)				
Absolute (g/day)	331.3 ± 19.3	367.9 ± 35.0	311.3 ± 20.3	346.7 ± 28.5
Relative (g/kg/day)	85.1 ± 5.8	91.5 ± 8.1	77.6 ± 5.2	88.6 ± 6.4
Average sodium fluoride intake (mg NaF/kg/day)				
From drinking water (GD 6 to 20)		10.3 ± 0.4	18.1 ± 0.5	29.2 ± 1.1
% Fluoride from water (GD 6 to 20)	<8.5%	84.4%	91.4%	95.1%
% Fluoride from feed (GD 6 to 20)	91.5–100%	15.6%	8.6%	4 9%
Average fluoride intake (mg F/kg/day)				
From water (GD 6 to 20)	—	4.67 ± 0.20	8.20 ± 0.23	13.21 ± 0.51
From feed (GD 6 to 20)	0.78 ± 0 03	0.84 ± 0.03	0.80 ± 0.04	0.67 ± 0.03
Total (GD 6 to 20)	0.78 ± 0.03	5.75 ± 0.26	8.79 ± 0.35	13.72 ± 0.66

[a] One doe delivered prior to scheduled euthanization.
[b] One doe aborted and two does delivered prior to scheduled euthanization.
[c] Three does delivered prior to scheduled euthanization
[d] Two does delivered prior to scheduled euthanization.
[e] Includes all does pregnant at euthanization; mean ± SEM; GD, gestational day.
[f] Body weights were recorded in the morning of each designated gestational day.
[g] Weight change during gestation minus gravid uterine weight.
[h] Body weights were recorded in the morning of each designated gestational day. Relative food and water intake (g/kg/day) were calculated using these weights.
* Significant at $p < 0.05$ for comparison with the control group.
§ Significant trend at $p < 0.05$.

DISCUSSION

These developmental toxicity studies found no adverse effect of sodium fluoride on the embryonal and fetal development of rats or rabbits given sodium fluoride in drinking water up to levels that resulted in decreased maternal water intake. The drinking water route of exposure was chosen to mimic human exposure and to reflect the impact on the developing fetus of the sustained blood levels of fluoride that would occur from water consumption throughout the

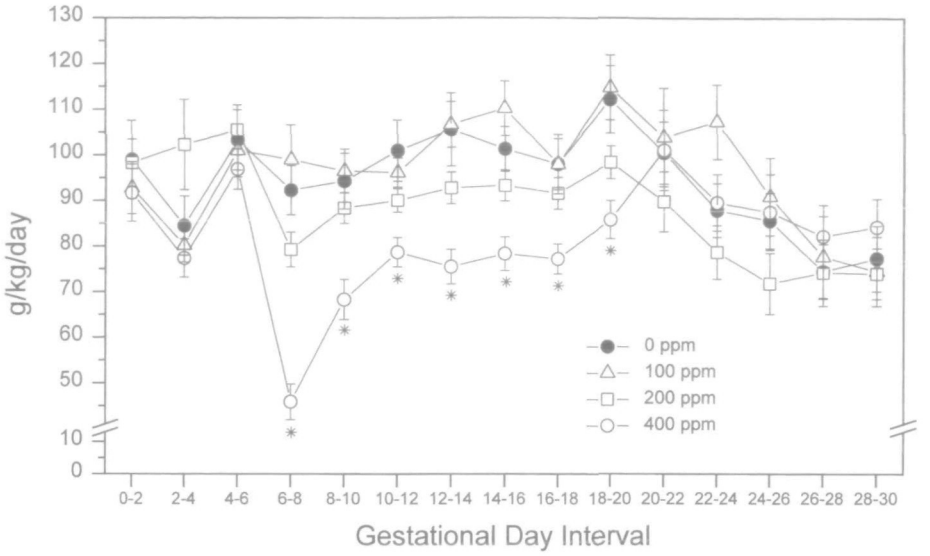

FIG. 3. Maternal relative water consumption of rabbits given sodium fluoride in drinking water GD 6 through 19. Data are presented as means ± SEM for each measurement period. *$p < 0.05$, pairwise comparison to the concurrent control group.

day. However, it is recognized that the diurnal patterns of rodent and lagamorph fluid consumption, as well as, the relative daily intake (g water/kg body weight) differs markedly from that of humans.

