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From: Fluoride Action Network

Appendix B:

Comments on Sprando and Collins et. al. studies: Effects in Control groups compared to NaF treated groups.

Tables Include:

Sprando, Collins et al. published 6 papers on fluoride's effects in Food and Chemical Toxicology (1995, 1996, 1997, 1998, June 2001, August 2001).

The 1997 and 1998 studies were two of the four studies cited by ATSDR 2003 Toxicological Profile for Fluorides, Hydrogen fluoride, and Fluorine to counter the many studies which reported adverse effects on the male reproductive system. FAN's concern with the Sprando and Collins et al. studies are:

- the high number of adverse effects found in the Control group vs. the Treated groups in the multigenerational studies
- the rats used in the 1997 and 1998 studies were bred from the rat group used in the multigenerational studies (published in 2001)
- the lack of published fluoride levels in blood, bone, organs, and tissues in all these studies. Without this data it is not possible to understand the high number of effects reported for the Control groups.

In March 2004, Ellen Connett of Fluoride Action Network spoke with Robert Collins, one of the authors, about these studies. Dr. Collins stated that samples of blood, bone, tissue, and organs, from all experiments were given to a FDA researcher for analysis of fluoride levels. However, the results of this analysis have not been published. This is regrettable as other researchers have reported fluoride levels, for example,

> Messer et al. (1973), used a "low fluoride" diet that varied from 0.1 to 0.3 ppm fluoride (compared to the Sprando & Collins et. al. "low fluoride" diet of 7.95 pm fluoride) and published the fluoride levels in the humeri of two generations. The authors state, "The concentration of fluoride in the humeri of mice fed the low fluoride diet was 70 to 80 times lower than in animals receiving 50 pppm fluoride in their drinking water." - (page 1325). Ref: Messer et al. (1973). Influence of fluoride intake on reproduction in mice. Journal of Nutrition 103:1319-1327

Table 1. August 2001 Study. Effects: Control versus NaF treated groups.
Developmental toxicity of sodium fluoride measured during multiple generations.
Collins TF, Sprando RL, Black TN, Shackelford ME, Olejnik N, Ames MJ, Rorie JI, Ruggles DI.
Food Chem Toxicol. 2001 Aug;39(8):867-76.

"This study was done to determine any generational effects of NaF on the development of the offspring." - page 87

• The treated groups were exposed to NaF in drinking water at levels of 25 ppm, 100 ppm, 175 ppm, and 250 ppm.
• No Fluoride levels in blood, bone and tissue are presented
• Skeletal variations are not identified by sex
• Incidence of specific soft -tissue variations are not identified by sex
• Runts are not identified by sex

Incidence of specific skeletal variations (excluding sternebral) are presented for F2 fetuses (Table 7) -
25 Parameters were listed:
- only the 175-ppm treated group had higher number of effects than the Control (16 vs 6 respectively)
- the Control had higher number of effects compared to the 250-ppm treated group (12 vs 10 respectively)
- the Control had higher number of effects compared to the 100-ppm treated group (14 vs 8 respectively)
- the Control had higher number of effects compared to the 25-ppm treated groups (12 vs 5 respectively)
- in 5 of the 25 parameters, the Control group had the highest number of effects
- in 3 of the 25 Parameters: 175-ppm treated group had no effects listed
- in 4 of the 25 Parameters: 100-ppm treated group had no effects listed
- in 5 of the 25 Parameters: Control group had no effects listed
- in 6 of the 25 Parameters: 25-ppm treated group had no effects listed
- in 6 of the 25 Parameters: 250-ppm treated group had no effects listed

Analysis of incidence of sternebral variations are presented only for F2 fetuses (Table 6)
Two of the 4 Parameters:
• **Fetuses with 2 + variation**: Control group had higher number of effects than 25-ppm and 250-pppm treated groups.
• **Fetuses with 1 + variation**: Control group had higher number of effects than 25-ppm treated group.

Incidence of specific sternebral variation are presented for F2 fetuses (Table 5)
Three of the 6 Parameters:
• **Bipartite**: Controls had higher number of effects than 250-ppm treated group.
• **Reduced Ossification**: Control group and 250-ppm treated group had same number of effects. Control group had higher number of effects than 25-ppm treated group.
• **Non-ossified**: Control group had higher number of effects than 25-ppm and 250-ppm treated groups
• The authors commented that one Control fetus with fused sternebrae was not presented in this Table.

