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CHRONIC TOXICITY OF DIETARY FLUORINE TO MINK[1]

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ABSTRACT

Seventy-two 3-mo-old pastel mink were fed diets that contained 0, 33, 60, 108, 194 or 350 ppm supplemental fluorine (F), as NaF, for 382 d to assess its effects on growth, fur quality, reproduction and survivability. The basal diet contained 35 ppm F as fed. No significant differences were observed in body weight gains or fur quality between the controls and any of the F-treated groups (P>.05). Some males fed 350 ppm supplemental F for a 4-mo period prior to pelting had weakened frontal, parietal and femoral bones that fractured during the pelting process. The F treatments had no measurable adverse effects on breeding, gestation, whelping or lactation, although only 14% of the kits whelped by females fed 350 ppm F survived to 3 wk of age. The survivability of the adult mink was adversely affected only at 350 ppm supplemental F. At the termination of the study, no differences were observed in hematologic parameters or serum calcium concentrations between the controls and treated mink (P>.05), but serum alkaline phosphatase activities were increased (P<.05) by the two highest dietary F levels. Serum F levels were elevated (P<.01) only in mink fed 194 and 350 ppm F, and urinary and femoral F concentrations in the treated animals were generally greater (P<.05; P<.01) than control values and were closely related with dietary F levels. Femoral ash contents of the 194 and 350 ppm F-treated mink were less than the control values (P<.05). Clinical signs of toxicosis included skeletal and dental lesions, general unthriftiness and hyperexcitability.
(Key Words: Minks, Fluorine, Toxicity.)

Introduction

The effects of excessive dietary fluorine (F) in many farm and laboratory species have been well documented (NRC, 1974, 1980). However, relatively little information is available on the effects of high dietary concentrations of this mineral on mink (Mustela vison). Sodium monofluoroacetate, a rodenticide and predacide containing an organic form of F, has been shown to be highly toxic to mink and European ferrets (Hornshaw et al., 1986). However, in nature, F is usually present in the inorganic form, and various environmental sources of this mineral may contribute to the total F intake of animals.

Chronic fluorosis in mink raised commercially would most likely occur from consumption of drinking water, feed ingredients and(or) mineral supplements with high F content. Inorganic phosphates, such as phosphate rock used in fertilizers, animal by-products containing bone, and mineral supplements often contain high levels of F (NRC, 1980; Shupe and Olson, 1982). In recent years, as phosphorus supplement prices have risen, there has been an increase in F toxicosis traceable to mineral supplements of inferior quality (Shupe and Olson, 1982). Fluorine is cumulative, and when quantities are ingested that exceed the critical level for a species, F toxicosis results (Shupe et al., 1963).

Recently, there has been an increased frequency of inquiries and reports of suspected fluorosis in mink. Because there is little information dealing with the effects of dietary F on mink production, the present study was conducted.

Experimental Procedure

On August 2, 1985, 72 pastel mink kits approximately 3 mo of age were randomly

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TABLE 1. COMPOSITION AND NUTRIENT ANALYSIS OF THE BASAL MINK DIET

Ingredient	Percentage
Commercial mink cereal[a]	20
Fish (cod, haddock and flounder) trimmings	15
Liver (beef)	10
Chicken (whole, ground)	24
Lungs and trimmings (beef)	12
Water	19
Nutrient analysis[b]	
Moisture	67.4
Protein	12.4
Fat	8.4
Ash	2.8
Fluorine, ppm	35

[a]XK-40, XK Mink Foods, Thiensville, WI.

[b]Proximate analysis by Aqualab Inc., Chicago, IL; fluorine analysis by Michigan State Univ., Anim. Health Diagnostic Lab., East Lansing.

allocated into six groups (each containing six males and six females) and placed on diets that contained 0, 33, 60, 108, 194 or 350 ppm supplemental F, as fed. The diets were prepared by dissolving appropriate quantities of NaF[3] in water and mixing the solutions with a basal mink feed (table 1) to yield the desired concentrations of supplemental F. Composites of several grab samples from the drinking water and from the 0, 33, and 350 ppm F-supplemented diets were analyzed[4] for F content. Feed and water were provided ad libitum.

