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EFFECTS OF FLUORIDE ON SCREECH OWL REPRODUCTION: TERATOLOGICAL EVALUATION, GROWTH, AND BLOOD CHEMISTRY IN HATCHLINGS

(Fluoride; *Otus asio*; teratology; reproduction; growth; blood chemistry)

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SUMMARY

The effects on reproduction in screech owls (*Otus asio*) of chronic dietary sodium fluoride administration at 0, 40, and 200 ppm were examined. Fluoride at 40 ppm resulted in a significantly smaller egg volume, while 200 ppm also resulted in lower egg weights and lengths. Day-one hatchlings in the 200 ppm group weighed almost 10% less than controls and had shorter crown–rump lenghts. No gross abnormalities were apparent. Skeletal clearing and staining revealed significantly shorter tibiotarsus lengths in the 40 ppm and 200 ppm groups and a shorter radius-ulna length in the 200 ppm group. By 7 days of age, body weights and lengths did not differ from controls, but the tibiotarsus in the 200 ppm group remained shorter. No significant differences were detected in hematocrit, hemoglobin, plasma calcium or alkaline phosphatase. Plasma phosphorus levels were higher in the 40 ppm group than in controls.

These results, in combination with the findings of Pattee et al. [25], revealed significant impairment of overall reproduction, suggesting that sodium fluoride could cause slight to moderate reproduction disorders in owls in fluoride-polluted areas.

INTRODUCTION

Fluoride is present in the natural environment, constituting between 0.06% and 0.09% by weight of the upper layers of the lithosphere [1,2]. It is also a major environmental pollutant, entering from such sources as steel/iron production, coal power production, aluminum smelting, and phosphate fertilizer production [3]. Chronic fluoride toxicosis has often been reported in cattle [4,5]. Structural bone changes may also occur under chronic exposure conditions [6]. Fluorides at 100 ppm or greater in drinking water caused impaired reproduction and retarded growth in mice [7] and altered bone, liver, and kidney weights as well as altered ascorbic acid metabolism in growing chickens [8]. Yolk-sac injection studies with chicken eggs showed sodium fluoride to be nearly 10 times more toxic than aluminum chloride

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[9] suggesting that fluoride pollution in the vicinity of aluminum smelters could be of equal or greater concern than aluminum pollution. Chick embryo limb-culture studies have revealed that fluoride can influence the rate of bone mineralization [10]. High levels of fluoride (700–1000 ppm) in the diet of chickens resulted in a reduction in egg size and increased mortality [11].

There is little data on the effects of fluorides on wild birds. However, in the vicinity of aluminum smelters, fluoride emissions were reported to result in elevated yolk and albumen fluoride levels in eggs of chickens and wild birds [12]. It has been suggested that house martins (*Delichon urbica*) [13] and tawny owls (*Strix aluco*) [12] near an aluminum smelter were adversely affected, with evidence of impaired reproduction in the owls. Sodium fluoride in the diet of captive American kestrels had few adverse effects on reproductive performance at 50 ppm [14]. The present study was undertaken to assess the overall effect of sodium fluoride on reproduction in screech owls (*Otus asio*). This paper reports the portion of this study that was designed to assess potential teratogenic effects, growth, and blood chemistry in 1- and 7- day-old hatchlings derived from breeding pairs that were subjected to chronic dietary fluoride.

METHODS

60 Breeding pairs of screech owls from a captive colony at the Patuxent Wildlife Research Center were randomly assigned to 1 of 3 treatments: 0, 40, or 200 ppm of sodium fluoride in the diet. Birds were housed in outdoor pens (14.8 × 3.2 × 2.0 m high) equipped with nest boxes, perches and food shelters. Birds were fed a commercial bird-of-prey diet (Nebraska Brand, North Platte, Nebraska) mixed with the appropriate concentration of fluoride, starting on 30 November 1981. Water was provided ad lib. Nest boxes were monitored daily. Eggs were numbered as they were laid and the date and time of laying was recorded. The third egg laid was selected from 13–14 different nests for each treatment. The entire egg was weighed, its volume was determined by water displacement, and length and width were measured. Hatching was monitored, and unhatched eggs were removed. As soon as hatching occurred, clean food without fluoride was provided, consisting of 1-day-old chicks (*Gallus gallus*); previous experience has shown that young birds of prey do not survive well on the commercial bird-of-prey diet.

