Bull. Environ. Contam. Toxicol. (1987) 39:945–952

Bulletin of Environmental Contamination and Toxicology

Fluoride Pollution in a Salt Marsh: Movement Between Soil, Vegetation, and Sheep

A. J. Baars,[1] H. van Beek,[1] Th. J. Spierenburg,[1] G. J. de Graaf,[1]
W. G. Beeftink,[2] J. Nieuwenhuize,[2] J. Boom,[3] and J. J. Pekelder[4]

[1]Central Veterinary Institute, Department of Analytical Chemistry & Toxicology, P.O. Box 65, 8200 AB Lelystad; [2]Delta Institute for Hydrobiological Research, Vierstraat 28, 4401 EA Yerseke (communication no. △ 366); [3]Saeftinge Sheep Farm, Koninginnestraat 24, 4568 PT Nieuw Namen; and [4]Regional Animal Health Service, P.O. Box 87, 2800 AB Gouda, The Netherlands

The river Scheldt (southwestern part of The Netherlands) is responsible for a considerable pollution of its estuary with organic and inorganic waste (Salomons and Eysink, 1981; Van der Kooij, 1982; Valenta *et al.*, 1984), which becomes manifest particularly in the salt marshes (Beeftink *et al.*, 1982; Beeftink and Nieuwenhuize, 1986). Of these marshes, the 3400 ha nature reserve of the Saeftinge salt marsh (figure 1) constitutes a representative example of such a valuable tidal brackish ecosystem. This marsh is partly grazed by sheep, thus contributing to its original character and assisting in the preservation of the local flora and fauna. Preceeding reports (Beeftink *et al.*, 1982; Beeftink and Nieuwenhuize, 1986; Baars *et al.*, 1986) indicated a significant degree of contamination with heavy metals, which were shown to enter food chains.

The present study focusses on fluoride, an environmental contaminant known to be spread by water and air, and, although assumed to be beneficial in small quantities (Rich and Ensinck, 1961), a potential threat for plants and animals, particularly herbivores (Shupe, 1980; NAS-USA, 1980).

MATERIALS AND METHODS

Soil and vegetation were sampled monthly from May, 1983, to July, 1984. Selected samples were also taken in the period October, 1984, to May, 1985. Soil samples were taken from 0–20 cm depth on high, middle and low locations on the marsh, based upon tidal submergence. Plants were taken from species commonly consumed by sheep: *Aster tripolium, Elymus pycnanthus* (formerly called *Elytrigia pungens*), *Festuca rubra* and *Puccinellia maritima*. During the second period of investigation, also samples of meadow grass were taken at three locations on the landside of the dike. Sheep faeces and urine were sampled monthly. Rib material was removed from sheep that died or were slaughtered during the study. Regularly, a veterinary inspection of the flock was done.

Send reprint requests to dr. A.J. Baars at the above address.

