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FLUOROSIS RISKS TO RESIDENT HISPID COTTON RATS ON LAND-TREATMENT FACILITIES FOR PETROCHEMICAL WASTES

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ABSTRACT: Land-treatment of petroleum wastes is a widely used industrial practice, yet there has been no comprehensive evaluation of the long-term risks to human or terrestrial ecosystems from such practices. We evaluated cotton rat (*Sigmodon hispidus*) populations on three sites in Oklahoma (USA) that historically used land-treatment for disposal of various petroleum wastes (July 1995–March 1997). Average concentrations of fluoride in soil from these sites ranged from 878 to 4317 mg/kg. A census of resident cotton rats on land-treatment sites revealed a high incidence (40% overall) of dental lesions compared to reference populations (<1% dental lesions). During winter there was a 34% to 65% increase compared to summer in frequency of dental lesions in cotton rats on two of the three land-treatment sites. Incidence of dental lesions on two land-treatment sites was greater (9–16%) in female cotton rats compared to males. Cotton rats from land-treatment sites had higher concentrations of fluoride in bone and greater severity of dental lesions compared to reference animals. Dental lesions were considered to be most consistent with dental fluorosis because of elevated fluoride in bone. Neither concentration of fluoride in soil nor level of fluoride in bone was a good predictor of severity of dental lesions in cotton rats on land-treatment sites.

Key words: Cotton rat, fluoride, fluorosis, dental disease, petrochemical contamination, *Sigmodon hispidus,* wildlife toxicology.

INTRODUCTION

Excessively high concentrations of fluoride from both natural and industrial sources are a continuing environmental health problem for human and wildlife populations throughout the world (WHO, 1984). Compared to human populations, wildlife and domestic livestock tend to display more severe signs of fluoride toxicity due to their higher consumption and reliance on natural vegetation and water in areas with high concentrations of fluoride (Dwivedi et al., 1997). Because of their greater exposure risks, small mammals have been useful biomonitors for fluoride contamination. For example, Boulton et al. (1994a) reported dental lesions in field voles (*Microtus agrestis*) and bank voles (*Clethrionomys glareolus*) in association with environmental exposure to contaminated soils around aluminum smelters, fluorochemical industrial works, and mine tailings. A high incidence of dental lesions consistent with dental fluorosis has been reported in cotton rats (*Sigmodon hispidus*) on petroleum waste sites (Paranjpe et al., 1994).

Many refineries of crude petroleum use hydrofluoric acid in the alkylation process for production of higher-octane fuels. Waste products generated from this process are thought to be the most likely source of fluoride in soils where such wastes are land-treated for disposal. This managed technology involves controlled application of waste onto or into soil for the purpose of biodegradation of organic wastes, immobilization of inorganics, and avoiding bioaccumulation of hazardous compounds (Loehr and Malina, 1986). Over 50% of the hazardous wastes generated by the petroleum industry have been disposed of through land-treatment operations (American Petroleum Institute, 1984), yet no comprehensive assessment of their impact on terrestrial ecosystems has been completed. Initial surveys by Paranjpe et al. (1994) and McMurry (1993) suggested that fluoride toxicity risks might be high for land-treatment sites. New techniques to monitor environmental contamination that are biologically based have the potential to identify unknown problems. Substantiation that fluorosis is impacting wildlife on these sites could influence remediation and future land-treatment of these wastes. In addition, new techniques to monitor environmental contamination that are biologically based have the potential to identify unknown problems.

We explored the hypothesis that various sites where treatment of petrochemical wastes was facilitated by land-treatment techniques pose a fluorosis risk with seasonal variation to resident populations of cotton rats. We also explored the hypothesis that soil fluoride concentrations are predictive of cotton rat bone concentrations as well as incidence and severity of dental fluorosis. To test these hypotheses, we surveyed resident cotton rat populations for incidence and severity of dental lesions, accumulation of fluoride in bones, and soil concentrations on three land-treatment facilities and reference sites during summer and winter in Oklahoma (USA).

MATERIALS AND METHODS

Site selection

We selected three individual units (herein referred to as land-treatment units 1, 2, and 5) in Oklahoma for study. Each unit (unit 1 at 34°54.18′N, 98°12.24′W; unit 2 at 35°53.79′N, 96°2.94′W; and unit 5 at 35°54.00′N 96°2.54′W) was inhabited by a population of cotton rats that resided directly on the land-treatment waste site and another matched-reference population that resided in similar habitat <16 km from the land-treatment site. All land-treatment sites were characteristic of a disturbed prairie ecosystem, with early successional species dominating the vegetation community. Reference sites were selected based on a visual assessment of similarity of vegetation structure and composition with their paired land-treatment site; sites were also similar with respect to topography.