Hatchlings were selected on the day of hatching from 7 nest boxes from each treatment group, providing a total of 23 controls, 24 from the 40 ppm fluoride group, and 18 from the 200 ppm fluoride group. Each hatchling was examined for overt teratogenic defects, weighed and measured for crown-rump length. Blood from jugular veins was collected in ammonium heparinized caroway tubes. Hematocrits were recorded and hemoglobin was determined by the cyanomethemoglobin method. Plasma was used for determinations of uric acid [15] which is the main excretory product in adult birds [16] and in embryos past 8 days

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of development [17]. Plasma alkaline phosphatase (EC 3.1.3.1) [18] was determined spectrophotometrically by micromethods, in conjunction with a centrifugal analyzer (Centrifichem 500, Union Carbide Corp.). Plasma calcium [19] and phosphorus [20,21] were also determined in conjunction with the centrifugal analyzer. Alkaline phosphatase, calcium, and phosphorus are all involved in bone mineralization and can be altered after fluoride exposure [22,23]. Soft tissues were carefully removed and examined and the hatchlings were then cleared and stained with alizarin red S for skeletal examination by the method of Karnofsky [24]. The cleared and stained radius-ulna of the right wing and the tibiotarsus of the right leg were measured. Similar morphological, hematological, and plasma chemistry measurements were made in 7-day-old nestling owls.

Egg weight and volume, morphological measurements, hematocrit, hemoglobin and plasma chemistries were compared by one-way analysis of variance ($P < 0.01$) and Duncan's multiple range test ($P < 0.05$) when comparison was made among treatment groups. Control values between day 1 and day 7 were compared by the t-test ($P < 0.05$).

TABLE I
EFFECTS OF SODIUM FLUORIDE ON EGG SIZE AND GROWTH IN NESTLING SCREECH OWLS

	Controls	NaFl, 40 ppm	NaFl, 200 ppm
Egg weight (g)	18.2 ± 1.1[a]	17.4 ± 1.6	17.1[b] ± 1.2
Egg volume (ml)	18.1 ± 1.4	17.1[b] ± 1.2	17.0[b] ± 1.1
Egg length (mm)	36.3 ± 1.1	35.6 ± 1.2	35.0[b] ± 0.9
Egg width (mm)	30.9 ± 1.1	30.5 ± 1.3	30.1 ± 0.8
Body weight (g)			
Day 1	13.3 ± 1.4	12.7 ± 1.5	12.1[b] ± 1.1
Day 7	61.9[c] ± 10.6	62.6 ± 3.8	61.6 ± 11.9
Crown-rump length (mm)			
Day 1	63.7 ± 1.5	64.2 ± 2.2	62.8[d] ± 2.2
Day 7	95.1[c] ± 5.2	98.8 ± 3.2	96.8 ± 6.6
Radius-ulna length (mm)			
Day 1	8.5 ± 0.3	8.3 ± 0.4	8.2[b] ± 0.3
Day 7	22.5[c] ± 2.8	21.1 ± 1.2	20.9 ± 2.6
Tibiotarsus length (mm)			
Day 1	12.1 ± 0.6	11.7[b] ± 0.5	11.4[b] ± 0.4
Day 7	31.1[c] ± 1.5	30.0 ± 1.7	28.3[b] ± 2.4

[a]Means ± SD.
[b]Significantly different from controls by one-way ANOVA ($P < 0.01$) and Duncan's multiple range test ($P > 0.05$).
[c]Significantly different from corresponding one-day-old controls by Student's t-test ($P > 0.05$).
[d]Significantly different from 'NaFl, 40 ppm' group by one-way ANOVA ($P > 0.01$) and Duncan's multiple range test ($P > 0.05$).

