# FLUORIDE ACCUMULATION IN DOG BONES

Mirona Palczewska-Komsa,[a] Elżbieta Kalisińska,[a] Danuta I Kosik-Bogacka,[a] Natalia Łanocha,[a] Halina Budis,[a] Irena Baranowska-Bosiacka,[b] Izabela Gutowska,[c] Dariusz Chlubek[b]

Szczecin, Poland

SUMMARY: Since the beginning of the 20th century the environment has been burdened with increasing emissions of fluorine (F) compounds due to the development of industry, fluoridation of drinking water, and the widespread use of fluoride-containing toothpaste. All these factors have resulted in an intensive accumulation of F in the bodies of vertebrates, mainly in the bones, and have created a significant threat of intoxication among animals, including mammals. Ecotoxicologists worldwide have looked for various mammalian species that may serve as good bioindicators of environmental F pollution, also potentially harmful to humans. Unlike ungulates, the dog (along with other long-lived domestic mammals or wild carnivores) has rarely been subject to such studies. In this study, we aimed to investigate the fluoride content in dog bones from northwestern Poland and to examine the relationship between the F content in bones and the age category of the studied dogs. The study material comprised hip joint bones obtained from 43 dogs from veterinary clinics in Szczecin, NW Poland. The determinations of F (using potentiometry with Thermo Orion ion-selective electrodes) were performed in triplicate. The F content is expressed on a dry weight basis. The highest average F content was found in adult dogs (>2 years old), with the lowest in young dogs (<2 years old). Older dogs had significantly higher F content than one-day old puppies and young dogs. F content in the bones of dogs significantly correlated with their age (p<0.001). There was no significant difference between adult females and males. Our results do not differ from other reports and prove to be typical of F content in the bones of dogs not supplemented with F.

Keywords: Bone; Dog; Environmental exposure; Fluoride; Poland.

## INTRODUCTION

Fluorine (F) ranks as the 13th most common element in the Earth's crust.[1-3] Its natural sources include inorganic fluorine minerals, volcanic gases and dust, and marine aerosols.[4-6] Anthropogenic F sources include factories producing phosphoric acid and fertilizers (superphosphates), which are a source of soil and water contamination.[7] In addition, F enters the atmosphere as a by-product in the production of process gases and by the combustion of coal, wood, oil, and peat.[8] Other sources of F are in the chemicals used in toothpaste (for caries prevention), in lacquer, varnishes, and rinses, all which enter the water with sewage.[3,9]

The constant emission of F to the environment, both natural and anthropogenic, results in a threat of intoxication to mammals. From the 20th century until now, the supply of anthropogenic F in the environment has been doubling each decade. Therefore, it seems reasonable to control the concentration of this element in mammals, where it enters with water, food, healthcare products and inhaled air. It is absorbed through the intestinal tract, respiratory system and skin, and excreted

[a]Department of Biology and Medical Parasitology, [b]Department of Biochemistry and Medical Chemistry, [c]Department of Biochemistry and Human Nutrition, Pomeranian Medical University, Szczecin, Poland. For correspondence: D Chlubek; E-mail: dchlubek@pum.edu.pl

99 Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones 99
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek

in urine, feces and perspiration.[10-11] In mammals, about 99% of F is accumulated in the bones and other mineralized tissues by replacing hydroxyl ions in apatites constituting the bone structure, in a process of ion exchange.[9,11-13]

The physiological role of F is associated with the mineralization of hard tissue. A deficiency may lead to disturbances in the binding of calcium, magnesium, and phosphorus in bones, leading to hypomagnesemia and bone demineralization. F is also involved in the biosynthesis of enzymes (e.g., cyclases), and the mineralization of cartilage to increase its mass.[14,15] Excess F adversely affects cartilage. Electron microscopy study of cartilage from young dogs and rabbits shows a deformation of chondrocytes, disrupted proliferation, and more rigid cytoplasm due to increased glycogen deposition within the cells.[16] Cell necrosis has also been observed with the disrupted architecture of chondrocytes. X-ray images of elbow joints has revealed the formation of cysts and sclerosis of the cartilage. Furthermore, the thickness of cartilage increases in response to chronic exposure to fluoride, resulting in the inhibition of glycolysis in cartilage cells.[17]

Research on F in living organisms is an important part of biomonitoring and bioindicative studies. Among the various types of biological materials used in the assessment of exposure to F, increasing importance is being attributed to mineralized tissues, mainly bones. With their periodic growth and continuous reconstruction they are a good material in which to study long-term accumulation. There is also the need for convenient and accurate bioindicators of environmental F pollution. In an indirect assessment of environmental exposure to F, the most suitable subjects seem to be medium-sized omnivorous mammals, including the domestic dog (*Canis lupis familiaris*). However, in Europe, canines have only occasionally been used for bioindicative purposes.