In the rat study the highest dose level of 300 ppm resulted in a sustained decrease in water consumption that returned to normal immediately after dosing was stopped on GD 16. This decreased water intake was not associated with any overall change in body, kidney, or liver weight or any other clinical signs of toxicity and was presumed to be due to palatability. Nonetheless, this precluded the use of higher levels of sodium fluoride and resulted in a maximal intake of 27 mg/kg/day at the highest dose.

Evaluation of the maternal rats in this study on GD 20 revealed that all pregnant animals had live litters. Furthermore, there were no effects of sodium fluoride intake on embryo/fetal survival. No significant effects of sodium fluoride were observed on fetal body weight or on the overall incidence of malformations (external, skeletal, and visceral) or variations (external, skeletal, and visceral). Increasing

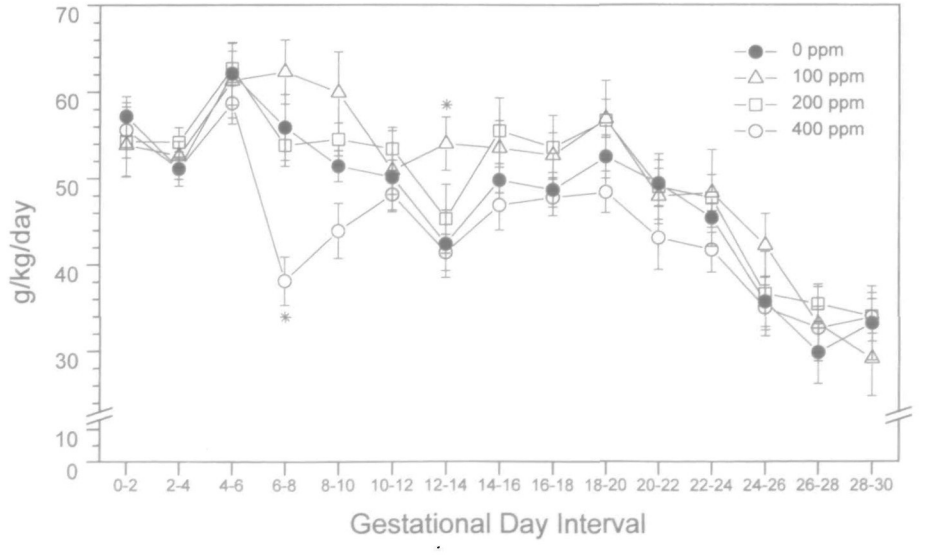

FIG. 4. Maternal relative food consumption of rabbits given sodium fluoride in drinking water GD 6 through 19. Data are presented as means ± SEM for each measurement period. *$p < 0.05$, pairwise comparison to the concurrent control group.

TABLE 5
Developmental Toxicity in NZW Rabbit Fetuses Following Maternal Inhibition of Sodium Fluoride on Gestational Days 6 through 19

	Sodium fluoride (ppm in water)			
	0	100	200	400
All litters[a,b]	21	20	18	20
No. corpora lutea/dam	9.9 ± 0.5	10.0 ± 0.5	10.1 ± 0.6	10.3 ± 0.5
No. implantations/litter	6.1 ± 0.7	6.3 ± 0.6	7.3 ± 0.8	6.9 ± 0.6
% Preimplantation loss/litter	37.4 ± 6.7	36.5 ± 5.7	27.4 ± 6.3	30.2 ± 5.9
% Resorptions/litter	16.1 ± 7.1	9.3 ± 5.0	3.8 ± 1.9	7.1 ± 2.7
% Litters with resorptions	33.3	35.0	22.2	35.0
% Late fetal deaths/litter	1.1 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 0.9
% Litters with late fetal deaths	4.8	0	0	10.0
Live litters[b,c]	19	19	18	20
No. live fetuses/litter	6.1 ± 0.7	6.2 ± 0.5	7.0 ± 0.7	6.3 ± 0.5
Avg male fetal body wt/litter (g)	55.8 ± 1.8	54.2 ± 1.7	51.9 ± 1.9	53.6 ± 1.4
Avg female fetal body wt/litter (g)§	56.3 ± 1.7	53.7 ± 1.2	51.1 ± 1.8	51.1 ± 1.9
% Male fetuses/litter	52.1 ± 4.3	54.1 ± 5.5	51.7 ± 4.3	47.6 ± 3.6
% Externally malformed fetuses/litter[d]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
% Viscerally malformed fetuses/litter[d]	0.0 ± 0.0	0.4 ± 0.4	1.7 ± 1.2	0.0 ± 0.0
% Skeletally malformed fetuses/litter[d]	1.8 ± 1.8	0.0 ± 0.0	1.2 ± 1.2	1.7 ± 1.7
% Malformed fetuses/litter[d]	1.8 ± 1.8	0.4 ± 0.4	3.0 ± 1.6	1.7 ± 1.7

