DOI 10.24425/pjvs.2021.136804

Original article

Fluoride concentration in teeth of the roe deer (Capreolus capreolus) and red deer (Cervus elaphus) from areas of Poland industrially uncontaminated with fluoride compounds

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Abstract

The last biomonitoring study in Poland on intoxication with fluoride compounds of deer was conducted almost two decades ago. Given the fact that fluoride level in air and water is not widely monitored in Poland, it is justified to undertake monitoring of F^- levels in people and other long-lived mammals. This paper provides the assessment of the present level of fluoride accumulation in mineralized tissue of large herbivorous mammals. The aim of the present study was to determine fluoride concentration in teeth of deer inhabiting the areas of Poland which are industrially uncontaminated with fluoride compounds, to establish possible correlations between the analysed parameters, and to provide a comparison of the present results with those obtained in other studies. Mean concentration of fluoride in all analysed samples amounted to 231.0 F mg/kg, with the minimum value of 22.0 F mg/kg and the maximum of 935.0 F mg/kg. This results from the development of industry and a widespread use of fluoride-supplemented caries prevention products which contributes to an intense accumulation of these substances in vertebrates, predominantly in mineralized tissue.

Key words: fluoride, biomonitoring, teeth, herbivorous, mammals

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Introduction

Over the last 20 years, the field of study mainly concerned with the problems resulting from exponential population growth, i.e. ecotoxicology has developed significantly. The demographic growth is consequential for land use and disturbs the natural geochemical cycle of numerous elements. This results in their large-scale extraction from the natural deposits due to economic activity and the release of anthropogenic pollutants to the environment. These environmental changes negatively affect not only humans but also other vertebrates living in terrestrial environment. Fluoride is the element which is constantly being used by humans in numerous industries. Various biological materials are used for the assessment of fluoride exposure, however, the priority is given to mineralized tissues as, due to their periodic growth and constant reconstruction, they constitute a significant study material with respect to long-term fluoride accumulation. Since large amounts of fluoride compounds are deposited in hard tissue, the concentration is analysed using highly mineralized biological tissues such as antler, teeth and bones (Machoy et al.1995, Kierdorf et al.1995, Bezerra de Menezes et al. 2003, Gutowska et al. 2004). For years, the biomonitoring studies on environmental contamination with fluoride are conducted on species of herbivorous ungulates including: red deer (*Cervus elaphus*), blacktail deer (*Odocoileus hemionus columbianus*), whitetail deer (*Odocoileus virginianus*), wapiti (*C. e. canadiensis*), roe deer (*Capreolus capreolus*), elk (*Alces alces*), bighorn sheep (*Ovis canadiensis*) and omnivorous wild boar (*Sus scofa*). These animals inhabit a given area all year long, therefore the amount of fluoride present in the environment correlates with the level of fluoride accumulated in their hard tissues as well as with their age (Newman and Yu 1976, Suttie et al. 1987, Dąbkowska et al. 1995, Kierdorf et al. 1995, 1996, 1999, 2000, Machoy et al. 1995, Schultz et al. 1998, Gutowska et al. 2004, Kierdorf et al. 2005, Zakrzewska et al. 2005, Zemek et al. 2006, Richter et al. 2010). It is established that in herbivorous mammals originating from areas anthropogenically contaminated with fluoride, concentration of F^- in mineralized tissue is higher in comparison with that found in animals originating from uncontaminated areas (Newman and Yu 1976, Suttie et al. 1987, Kierdorf and Kierdorf 1999, Kierdorf et al. 2000, Gutowska et al. 2004, Zakrzewska et al. 2005). In North-West Poland, the concentration of fluoride in the environment was closely monitored till the end of the 1990s, mainly due to the location of chemical plants producing chemical fertilizers and responsible for the emission of large amounts of gases and dust containing fluoride (Machoy et al. 1997, 2002). Following the modernization of such plants, the biomonitoring was ceased and currently the concentration of fluoride in air and water is not routinely studied in Poland. Therefore, it is vital to monitor the concentration of this toxic element in living organisms. The biomonitoring studies concerning pollution with fluorides were conducted in north-western Poland on bone formations (bone and antler) of family Cervidae (Machoy et al. 1995, Gutowska et al. 2004, Zakrzewska et al. 2005).