On the basis of the existing state of knowledge and scientific evidence, the main objectives of this study were to determine F content in bones obtained from dogs in NW Poland and determine the relationships between the bone F content and the dogs age categories.

## MATERIALS AND METHODS

*The study area:* North-western Poland lies in a region with a Baltic climate.[18] The environment in West Pomerania is or has been exposed to F emissions from a number of industrial plants including: the Chemical Plant "Police," FOSFAN (Formerly Szczecin Phosphate Fertilizer Plant), the Power Plant Company "Dolna Odra," Elektrim Kable – the cable factory Załom, Szczecin Shipyard Porta Holding (closed in 2009), the mill "Huta Szczecin" (closed in 2008), and Paper Mill Szczecin - Skolwin (closed in 2007). Near the German-Polish border in Schwedt (SW of Szczecin) there is also a German refinery that has no less impact on the environment than the above-mentioned plants, due to the prevailing winds in Western Pomerania.

Figure 1 shows the map of Poland and districts of West Pomerania including the locations of the aforementioned plants and the direction of the prevailing wind. Fluorinated waste from these factories is often discharged with waste water into

100 Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones 100
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek

rivers and thus reaches urban drinking water supplies. In the period 1993-1996, the annual average F level in wastewater near the chemical plant "Police" was 0.23 mg/L.[19] Currently, F in water in the river Gunica near the refinery is 0.28 mg/L[20], at least 10 times more than most Polish rivers where F does not exceed 0.02 mg/L.[21]
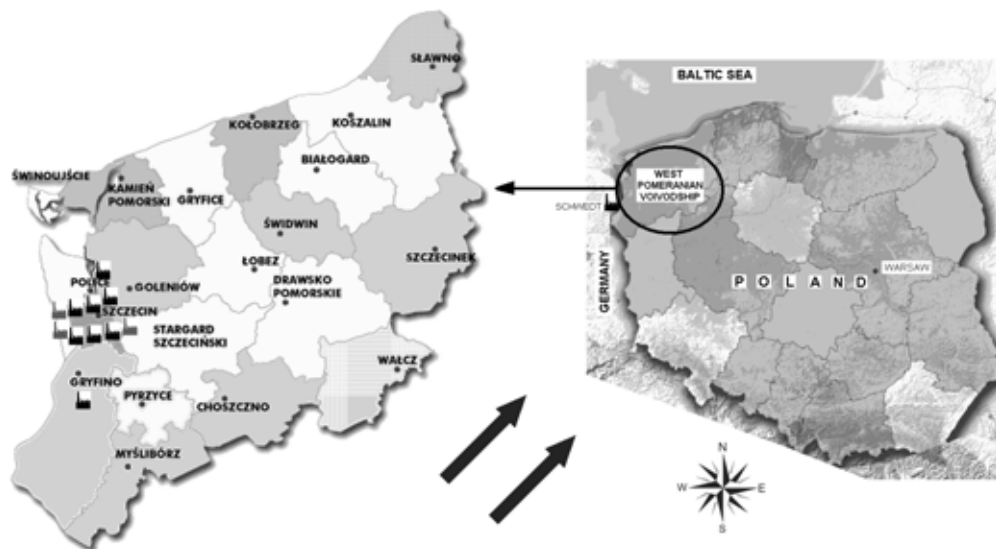


**Figure 1.** Map of Poland and districts of the province including the West Pomeranian Voivodship plants emitting fluoride and direction of the predominant winds (black factory icons: active plants; gray factory icons factories: closed plants, black arrows: direction of the wind)

*Material:* The studied material consisted of femurs obtained from 43 dogs *(Canis lupis familiaris)* obtained in the area of Szczecin in 2009–2012. Bone samples came from 32 young and adult dogs (13 females and 19 males) and 11 one-day-old puppies (5 males and 6 females). The age of the dogs was identified by veterinarians on the basis of information contained in medical records and originating from the dog owners. The average age of the dogs was 8 years (ranging from 1 day to 19 years). The dogs were divided into three age categories: Clf0: one-day-old puppies (n = 11), Clf1: <2 years of age (n=4), and Clf2: >2 years of age (n=28).

The dogs came from the clinics at the Animal Shelter in Szczecin and the Society for the Prevention of Cruelty to Animals branch in Szczecin. The main reasons for euthanasia included respiratory disorders, vascular diseases, and cancer. The dogs were not deliberately sacrificed for this study. The acquisition of biological material from the dogs was approved by the District Veterinary Officer in Szczecin. The study was approved by the Local Ethics Committee for Research on Animals (Resolution No. 5/2012 of 05.12.2012).