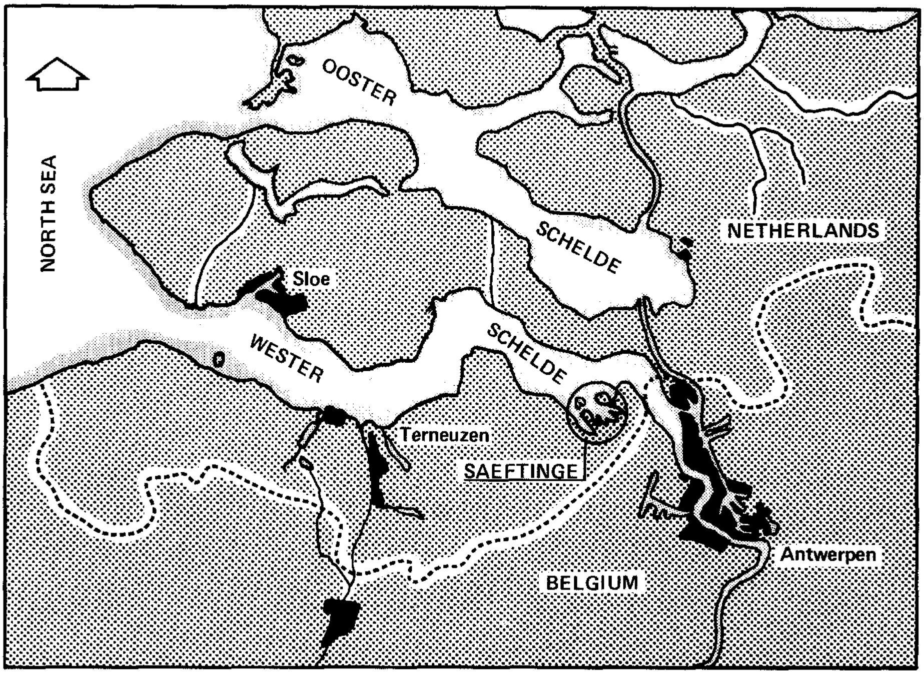

Figure 1. Location of the Saeftinge salt marsh in the Scheldt estuary, southwestern part of The Netherlands. Main industrial areas are depicted in black.

Table 1. **Soil characterization**

	Location		
	High	**Middle**	**Low**
Dry material (%)	73.1 ± 0.7	60.1 ± 2.2*	56.0 ± 1.4*
pH – KCl	7.59 ± 0.06	7.31 ± 0.06#	7.29 ± 0.05*
Clay particles < 16 μm (%)	15.8 ± 1.0	46.2 ± 2.6*	56.3 ± 0.9*
Particulate organic carbon (g per kg dry soil)	19 ± 1	47 ± 2*	49 ± 2*
Fluoride (mg per kg dry soil)	82 ± 2	121 ± 3*	139 ± 2**

Data are presented as mean ± SEM, n = 16 (for Dry material and Fluoride, n = 12).
Significantly different from high location, $P < 0.01$.
* Significantly different from high location, $P < 0.001$.
**Significantly different from high and middle locations, $P < 0.001$ (Student t-test).

For the analysis of fluoride, rib material was cleaned from fat and cartilage, boiled in distilled water and homogenized after drying overnight at 100°C. Soil samples were lyophilized and ground, plant material (unwashed) was minced. After that, all samples were extracted with 6N HCl and filtered after buffering with sodium citrate at pH 6.0. Fluoride was measured potentiometrically applying a fluoride-specific electrode. Urinary fluoride was measured directly in the sample after proper dilution with sodium citrate buffer pH 6.0.

RESULTS AND DISCUSSION

Soil samples taken during this study are characterized in table 1. The fluoride content ranges from 80 to 140 mg per kg dry matter, which does not particularly indicate pollution: soil in The Netherlands generally contains 100 to 400 mg F per kg (Health Council of The Netherlands, 1981), while internationally background values between 50 and 1000 mg F per kg were reported (NAS-USA, 1980). On the salt marsh significant differences with respect to the locations were observed. Apparently, the fluoride content is related to the amount of clay particles and particulate organic carbon in the soil. Also the frequency of flooding is known to influence these parameters (Beeftink *et al.*, 1977).

In contrast to soil, the fluoride content of vegetation shows a seasonal variation, as is illustrated in the upper part of figure 2: levels in early spring are about 4 times higher than in late summer. This indicates a relation with the growth cycle of the plants, as was also observed earlier (Beeftink and Nieuwenhuize, 1986). However, although this seasonal variation was seen in all species studied, there is also a considerable difference between species, as becomes clear from table 2. Obviously, this species difference is not related to the amount of dry matter. The relation between the content in soil and vegetation is very complex. The uptake of fluoride can proceed via the roots as well as via the stomata, the latter particularly during tidal submergence and rain. In general, uptake will also be influenced by e.g. the soil texture, the amount and nature of organic material in the soil, the degree of aeration and the pH. Furthermore, there might be a notable contribution of adherent sediment particles in tidal salt marshes (Baars *et al.*, 1986). As can be concluded from table 2, *Puccinellia maritima* accumulates the highest concentration of fluoride. Visible damage of the plants however was not observed, in agreement with Murray (1985) who suggested that some plant species can tolerate relatively large amounts of fluoride without being affected.