The mink were weighed at the start of the study and every two wk through December 12, 1985, when all but one of the males in each group were pelted. The females and remaining male in each group were retained on their respective diets through the breeding, gestation, and early kit growth periods to assess the effects of the supplemental F on reproduction, kit growth and survival. The females were mated between March 1 and 19, 1986 to the male in their respective dietary groups. When a successful mating was accomplished (as verified by the presence of normal-appearing, motile spermatozoa in vaginal aspirations taken immediately after copulation), the females were given opportunity to mate a second time, either 8 d after the initial mating or the next day if the initial mating occurred late in the breeding season. This procedure is in accordance with customary commercial mink breeding practices. Kits whelped by the females were counted and weighed on the day of birth and at 3 and 6 wk of age. Mink kits begin to consume solid feed at about 3 wk of age and are w[illegible]ed when about 6 wk old. The kit mink w[illegible]d the F-treated diets until they reached 12 [illegible] age in an effort to assess the effects [illegible] treatments on dentition. The deciduous [illegible]n of mink are replaced by the permanent teeth during the second and third month of life (Aulerich and Swindler, 1968).

During August 1986, urine was collected from the adult females for determination of urinary F levels. Urine samples were collected by catching the animals and immediately holding them over a clean beaker, into which they would usually urinate. At the end of the study (on August 19, 1986), blood samples were taken by heart puncture from the adult female survivors for determination of hematologic characteristics (hemoglobin; mean corpuscular hemoglobin; mean corpuscular hemoglobin concentration; mean corpuscular volume; hematocrit; and red, white and differential blood cell counts), serum calcium[5] and serum alkaline phosphatase activity[5]. Fluorine (serum, urine, bone, water and feed) concentrations were determined (AOAC, 1984) by the Michigan State University Animal Health Diagnostic Lab. The mink were then killed and necropsied. Brain, heart, liver, kidney, spleen, thyroid and adrenal gland weights were recorded and a femur was collected for determination of femoral F concentration and ash content.

Because of the variability in the data and small number of replications which could lead to false significance on regression analysis, where appropriate, the data were subjected to analysis of variance and differences ($P<.05$) were determined by Dunnett's method for comparison with a control (Gill, 1978).

Results

The drinking water consumed by the milk contained .345 ppm F. Analysis of the control, 33, and 350 ppm F-supplemented diets yielded F concentrations of 35, 75 and 360 ppm (as

[3]Analytical reagent, Mallinckrodt, Inc., Paris, KY.

[4]Analyses by Michigan State Univ., Anim. Health Diagnostic Lab.

[5]Sigma Chemical Co., St. Louis, MO 63178.

fed), respectively. The F content of the control diet is typical of conventional mink diets (W. L. Leoschke, personal communication).

During the mink growing period, from August to December, there were no significant differences in body weight gains between the control and any of the F-treated groups (table 2). No gross differences were observed in fur quality (hair texture, density, luster or color) among the groups at pelting (December), although the skulls of the males fed the highest concentration of F (350 ppm) were invariably crushed or fractured when placed in a clamping device used to hold the carcass while removing the skin. This fragility of the parietal, frontal and femoral bones was not noted in the control milk nor in the animals fed the lower concentrations of F. The thickness and gross appearance of the skull and femoral bones of the 350 ppm F-treated and control males appeared similar.

As shown in table 3, the levels of dietary F fed to the mink had no apparent adverse effects on breeding, gestation, or whelping. The only noteworthy differences were a marked decrease in survivability of the kits whelped by the females fed 350 ppm supplemental F and a significant increase in the 3- and 6-wk kit body weights in the 60 and 108 ppm F-supplemented groups.

The hematologic characteristics measured in the F-treated animals were all within the normal range for mink (Asher et al., 1976). As shown in table 4, serum F concentrations and alkaline phosphatase activities were elevated ($P<.05$) in mink fed the higher (194 and 350 ppm) F diets. A significant increase in F concentration from control values was observed in urine from mink at all levels of supplemental F (table 4). The concentrations of F in femurs of mink that received 60 ppm, or more, supplemental F were greater ($P<.05$) than the controls, and the ash contents of femurs from mink fed the two highest concentrations of F were reduced ($P<.05$) below controls (table 4).