*Preparation of material for chemical analysis and determination of fluoride:* The collected femurs were cleaned of any remnants of tendon and muscle, and then samples of compact bone were separated. The samples were dried to constant weight in an oven at 105ºC. The percentage of water content in the samples was determined by gravimetric method. Dried samples were ground, then mixed with

101 Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones 101
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek

perchloric acid and shaken. After cooling, sodium citrate and TISAB II were added to the sample.

Serum levels of F were determined using a potentiometric ion-selective electrode (Thermo Scientific Orion, USA) according to the works of Gutowska et al.[22-24] The F content in samples was calculated based on the difference of potentials measured in each sample, the sample weight, and the concentration of the added standard.

*Statistical analysis:* Statistical studies were performed using Stat Soft Statistica 10.0 and Microsoft Excel 2007. The arithmetic means (AM), standard deviations of the AM (SD), medians (Med) and coefficients of variation (CV) were calculated for each studied group. Compliance of distributions of F were checked using a Kolmogorov-Smirnov test with Lillefors correction (KS). F content distribution in the compact bone deviated from the expected normal distribution (KS=0.1, p<0.01); therefore, in the statistical analysis we used a Kruskal-Wallis test (KW) for the three age groups, and Mann-Whitney U test (MW) for pairwise comparisons. In determining the correlation between F content and age we used the Spearman's rank correlation coefficient for the groups of dogs where n>4.

## RESULTS

The F content in the femur compact bone from all dogs (n=43) was in the range of 77–960 mg/kg dry weight (dw) (Table 1). The median F content in the bones of all dogs was 443.4 mg/kg dw. After excluding the one-day-old puppies (n=11), the median F content was higher at 491.3 mg/kg dw (Table 1). The highest median content of F was found in the males of group Clf2, while the lowest was recorded for all dogs in group Clf1.

Due to the small number of data for males and females in the group Clf1 (n=<4) we did not compare F levels within this group. We did perform an analysis of differences between the genders in each group. There were no statistically significant differences between the content of F in the bones of male and female dogs of group Clf2 and between all females (Clf1 + Clf2) and all males (Clf1 + Clf2).

The KW test (H=11, p<0.003) indicated differences in F level between the age groups of dogs (excluding their sex) (Fig. 2). One-day-old puppies clearly had a lower F content in the compact femur bone than dogs from group Clf2, as confirmed by MW U test (U=57, p<0.003). There was a statistically significant difference between groups Clf1 and Clf2 (U=21, p<0.05) wherein older dogs (>2 yr-old) had an F content over 100% greater than group Clf1 dogs (<2 yr-old). The significant correlation between the F content in the bones of dogs and their age was highly significant according to the Spearman rank correlation coefficient (r= 0.51, p<0.001) (Fig. 3). The F content in the compact femur bone exhibited a clear upward trend, from postnatal ontogenesis to old age.

102 Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones 102
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek

**Table 1.** The fluoride (F) concentration (mg/kg dw) in the dog bones by gender and age
(%CV = percent coefficient of variation; Clf0 = one-day-old puppies;
Clf1 = young dogs <2 yr-old; Clf2 = adult dogs >2 yr-old)

| Gender | Age category (n) | Statistical parameter | F concentration |
|---|---|---|---|
| Female | Clf1 (n=1) | Mean | 201.6 |
| | Clf2 (n=12) | Mean±SD | 491.2±294.2 |
| | | Median | 466.7 |
| | | Range | 111.9–942.5 |
| | | %CV | 59.8 |
| | Clf1+Clf2 (n=13) | Mean±SD | 469.0±292.9 |
| | | Median | 457.3 |
| | | Range | 111.9–942.5 |
| | | %CV | 62.4 |
| Male | Clf1 (n=3) | Mean±SD | 284.0±204.0 |
| | | Median | 290.4 |
| | | Range | 76.9–484.7 |
| | | %CV | 71.8 |
| | Clf2 (n=16) | Mean±SD | 564.1±199.3 |
| | | Median | 538.6 |
| | | Range | 134.5–959.9 |
| | | %CV | 35.3 |
| | Clf1+Clf2 (n=19) | Mean±SD | 519.9±220.8 |
| | | Median | 512.5 |
| | | Range | 76.9–959.9 |
| | | %CV | 42.46 |
| Female + Male | Clf0 (n=11) | Mean±SD | 313.1±48.04 |
| | | Median | 317.6 |
| | | Range | 229.3–383.7 |
| | | %CV | 15.3 |
| | Clf1 (n=4) | Mean±SD | 263.4±171.6 |
| | | Median | 246.0 |
| | | Range | 76.9–484.7 |
| | | %CV | 65.1 |
| | Clf2 (n=28) | Mean±SD | 532.9±242.2 |
| | | Median | 518.6 |
| | | Range | 111.9–959.9 |
| | | %CV | 45.4 |
| | Clf1+Clf2 (n=32) | Mean±SD | 499.2±249.3 |
| | | Median | 491.3 |
| | | Range | 76.9-959.9 |
| | | %CV | 49.9 |
| | Clf0+Clf1+Clf2 (n=43) | Mean±SD | 451.6±230.6 |
| | | Median | 443.4 |
| | | Range | 76.9-959.9 |
| | | %CV | 51.1 |