The fluoride load in the marsh can be assumed to originate from air and water deposition. The Westerschelde was reported to contain 1.5 mg F per liter (Van der Kooij, 1982), which is comparable to the concentration in the river Seine, and about 30 times above the natural background (Martin and Salvadori, 1983). Airborne fluoride (Murray, 1985) generally originates from industrial exhausts, but also wind transportation with salt crystals has been reported (Lavado and Reinaudi, 1986). Additionally, transportation by salt spray might contribute as well. The contribution of airborne fluoride deposition on the Saeftinge salt marsh was studied by comparing 16 samples of marsh vegetation *(Puccinellia maritima)* with 24 grass samples taken from meadows on the landside of the dike

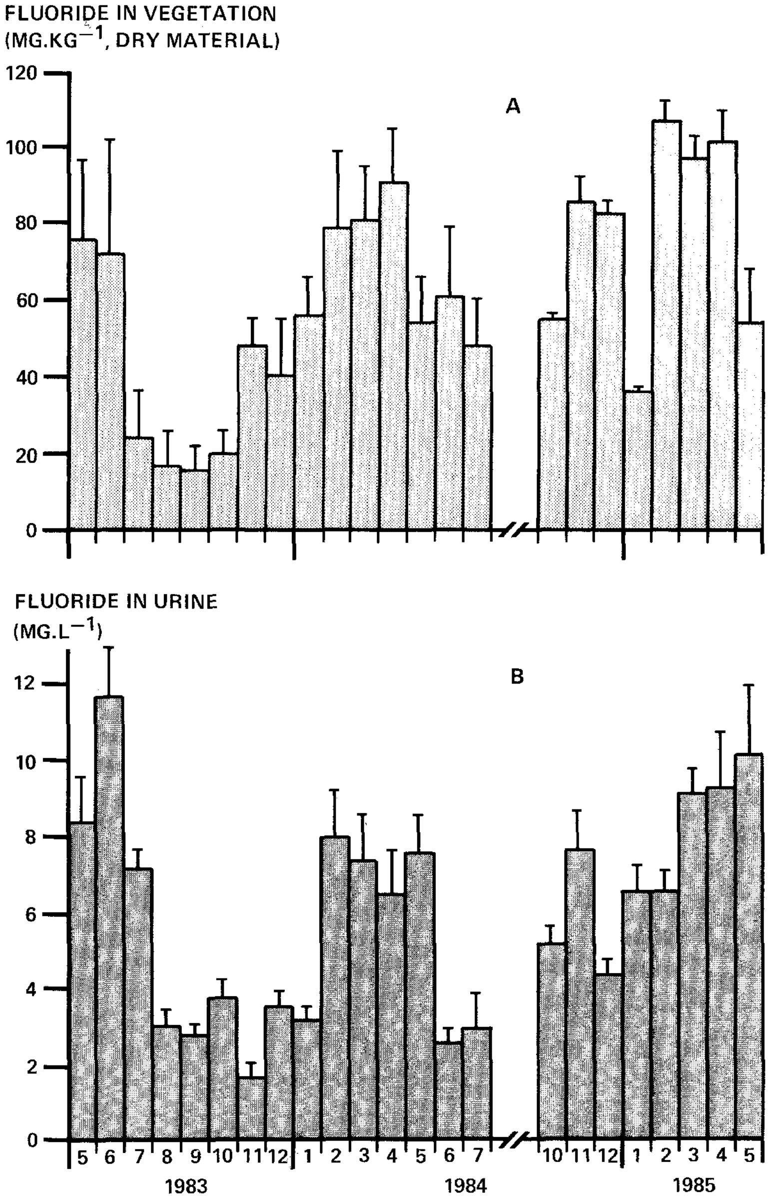

Figure 2. Monthly average of fluoride in plants (A: mg per kg dry matter ± SEM; n = 6), and in urine of sheep (B: mg per liter ± SEM; n = 16-26 for the period May 1983 to July 1984, n = 5 for the period October 1984 to May 1985).

adjacent to the marsh, in the period October 1984 to May 1985. The marsh vegetation contained 77 ± 7 mg F per kg dry matter, the grass contained 13 ± 2 mg F per kg dry matter (means ± SEM). Even taken into account that of the plant species studied, *Puccinellia* showed the highest fluoride concentration, it can be concluded that air deposition of fluoride is not a main contributor to the amounts

Table 2. **Fluoride content of plant species**

	Dry material (%)	Fluoride (mg per kg dry material)
Aster tripolium	10.6 ± 0.6	33.3 ± 4.7
Puccinellia maritima	24.0 ± 1.2	85.2 ± 8.3
Elymus pycnanthus	31.4 ± 1.8	23.0 ± 4.8
Festuca rubra	25.6 ± 1.4	54.4 ± 7.8

Data are presented as mean ± SEM. Number of samples for *Aster* and *Puccinellia* = 30, for *Elymus* = 15 and for *Festuca* = 14.

that were found in the marsh. Thus, the regular flooding of the marsh seems to be largely responsible for the observed rather high fluoride levels in the salt-marsh vegetation. This in turn suggests direct fluoride uptake by the plants from the water as a considerable contribution to their total fluoride content. Alternatively, adherent sediment might be an important contributor (Baars *et al.*, 1986).