RESULTS AND DISCUSSION

Sodium fluoride (40 ppm) in the diet of screech owls resulted in a significantly smaller egg volume, and at 200 ppm resulted in a significantly smaller egg weight and volume (Table I). Hatching success was found to be impaired by nearly 40% in the 200 ppm group, as discussed by Pattee et al. [25]; the reduction in hatching success was believed to be largely due to reduced fertility. There was no difference in the number of eggs laid after treatment. Day-one hatchlings in the 200 ppm group weighed 9% less than controls and had slightly, but significantly, shorter crown-rump lengths than the 40 ppm group (Table I). There were also small but significant differences in bone lengths as detected by measuring cleared and alizarin red S-stained radius-ulna and tibiotarsus lengths; the radius-ulna was significantly shorter in the 200 ppm group compared to control, while the tibiotarsus was significantly shorter in both the 40 and 200 ppm groups. There was no evidence of teratogenic effects in either treatment group. By 7 days of age there were no longer any significant differences between control and treatment groups in body weight,

TABLE II
EFFECTS OF SODIUM FLUORIDE ON BLOOD CHEMISTRY OF 1- AND 7- DAY-OLD SCREECH OWLS.

	Controls	NaFl, 40 ppm	NaFl, 200 ppm
Hematocrit (%)			
Day 1	22.5 ± 4.4	24.1 ± 3.8	24.2 ± 4.2
Day 7	30.7[c] ± 3.6	32.3 ± 2.9	32.1 ± 4.3
Hemoglobin (g/dl)			
Day 1	7.6 ± 1.4	8.1 ± 1.3	7.9 ± 1.7
Day 7	8.7[d] ± 1.9	8.2 ± 0.5	8.1 ± 1.6
Plasma calcium (mg/dl)			
Day 1	8.1 ± 1.2	8.2 ± 0.8	7.6 ± 0.6
Day 7	11.8[c] ± 3.8	10.9 ± 1.3	12.3 ± 6.3
Plasma phosphorus (mg/dl)			
Day 1	4.7 ± 1.7	3.8 ± 1.7	4.5 ± 1.4
Day 7	7.4[c] ± 1.0	9.5[b] ± 2.8	7.6 ± 1.4
Plasma uric acid (mg/dl)			
Day 1	7.7 ± 2.5	8.0 ± 5.1	8.4 ± 5.4
Day 7	13.9[c] ± 4.8	17.3 ± 10.8	17.0 ± 5.6
Plasma alkaline phosphatase (U/l)			
Day 1	313 ± 77	325 ± 111	283 ± 107
Day 7	406[c] ± 109	344 ± 185	475 ± 130

[a]Mean ± SD.
[b]Significantly different from untreated controls by one-way ANOVA ($P < 0.01$) and Duncan's multiple-range test ($P < 0.05$).
[c]Significantly different from corresponding 1-day-old controls by Student's t-test ($P < 0.01$).
[d]Significantly different from corresponding 1-day-old controls by Student's t-test ($P < 0.05$).

crown-rump length, or radius-ulna length. However, the tibiotarsus in the 200 ppm group remained significantly shorter than control.

No significant differences were detected in hematocrit, hemoglobin, plasma calcium, phosphorus, uric acid or alkaline phosphatase in 1-day-old hatchlings (Table II). At 7 days of age, plasma phosphorus was significantly greater in the 40 ppm group than in controls. Developmental age related differences were apparent when day-one controls were compared with 7-day-old controls. Mean weight increased by nearly 5-fold and crown-rump length by 1.5-fold by day 7. Hematocrit was 36% greater and hemoglobin 14% greater in 7-day-olds. Plasma calcium was 46% higher, plasma phosphorus 57% higher, uric acid 81% higher, and plasma alkaline phosphatase activity 30% greater in 7-day-olds compared to 1-day-old controls.

Other studies involving yolk-sac injections of sodium fluoride into chicken eggs did not report teratogenic effects [9,26], in agreement with our findings. Fluoride (150 ppm) in the diet of 1-day-old chickens did not result in differences in body weight but caused alterations in organ weights and ascorbic acid metabolism. Fluoride in the drinking water of mice at 100 ppm or more caused infertility and stillbirths, as well as retarded growth in weanling mice [7]. Embryonic death, growth retardation, and incomplete ossification without gross abnormalities were reported in rats [27].

In conclusion, this study is one of the few reproductive studies designed to assess the effects of fluoride in birds, and in combination with the findings of Pattee et al. [25] suggests that sodium fluoride could cause slight to moderate reproductive impairment in owls nesting in polluted areas. It is possible that continuous exposure to fluoride after hatching could cause further growth retardation in nestlings, similar to that reported in young mammals.

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