103  Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones  103
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek


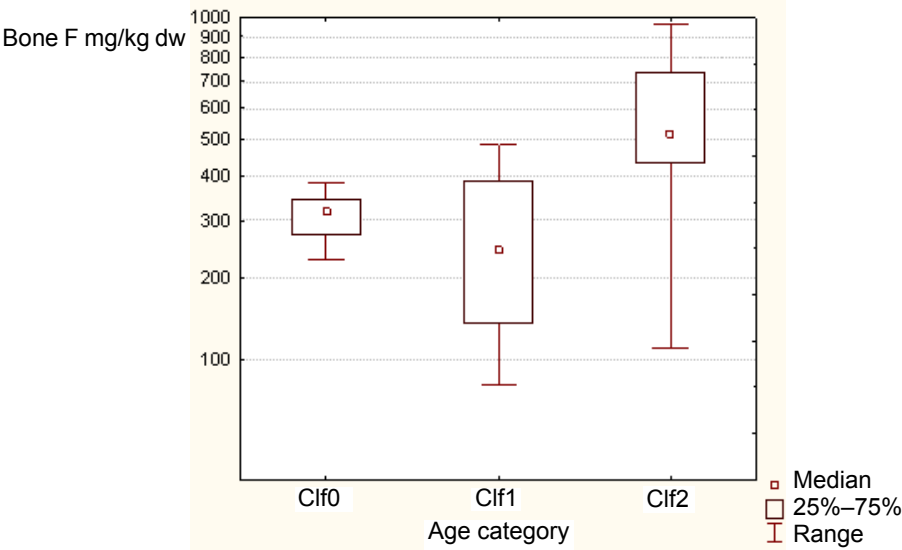
**Figure 2.** Fluoride level in dog bones in relation to age.
(Clf0 = 1-day-old puppies; Clf1 = young dogs <2 yr-old; Clf2 = adult dogs >2 yr-old).
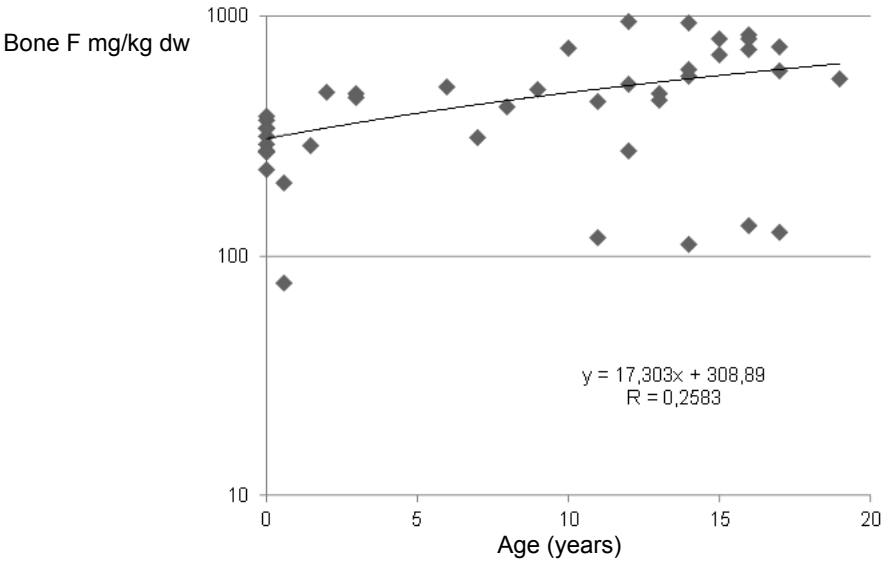


**Figure 3.** The relationship between fluoride in dog bones and age.

### DISCUSSION

In the literature, F levels in bone samples are denoted in different ways—in terms of wet or dry body weight, or ash. To be able to make comparisons between our results (expressed in mg/kg dw) and other authors, we used conversions based on our calculations and the works of Łanocha et al.[25] and Budis et al.[26] We assumed that the average water content in the dog compact bone was 23%. Based on the work of Suzuki,[27] we assumed that an F level at dry weight is 59% of bone ash level. In most publications, F content in bone is given as the arithmetic mean

104 Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones 104
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek

or range of values, therefore in comparisons we did not use median but arithmetic means or ranges of values.