Concerning the avoidance of potential toxic effects on animal husbandry, a maximum level in cattle fodder is advised of 30 to 40 mg F per kg dry matter on a yearly base, while the maximum during one month should not exceed 75 mg per kg (Health Council of The Netherlands, 1981). Sheep are slightly more tolerant to fluoride: tolerable levels of 60 (during breeding) to 150 (during fattening) mg F per kg were reported (NAS-USA, 1980). In this respect the observed levels in the marsh vegetation, though not alarming, cannot be considered entirely safe.

Hazardous situations for animals can be diagnosed by considering the urinary excretion of fluoride. Urinary contents up to 10 mg per liter are agreed to be normal, and above 15 mg per liter it indicates ingestion of abnormal amounts of fluoride. A urinary level between 10 and 15 mg per liter is regarded as suspicious (Clarke *et al.*, 1981). It has to be noted however that urinary levels not always reflect current intake. The time course of excretion is related to the height and duration of fluoride uptake (Shupe, 1980). The fluoride content of urine from sheep grazing on the salt marsh studied is presented in the lower part of figure 2. Only in early summer the average levels slightly exceeded 10 mg F per liter. In the period May, 1983 to May, 1985, 380 urine samples were analyzed; 3.7% had levels above 15 mg F per liter, and 9.7% of the samples contained between 10 and 15 mg F per liter. The highest level observed was one sample containing 27 mg F per liter. In no case urine samples from individual animals contained amounts above the limits for a period of more than three serried months. It must be noted however, that during the winter period (generally from December to March/April) the marsh is not grazed by the sheep due to climatological conditions.

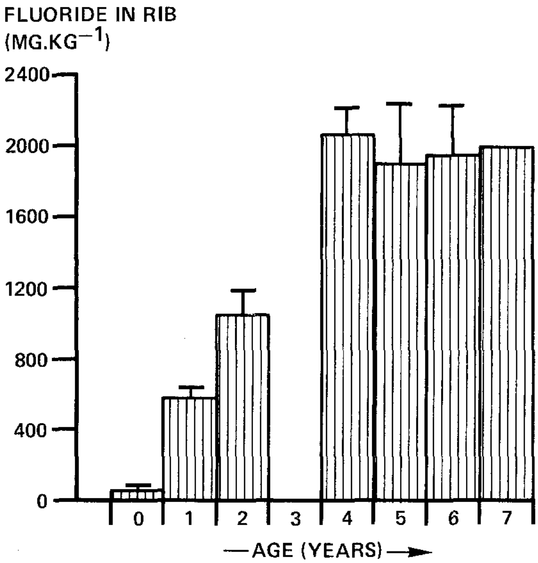

Figure 3. Average fluoride amounts in ribs of sheep of different age. Vertical bars indicate SEM. Number of samples: 33 (age 0), 2 (age 1), 4 (age 2), 5 (age 4), 3 (age 5), 3 (age 6), and 1 (age 7 years).

It is well known that fluoride, once absorbed, is partly incorporated in calcifying tissues; soft tissues, including the edible parts, do not accumulate fluoride (Clarke *et al.*, 1981). The resulting accumulation in e.g. bones is also observed in the present study, as shown in figure 3, illustrating the fluoride content of rib material in relation to age. The results suggest a steady state level which is reached at the age of 4 years. However, because only sheep which died during the study were analyzed on rib fluoride content, far more young than older animals became available, mainly due to the rather hostile character of the marsh particularly for young animals. Limit values for bone fluoride content are given as about 1100 mg F per kg for one-year-old cattle (including sheep), 2100 mg F per kg at the age of 4 years and up to 2900 mg F per kg (all on a dry matter base) at 7 years of age (Shupe, 1980). The analytical results (figure 3) show clearly that the fluoride contents of rib material from sheep grazing the salt marsh approach these limits, but do not exceed them. This agrees with the observed urinary fluoride levels: both findings suggest a fluoride uptake above normal, but not to a level that would indicate fluorosis. Also the veterinary inspections did not reveal indications for this disease.