[a] Includes all dams pregnant at euthanization; litter size, number of implantation sites per dam.
[b] Reported as the mean ± SEM.
[c] Includes only dams with live fetuses; litter size equals number of live fetuses per dam.
[d] Fetuses with one or more malformations.
§ $p < 0.05$, linear trend test.

dose-related trends were observed for the percentage of litters of rats with one or more externally malformed fetuses, and for the percentage of externally or skeletally malformed fetuses per litter, but these trends occurred in the absence of significant groupwise differences (ANOVA) among the control and treated groups.

Examination of individual fetal findings indicated that no fetuses with external malformations were observed in the control or low-dose groups. The external malformations observed in the mid- and high-dose groups are given as follows and other types of malformations in the same fetus are shown in brackets: (1) at 150 ppm, one male fetus (1/362 fetuses examined) exhibited gastroschisis [and cleft sternum], the fetus weighed 3.0 g; (2) at 300 ppm, three fetuses (3/373 fetuses examined) exhibited multiple malformations as follows: one male fetus (3.1 g) exhibited anasarca [and displaced and small kidney, displaced testis, and bipartite thoracic centrum], one male fetus (1.7 g) displayed craniorachischisis and microphthalmia [as well as fused ribs and bipartite thoracic centrum]; and one female fetus (1.3 g) exhibited bilateral anophthalmia, craniorachischisis, gastroschisis, and ectocardia [as well as renal agenesis, right side]. At 300 ppm, two of the three fetuses with malformations were clustered in one litter. All of these external malformations, as well as the associated visceral and skeletal malformations, occur spontaneoulsy in this species and strain with a low incidence (Charles River, 1993), with the possible exception of ectocardia. The one rat fetus with ectocardia (externalization of the heart or thoracoschisis) in this study was also reported to show gastroschisis. Thus, fissure of the ventral wall was quite extensive, and ectocardia was not an isolated defect. Considering the low incidence of these external malformations, and the lack of a statistically significant difference among groups, there was insufficient evidence to establish a clear cause and effect relationship between the occurrence of these external malformations and sodium fluoride exposure.

In light of these considerations, as well as the absence of any effect on fetal growth or viability, there was insufficient evidence to clearly characterize the group at 300 ppm as an adverse effect level for developmental toxicity in the rat. Higher concentrations of sodium fluoride in drinking water were not evaluated in the rat study due to reported toxicity in adult rats at >300 ppm in drinking water (NTP, 1990). Thus, it was not feasible to determine whether the finding of uncommon malformations in a small number of rodent fetuses at the high dose might constitute the lower tail of a dose–response curve. As also noted above, administration of higher concentrations of sodium fluoride throughout gestation is not feasible due to the unpalatability of the solution.

TABLE 6
Morphological Abnormalities Observed in NZW Rabbit Fetuses Following Maternal Inbibition of Sodium Fluoride on Gestational Days 6 through 19: Listing by Defect Type[a]

	Sodium fluoride (ppm in water)			
	0	100	200	400
No. fetuses examined[b]	115	117	126	125
No. litters examined[c]	19	19	18	20
All malformations				
No. of fetuses with any malformations[d]	1	1	4	1
% fetuses with any malformations	0.9%	0.9%	3.2%	0.8%
No. of litters with any malformations[e]	1	1	3	1
% litters with any malformations	5.3%	5.3%	16.7%	5.0%
External malformations				
No. of fetuses with external malformations[b]	0	0	0	0
% fetuses with external malformations	0.0%	0.0%	0.0%	0.0%
No. of litters with external malformations[e]	0	0	0	0
% litters with external malformations	0.0%	0.0%	0.0%	0.0%
Visceral malformations				
No. of fetuses with visceral malformations[d]	0	1	2	0
% fetuses with visceral malformations	0.0%	0.9%	1.6%	0.0%
No. of litters with visceral malformations[e]	0	1	2	0
% litters with visceral malformations	0.0%	5.3%	11.1%	0.0%
Left common carotid arises from innominate Artery			1 (1)	
Gall bladder half normal size and round			1 (1)	
Gall bladder twice normal size		1 (1)		
Skeletal malformations				
No. of fetuses with skeletal malformations[d]	1	0	2	1
% fetuses with skeletal malformations	0.9%	0.0%	1.6%	0.8%
No. of litters with skeletal malformations[e]	1	0	1	1
% litters with skeletal malformations	5.3%	0.0%	5.6%	5.0%
Branched rib			2 (1)	
Extra rib cartilage attached to sternum			1 (1)	
Fused ribs			1 (1)	
Bipartite cartilage, normal ossification center Thoracic centrum				1 (1)
Unilateral cartilage, unilateral ossification center Lumbar centrum	1 (1)			
Misaligned centrum				
Lumbar	1 (1)			
Thoracic	1 (1)			
All variations				
No. of fetuses with any variations[d]	61	64	76	76
% of fetuses with any variations	53.0%	54.7%	60.3%	60.8%
No. of litters with any variations[e]	18	17	17	20
% litters with any variations	94.7%	89.5%	94.4%	100.0%
External variations				
No. of fetuses with external variations[d]	0	0	0	0
% of fetuses with external variations	0.0%	0.0%	0.0%	0.0%
No. of litters with external variations[e]	0	0	0	0
% litters with external variations	0.0%	0.0%	0.0%	0.0%