On the basis of many papers, we assumed that in dogs from uncontaminated areas, the typical average F content in bone (reflecting the geochemical background) does not exceed 550 mg/kg dw.[13,27-32] In the present study, in 72% of dogs from the area of Szczecin this content was not exceeded in the femoral compact bone.

Based on available reports, the average F content in the femur of puppies ranges from 5 to 258 mg/kg dw, while in young dogs (ages 1–2 years), it is slightly higher and may even reach 270 mg/kg dw (Tables 2A and 2B). In adult dogs, it ranges from 230 to 450 mg/kg dw. Sometimes concentrations exceeding 1700 mg/kg dw are detected, but with no visible signs of intoxication.[33-36] The results presented in this paper concerning the F content in one-day-old puppies and adult dogs (313 and 533 mg/kg dw, respectively) slightly exceeded the upper range of values typical for these age groups presented by other authors. In contrast, the mean F content in the bones of young dogs, at 263 mg/kg dw, was within the typical range for this age group (Tables 2A and 2B).

The F content in dog bone depends on the type of bone tested (Table 2A). Simons[35] found a trend based on the findings of other authors to a greater accumulation of F in the ribs than the femurs of dogs (380 and 230 mg/kg dw, respectively). In addition, there are differences in the accumulation of F depending on the structural element of bones; in spongy bone F content is on average about 60% higher than compact bone and periosteum, which is associated with a better blood supply to the bone surface layers.[34,37]

Gardner et al.[34] observed that in the femur of healthy young dogs from New York, USA, F content was 6 times lower than in adult dogs from the same area (270 and 1780 mg/kg dw). Also in this present study, in one-day-old puppies in Szczecin, the F content was more than 6 times lower compared to dogs aged 8 months. Many authors have emphasized the relationship between the F content and the age of the dogs, for example Greenwood et al.[33] In the present work, puppies and young dogs had significantly lower content of F in bones than adult dogs (p<0.003 and p<0.05), and there was a significant relationship between the F content and the age of the animals, confirmed by a significant Spearman's rank correlation coefficient (r=0.51, p<0.001).

F strongly affects bone mineralization. Chavassieux,[38] in dogs receiving 0.7 mg/ kg NaF per day for a period of six months, found a reduction in bone mineralization and bone formation, and an increase in resorption in the spongy bone. In addition, he noted exostosis and changed characteristics of skeletal fluorosis, which could be related to hyperparathyroidism. Henrikson et al.[36] observed in dogs that received 40 weeks of NaF in different doses (from 26 to 1125 mg/kg), the accumulation of F in the bone depended on the dosage of F. Furthermore, the increase in bone F was accompanied by a reduction in bone calcium, although no hyperparathyroidism was observed.

105 Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones 105
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek

**Table 2A.** The fluoride concentration (mg/kg dw or ash) in dog bone based on the work of various authors
(n = number of animals; yr = years; mo = months; CB = compact bone; SB = spongy bone;
dw = dry weight; bw = body weight; F = female; M = male)