Analysis of incidence of skeletal variations in F2 fetuses (Table 8).
The six Parameters:
• **Litters with fetuses with 1 + variations**: Control group had highest number of effects compared to all treated groups.
• **Fetuses with 2 + variations**: Control group had higher number of effects compared to 25-ppm, 100-ppm, and 175-ppm treated groups.
• **Fetuses with 1 + variations**: Control group had higher number of effects compared to 25-ppm and 100-ppm treated groups.

Table 1 continued: Analysis of incidence of skeletal variations in F2 fetuses (Table 8).

- **Litters with fetuses with 2 + variations**: Control group had higher number of effects compared to 25-ppm and 100-ppm treated groups.
- **Fetuses with 3 + variations**: Control group had same number of effects as 100-ppm treated group.
- **Litters with fetuses with 3 + variations**: Control group had higher number of effects compared to 25-ppm and 100-ppm treated groups.
- **of 6 Parameters**: Control group had higher number of effects than 25-ppm treated group
- **of 5 Parameters**: Control group had higher number of effects than 100-ppm treated group (for 6th parameter both Control and 100-ppm treated group had same number of effects)

Incidence of specific soft-tissue variations in F2 fetuses (Table 9):
Three of the 7 Parameters:

- **Severe Enlarged ureter or kidney**: Control group had same number of effects as 100-ppm treated group.
- **Moderate Enlarged ureter or kidney**: Control group had higher number of effects compared to 250-ppm treated group.
- **Hemorrhage, internal**: Control group had higher number of effects compared to 25-ppm and 250-ppm treated groups.

While the authors give definitions for early and late deaths, the statistics presented are grouped as one.

Early deaths were defined as Deciduomas (brownish impantation sites without placenta)
Late Deaths are defined: Implantation sites with placentas and with complete but non-viable fetuses that were of subnormal size, that showed retarded development, or were in a macerated condition.

The authors state

- **The single statistically significant decrease in crown-rump length of F2 females at 175 ppm was considered random**.

Note: In a 2003 sodium fluoride teratogen study, Goh and Neff reported:
"The most prominent malformations caused by sodium fluoride are reduction in the head-tail lengths and dysfunction of the neuromuscular system of the tadpoles... the observed teratogenic action of sodium fluoride on frog embryos would indicate a strong possibility that sodium fluoride may also act directly on developing mammalian fetuses to cause malformation."
Ref: Effects of fluoride on Xenopus embryo development. Food Chem Toxicol. 2003 Nov;41(11):1501-8.

Table 2. 1998 Study. Effects: Control versus NaF treated groups.
Testing the potential of sodium fluoride to affect spermatogenesis: a morphometric study.
Sprando RL, Collins TX, Black T, Olejnik N, Rorie J.
Food and Chemical Toxicology 36 (1998) 1117-1124.

"This study provides quantitative information on the effect of sodium fluoride (NaF) on the testes of F1 generation male rats exposed in utero and during lactation to NaF at one of four concentraitons (25, 100, 175, 250 ppm)..." - page 1117

In this study, the authors cite the lack of statistical signficance for several parameters for NaF-treated groups compared to the control. The public have no way to determine the lack of significance without the blood fluoride levels in the control and treated groups.

- Statistically significant differences in testis weight, testicular volume or testicular specifric gravity were not observed between the control and NaF-treated animals.

- "The seminiferous tubules from the controls and NaF-treated groups occupied approximately 87-88% of the total testis volume and the seminiferous tubular lumen occupied approximately 12-14% of the testicular volume for controls and the NaF-treated groups. The interstitial space occupied approximately 9-11% of the total testis volume, the Leydig cells occupied 3-4%, blood vessels occupied 3%, macrophages occupied 0.40-0.60% for the controls and the 25 ppm, 100 ppm, 175 ppm and 250 pp m NaF treatment groups. No statistically significant differences were observed between the controls and NaF-treated groups for the above parameters."

- "Statistically significant differences were not observed in the mean numbers of Sertoli cell nucleoli counted per cross-sectional seminiferous tubles, the seminiferous tubule diameters or the height of the seminiferous epithelium between the control and NaF-treated group."