These sites were chosen based on historic but not current use for land-treating oil refinery wastes, vegetation adequate to provide suitable cover to support a resident small mammal population, and accessibility to researchers. All sites were previously or currently owned by corporations and only one land-treatment site was a designated Superfund Waste Site (land-treatment unit 1, Oklahoma Refining Company, Cyril, Oklahoma) on the U.S. Environmental Protection Agency's National Priorities List. Treatment of petroleum wastes was discontinued on all sites during the early to mid 1980's. Little or no prior information existed as to duration, loading rates, and types of refinery waste products incorporated into soils on these sites.

Population assessment

Sampling occurred (July 1995–March 1997) on all test units across two seasons, winter (January–March) and summer (July–October), to evaluate whether season influenced susceptibility or sensitivity to fluoride contamination. A permanent 70 by 70 m trapping grid with 64 trap stations evenly spaced at 10 m intervals was established on each land-treatment site and its matched-reference site. One Sherman live trap (H.B. Sherman Trap Co., Tallahassee, Florida, USA) was placed at each trap station and baited with rolled oats. Cotton rats were censused for 8 periods (4 during each season) with 3 wk separating each period within a season. Traps were set in the evening and checked between 0600 and 1000 hr for the next 4 days. Cotton rats were toe clipped with a unique identification number and information recorded on capture location, species, sex, body weight, presence or absence of dental lesions, female reproductive status (pregnant, lactating, vaginal orifice perforate or closed), and male reproductive status (scrotal or non-scrotal). We used a slight modification of the suggested method of Stafford and Stout (1983) for aging cotton rats: <50 g = juvenile, 50–79.9 g = subadult, and >80 g = adult.

Cotton rat incisors are normally deep, glossy, and yellow-orange in color. Presence of dental lesions was indicated by a lack of pigmentation on the upper and or lower incisors, which varied in degree from either complete (totally white) or incomplete (striations of white) loss of pigmentation. Departures from normal glossy appearance are described as chalky, indicating a loss of glossy surface and slight pitting. During each population census, we inspected the incisors of each cotton rat that was captured and recorded the appearance of abnormal tooth color.

Laboratory evaluation

Twelve adult cotton rats (6 males, 6 females) were removed from each land-treatment and reference site at the end of the last trapping period in summer and again in winter. Animals were returned to Oklahoma State University (Stillwater, Oklahoma, USA) campus for further processing within 48 hr after capture. Severity of dental lesions in each cotton rat returned to the laboratory was determined under a magnifying glass using a slightly modified method of Boulton et al. (1995): 0 = normal incisor color, 1 = lower incisor mildly striated; 2 = lower and upper incisor mildly to moderately striated; 3 = lower incisor severely striated to white and chalky, upper incisor mildly striated, 4 = lower incisor white and chalky, upper incisor moderate to severely striated, 5 = lower and upper incisor white and chalky.

For soil sampling, land-treatment sites were sub-divided into six sample stations and matched-reference sites were sub-divided into two sample stations. At each sample station, a composite sample of soil was made from three random surface (0 to 3 cm) samples of soil obtained from within a 1-m radius of the sample station. Composite soil samples were mixed and sealed in acid-washed glass jars and transported to the laboratory for analysis. Total soil fluoride was analyzed for each composite soil sample by using fusion methods as described by McQuaker and Gurney (1977) and Schroder (1998). Briefly, 0.5 g of soil was placed in a 100 ml nickel crucible and slightly moistened with deionized distilled water. Concentrated NaOH (19M) was added to the sample or an empty crucible (blank control) and fused in a muffle furnace at 600 C. The fusion cake was dissolved in deionized distilled water and neutralized with concentrated HCl to pH 8–9. The cooled sample was then transferred to a 100 ml volumetric flask, diluted to volume, and filtered through a 0.45 membrane filter. Sample digest (5.0 ml) was combined with 5.0 ml of TISAB II and fluoride was determined with a combination ion selective electrode (Orion 960900, Orion Research Incorporated, Beverly, Massachusetts, USA). Values were reported as mg/kg on a dry weight basis. All blanks were below the detection limit of 0.025 mg/kg of fluoride.