Thus, in this rat study, the maternal NOAEL was 150 ppm (18 mg/kg/day) and the LOAEL was 300 ppm (27 mg/kg/day) based on a transient decrease in body weight. This study established a NOAEL for developmental toxicity of 27 mg/kg/day or higher for sodium fluoride administered in drinking water at 300 ppm to pregnant rats during organogenesis. Recently, Collins and co-workers (FDA, 1993; Collins *et al.*, 1994) reported a sodium fluoride developmental toxicity study in CD rats where the highest dose levels, 175 and 250 ppm in drinking water throughout pregnancy (GD 0–20), resulted in intakes of 24.7 and 25.1 mg/kg/day. As with the studies reported herein, the animals consuming

TABLE 6—*Continued*

	Sodium fluoride (ppm in water)			
	0	100	200	400
Visceral variations				
No. of fetuses with visceral variations[d]	3	1	6	3
% fetuses with visceral variations	2.6%	0.9%	4.8%	2.4%
No. of litters with visceral variations[e]	3	1	5	3
% litters with visceral variations	15.8%	5.3%	27.8%	15.0%
Abnormal number of papillary muscles				
4 in left ventricle		1 (1)	1 (1)	
2 in right ventricle	1 (1)			
4 in right ventricle			1 (1)	
Bifurcated papillary muscle(s)				
Left ventricle			1 (1)	
Right ventricle				1 (1)
Liver like tissue attached to gall bladder	2 (2)		3 (3)	2 (2)
Skeletal variations				
No. of fetuses with skeletal variations[d]	59	64	73	75
% fetuses with skeletal variations	51.3%	54.7%	57.9%	60.0%
No. of litters with skeletal variations[e]	18	17	17	20
% litters with skeletal variations	94.7%	89.5%	94.4%	100.0%
Rib on lumbar 1				
Bilateral full	45 (14)	42 (16)	50 (14)	54 (19)
Left full	7 (6)	4 (4)	6 (4)	10 (7)
Right full	2 (2)	3 (3)	4 (4)	4 (4)
Bilateral rudimentary	3 (3)	8 (6)	6 (4)	3 (3)
Left rudimentary	1 (1)	5 (3)	4 (4)	1 (1)
Right rudimentary	4 (4)	2 (2)	4 (4)	5 (5)

[a] The incidence of individual defects is expressed as the number of individual fetuses exhibiting that defect. Thus, a single fetus may be represented more than once in listing individual defects. Approximately 50% of the fetuses were decapitated prior to skeletal staining; heads were fixed in Bouin's solution and examined using a free-hand section method.

[b] Only live fetuses were examined.

[c] Includes only litters with live fetuses.

[d] Fetuses with one or more malformations/variations.

[e] Litters with one or more malformed/variant fetuses.

these levels of sodium fluoride drank less fluid. Animals in the high dose also ate less food than control animals. However, there were no definitive treatment-related effects on fetal growth or on the incidence of external, visceral, or skeletal abnormalities at levels of sodium fluoride up to and including 25.1 mg/kg/day. Thus, the findings of Collins *et al.* (1994) corroborate the findings and interpretation of the NTP rat study reported herein.