Survivability of mink fed supplemental F for 382 d was severely impaired only at 350 ppm (table 5). Mean survival time of the mink that died in this group was 316 d. The only clinical signs of toxicosis noted in these animals were a general unthriftiness accompanied by hyperexcitability. A few days before they died, these mink became lethargic and were reluctant to move. No signs of lameness, as reported in other species exposed to toxic concentrations of F (Maplesden et al., 1958; Hillman et al., 1978; Osweiler et al., 1985), were noted in the mink.

At necropsy, it was observed that the sagittal crests of three of the six adult females fed 350 ppm supplemental F were enlarged and(or) deformed, as shown in figure 1. No significant differences were observed in absolute organ weights between the control and any of the F-treated groups. However, when organ weight was expressed as a percentage of brain weight, increases ($P<.05$) over control values in heart and adrenal gland were noted for mink fed the higher concentrations of F (table 5). No signs of dental lesions were observed in the adult mink at necropsy, although many kits whelped and nursed by females that were fed the 194 and 350 ppm F diets and fed the same diets post-weaning until 12 wk of age showed a dark mottling of the teeth, particularly the canine teeth (figure 2). Several of these kits had broken canines and exostotic lesions (figure 2).

TABLE 2. MEAN INITIAL BODY WEIGHTS AND BODY WEIGHT GAINS OF MALE AND FEMALE MINK FED VARIOUS CONCENTRATIONS OF SUPPLEMENTAL FLUORINE FROM AUGUST 2, 1985 TO DECEMBER 12, 1985

	Supplemental fluorine, ppm						
Item	0	33	60	108	194	350	SE[a]
Initial body wt, g							
Males	1,105	1,016	1,146	1,178	1,063	1,110	40
Females	710	777	747	728	699	737	30
Body weight gain, g							
Males	733	652	734	888	602	624	63
Females	332	333	247	353	272	277	47

[a]Standard error of the means; n=six males and six females per treatment.

TABLE 3. EFFECT OF SUPPLEMENTAL FLUORINE ON REPRODUCTIVE PERFORMANCE OF FEMALE MINK AND ON BODY WEIGHTS AND SURVIVAL OF THEIR KITS

Item	Supplemental fluorine, ppm					
	0	33	60	108	194	350
No. ♀ whelped/no. ♀ mated	4/5[a]	2/4[b]	5/5[a]	5/5	6/6	5/5
Mean gestation[c], d	47 ± 2.8	52 ± 3.9	51 ± 2.5	50 ± 2.5	50 ± 2.2	49 ± 2.5
Mean litter size[d]	5.5	5.5	4.4	4.6	6.2	5.8
Mean kit body wt[c], g						
Birth	9.2 ± .3	9.2 ± .5	8.3 ± .3	10.2 ± .3	9.2 ± .2	7.3 ± .3
3 wk	77 ± 4.3	95 ± 6.8	99 ± 48**	104 ± 4.1**	98 ± 3.3	89 ± 10.4
6 wk	2.11 ± 9.9	235 ± 15.9	265 ± 11.7**	245 ± 9.9*	217 ± 7.7	219 ± 24.3
Kit survival, %						
3 wk	86	78	74	100	83	14
6 wk	86	78	68	95	83	14

[a]One female died from injuries unrelated to the diet.

[b]Two females died from injuries unrelated to the diet.

[c]Mean ± standard error.

[d]Total number of kits whelped per number of females that whelped.

*Different from control (P<.05).

**Different from control (P<.01).

TABLE 4. MEAN SERUM ALKALINE PHOSPHATASE ACTIVITY; FEMORAL ASH CONTENT; AND SERUM, URINARY AND FEMORAL FLUORINE CONCENTRATION OF FEMALE MINK FED DIETS THAT CONTAINED VARIOUS LEVELS OF SUPPLEMENTAL FLUORINE