Monitoring of the natural environment is a key issue in the undertaken environmental protection measures. It allows adequate assessment of the threat and, consequently, the selection of appropriate preventive measures in a constantly increasing environmental pollution due to civilisation progress. The original aspect of the present study is based on two elements. The first being the continuation of the previously conducted bioindicatory studies in north-west Poland, and the second – the practical application of the rarely used bioindicator. The last biomonitoring study in Poland on intoxication with fluoride compounds of deer was conducted almost two decades ago. This paper provides the assessment of the present level of fluoride accumulation in the mineralized tissue of large herbivorous mammals. The bone tissue is most frequently used in bioindicatory studies. However, this tissue is relatively difficult to obtain. Therefore, the present study used readily available and reliable bioindicatory material, i.e. teeth, which resembles the bone tissue in its structure.

The aim of the present study was to determine fluoride concentration in teeth of deer inhabiting the areas of Poland which are industrially uncontaminated with fluoride compounds, to establish possible correlations between the analysed parameters, and to provide a comparison of the present results with those obtained in other studies.

Materials and Methods

The study was conducted using 22 molars of red deer (*Cervus elaphus*) obtained from authorised shooting in the area of the Landscape Park Zielonka (wielkopolskie voivodship, Poland). Additionally, 54 molars of roe deer (*Capreolus capreolus*) obtained from authorised shooting in the area of the Drawskie Lake District (zachodniopomorskie voivodship, Poland) were used in the study. The age of the animals was identified in the presence of a representative of the Polish Hunting Association on the basis of the developmental stage and the condition of teeth. In total, for the purpose of the analysis, the following were obtained: 26 teeth of young roe deer, 28 teeth of adult roe deer and 22 teeth of adult red deer.

Table 1. Fluoride concentration (mg/kg) in tissue material of molars per deer species.
(number of samples- n, arithmetic mean- AM, standard deviation from AM- SD, minimum- Min and maximum- Max value, median value- Med, statistically significant difference- p)

Tissue material	Species	F^- mg/kg					
		n	AM	SD	Min	Max	Med
Enamel	young roe deer	26	**272.1** [a]	148.2	106.7	795.0	239.1
	adult roe deer	25	**178.6** [a, b]	115.1	58.4	580.4	166.7
	adult red deer	22	**92.2** [b]	49.4	37.5	199.9	83.5
Dentin	adult roe deer	25	**251.6** [c]	124.3	22.4	559.0	229.3
	adult red deer	21	**119.9** [c]	75.4	31.4	319.5	110.0
Cementum	adult roe deer	25	**390.1** [d]	187.7	177.1	935.2	344.2
	adult red deer	19	**201.7** [d]	85.2	89.2	386.0	182.5
Whole tooth	adult roe deer	25	**820.3** [e]	297.0	458.1	1744.9	746.3
	adult red deer	19	**410.2** [e]	156.3	98.4	808.5	351.6

[a] p<0,005, [b] p<0,001, [c] p<0,0002, [d] p<0,00001, [e] p<0,005

The teeth selected for the analysis were stored in a fridge at the temperature of 4°C until analysis. Then, the teeth were cleansed, dried and degreased with acetone. In the next stage, using a dental drill, coronal dentin including the chamber and pulp residue was prepared from each tooth. Each tooth fragment selected for the analysis was weighed (with the accuracy of 0.0001 g) to identify air-dry weight which was determined by means of weighing method. Next, the teeth were dried to constant weight in a laboratory drier at 105°C for 4 days. The teeth were then ground in an agate mortar and test portions of approx. 1 g each were prepared. Each sample was weighed with the accuracy of 0.0001 g and treated with 1 ml of perchloric acid and shaken at the temperature of 90°C for 1 hour. When cooled, 0.5 ml of sample was transferred to a plastic container and supplemented with 2 ml of sodium citrate solution and 2.5 TISAB II solution. After mixing, the potential was measured for 10 minutes in each sample: 5 minutes before and 5 minutes after addition of a respective standard. Fluoride concentration in a sample was calculated by means of differences between the determined potentials, mass of the sample and concentration of the respective standard added. The correctness of the analytical procedure was controlled by determining fluoride ions in reference materials of known concentration, i.e. standard NaF solution at concentrations: 0.1, 1, 10 ppm by Orion. The statistical analysis was developed using Statistica 10.0 Stat Soft and Microsoft Excel 2007 and consisted of the following steps: determination of the mean fluoride concentration in teeth of the analysed species; calculation of the arithmetic mean (AM), standard deviation from AM (SD), median value (Med) as well as the minimum (Min) and maximum (Max) value of fluoride concentration in teeth. Conformity of distribution of fluoride concentration was verified using Kolmogorov-Smirnov test with Lilliefors modification. The nonparametric Mann--Whitney U test was used to compare the means.