Both humeri were removed from each cotton rat and adhering muscle tissue teased away. Humeri were dried to constant weight by lyophilization. Fat was removed from bones by soaking over night in petroleum ether. Bones were dried, wet digested with 5 ml trace metal grade HNO_3 (16N) in a 25 ml Teflon beaker, and refluxed on a hotplate at 95 C for 1 hour. A 1 ml aliquot was diluted to 5.0 ml with deionized distilled water and combined with 5.0 ml of TISAB II for fluoride determination with an combination ion selective electrode (Orion 960900, Orion Research Incorporated, Beverly, Massachusetts, USA) and reported as mg/kg on a dry weight basis. Aliquots of HNO_3 bone digest were analyzed for heavy metals and quantified by inductively coupled plasma emission spectroscopy (ICP; American Resources Laboratory, Fisons Maxim, Boston, Massachusetts, USA): Samples were analyzed for Ba, Cr, Pb, Sr, Ti, and Zn concentrations and reported as mg/kg on a dry weight basis (Schroder, 1998). Blanks containing just HNO_3, spiked samples, and standard reference materials were used to evaluate laboratory procedures.

Analysis

All data were tested for normality (PROC NORMAL; SAS, 1994) and homogeneity of variances (Levines test; Steel and Torrie, 1980). Data not meeting these assumptions were transformed (arcsine square-root or log normal) prior to further statistical analyses.

Bone concentrations of fluoride and heavy metals were analyzed using a 2×2 factorial treatment structure (two treatments, land-treatment and reference sites; two seasons, summer and winter) in a randomized complete block design (each unit as a block) to evaluate whether land-treatment of petrochemical wastes was associated with increased risk of fluorosis for resident cotton rat populations. Comparisons were made using PROC MIXED (SAS, 1994) with sources of variation distributed among the main factor effects (treatment and season) and the interaction terms (land-treatment unit by treatment interaction, season by treatment interaction, and land-treatment unit by season interaction); we used the treatment by land-treatment unit by season interaction as the error term in our analysis. If there were no significant interaction terms, main effects were compared with the PDIFF option for the LSMEANS statement. Significant interaction term effects were compared using the SLICE option for the LSMEANS statement. Satterthwait's approximation was used in calculating degrees of freedom for the error term.

Chi-squared analysis (PROC FREQ; SAS, 1994) was used to determine if population parameters were significantly different for presence or absence of dental fluorosis. A logistic regression model (PROC LOGISTIC; SAS, 1994) examined the relationship between body weight and presence or absence of dental fluo-

TABLE 1. Prevalence of dental fluorosis in populations of wild cotton rats surveyed by mark-recapture census. Three land-treatment units consisted of a land treatment site and matched reference site surveyed in Oklahoma. Number of cotton rats surveyed (*n*) and percentage (%) of cotton rats with dental fluorosis are indicated for season and sex categories.

	Land treatment unit											
	Unit 1				Unit 2				Unit 5			
	Reference		Land-treatment		Reference		Land-treatment		Reference		Land-treatment	
Category	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Season												
Summer	179	0	91	73[b]	148	3	263	10	198	2	243	51[b]
Winter	166	0	60	98	61	0	191	13	139	0	100	84
Sex												
Female	184	0	76	90[c]	116	2	259	12	147	1	194	64[d]
Male	161	0	75	74	93	2	195	10	190	1	149	55
Total[a]	345	0	151	83*	209	2	454	11*	337	1	343	61*

* Land-treatment significantly different ($P < 0.0001$) from reference site for that unit.
[a] Land-treatment sites differ across land-treatment units ($P < 0.0001$).
[b] Summer significantly different ($P < 0.001$) from winter for that unit.
[c] Females significantly different ($P < 0.009$) from males for that unit.
[d] Females marginally different ($P < 0.06$) from males for that unit.

rosis for census data. A normal regression model (PROC PROBIT; SAS, 1994) was used to describe the relationship between severity of fluorosis and bone concentrations of fluoride. An analysis of variance (PROC CATMOD; SAS, 1994) was used to evaluate whether land-treatment, season, or sex were associated with increased risk of severity of dental fluorosis. Statistical significance for all hypotheses tests were set *a priori* at $P \leq 0.05$ and all means are reported as mean (±SE).