- "Statistically significant differences were not observed in the mean absolute seminiferous tuble lengths or mean absolute surface areas between the control and the sodium fluoride treated groups."

- "The number of Sertoli cell nucleoli was not statistically different when the control and NaF-treated rats were compared..."

- "The mean diameters of the seminiferous tubules from the treatment groups were not significantly different from the control groups... "

- "The numbers of homogenization resistant spermatids per testis from the F1 generation NaF-treated male rats used in the present study were not statistically different from their F1 generation controls."

Statistificantly significant effects the authors reported in their 1998 paper:

- "... there was a significant reduction in the absolute volume of the testicular capsule in the 100 ppm NaF treatment group compared to the controls. This finding was not considered a treatment-related effect because no dose-response relationship was observed and it is probably a result of sampling error for this parameter..."

- "A statistically significant difference in body weight between the controls and the NaF-treated rats in the 100 ppm, 175 ppm and a borderline statistically significant effect was observed in the 250 ppm NaF treatment groups: however, this decrease in body weight was not dose related."

- "A statistically significant decrease in the absolute volume and volume percent of the lymphatic endothelium was observed in the 175 and 250 ppm NaF-treated groups and in the testicular capsule in the 100 ppm NaF-treated groups. The significance of this finding is unknown at the present time..."

Table 3. 1997 Study. Effects: Control versus NaF treated groups.
Testing the potential of sodium fluoride to affect spermatogenesis in the rat.
Sprando RL, Collins TFX, Black TN, Rorie J, Ames MJ, O'Donnell M.
Food and Chemical Toxicology 35 (1997) 881-890.

Again, in this study the authors cite the lack of statistical signficance for several parameters for NaF-treated groups compared to the control. The public has no way to determine the lack of significance without the blood, tissue and organ fluoride levels in the control and treated groups.

Body weight: "The mean body weights of the P and F1 generation rats (Table 1) from all sodium fluoride treatment groups were not statistically different than their respective controls..."

Testis weight: "Dose-related effects were not observed. No statistically significant differences were observed in mean testes weights (Table 2) between P and F1 controls and their respective treatment groups."

Epididymal weight: "Mean epididymal weights of treated and control rats are presented in Table 3. Statistically significant differences in mean epididymal weights were not observed between control and treatment groups of the P generation however; within the F1 generation the right epididymal weight of the 175 ppm group was significantly lower than the F1 control. No dose-related effects were observed...."

Prostate/seminal vesicle weight: "No statistically significant difference in prostate/seminal vesicle weights was observed between treated and control rats in either the P or F1 generation. No significant differences in prostate/seminal vesicle weights were observed between generations when similar treatment groups were compared."

Spermatid numbers: "Mean spermatid counts per testis, per gram of testis, and per gram of testis per day of P and F1 generation male rats are shown in Table 5. No statistically significant differences in spermatid numbers between controls and sodium fluoride treatment groups in either generation were observed. Similarly, no statistically significant differences were observed when the same treatment groups were compared between generations."

Serum testosterone, LH and FSH: "Mean serum testosterone concentrations, serum LH and serum FSH concentrations of treated and control rats from the P and F1 generations are shown in Table 5. No statistically significant differences were observed between the treated and control groups in either the P or F1 generation. P v. F1: No significant differences were observed in mean testosterone, LH or FSH values between generations when the same dose levels were compared."

Non-reproductive organ weights: "Mean organ weights of treated and control rats from the P and F1 generation are presented in Table 7... F1 generation: Liver weight in the 100 and 250 ppm treatment groups were significantly lower than controls. This was considered a random occurrence and not biologically significant because no dose-related effects were observed. Otherwise, organ weights in the F1 generation treatement groups were not significantly different than F1 control values. P v F1: There were no significant differences observed in heart, liver, kidney or spleen weights between generations for any dose groups."

• At injection site ... Occasionally, free germ cells (spermatocytes or round spermatids) were found in the tubular lumen; their presence was probably due to mechanical trauma to the testis (handling or cutting the testis; not shown)...