Item	Supplemental fluorine, ppm					
	0	33	60	108	194	350
Serum						
N	4[a]	3[b]	5[a]	4	6	1
Alkaline phosphatase IU/liter[c]	43 ± 8.7	36 ± 11.2	43 ± 8.7	69 ± 9.7	95 ± 7.9*	168
Fluorine, μg/ml[c]	.32 ± .025	.48 ± .090	.44 ± .055	.66 ± .102	.99 ± .137**	.88
Urinary fluorine						
N	4	2	5	2	2	0
μg/ml[c]	6.8 ± 1.12	25.4 ± 4.05**	32.5 ± 3.03**	76.3 ± 2.55**	68.9 ± 1.95	
Femoral fluoride						
N	5	3	5	4	6	4
μg/g ash[c]	1,362 ± 118	3,511 ± 564	5,202 ± 553*	6,425 ± 1,136**	9,177 ± 1,244**	13,706 ± 317**
Ash, % of dry, ash-free bone[c]	57 ± 1.5	56 ± 1.6	57 ± 1.6	60 ± .6	49 ± 2.9*	45 ± 1.3**

[a]One female died from injuries unrelated to the diet.

[b]Two females died from injuries unrelated to the diet.

[c]Mean ± standard error.

*Different ($P<.05$) from control.

**Different ($P<.01$) from control.

TABLE 5. MEAN ORGAN WEIGHTS, SURVIVABILITY AND DAYS TO DEATH OF FEMALE MINK FED VARIOUS CONCENTRATIONS OF SUPPLEMENTAL FLUORINE

Item	Supplemental fluorine, ppm					
	0	33	60	108	194	350
N	5[a]	3[b]	5[a]	4	6	1
Organ wt						
Heart						
g[c]	4.7 ± .20	5.1 ± .26	5.1 ± .19	5.4 ± .20	4.9 ± .89	4.9
As % brain wt	55.4	59.1	60.8	69.4*	64.9*	
Adrenals						
g[c]	.039 ± .0047	.038 ± .0070	.044 ± .0043	.049 ± .0061	.049 ± .0041	.055
As % brain wt	.45	.44	.52	.64	.67*	
Survivability, % survival to end of trial; 382 d	100	100	100	83.3	100	16.7
No. days to death				370		316

[a]One female died of injuries unrelated to diet.

[b]Two females died of injuries unrelated to the diet.

[c]Mean ± standard error.

*Different ($P<.05$) from control.

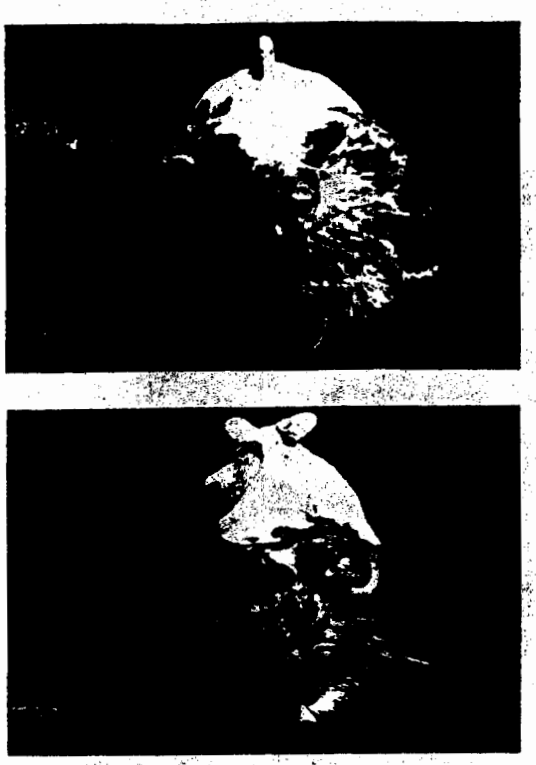

Figure 1. Exostosis of the saggital crests of adult female mink exposed to 350 ppm supplemental dietary fluorine for 382 d.

Discussion

Ingested F retained by animals is primarily sequestered in skeletal and dental tissues, where it induces morphologic changes resulting in weakened, sclerotic bones and hypomineralized and mottled teeth (Kick et al., 1933; Shupe et al., 1963; Osweiler et al., 1985). The observed fragility of the parietal, frontal and femoral bones of the adult males and canine teeth of the kits exposed to the higher concentrations of supplemental dietary F was probably due to a disruption in normal calcification and mineralization of these tissues. The exostotic anomalies of the sagittal crests (figure 1) and jaw bones (figure 2) in some of the mink in the 194 and 350 ppm F-treated groups may be attributable to increased osteoblastic activity, resulting in abnormal mineralization of these bones. Fluorine-induced hyperostosis and dental lesions are commonly observed signs of fluorosis reported in other species (NRC, 1980; Shupe and Olson, 1982).