RESULTS

Soils from both reference and land-treatment sites had detectable levels of fluoride. All land-treatment sites had mean concentrations of fluoride in soil that far exceeded the mean of three matched-reference sites, which was 121 mg/kg. Soils of land-treatment unit 5 had the highest concentrations of fluoride in soils with a mean of 4,317 mg/kg; range (6 composite samples) of 2,544 to 6,610 mg/kg. Concentration of fluoride in soil of land-treatment unit 1 averaged 2,672 mg/kg with a range between 1,001 to 5,077 mg/kg. Compared to the two other land-treatment sites in this study, land-treatment unit 2 had considerably lower concentrations of fluoride in soil with a mean of 878 mg/kg; range between 669 to 1,100 mg/kg.

Analysis of cotton rat populations revealed an extremely high ($P < 0.0001$) prevalence of dental lesions in animals from land-treatment sites compared to those from reference sites. Overall, 40% of 948 cotton rats caught in populations from land-treatment sites had dental lesions, whereas only 0.8% of 884 cotton rats from populations at reference sites had lesions characteristic of dental fluorosis (Table 1). Land-treatment sites differed significantly ($P < 0.0001$) among each other in prevalence of dental lesions in cotton rat populations. Prevalence was greatest in land-treatment unit 1 (83%) and lowest in land-treatment unit 2 (11%). Prevalence of dental lesions in populations of cotton rats was significantly ($P < 0.001$) influenced by season in two of the three land-treatment sites surveyed. On land-treatment unit 1 we observed a 34% increase in prevalence of lesions in cotton rat populations from summer to winter. On land-treatment unit 5 there was a 65% increase in prevalence from summer to winter.

Dental lesions in cotton rats were significantly more prevalent in females than males on land-treatment sites, especially on land-treatment unit 1 ($P < 0.009$) and marginally ($P < 0.06$) on land-treatment unit 2 (Table 1). Differences between females and males for prevalence of dental lesions was greatest during summer on land-treatment sites with 84% females compared to 64% males from land-treatment unit 1 and 60% females compared to 39% males on land-treatment unit 5. We found a significant correlation ($P < 0.013$) between age of cotton rats and presence of dental lesions. However, this correlation was largely attributed to land-treatment unit 5 ($P < 0.001$), where 70% adults, 58% sub-adults, and 45% of juveniles were positive for dental lesions.

Cotton rats returned to the laboratory for more detailed examination of dental lesions indicated severity of lesions differed significantly ($P < 0.0001$) among land-treatment units (Fig. 1). Based on dental severity scores (0 = none to 5 = severe lesions) cotton rats from the three reference sites had an average severity score <0.13; cotton rats from land-treatment sites had average severity scores ranging from 0.88 on unit 2 to 3.1 for animals on land-treatment unit 1. Average severity scores in cotton rats on land treatment sites differed significantly ($P < 0.0002$) between seasons with a 72% increase in severity from summer to winter.

Concentrations of fluoride in bone of cotton rats demonstrated a significant treatment by unit interaction ($P < 0.0001$). Mean concentrations of fluoride in bone of cotton rats from land-treatment sites was greater ($P < 0.0001$) than those from matched-reference sites for all land-treatment units (Fig. 1). Fluoride concentrations were greatest in animals from land-treatment unit 5, which was 1.8- to 2-fold greater than levels observed in animals from land-treatment units 1 and 2. We observed a significant seasonal effect ($P < 0.0001$) in bone fluoride as well, with cotton rats on land-treatment sites accumulating 89% more fluoride in winter compared to summer.

Regression analysis revealed a positive relationship ($P < 0.0001$) between concentration of fluoride in bone and severity of dental lesions in cotton rats, indicating that in general, high fluoride leads to greater severity of dental fluorosis (Figure 2). However, this relationship was not strong, especially for animals with severity scores of 4 or 5. The ability to predict severity of dental lesions from concentrations of fluoride in bone was poor as indicated by the wide variation in Figure 2.