Table 4. 1995 Study. Effects: Control versus NaF treated groups.
Developmental toxicity of sodium fluroide in rats.
Collins TFX, Sprando RL, Shackelford ME, Black TN, Ames MJ, Welsh JJ, Balmer MF, Olejnik N, Ruggles DI.
Food and Chemical Toxicology (1995) 33:11 951-960

"This study was conducted to determine the effects of sodium fluoride (NaF) on foetal development..." - page 951

Treated groups with sodium fluoride levels in drinking water of:
10 ppm, 25 ppm, 100 ppm, 175 ppm, and 250 ppm (1.4, 3.9, 15.6, 24.7 or 25.1 mg/kg body weight)

Rats: Caesarean-derived, viral antibody-free (CD-CRL: CD-BR, VAF+) rats (Charles River Laboratories, Inc., Wilmington, MA, USA) were used. On receipt, the males weighed 351-375 g, and the females weighed 175-200 g. The males were used as sires onlly, and were not treated.

Rat Chow: Female rats were fed low-fluoride NIH-07 diet (7.95 ppm fluroide).

Animals were mated. Caesarean sections were performed on gestation day 20.

Analysis of incidence of **soft-tissue variations** in foetuses of rats treated with sodium fluoride (Table 9). 6 of the 6 parameters:

- **Foetuses with 1+ variations:** Control group had higher number of effects compared to all treated groups
- **Litters with foetuses with 1+ variations:** Control group had higher number of effects compared to all treated groups
- **Foetuses with 2+ variations:** Control group had higher number of effects compared to all treated groups
- **Litters with foetuses with 2+ variations:** Control group had higher number of effects compared to all treated groups
- **Foetuses with 3+ variations:** Control group had higher number of effects compared to all treated groups
- **Litters with foetuses with 3+ variations:** Control group had higher number of effects compared to all treated groups

Incidence of specific **sternebral variations** in foetuses of rats treated with sodium fluoride (Table 4). 4 of the 6 parameters:

- **Incomplete ossification:** Control group had higher number of effects compared to 10 ppm, 25 ppm, and 175 ppm treated groups.
- **Non-ossified**: Control group had higher number of effects compared to 10 ppm, 100 ppm, and 175 ppm treated groups.
- **Malaligned**: Control group had higher number of effects compared to 10 ppm, 25 ppm, 100 ppm, and 175 ppm treated groups.
- **Misshapen**: Control group had higher number of effects compared to all treated groups.

Analysis of incidence of **sternebral variations** in foetuses of rats treated with sodium fluroide (Table 5). 3 of the 4 parameters:

- **Foetuses with 1 + variations**: Control group had higher number of effects compared to 10 ppm, 25 ppm, and 175 ppm treated groups.
- **Litters with foetuses with 1 + variations**: Control group had higher number of effects compared to 10 ppm, 25 ppm, and 175 ppm treated groups.
- **Foetuses with 2 + variations**: Control group had higher number of effects compared to 10 ppm ad 25 ppm treated groups.

Table 4 continued.

Incidence of specific skeletal variations in foetuses of rats treated with sodium fluroide (Table 6). 22 of the 38 parameters:

- **Ribs, wavy:** Control group had higher number of effects compared to 10 ppm, 25 ppm, and 175 ppm treated groups.
- **14th rib (C7):** Control group had higher number of effects compared to 10 ppm, 25 ppm, and 100 ppm treated groups. Control group had same number of effects as the 250 ppm treated group. (No effects in 10 ppm and 100 ppm treated groups.)
- **14th rib bud (L1):** Control group had higher number of effects compared to all treated groups.
- **Ossified bud off L4**: Control group had same number of effects as 250 ppm group. (No effects in 10 ppm, 25 ppm, and 175 ppm treated groups).
- **Rib missing**: Control group had same number of effects as 25 ppm and 175 ppm treated groups. (No effects in 10 ppm, 100 ppm, and 250 ppm treated groups.)
- **Centra. red. oss**.: Control group had higher number of effects compared to all treated groups.
- **Centra. misshapen:** Control group had higher number of effects compared to 25 ppm and 175 ppm treated groups.
- **Centra. bipartite**: Control group had higher number of effects compared to 10 ppm, 25 ppm, 100 ppm and 250 ppm treated groups. Control group had same number of effects as the 175 ppm treated group.
- **Centra. not ossified**: Control group had same number of effects as the 250 ppm treated group.
- **Dorsal arches. red. oss**: Control group had higher number of effects than 10 ppm, 175 ppm and 250 ppm treated groups. (Note: these treated groups had no effects listed).
- **Parietal red. oss**: Control group had same number of effects as the 175 ppm and 250 ppm treated groups. (No effects listed for 10 ppm and 25 ppm treated groups.)
- **Frontal. red. oss**: Control group had same number of effects as 100 ppm treated group. (All other treated groups had no effect listed.)
- **Nasal. red. oss**: Control group had same number of effects as 175 ppm treated group. (No effect listed for 10 ppm treated group.)
- **Supraoccipital. red. oss**: Control group had higher number of effects than 10 ppm treated group.
- **Hyoid. red. oss**: Control group had higher number of effects than 10 ppm and 175 ppm treated groups.
- **Zygomatic. red. oss:** Control group had same number of effects as 10 ppm treated group.
- **Ischicm. red. oss**: Control group had same number of effects as 25 ppm treated group. (No effects listed for 10 ppm and 175 ppm treated groups.)
- **Pubis. red. oss**: Control group had higher number of effects compared to 10 ppm and 175 ppm treated groups (these treated groups had no effects listed).
- **Metacarpals red. oss**: Control group had same number of effects as 100 ppm treated group. (No effects listed for 10 ppm and 175 ppm treated groups.)
- **Metatarsals. red. oss**: Control group had higher number of effects compared to 10 ppm treated group. (No effects listed for 175 ppm treated group.)
- **Basisphenoid. red. oss**: Control group is only group listed with effects.
- **Centrium. unilateral oss**: Control group and 175 ppm treated group had same number of effects. (All other treated groups had no effects listed.)

- in 14 of the 38 parameters: the 100 ppm treated group had no effects listed
- in 15 of the 38 parameters: the Control group had no effects listed
- in 15 of the 38 parameters: the 25 ppm treated group had no effects listed
- in 15 of the 38 parameters: the 250 ppm treated group had no effects listed
- in 20 of the 38 parameters: the 175 ppm treated group had no effects listed
- in 26 of the 38 parameters: the 10 ppm treated group had no effects listed

Table 4 continued.

- 175 ppm treated group: the Control group had higher number of effects: 14 vs. 8
- 10 ppm treated group: the Control group had higher number of effects: 20 vs. 4
- 250 ppm treated group: in 8 parameters the Control group had higher number of effects:
- 100 ppm treated group: in 7 parameters the Control had higher number of effects
- 25 ppm treated group: in 11 parameters the Control group had higher number of effects.

- 10 ppm treated group: in 1 parameter the Control had same number of effects.
- 25 ppm treated group: in 2 parameters the Control group had same number of effects.
- 100 ppm treated group: in 1 parameter the Control group had same number of effects
- 175 ppm treated group: in 5 parameters the Control group had same number of effects
- 250 ppm treated group: in 4 parameters the Control had same number of effects

Analysis of incidence of **skeletal variations** in foetses of rats treated with sodium fluoride (Table 7) . 5 of the 6 parameters:

* **Foetuses with 1+ variations**: Control group had higher number of effects compared to 10 ppm, and 175 ppm treated groups. Control group and 250 ppm treated group had same number of effects.
• **Litters with foetuses with 1+ variations**: Control group had higher number of effects compared to all treated groups.
• **Foetuses with 2+ variations**: Control group had higher number of effects compared to 10 ppm treated group.
• **Litters with foetuses with 2+ variations**: Control group had higher number of effects compared to 10 ppm and 175 ppm treated groups.
• **Litters with foetuses with 3+ variations**: Control group had higher number of effects compared to 10 ppm treated group.