In our rabbit study, palatability was also a problem at the highest concentration of 400 ppm. Thus, the average sodium fluoride exposure from drinking water was 10, 18, and 29 mg/kg/day for the groups at 100, 200, and 400 ppm. Evaluation of the maternal rabbits in this study on GD 30 revealed no effect of sodium fluoride intake on embryo/fetal survival. No effect was observed on the number of live fetuses per litter or on the morphological development of the fetuses as determined by external, visceral, and skeletal examinations. No biologically relevant effects on fetal body weight were observed, although there was a statistical trend reported for decreasing body weight among female fetuses. The low-, mid-, and high-dose mean fetal body weights for female fetuses were 95, 91, and 91% of the mean control weight, respectively. The absence of any further decrease in the dose response between 200 and 400 ppm, and the lack of statistical significance for the overall comparison across groups by ANOVA (fetal body weight for males, females or both sexes together) indicate that the significant trend for female fetal weight may have been a spurious result. This study established a NOAEL for maternal toxicity at 200 ppm sodium fluoride or greater in drinking water (approximately 18 mg/kg/day) and a NOAEL for developmental toxicity of 400 ppm sodium fluoride or greater in drinking water (approximately 29 mg/kg/day) administered to pregnant rabbits during organogenesis.

Fluoride crosses the placenta of humans and animals, including rats and rabbits (Ericsson and Malmnas, 1962; Zipkin and Babeaux, 1965). Thus, maternal supplementation during pregnancy results in increased fluoride concentrations

TABLE 7
Comparison of Sodium Fluoride (NaF) Comparison in Drinking Water, Dosage Equivalents, and Serum Levels for Rats and Rabbits

	Rats				Rabbits			
Nominal concentrations in drinking water during major organogenesis								
NaF (ppm or mg/liter or μg/ml	<0.6	50	150	300	<0.6	100	200	400
F (ppm or mg/liter or μg/ml)[a]	<0.27	22.6	67.9	135.7	<0.27	45.24	90.48	180.96
Calculated dose from drinking water								
Mg NaF/kg body weight/day[b]	—	6.6	18.3	27.1	—	10.3	18.1	29.2
mgF/kg body weight/day[a]	—	2.99	8.30	12.26	—	4.67	8.20	13.21
Calculated dose from food[c,d]								
mg NaF/kg body weight/day	2.21	2.17	2.19	2.12	1.72	1.86	1.77	1.48
mg F/kg body weight/day	1.00	0.98	0.99	0.96	0.78	0.84	0.80	0.67
Calculated total dose from food and water								
mg NaF/kg body weight/day	2.21	8.78	20.53	29.20	1.72	12.70	19.43	30.33
mg F/kg body weight/day	1.00	3.97	9.29	13.21	0.78	5.75	8.79	13.72
Maternal serum concentration[e]								
F (ppm or mg/liter or μg/ml	0.007	0.035	0.039	0.187	0.06	0.24	0.39	0.70

[a] NaF contains 45.24% F by weight (MW of F, 18.994; MW of Na, 22.9898).

[b] Calculated intake of NaF was based on measurement of maternal relative water consumption (g/kg body weight/day) and the nominal concentration of NaF added to the drinking water for each experimental group. Control drinking water was below the method detection limit of 0.6 ppm NaF.

[c] NIH-07 rodent chow contained 11.6–13.4 ppm F (average = 12.4 ppm F, equivalent to 27.41 ppm NaF). Purina rabbit chow contained 14.6–16.6 ppm F (average = 15.6 ppm F, equivalent to 34.48 ppm NaF).

[d] Calculated intake of fluoride or of NaF equivalents was based on measurement of maternal relative food consumption (g/kg body weight/day) during the treatment period, as well as average concentration of fluoride in NIH-07 rodent chow or Purina rabbit chow.

[e] Maternal serum concentrations of fluoride were determined for GD 16 (rats) or GD 20 (rabbits).

not only in maternal blood, but also in cord blood and offspring tissues, especially bones and teeth (Theuer *et al.*, 1971; Katz and Stookey, 1973; Drinkard *et al.*, 1985; Speirs, 1986; Caldera *et al.*, 1988; Gedalia and Shapira, 1989; Chan *et al.*, 1989). Transplacental passage of fluoride probably occurs by passive diffusion at low maternal plasma concentrations, but the human placenta serves as a partial barrier to embryo/fetal exposure when maternal plasma concentrations exceed 0.4 ppm (Armstrong *et al.*, 1970; Shen *et al.*, 1974; Louw and Van Wyk, 1984; Ron *et al.*, 1986; Gupta *et al.*, 1993). Dynamic interactions among the maternal, fetal, placental, and amniotic compartments may contribute to interindividual variability in maternal/fetal ratios for circulating fluoride levels (Caldera *et al.*, 1988).