Concentrations of Ba and Cr in bone of cotton rats did not differ by treatment with an overall mean ($n = 144$) concentration of 38 ± 1.6 mg/kg for Ba and 1.3 ± 0.34 mg/kg for Cr. Concentrations of Zn in bone demonstrated a significant treatment by season interaction ($P < 0.008$; Fig. 1) with 23% greater concentrations of Zn in cotton rats from land-treatment sites compared to reference animals in winter. Bone concentrations of Sr ($P < 0.0001$), Pb ($P < 0.02$), and Ti ($P < 0.02$) had a significant treatment by land-treatment unit interaction. Least squared means revealed that this significant interaction was attributed to land-treatment unit 1 which differed from its matched-reference site for concentrations of Sr, Pb, and Ti in bone (Fig. 1). Concentrations of these metals in bones of cotton rats from land-treatment unit 1 were higher compared to reference site 1 with 4-fold higher levels of Sr ($P < 0.0001$), 2.4-fold higher levels of Pb ($P < 0.0001$), and 2.3-fold higher levels of Ti ($P < 0.002$).

DISCUSSION

Population surveys for dental lesions in rodents residing on waste sites contaminated with fluoride are limited and most published reports of fluoride toxicity have dealt with documenting concentrations in soil, plant, and animal tissue. Few studies have attempted to document consequences of exposure to excessive levels of fluoride in soil for wild rodent communities. Exceptions include reports of Paranjpe et al. (1994) for cotton rats residing on petroleum waste sites and Boulton et al. (1994a) for field voles and bank voles re-