| Place and reference | Tissue | Age | Gender | n | Fluoride mg/kg dw or ash | Supplementation |
|---|---|---|---|---|---|---|
| Szczecin, Poland This study | Femur CB | >2 yr | F+M | 28 | 5323±242 mg/kg dw | – |
| | Femur CB | <2 yr | F+M | 4 | 263±172 mg/kg dw | – |
| | Femur CB | 1 day | F+M | 11 | 313±48 mg/kg dw | – |
| Hyderabad, India 41 | Femur | 2.5–3 mo | F+M | 4 | 466±128 mg/kg ash | Control |
| | Femur | 2.5–3 mo | F+M | 4 | 274±75 mg/kg dw | |
| | Femur | 2.5–3 mo | F+M | 5 | 3397±240 mg/kg ash | NaF 10 mg F/dog/day for 3 months |
| | Femur | 2.5–3 mo | F+M | 5 | 1998±141 mg/kg dw | |
| | Femur | 2.5–3 mo | F+M | 5 | 2000±435 mg/kg ash | NaF 10 mg F/dog/day + tamarind paste for 3 months |
| | Femur | 2.5–3 mo | F+M | 5 | 1176±256 mg/kg dw | |
| Hohenheim, Germany 37 | Femur CB | Adult | – | 1 | 485 mg/kg ash | – |
| | Femur CB | Adult | – | 1 | 285 mg/kg dw | – |
| | Femur SB | Adult | – | 1 | 781 mg/kg ash | – |
| | Femur SB | Adult | – | 1 | 495 mg/kg dw | – |
| Sztokholm, Sweden 36 | Femur | 1 yr | F+M | 2 | 765 mg/kg ash | Control |
| | Femur | 1 yr | F+M | 2 | 450 mg/kg dw | |
| | Femur | 1 yr | M | 2 | 940 mg/kg ash | 26 µg F/kg bw/day for 287 days |
| | Femur | 1 yr | M | 2 | 553 mg/kg dw | |
| | Femur | 2 yr | F | 2 | 845 mg/kg ash | 85–88 µg F/kg bw/day for 287 days |
| | Femur | 2 yr | F | 2 | 497 mg/kg dw | |
| | Femur | 2 yr | F | 2 | 1570 mg/kg ash | 295 µg F/kg bw/day for 287 days |
| | Femur | 2 yr | F | 2 | 923 mg/kg dw | |
| | Femur | 1 yr | F | 2 | 2315 mg/kg ash | 825–1125 µg F/kg bw/day for 287 days |
| | Femur | 1 yr | F | 2 | 1362 mg/kg dw | |
| | Humerus | 1 yr | F+M | 2 | 720 mg/kg ash | Control |
| | Humerus | 1 yr | F+M | 2 | 423 mg/kg dw | Control |
| | Vertebrae | 1 yr | F+M | 2 | 715 mg/kg ash | Control |
| | Vertebrae | 1 yr | F+M | 2 | 420 mg/kg dw | Control |
| | Frontal bone | 1 yr | F+M | 2 | 680 mg/kg ash | Control |
| | Frontal bone | 1 yr | F+M | 2 | 400 mg/kg dw | Control |

106  Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones  106
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek

**Table 2B.** The fluoride concentration (mg/kg dw or ash) in dog bone based on the work of various authors
(n = number of animals; yr = years; mo = months; CB = compact bone; SB = spongy bone;
dw = dry weight; bw = body weight; F = female; M = male)

| Place and reference | Tissue | Age | Gender | n | Fluoride mg/kg dw or ash | Supplementation |
|---|---|---|---|---|---|---|
| New York, USA 34 | Femur | 1 yr | – | 1 | 464 mg/kg ash | – |
| | Femur | 1 yr | – | 1 | 272.7 mg/kg dw | – |
| | Femur | 3–6 yr | – | 1 | 3030 mg/kg ash | – |
| | Femur | 3–6 yr | – | 1 | 1782 mg/kg dw | – |
| Chicago, USA 33 | Femur | 1 day | F+M | 9 | 5–258 mg/kg dw | Control |
| | Femur | 2 days | F+M | 15 | 12–310 mg/kg dw | Purified bone meal powder 5 mg F/kg bw/day for 110 days |
| | Femur | 2 days | F+M | 6 | 22–258 mg/kg dw | Defluorinated phosphate 5 mg F/kg bw/day for 98 days |
| | Femur | 1 day | F+M | 14 | 39–2150 mg/kg dw | NaF 5 mg F/kg/bw/day for 102 days |
| USA 35 | Femur | – | – | – | 250–269 mg/kg dw | – |
| | Femur | 6 yr | F | – | 230 mg/kg dw | – |
| | Femur | 6 yr | F | – | 280 mg/kg dw | – |

Those authors observed no pathological changes in bones or teeth dependent on the different doses of F received by the dogs. Similarly, Greenwood et al.[33] and Heyroth[39] observed no pathological changes in the bones of dogs treated with F in their food for three months at a dose of 5 mg/kg dw or for 5 years at a dose of 3 to 5 mg/kg dw, respectively. Similarly, Mulvhill et al.[40] found no pathological changes in the bones of dogs fed with NaF at a concentration of 9.5 to 19 mg/L. They found that both dose rate and duration of exposure to NaF had no toxic effect on the skeletal system of the dog.

The F content in the bone depends on the dose of the compounds obtained. In the paper by Khandare et al.,[41] in ash from the femurs of 2.5–3 month old puppies supplemented daily with 10 mg NaF for 3 months, the average F content was more than 7 times higher than in the control group without F supplementation (Table 2A).

Some researchers also examined the relationship between F supplementation and F metabolism in the dog. Heyroth[39] found that in dogs that received, for a few months, feed dosed with 10 mg F per kg body weight, 84% of F was excreted in the urine, while the remaining 16% of F was deposited in the bones.