Based on veterinary practice, a rough estimation of the fluoride balance of the sheep can be made assuming a daily intake of 1.5 kg plant material (on a dry matter base) per animal, a manure production of 0.5 kg dry material per day and a urine production of 0.5 – 4 liters per day. The average fluoride content of the plant material was 52 mg per kg, the faeces contained 14 mg per kg (range < 5 –

50, 36 samples) and the mean urine concentration was 5 mg per liter (data from May 1983 to May 1984). Hence the bioavailability of the current fluoride appears to be about 65 – 90%, which is well in agreement with the values of 50 – > 90% reported by the NAS-USA (1980). Apparently, the rather high levels of fluoride in the vegetation are not directly hazardous to sheep consuming these plants, although sheep farming in the present situation continues to ask great care and attention. Herbivores generally seem to be able to avoid heavily contaminated feed (Spedding, 1975). Also in the present study we observed e.g. that plants with much adherent sediment were not consumed (Baars *et al.*, 1986). Hence the actual consumption pattern could have resulted in a smaller uptake than indicated by the average fluoride levels in the vegetation. If so, and if the assumptions in the above estimation are justified, then also the bioavailability of the current fluoride would be relatively low.

Acknowledgments. The authors acknowledge the contributions of H.C. Buysse, A. Goud, M.C. Daane, H.C.A. Greefkes, A.M.C. Paulissen, H.J. Over and C.H. Vos.

REFERENCES

Baars AJ, van Beek H, de Graaf GJ, Spierenburg ThJ, Beeftink WG, Nieuwenhuize J (1986) Metal pollution in a salt marsh and its effect on sheep. Arch Toxicol, suppl **9**:410-413.

Beeftink WG, Daane MC, van Liere JM, Nieuwenhuize J (1977) Analysis of estuarine soil gradients in salt marshes of the southwestern Netherlands with special reference to the Scheldt estuary. Hydrobiologia **52**:93-106.

Beeftink WG, Nieuwenhuize J (1986) Monitoring trace metal contamination in salt marsh ecosystems, with special reference to the Westerschelde estuary. Environm Monit Assessm **7**:233-248.

Beeftink WG, Nieuwenhuize J, Stoeppler M, Mohl C (1982) Heavy metal accumulation in salt marshes from the Western and Eastern Scheldt. Science Total Environm **25**:199-223.

Clarke ML, Harvey DG, Humphries DJ (1981) Veterinary Toxicology. Baillière Tindall, London; pp 48-54.

Health Council of The Netherlands (1981) Advies inzake advieswaarden voor anorganische fluoriden in de buitenlucht. Report VAR 50, Ministry of Wellfare, Public Health and Cultural Affairs; Government Publishing Office, Den Haag.

Lavado RS, Reinaudi NB (1986) Wind-blown dust from salty areas, a source of fluoride in plants. Fluoride **19**:14-18.

Martin JM, Salvadori F (1983) Fluoride pollution in French rivers and estuaries. Estuarine, Coastal & Shelf Sci **17**:231-242.

Murray F (1985) Cycling of fluoride in a mangrove community near a fluoride emission source. J Appl Ecology **22**:277-285.

NAS-USA (1980) Mineral tolerance of domestic animals. National Academy of Science, National Research Council, Subcommittee on Animal Nutrition; Washington DC.

Rich C, Ensinck J (1961) Effect of sodium fluoride on calcium metabolism of human beings. Nature **191**:184-185.

Salomons W, Eysink WD (1981) Pathways of mud and particulate trace metals from rivers to the southern North Sea. In: Nio SD, Schüttenhelm RTE, Weering TCE (eds) Holocene marine sedimentation in the North Sea basin. Spec Publ Intern Assoc Sedimentol **5**:429-450.

Shupe JL (1980) Clinicopathological features of fluoride toxicosis in cattle. J Animal Sci **47**:746-758.

Spedding GRW (1975) The biology of agricultural systems. Acad. Press, New York.

Valenta P, Nürnberg HW, Rützel H, Merks AGA (1984) Die Belastung der Ästuare der Wester- und Oosterschelde mit ökotoxische Metallen. Vom Wasser **62**:295-306.

Van der Kooij LA (1982) De waterkwaliteit van de Westerschelde in de periode 1964-1981. Report no. 82.063, National Institute for Inland Water Management and Waste Water Treatment, Lelystad.

Received August 8, 1987; accepted September 30, 1987.