Transfer of fluoride to fetal tissues has previously been demonstrated in pregnant Sprague–Dawley rats fed diets containing ⩾2 (control), 50, 100, or 200 ppm fluoride (as sodium fluoride) throughout gestation (Theuer *et al.*, 1971). The ingested doses (0.1, 2.5, 5, or 10 mg F/kg/day by our estimation) were similar to those in the present rat study (Table 7). At termination on GD 20, whole fetal fluoride levels (i.e., 0.37 ± 0.14, 0.65 ± 0.07, 1.15 ± 0.09, 3.02 ± 0.30 ppm, respectively) reflected the maternal ingested doses, but maternal blood levels were not measured (Theuer *et al.*, 1971). As in this rat study, no adverse effects of sodium fluoride were noted on litter size, fetal weight, or prenatal mortality, but fetal morphological development was not evaluated in that study (Theuer *et al.*, 1971).

In addition to the negative findings from developmental toxicity studies in animals (above), the DHHS (1991) has summarized several studies of human development in relation to fluoride exposure during pregnancy. The birth defects registry of the metropolitan Atlanta, Georgia area has been evaluated for evidence of a possible adverse effect of fluoridation. Erickson and co-workers (1976) studied data from hospital records of 120,000 live births (1967 to 1973) in the five counties of the metropolitan Atlanta area, and birth-certificate diagnoses for more than 1.25 million live births in the 29 states and 2 cities covered by the National Cleft Lip and Palate Intelligence Service. The incidence of congenital malformations among live-born infants in fluoridated areas did not differ from the nonfluoridated areas.

Total daily fluoride intake from food and water sources in our studies (Table 7) may be compared to the estimated range of intake from food and fluid sources (including fluoridated drinking water) for adult humans, i.e., 0.014 to 0.080 mg fluoride/kg/day (IPCS, 1984). Thus, the low-, mid-, and high-dose groups in the rat study showed fluoride intakes (mg fluoride/kg/day) which were 50-, 116-, and 165-fold higher than the upper estimate for total human exposure from food and water in areas with fluoridated drinking water. Similarly, total doses consumed in the rabbit study represent

approximately 72-, 110-, and 172-fold increases relative to the upper estimate for total human exposure from food and water in areas with fluoridated drinking water. Interestingly, the control animals in these studies may have ingested 10 times (rabbits) and 13 times (rats) more fluoride (mg fluoride/kg/day) from food alone (Table 7) than the upper estimated human intake from food and fluid sources combined (IPCS, 1984).

A comparison of fluoride consumption and serum fluoride levels across species is also of interest in this context. In the present study, pregnant rats exposed to 300 ppm sodium fluoride for 10 days consumed an average dose of 12 mg fluoride/kg/day from drinking water and exhibited serum fluoride levels of 0.187 ppm fluoride at the end of the exposure period (see Table 7). Pregnant rabbits exposed to 400 ppm sodium fluoride for 14 days consumed an average dose of 13 mg fluoride/kg/day from drinking water and exhibited serum fluoride levels of 0.70 ppm fluoride at the end of the exposure period. Thus, rabbits had a higher serum fluoride level than rats when both species consumed a similar dose of fluoride. By comparison, an adult (70 kg) human who drinks one-half gallon of water per day would consume approximately 27 g/kg/day of water containing 0.027 mg fluoride/kg/day from a fluoridated water supply containing 1 ppm fluoride. Human serum or plasma concentrations of 0.010–0.015 ppm fluoride were reported from four studies in which the drinking water either contained $\leq$1 ppm or the drinking water level of fluoride was not reported (IPCS, 1984). These results suggest that the circulating levels of fluoride in our high-dose animals were about 12–19 times (rats) or 47–70 times (rabbits) higher than the estimated circulating levels of an adult human consuming fluoridated water. Considering the many variables which may have influenced the assay values in both the animal and human studies, these data provide a relatively crude basis for comparison of serum concentrations across species.

In summary, sodium fluoride in drinking water at doses up to 27 mg/kg/day in the rat or 29 mg/kg/day in the rabbit throughout major organogenesis caused no definitive developmental toxicity. The total ingested doses of fluoride from food and drinking water combined (mg F/kg body weight/day) for the mid- and high-dose groups in both studies were >100-fold higher than the range of 0.014–0.08 mg/kg/day fluoride intake estimated for a 70-kg person from food and fluoridated (1 ppm) drinking water (IPCS, 1984).

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