Dogs ingest fluorine compounds primarily with water and food. In 8 out of 8 different dog foods tested in the USA, F compound levels were 2.5 times higher than EPA permissible F levels in drinking water.[42] In addition, high doses of F in

107 Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones 107
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek

dog feeds were associated with a high content of this element in their bones, which is largely the result of their widespread use in ready-made commercial dog feeds in the United States. There is evidence suggesting that the increased F content in the bones of dogs may be responsible for the increased incidence of osteosarcoma, the most common primary bone tumor in dogs, which makes up 85% of all tumors of the skeleton.[42-45]

Our results do not differ from the corresponding results reported by other authors and are typical for F content found in the bones of dogs not supplemented with F (unproven given that the feeds referred to in the previous paragraph were not taken into consideration). Results of this study may contribute to increased knowledge about the burden of F compounds on the biotic and abiotic environment, as well as demonstrate the usefulness of the dog for bioindicative purposes.

## ACKNOWLEDGMENT

## REFERENCES

1   Weinstein LH, Davison AW. Fluorides in the environment; effects on plants and animals. Wallingford, Oxon: CABI Publishing; 2004.
2   Fordyce FM, Vrana K, Zhovinsky E, Povoroznuk V, Toth G, Hope BC, et al. A health risk assessment for fluoride in Central Europe. Environ Geochem Health 2007;29:83-102.
3   Ghosh A, Mukherjee K, Ghosh SK, Saha B. Sources and toxicity of fluoride in the environment. Res Chem Intermed 2013;39:2881-915.
4   Camargo JA. Fluoride toxicity to aquatic organisms: a review. Chemosphere 2003;50:251-64.
5   Kockum PC, Herbert RB, Gislason SR. A diverse ecosystem response to volcanic aerosols. Chem Geol 2006;231:57-66.
6   Ozsvath DL. Fluoride and environmental health: a review. Rev Environ Sci Biotech 2009;8:59-79.
7   Seńczuk W, editor. Toxicology. Warsaw: PZWL; 1994.
8   WHO. Air Quality Guidelines for Europe, 2nd ed. Geneva: WHO Regional Publications, European Series [Opera Internet Browser 11.60]. 2000 [cited 2013 Jan 23];91:1-252.Available from: http://www.euro.who.int/__data/assets/pdf_file/0005/74732/E71922.pdf
9   Fawell J, Bailey K, Chilton J, Dahi E, Fewtrell L, Magara Y. Fluoride in drinking-water. WHO: IWA publishing; 2006.
10  Indulski JA. Fluorine and fluorides. Warsaw: PZWL; 1989.
11  Whitford GM. Intake and metabolism of fluoride. Adv Dent Res 1994;8:5-14.
12  Inkielewicz I, Krechniak J. Fluoride content in soft tissues and urine of rats exposed to sodium fluoride in drinking water. Fluoride 2003;36:263-6.
13  Vieira APGF, Mousny M, Maia R, Hancock R, Everett ET, Grynpas MD. Assessment of teeth as biomarkers for skeletal fluoride exposure. Osteoporosis Int 2005;16:1576-82.
14  Dobrzański Z, Górecka H. Fluorine in poultry nutrition. Drob Pol 2001:3:13-5.
15  Inoue M, Le Geros RZ, Inoue M, Riviera RS, Sathi GA, Tsujigiwa H, et al. Fluoride supplement affects bone mineralization in young rats. J Hard Tissue Biol 2006;15:61-4.
16  Wang YZ. The cartilage damage of fluorosis [abstract]. Fluoride 1995;28:39.
17  Harbow DJ, Robinson MG, Monsour PA. The effect of chronic fluoride administration on rat condylar cartilage. Aust Dent J 1992;37:55-62.
18  Szmuck A. Meteorology and climatology for the WSR. Warsaw: PWN; 1969.
19  Zabłocki Z. Changes in the concentration of fluoride in some components in the area of environmental effects of the emissions of chemical plants "Police" in the years 1977-1996. In: Ogoński T, Samujło D, Machoy Z. Fluoride and micronutrients in biology and medicine. VIII Fluorine Symposium; 1998 Apr 23-24; Szczecin, Poland. pp. 16-23.
20  Telesiński A, Śnioszek M, Środa E. Fluoride accumulation in chosen hydromacrophytes species depending on their content in water and sediments of Gunica river. Ochr Środ Zas Nat 2011;49:345-52.

108 Research report
Fluoride 47(2)98–108
April-June 2014

Fluoride accumulation in dog bones 108
Palczewska-Komsa, Kalisińska, Kosik-Bogacka, Łanocha, Budis,
Baranowska-Bosiacka, Gutowska, Chlubek

21 Dąbrowska E, Balunowska M, Letko R. Dangers connected with excessive supply of fluorine: a review. Nowa Stomat 2001;4:22-7.