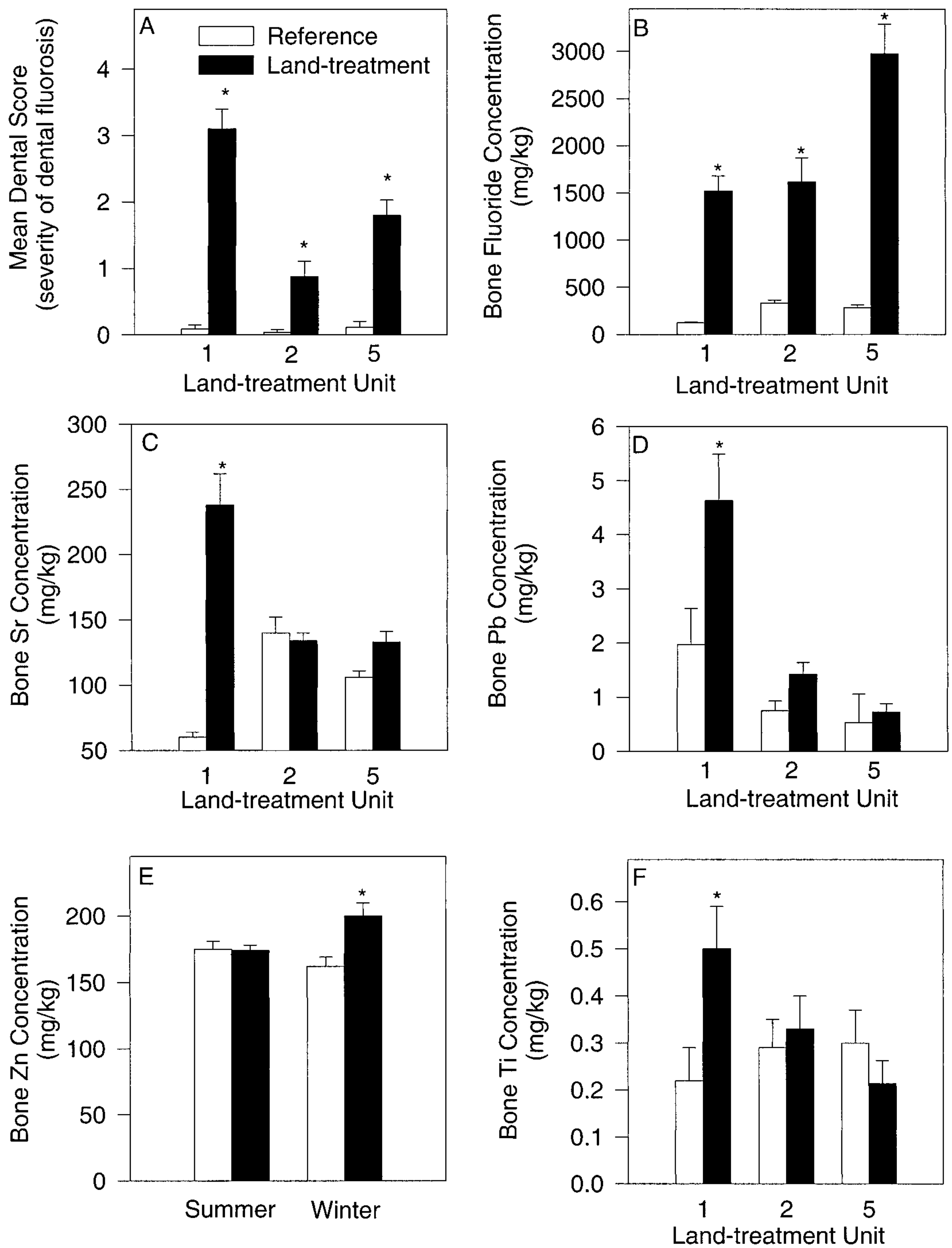

FIGURE 1. Mean (±SE) severity scores of dental fluorosis for incisors (0–5) and concentrations of contaminants in bone (mg/kg) of adult cotton rats collected from land-treatment sites (n = 3) and matched-reference sites (n = 3) in Oklahoma. For graphs A (mean severity of fluorosis), B (bone fluoride concentration), C (bone Sr concentration), D (bone Pb concentration), and F (bone Ti concentrations), values were pooled across seasons (summer and winter) and differences between land-treatment and reference sites within a land-treatment unit are indicated by an asterisk ($P < 0.05$). For graph E (bone Zn concentrations), values were pooled across treatments (land-treatment and reference) and differences between land-treatment and reference sites for season are indicated by an asterisk ($P < 0.05$).

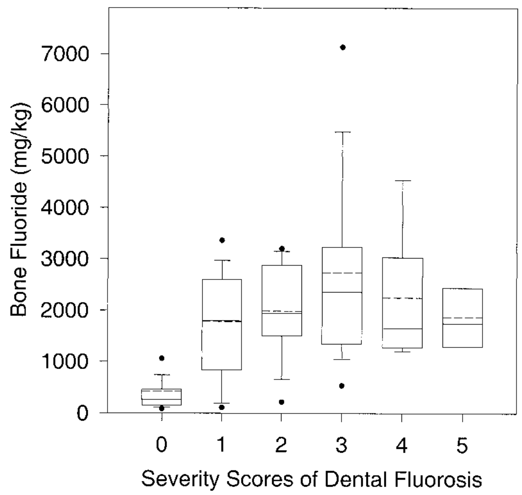

FIGURE 2. A Tukey's box plot depicting the relationship between severity scores of dental fluorosis and concentration of fluoride in bone of adult cotton rats (n = 144) collected from land-treatment sites (n = 3) and matched-reference sites (n = 3) in Oklahoma. The box represents the 25th and 75th percentiles of the column while the capped bars represent the 5th and 95th percentiles. The solid line within the box is the 50th percentile; the dashed line is the arithmetic mean. Data outside of the 5th and 95th percentiles are depicted as a black circle.

siding on fluoride-contaminated soils in Europe.

Results of this study clearly establish that cotton rats have a substantial risk of developing dental lesions consistent with dental fluorosis in habitats where petroleum wastes were land-treated. Less than 1% of cotton rats from reference sites showed any sign of fluorosis and those that did had mild dental lesions. Taken alone, this indicates that cotton rats are a good in situ biomonitor for fluoride contamination at the levels experienced on the three land-treatment sites in this study and concurs with previous studies (Paranjpe et al., 1994).

Cotton rats on the three land-treatment sites demonstrated a strong seasonal influence on uptake of fluoride and prevalence and severity of dental fluorosis. This seasonal relationship was evident in cotton rats from the field census as well as those returned to the laboratory with greater risk associated with winter. Wang et al. (1995) noted a similar seasonal pattern of fluoride toxicity in Baotou goats, which they attributed to higher rates of dust-borne fluoride on, and uptake by, plants during the winter. A survey of a waste site containing fluorspar tailings in the UK revealed higher fluoride concentrations in herbivorous rodents and plants sampled in later winter (April) compared to July and October (Andrews et al., 1989). We hypothesize that seasonally-induced changes in the feeding habits of cotton rats contribute to greater rates of soil ingestion and risks of fluorosis during winter. Modeling efforts by Schroder (1998) indicate that most uptake of fluoride compounds by cotton rats is from soil ingestion and dry matter deposition on plant material, and cool season grasses such as *Bromus* sp. contained higher concentrations of fluoride compared to warm season grasses. Schetter et al. (1998) revealed a tendency for cotton rats to forage on germinating cool-season grasses in winter, which may inherently involve greater rates of soil ingestion. In addition to potentially higher exposures to fluoride, cotton rats are in poorer overall condition during the winter (Fleharty and Choate, 1973; Cameron et al., 1979). Stresses from nutritional and caloric restrictions may contribute to the severity of dental lesions.