Incidence of specific **soft-tissue variations** in foetuses of rats treated with sodium fluroide (Table 8). 6 of 11 parameters:

• **Haemorrhages, internal**: Control group had higher number of effects compared to 175 ppm and 250 ppm treated groups. Control group had same number of effects as the 100 ppm treated groups.
• **Hydroureter severe**: Control group had higher number of effects compared to 10 ppm, 25 ppm, 175 ppm, and 250 ppm treated groups.
• **Hydroureter moderate**: Control group had higher number of effects compared to all treated groups.
• **Enlarged renal pelvis - moderate**: Control group had higher number of effects compared to 10 ppm, 25 ppm, 175 ppm, and 250 ppm treated groups. (Note: 25 ppm and 250 ppm treated groups had no effects listed.)
• **Enlarged uretal kidney - severe**: Control group had the same number of effects as the 25 ppm, 175 ppm, and 250 ppm treated groups.
• **Enlarged uretal kidney - moderate**: Control group had higher number of effects compared to all treated groups.

- in 5 of the 11 parameters: Control group had no effects listed
- in 5 of the 11 parameters: 250 ppm treated group had no effects listed
- in 4 of the 11 parameters: 10 ppm treated group had no effects listed
- in 4 of the 11 parameters: 100 ppm treated group had no effects listed
- in 4 of the 11 parameters: 175 ppm treaed group had no effects listed
- in 3 of the 11 parameters: 25 ppm treated group had no effects listed

Some comments by the authors:

• The data from our study can be compared with the data available from other studies only to a limited degree, owing to the differences in procedures, or species, or both.

Table 4 continued.

• In the control group, one foetus had a cleft palate.

• The control group showed the highest occurrence of in utero deaths per litter (Table 3), and there was no statistically significant difference between the control and treated groups.

• The average number of foetuses with at least one, at least two and at least three sof-tissue variations was less in all treated groups.

• The average number of litters with foetuses with one or more and two or more soft-tissue variations was less in the treated groups than in the control group, with no indication of dose relationship.

• The average number of litters containing foetuses with three or more soft-tissue variations was similar in the control and treated groups.

• Significant decreases in body weight gain were seen in the females of the 250-ppm group on days 0-3 and 6-9 and overall on days 0-20; these decreases are probably related to decreased feed consumption.

• A significant decrease in the mean number of corpora lutea per female was seen in the dams of the 250-ppm group. Because the number of corpora lutea is determined at birth, this decrease is considered to be random.

• The mean number of viable male foetuses per litter was significantly decreased in the 175-ppmn group when compared with the control group, but this decrease was not dose-related and is considered to be random.

• Vertebral ossification, also considered a measure of fetal ossification, showed no-dose-related effects. In the control group, 8.2 vertebrae were ossified, and 8.6 [25 ppm] , 8.3 [100 ppm], 8.2 [175 ppm], and 8.2 [250 ppm] ossified vertebrae, respectively, were seen per fetus in the treated groups.

• the foetuses were taken just before parturition: hence there were no teeth to observe.

• In the 2001 study the average number of fetuses per litter with three or more skeletal variations in the 250 ppm group was double that of the control group (1.0 vs 0.5 per litter) but this increase was insufficient to make the value statistically significant.

Table 5. 1996 Study. Effects: Control versus NaF treated groups.
Effect of intratesticular injection of sodium fluoride on spermatogenesis
Sprando and Black et al.
Food and Chemical Toxicology (1996) 34:377-384

The left testis of each rat was injected with either vehicle (0.9% sodium chloride; vehicle-injected testis) or 50 ul sodium fluoride (99% pure;p sodium fluoride-injected testis) in vehicle at the following concentrations: 50, 175 and 250 ppm. The contralateral (right) testis of each rat was not injected and served as an intact control (non-injected control). Testicular tissues were collected at 24 hr and 1, 2 and 3 wk post injection and processed.

Discussion (excerpt, page 383):

... The only significant finding in the present study was a local infilitraiton of leucocytes and free germ cells into the inertubular space at the injection site in both the vehicle- and fluoride-injected testes. Leucocyte infilitration in these groups was not considered a treatment-related effect. Leucocyte infilitration is not an uncommon occurrence when it injections are used (R.L. Sprando, unpublished observation, 1990). A slight increase in the amount of leucocyte infilitration was observed in the 175 ppm fluoride treatment groups (25% of sections observed) compared with that in the vehicle-injected control group, the non-injected control group (13% and 0% of sections observed, respectively) and the other fluroide treatment groups (18% and 17% of the sections observed for the 50 and 250 ppm treatment groups, respectively). At present it is not known why this increase was observed; however, it may have been a result of the tissue sampling procedure. The appearance of both germ and Sertoli cells in the interstitial space among the leucocytes was most likely caused by trauma to the seminferous tubules at the time of injection, since free germ cells were not observed in the intertubular space distal to the site of injection or in non-injected controls.