22 Gutowska I, Baranowska-Bosiacka I, Rybicka M, Dudzińska W, Marchlewicz M, Noceń I, et al. Changes in the concentration of fluoride and biogenic elements in the serum and bones of female rats with streptozotocin-induced diabetes. Fluoride 2009;42:9-16.

23 Gutowska I, Baranowska-Bosiacka I, Rybicka M, Noceń I, Dudzińska W, Marchlewicz M, et al. Changes in the concentration of microelements in the teeth of rats in final stage of type 1 diabetes, with an absolute lack of insulin. Biol Trace Elem Res 2011;139:332-40.

24 Gutowska I, Baranowska-Bosiacka I, Noceń I, Piotrowska K, Marchlewicz M, Wiernicki I, et al. Soy isoflavones administered pre- and postnatally may affect the ER and ER expression and elements content in bones of mature male rats. Hum Exp Toxicol 2012;31:346-54.

25 Łanocha N, Kalisińska E, Kosik-Bogacka DI, Budis H, Sokołowski S, Bohatyrewicz A. Comparison of metal concentrations in bones of long-living mammals. Biol Trace Elem Res 2013;152:195-203.

26 Budis H, Kalisińska E, Lanocha N, Kosik-Bogacka DI. The concentration of manganese, iron and strontium in bone of red fox *Vulpes vulpes* (L. 1758). Biol Trace Elem Res 2013;155:361-9.

27 Suzuki Y. The normal levels of fluorine in the bone tissue of Japanese subjects. Tohoku J Exp Med 1979;129:327-36.

28 Jackson D, Weidmann SM. Fluorine in human bone related to age and the water supply of different regions. J Pathol Bacteriol 1958;76:451-9.

29 Franke J. Fluoride and ash content of bone in various stages of human fluorosis. Fluoride 1984;22:195-203.

30 Kaminsky LS, Mahoney MC, Leach J, Melius J, Miller MJ. Fluoride: benefits and risks of exposure. Crit Rev Oral Biol Med 1990;1:261-81.

31 Reddy DR. Neurology of endemic skeletal fluorosis. Neurol India 2009;57:7-12.

32 Tankersley KB, Wells DH. Further evaluation of fluoride dating by ion selective electrode analysis. North American Archeologist 2011;32:247-65.

33 Greenwood DA, Blayney JR, Skinsnes OK, Hodges PC. Comparative studies of the feeding of fluorides as they occur in purified bone meal powder, defluorinated phosphate and sodium fluoride, in dogs. J Dent Res 1946;25:311-26.

34 Gardner DE, Smith FA, Hodge HG, Brudevold F, Eldredge DM. Distribution of fluoride in the normal dog femur. J Appl Physiol 1959;14:427-30.

35 Simons JH. Fluoride chemistry. Vol 4. New York: Academic Press; 1965.

36 Henrikson PA, Lutwak L, Krook L, Skogerboe R, Kallfelz F, Belanger LF, et al. Fluoride and nutritional osteoporosis: physiochemical data on bones from an experimental study in dogs. J Nutr 1970;100:631-42.

37 Loeffler K, Brosi C, Oelschläger W, Feyler L. Fluorosis in the dog. Kleiner-Praxis 1979;24:157-204.

38 Chavassieux P. Bone effects of fluoride in animal models in vivo. A review and a recent study. J Bone Miner Res 1990;5 Suppl 1:S95-9.

39 Heyroth F. Toxicological evidence for the safety of the fluoridation of public water supplies. Am J Public Health Nations Health 1952;42:1568-75.

40 Mulvihill JE, Goldhaber P, Zager NI. Toxicity of fluoride applied topically to the alveolar bone od dogs and monkeys. J Dent Res 1972;51:875.

41 Khandare AL, Kumar PU, Lakshmaiah N. Beneficial effect of tamarind ingestion on fluoride toxicity in dogs. Fluoride 2000;33:33-8.

42 Naidenko O. Dog food comparison shows high fluoride levels. Environmental Working Group. Available from: http://www.ewg.org/research/dog-food-comparison-shows-high-fluoride-levels

43 Bassin EB, Wypij D, Davis RB, Mittelman MA. Age-specific fluoride exposure in drinking water and osteosarcoma (United States). Cancer Causes Control 2006;17:421-8.

44 Mueller F, Fuchs B, Kaser-Hotz B. Comparative biology of human and canine osteosarcoma. Anticancer Res 2007;27:155-64.

45 Hannah H, Bachand A, Lana S, Reif J. Water fluoridation and canine osteosarcoma. Epidemiology 2004;15:S83.