An additional factor that may have contributed to seasonal differences was that cotton rats trapped in winter are relatively older than animals trapped during the summer breeding season. This may have permitted a longer exposure period for greater accumulations of fluoride in hard tissues. Previous studies have indicated that older animals can accumulate greater burdens than younger animals, while younger animals tend to accumulate fluoride at a faster rate than adults (Boulton et al., 1994b; Kierdorf et al., 1995). However, we only observed an age-related relationship with dental fluorosis on one land-treatment site, where as expected, younger animals had lower prevalence of dental fluorosis than older animals. Prevalence of dental fluorosis was so high on one land-treatment site that animals of all ages had some fluorosis. Cotton rat populations we trapped were typically not catchable until well after weaning, so it remains possible that dental fluorosis is less common among the very young. Boulton et al. (1994b) indicated that small mammals generally demonstrate rapid rates of fluoride uptake immediately after weaning.

The prevalence of dental fluorosis in field populations, degree of uptake of fluoride, and severity of dental fluorosis varied substantially among the three land-treatment sites surveyed in this study. Observations during population monitoring and evaluation of animals returned to the laboratory collectively indicated that levels of fluoride in soil did not directly reflect bone burdens of fluoride or severity of dental fluorosis. This was clearly demonstrated in cotton rats from land-treatment unit 5 which had the highest (4,317 mg/kg) levels of fluoride in soil yet ranked far below unit 1 (2,672 mg/kg in soil) in prevalence of dental fluorosis in the population. Examining concentrations of fluoride in soil and bone alone, without examining animals for dental fluorosis, would have incorrectly led us to predict that risks to cotton rats on land-treatment unit 5 were greater than on land-treatment unit 1. Animals from land-treatment unit 2 had the lowest frequency and severity of dental fluorosis, the concentration of fluoride in bones was comparable to levels on land-treatment unit 1 where prevalence and severity was greatest.

Contrary to our results, Boulton et al. (1994a,b, 1995) found a much stronger relationship among soil fluoride levels, concentrations of bone fluoride, and severity of dental fluorosis for other rodent species. Exposure, disposition, and elimination may differ between species. Cotton rats with dental severity lesions in the 4 and 5 category could have been representatives of tolerant individuals contributing to the poor relationship in our study at the higher dental severity scores. Also, concentration of fluoride in the bone of cotton rats reflects a life time exposure to fluoride while exposure to fluoride affecting the incisors is recent because their incisors are continuously erupting. Lack of other contaminants present in the soils in the above mentioned studies may also have contributed to these apparent contradictions. Soils on land-treatment sites contained numerous other contaminants besides fluoride (Loehr et al., 1992; Rafferty, 1998). Sulfur and aluminum compounds have been implicated in potentiating toxic effects of fluoride (Chen et al., 1996; Caverzasio et al., 1996). Metals implicated in the enhancement of the toxic action of fluoride such as Al were not quantified in this study, but are known to occur at high levels (7,000–12,000 mg/kg) in refining wastes that are land-treated (Loehr et al., 1992). In addition, factors such as nutritional deficiencies (Harris and Navia, 1980) and exposure to Cd (Katsuta et al., 1996) also may induce dental lesions. Cotton rats from land-treatment unit 1 where the most severe dental fluorosis was observed had significantly higher bone concentrations of Sr, Pb, and Ti compared to animals from other land-treatment units.

It is apparent from this study that cotton rats have a substantial risk of developing dental fluorosis from exposure to soils and vegetation of former land-treatment facilities where petroleum-refining wastes were disposed for biodegradation. Dental abnormalities and uptake of fluoride can interfere with the acquisition and absorption of essential food items, interfering with the overall health and survival of small mammal populations. It was not uncommon to witness individual cotton rats with broken and chipped teeth on fluoride contaminated sites. As indicated by our field survey of cotton rats residing directly on contaminated sites, a population monitoring program would be adequate to determine exposure to fluorides. Season of the year was observed to be a powerful determinant of risk and should be incorporated into any monitoring program. It also appears important to consider possible interactions with other contaminants that may contribute to the severity of dental fluorosis.

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