The F content of the femurs of mink fed supplemental F was directly related to the concentration of dietary F (table 4) and roughly comparable to femoral F levels reported for white-tailed deer (Suttie et al., 1985) and dairy cattle (Shupe et al., 1963) that were fed F-supplemented diets. It would appear that femoral F levels might, therefore, serve as a useful index of the degree of F exposure in mink. The ash content of the femurs was reduced ($P<.05$) only in the mink fed the higher (194 and 350 ppm) F diets. These results are in agreement with the findings of Kick et al. (1933), which indicated a reduction in the percentage of bone ash in the femurs of swine fed diets high in F.

The serum F concentrations and alkaline phosphatase activities of the mink were also roughly related to the amounts of F in the diet, although significant increases over control values in both measures were not observed in mink fed less than 194 ppm supplemental F. Burnell et al. (1986) also reported significant increase in serum F and alkaline phosphatase concentrations in swine fed 132 ppm or more of dietary F. However, because the concentration of F in sera of different species administered the same dose of F can vary markedly (Patterson et al., 1977), care should be exercised in making comparisons among species with respect to F levels in the blood.

The major excretion route of F from the body is via urine (Shupe and Olson, 1982; Osweiler et al., 1985). The rate of excretion is influenced by the type of F ingested, rate of absorption, level of F saturation of osseous tissues, age and condition of the animal, as well as other factors (NRC, 1974). Fluorine concentrations in the urine of the F-treated mink were comparable to concentrations (16 to 68 ppm) cited by Maplesden et al. (1958) for cattle with advanced fluorosis.

Fluorine fed to mink as NaF at up to 350 ppm for 7 mo prior to breeding did not produce adverse effects on reproduction or lactation. The high (86%) kit mortality that occurred in the 350 ppm F-treated group did not appear to be related to the lactational performance of the females, because there was no significant difference from control values in body weight of the F-treated kits at 3 wk postpartum (table 3). Chronic fluorosis has generally been found to have no direct adverse effects on the reproductive performance of animals (Osweiler et al., 1985). The significant increase in kit body weight at 3 and 6 wk of age over control values noted in the 60 and 108

ppm F-supplemented groups suggests that these levels of F supplementation may have had a beneficial effect on early kit growth. Further studies should be conducted to con[illegible] observation.

The long latent period that occurred be-

Figure 2. Dental lesions in mink kits associated with in utero, lactational and dietary exposure to supplemental fluorine. A) Mottled canines of kit exposed to 350 ppm fluorine. B) Slight mottling of upper canines and broken lower canines of kit exposed to 350 ppm fluorine. C) Exostotic lesions in the area of the canine alveoli in kit exposed to 194 ppm fluorine.

tween exposure to the F-treated diets and the development of the clinical signs and mortality (table 5) in the mink is in agreement with accounts of chronic fluorosis in other species (NRC, 1942).

The importance of the increased heart and adrenal weight (when expressed as a percentage of brain weight) in the mink fed the higher F-supplemented diets (table 5) is unknown and may reflect subtle secondary effects of F toxicosis. No primary lesions attributable to chronic fluorosis have been consistently found in soft tissues or organs (NRC, 1980; Shupe and Olson, 1982). However, the F ion is reactive, and ingestion of excessive levels for extended periods may indirectly have had an effect on these organs.

Based on the numerous reports in the literature, it is evident that within a species the toxicity of dietary F depends upon the age of the animals, type of compound ingested, dosage, duration of exposure, and the concentration of other minerals, vitamins, and other dietary constituents. Thus, it is difficult to estimate F tolerance by mink from the results of this study. The data indicate that prolonged dietary exposure of mink to 100 ppm F, as NaF, caused increased heart weight. Two F levels >200 ppm resulted in skeletal and dental lesions in immature mink, and 350 ppm produced high mortality in both kits and adults. These findings suggest that, compared with other species of livestock (NRC, 1974; 1980), mink are relatively tolerant of dietary F.

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