Results

Mean concentration of fluoride in all analysed samples amounted to 231.0 F^- mg/kg (SD=156.0 F^- mg/kg), with the minimum value of 22.0 F mg/kg and the maximum of 935.0 F^- mg/kg. Fluoride concentration distribution values, analysed using teeth of roe deer and red deer, deviated from the normal distribution (K-S=0.2; $p<0.05$), and for statistical analysis a nonparametric U Mann-Whitney test was used. Age estimation of roe deer and red deer was based on teeth development and allowed for categorisation of roe deer into: young (*immaturus*) and adult (*adultus*) samples, for red deer only the adult samples (*adultus*) were identified. Descriptive statistics concerning fluoride concentration in tissue material of particular species is presented in Table 1. Young roe deer teeth samples were partly excluded from the statistical analysis due to difficulties with obtaining dentine and cementum from very small teeth. Therefore, in the case of young roe deer samples, only the enamel was statistically analysed. For adult roe deer (n=25), mean fluoride concentration, determined for the whole tooth, was 820.3 F^- mg/kg, and for adult red deer (n=19) fluoride concentration level for the whole tooth, was two times lower and amounted to 410.2 F^- mg/kg ($p<0.005$). Fluoride concentration values determined in the enamel of young roe deer (n=26) ranged from 106.7 to 795.0 F^- mg/kg. The levels

Table 2. Fluoride concentration (mg/kg) in teeth of selected mammals, with regard to their age and origin (dw, dry weight).

Age m, month y, year	Material		Location	Source
	tooth	bone		
White-tailed deer *Odocoileus virginianus*				
all age classes, before 1979 after 1980	enamel, dw 149 457		USA, South Carolina, polluted	Suttie et al. 1987
1.5 y before 1979 after 1983 ≥2.5 y before 1979 after 1983		mandible, ash 182±69 586±215 286±100 1.275±624		
Red deer *Cervus elaphus*				
1-2 y	whole tooth, dw I: 159.0 II: 114.0	mandible, dw (alveolus) I: 231.8±30.8 II: 150.2±36.0	NW Poland I: polluted II: unpolluted	Zakrzewska et al. 2005
6-8 y	I: 470.1 II: 211.1	I: 637.8±121.8 II: 341.0±91.4		
1-2 y	molar: crown, dw I: 147.5 II: 98.1			
6-8 y	I: 342.7 II: 179.2			
1-2 y	molar: root, dw I: 212.9 II: 144.1			
6-8 y	I: 493.3±190.9 II: 264.0±119.8			
Red deer *Cervus elaphus*				
adults	whole tooth, dw 410.2 molar: enamel 92.2 molar: dentin 119.9 molar: cement 201.7		W Poland unpolluted	this paper
Roe deer *Capreolus capreolus*				
Immaturus	molar: enamel 272.1 molar: dentin, 398.3 molar: cement 367.5		NW Poland unpolluted	this paper
Adultus	whole tooth, dw 820.3 molar: enamel 178.6 molar: dentin 251.6 molar: cement 390		NW Poland unpolluted	this paper

determined in the enamel of adult roe deer (n=25) ranged from 58.4 to 580.4 F^- mg/kg. A statistically significant difference between the concentration of fluoride in the enamel of young and adult roe deer was found, with higher concentration determined in the young samples (mean 272.1-young and 178.6-adult F^- mg/kg, p<0.005 respectively). IN adult red deer species, fluoride concentration in the enamel ranged from 37.5 to 199.9 F^- mg/kg. It was found that the enamel of adult roe deer accumulated a markedly higher amounts of fluoride in comparison with adult red deer (respective mean: 178.6 and 92.2 F^- mg/kg, p<0.001). Statistically significant difference in fluoride level in the dentin was identified only with respect to adult roe deer and red deer, with adult roe deer accumulating higher amounts of fluoride than red deer (respective mean: 251.6 and 119.9 F^- mg/kg, p<0.0002). Statistically significant differences in fluoride level were obtained with respect to fluoride concentration in the cementum – the concentration determined in adult roe deer was almost twice that identified for red deer (respective mean: 390,1 and 201,7 F^- mg/kg p<0.00001).