Table 6. June 2001 Study. Control versus NaF treated groups.
Multigenerational evaluation of sodium fluoride in rats.
Collins and Sprando et al.
Food Chem Toxicol. 2001 Jun;39(6):601-13.

"This study evaluated the effects of sodium fluoride exposure on reproductive function in three generations of rats." - page 611

• The treated groups were exposed to NaF in drinking water at levels of 25 ppm, 100 ppm, 175 ppm, and 250 ppm.
• No Fluoride levels in blood, bone and tissue are presented
• Incidence of specific soft -tissue variations are not identified by sex
• Spontaneous disease lesions and incidental findings not defined

• Overall mean feed consumption of **F1 females** showed a significant negative linear regression for days 0-70, although none of the values was significantly less than the control value (Table 1). During the same period, F1 males in the treated groups consumed less feed than did the control group, but the decreases were neither dose-related nor significant. (page 606)

• Thymus, heart, liver, spleen, kidney, adrenal and brain weights were measured in adult females (150 days old) and males (129 days old), as well as reproductive organs (ovary wiehgt in females, and testis, epididymis, seminal vesicle and prostate weights in males). No significant differences were observed between control and treated groups. (page 609)

• Thymus, heart, liver, spleen, kidney, adrenal and brain weights were also measured in weanling females and males (25-27 days old), as well as reproductive organs (ovary weight in females, and testis, epididymis, seminal vesicle and prostate weights in males). No significant differences were observed between control and treated groups. (page 609-610).

• Mating indices of F1 females were also over 90%, indicating lack of compound-related effects. The fertility indices of the females in the 25 and 250 ppm groups were slightly less than the control animals, but the differences were not statistically significant (Table 4) and are probably due to random variation.

• Spontaneous disease lesions and incidental findings in other organs and tissues were of the usual number and type commonly observed in Sprague-Dawley rats at each age. These lesions and findings occurred at similar incidence rates in control and treated rats.

Significant Effects:

• **Body weights** ... Weight gain of F0 females and males during days 0-70 showed a significant negative linerar regression. Only the individual weight gain of F0 males in the 250 ppm was statistically less than the control. In the F1 generation, weight gain of females and males were similar. No significant dose-related effects

• ... During development, the teeth of **F1 adult females and males** (34-35 animals per group) were examined for color variations. No stained or mottled teeth were observed in either sex of any group. Mild whitening of the teeth was observed in both females and males at 100, 175 and 250 ppm NaF. Although the effect was mild, the number of affected females and males was **increased in a dose-related, statistically signficant manner**. (page 610-611) ... Rats normally have orange-brown pigments in their teeth. Loss of this pigment and the appearance of whitened teeth is a known response to fluoride consumption in rats. In the study reported here**, adult F1 females and males** (34-35 per group) were observed for tooth color. No dark discrete discolorations (i.e. mottling) were observed in any of the animals in our study at any time. The incidence of whitened teeth was increased in a dose-related manner in females and males from the groups given 100, 175 and 250 ppm sodium fluoride. The effect was mild and of a cosmetic nature. (page 612)

Table 7. Rat chow used in the 6 Sprando and Collins et al. studies

Note on "low fluoride diet"
Messer et al. (1973) state: "The diet used for all experimental groups was the low fluoride diet of Taylor et al. ... The fluoride content varied from 0.1 to 0.3 ppm fluoride, on a dry weight basis. Growth rates of mice and rats fed this diet were found to be at least equal to those of animals fed a standard laboratory ration." - (page 1320)

Unlike Sprando and Collins et. al, the Messer et al. 1973 study published the fluoride levels in the humeri for 2 generations. The authors state, "The concentration of fluoride in the humeri of mice fed the low fluoride diet was 70 to 80 times lower than in animals receiving 50 pppm fluoride in their drinking water." - (page 1325)

References:
Messer et al. (1973). Influence of fluoride intake on reproduction in mice. Journal of Nutrition 103:1319-1327
Taylor et al. (1961). Toxic effects of fluoride on the rat kidney. Toxicol. Appl. Pharmacol. 3, 290.