Discussion

The eco-toxicological studies which, among others, are aimed at providing an implicit assessment of environmental contamination with various substances including fluoride, rely on determining the concentration of the said pollutants in living organisms. Concentration of toxic substances is established mainly in organsresponsible for detoxification of the organism – liver and kidneys of birds and mammals. However, some harmful substances, such as fluoride compounds, with time accumulated in greater amounts in highly mineralized tissues. Therefore, for several decades, the bone tissue is being used for this purpose thus allowing the assessment of long-term environmental contamination with fluoride (Bezerra de Menezes et al. 2003).

In mammals, approx. 99% of fluoride is accumulated in bones and other mineralized tissues (Whitford 1994, Vieira et al. 2005, Fawell et al. 2006). Fluoride ions are easily deposited in a mineralized tissue due to hydroxyl ions (comprising the apatite forming the bone structure) exchange to fluorides which are integrated into apatite crystals in the ion exchange process. Hydroxyapatite contains carbonates and citrates. Carbonate content indicates enamel maturity – the lower the content, the younger the enamel. Carbonate groups show weak bonds with apatite and are more easily exchanged to fluoride ion (Dzidziul et al. 2006). In the present study the highest values of fluoride concentration was determined in the enamel of young roe deer (compared to adults of both species). This is probably related to the abrasion of the enamel and hence the loss of minerals with age. Moreover, according to some authors, the enamel does not constitute a good bio-indicator for long-term studies on the evaluation of fluoride exposure since fluorosis in the enamel reflects fluoride exposure mainly during tissue maturation (Den Besten et al. 1994, Vieira et al. 2004). However, the relationship between fluoride concentration in dentine and the occurrence of teeth fluorosis was demonstrated (Den Besten et al. 1994, Vieira et al. 2004). It was also found that the degree of fluorosis depends not only on the total fluoride dose, but also on the exposure time (Den Besten 1994, Vieira et al. 2004). Moreover, there is a relationship between the presence of fluoride in the dentine and bone due to similar structures of these tissues. The dentine is deposited throughout the entire life and fluoride concentration increases with age (Whitford 1994, Vieria et al. 2005). Therefore, the dentine, and particularly the coronal dentine, may be a superior indicator of total fluoride load. It contains only F^- which is accumulated in tissues due to systemic intake (Den Besten 1994). Furthermore, the dentine does not undergo resorption, it is easier to obtain than bone and protected against fluoride action from the oral cavity and surrounding bone by a layer of cementum (De Besten 1994). According to Vieira et al. (2005), fluoride concentration in the inner layers of the dentine is higher than that in the outer layers (Vieira et al. 2005). The fluoride level in the dentine gradually decreases from the surface of the pulp to the dentine-enamel junction (DEJ). High fluoride concentration in the enamel (whole tooth enamel) generally reflects fluoride exposure during tooth formation, whereas fluoride concentration in the dentine and a bone's generally proportional to a long-term intake of this element (Vieira et al. 2005). In most cases, the dentine is an easily accessible tissue and, as has already been mentioned above, the profile of fluoride concentration and its changes with age seem to be comparable to those of the cortical bone. However, bone biopsy is invasive and impractical from the perspective of epidemiological studies (Vieira et al. 2005).

For the purpose of an implicit assessment of the environmental contamination with fluoride, mineralized tissue samples (the mandible in particular) obtained from large long-lived ungulates (generally herbivorous) are widely used (Kierdorf et al. 1995, 1996, Schultz et al. 1998, Zemek et al. 2006, Richter et al. 2010). Table 2 provides a comparison of the present results with the results obtained by other authors with respect to fluoride concentration in deer teeth. In ungulates, fluoride concentration in bone samples depends on the area of the animal's habitat and its age, less so on the species and the type of bone selected for the analysis. It was found that F^- concentration in bones determined for extremely herbivorous mammals living in areas anthropogenically contaminated with fluoride is higher in comparison with bones of the animals from areas not contaminated with these substances (Newman and Yu 1976, Suttie et al. 1987, Kierdorf and Kierdorf 1999, Kierdorf et al. 2000, Gutowska et al. 2004, Zakrzewska et al. 2005). It is widely believed that dentin shows structural similarities with cancellous bones. Therefore, it can be assumed that the level of fluoride accumulation is comparable in both types of tissues (Vieira et al. 2005). The study by Gutkowska et al. (2004) conducted on the bones of red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) obtained from a hunted game in the areas distant from the Szczecin agglomeration (Forest Divisions: Szczecinek, Połczyn Zdrój and Świdwin), identified fluoride concentration below 300 F^- mg/kg. Fluoride Concentration in teh dentin of adult roe deer and red deer analysed in the present study showed smaller concentration than that identified by Gutkowska et al. 2004, and ranged from 120-250 F- mg/kg. Both in the analysed samples obtained from roe deer as well as red deer, fluoride concentration in the enamel was lower in comparison with that in the dentine and cementum. However, fluoride concentration in the enamel reflects a temporary fluoride intake by the analysed animals. Moreover, in the case of only one adult roe deer, mottled enamel was observed (concentration 580 F^- mg/kg). Similar changes were reported by Suttie et al. (1987) concerning the teeth of red deer at lower fluoride concentration in the enamel which amounted to 300 F^- mg/kg (Suttie et al. 1987). In general, changes in the enamel of the first molars of deer due to fluoride compounds indicate only mild fluorosis (Richter et al. 2010). Data concerning fluoride concentration in the cementum of the red deer in the study by Zakrzewska et al. (2005) are comparable to the results obtained herein and are close to ~250 F^- mg/kg. Zakrzewska et al. (2005) conducted a study on teeth of red deer from the areas of northern-west Poland in the late 1990s, i.e. in the initial period of modernisation of the chemical plant in Police. The teeth were obtained from the animals living in the area most exposed to fluoride (I, western part of the zachodniopomorskie voivodship comprising Szczecin and its vicinity, including Police county) and the areas with markedly smaller industrial contamination (II, eastern part of the said voivodship). In general, fluoride concentration determined in the teeth of adult red deer from area II was more than 45% smaller (~214 F^- mg/kg) than that identified in the animals from area I (~ 470 F^- mg/kg). Mean fluoride concentration in the teeth of adult red deer from Western Poland (410.2 F^- mg/kg) being the subject of the present analysis, was found to be comparable to that identified by Zakrzewska et al. (2005) almost two decades ago. However, it must be emphasized that the results of fluoride concentration in teeth of adult red deer from the uncontaminated areas (the Landscape Park Zielonka) are comparable to the results by Zakrzewska et al. (2005) for adult red deer, however from the areas industrially contaminated with fluoride compounds. The region of Wielkopolska is bordered to the north by non-industrialised forest and agricultural areas of Pomorze Zachodnie, however, on its southern borders there are industrialised areas of Dolny Śląsk, i.e. the area bordering the so-called "black triangle" (ecologically contaminated tri-border region of the Czech Republic, Poland and Germany). There are two main sources of fluoride emission in zachodniopomorskie voivodship (the habitat of roe deer being the subject of the present analysis): Dolna Odra power plant and Police chemical plant (Gutkowska et al. 2004). It was found that industrial emission of fluoride results in a sustained contamination with this element in all components of the environment (Bombik et al. 2020). Additional sources of fluoride are residues of caries prevention products (toothpaste, dental lacquer, varnish and mouthwash) which are released to the environment with water (Fawell et al. 2006). In Poland, the admissible fluoride level in water is 1.5 mg/l, whereas the reference value is 0.06 mg/kg (EPA 2012). Similar norms are applicable in other EU member countries. However, fluoride concentration in groundwater frequently reaches as much as 10 (mg/l)/day (Koc et al. 2006). In line with applicable law, fluoride level in air is not subject to analysis in Poland, nor in the EU. Typically, an increased fluoride concentration in air does not persist for long because a share of the pollution falls to the ground or water (Dobrzański and Górecka 2001). However, it needs to be observed that the extent of fluoride contamination of air, dependent on the size of the emission, is also determined by wind speed and direction, land relief and atmospheric precipitation. Given the prevalence of north-west winds in the area under analysis, it can be assumed that fluoride emission predominantly spreads towards north-east area encompassing the non-industrialised areas.

Conclusion

On the basis of the literature data and the results of the present analysis, it can be cautiously concluded that there has been an increase in the level of this element in the environment. This results from the development of industry and a widespread use of fluoride-supplemented caries prevention products which contributes to an intense accumulation of these substances in vertebrates, predominantly in mineralized tissues. Given the fact that fluoride level in air and water is not widely monitored in Poland, it is justified to undertake monitoring of F^{-} levels in people and other long-lived mammals.

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