Title of Study	Authors comments on Rat Chow used in study
Developmental toxicity of sodium fluroide in rats. Collins and Sprando et al. Food and Chemical Toxicology 33:11 951-960. 1995.	The males were used as sires only, and were not treated. Female rats were fed low-fluoride NIH-07 diet (7.95 ppm fluoride) to minimize interference with fluoride in drinking water. The diet was prepared by Ziegler Bros. Inc. (Gardiners, PA, USA), the company that provided the diet for the fluoride studies conducted by the NTP (NTP, 1990).
Effect of intratesticular injection of sodium fluoride on spermatogenesis Sprando and Black et al. Food and Chemical Toxicology 34:377-384. 1996.	No mention of rat chow in this study.
Testing the potential of sodium fluoride to affect spermatogenesis in the rat. Sprando and Collins et al. Food and Chemical Toxicology 35: 881-890. 1997. Testing the potential of sodium fluoride to affect spermatogenesis: a morphometric study. Sprando and Collins et al. Food and Chemical Toxicology 36: 1117-1124. 1998.	During the course of this study... fed a low fluoride NIH-07 diet (7.95 ppm fluoride) to minimize interference with fluoride in the drinking water. The diet was prepared by Ziegler Bros., Inc. (Gardiners, PA, USA) and is the same formulation as that used in the NTP chronic study (NTP, 1990).
Multigenerational evaluation of sodium fluoride in rats. Collins and Sprando et al. Food Chem Toxicol. Jun;39(6):601-13. June 2001. Developmental toxicity of sodium fluoride measured during multiple generations. Collins and Sprando et al. Food Chem Toxicol. Aug;39(8):867-76. August 2001.	Throughout the study, males and females were fed low-fluoride NIH-07 diet (7.95 pm fluoride) to mimize interference with fluoride in drinking water. The diet was prepared by Ziegler Bros. Inc. (Gardiners, PA, USA), the company that provided the low fluoride diet for the National Toxicology Program (1990) studies, and the diet was the same formulation as that used in the NTP studies.

Table 8. Rats used in the 6 Sprando and Collins et al. studies		
Title	Authors comments on Rats used in study	Comments
Developmental toxicity of sodium fluroide in rats. Collins and Sprando et al. Food and Chemical Toxicology (1995) 33:11 951-960.	Caesarean-derived, viral antibody-free (CD-CRL:CD-BR, VAF +) rats (Charles River Laboratories, Inc., Wilmington, MA, USA) were used. On receipt, the males weighed 351-375 g, and the females weighted 175-200 g.	The assumption is that these rats were obtained directly from Charles River Laboratories.
Effect of intratesticular injection of sodium fluoride on spermatogenesis Sprando and Black et al. Food and Chemical Toxicology (1996) 34:377-384	96 adult male Sprague-Dawley rats weighing 225-460 g were used.	Authors do not state where rats came from.
Testing the potential of sodium fluoride to affect spermatogenesis in the rat. Sprando and Collins et al. Food and Chemical Toxicology 35 (1997) 881-890.	The 124 male rats (P generation: n = 64 rats; F1 generation: n = 60 rats) utilized in this study were obtained from a larger two generation reproduction study.	
Testing the potential of sodium fluoride to affect spermatogenesis: a morphometric study. Sprando and Collins et al. Food and Chemical Toxicology 36 (1998) 1117-1124	The 25 male rats utilized in this study were obtained from a larger two-generation reproduction study (see Sprando et al, 1997).	
Multigenerational evaluation of sodium fluoride in rats. Collins and Sprando et al. Food Chem Toxicol. 2001 Jun;39(6):601-13.	Caesarean-derived (CD CRL:CD-BR). viral anti-body-free (240 males and 240 females) were obtained from Charles River Laboratories Inc. (Wilmington, MA, USA). The males and females weighed 51-75 g at the time of receipt.	
Developmental toxicity of sodium fluoride measured during multiple generations. Collins and Sprando et al. Food Chem Toxicol. 2001 Aug;39(8):867-76.	Caesarean-derived (CD CRL-CD-BR). viral anti-body-free rats were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA). the males and females weighed 51-75 g at the time of receipt.	