FLUORIDE IN THE COMPACT BONE AFTER FEMORAL HEAD ARTHROPLASTY IN PATIENTS FROM NORTH-WESTERN POLAND

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SUMMARY: Fluoride ions (F^-) may have a toxic effect, depending on dose, duration of exposure, age, sex, metabolism, concomitant renal and bone diseases, and supplementation. In north-western Poland industrial plants still emit considerable amounts of fluorine compounds and therefore F^- concentration must be monitored in the local population, particularly in bone tissue which reflects long-term F^- accumulation. The aim of this study was to determine the concentration of F^- in the bones of patients after femoral head arthroplasty, hospitalized in the Department of Orthopedics and Traumatology of the Pomeranian Medical University in Szczecin, Poland. The concentration of F^- was determined by potentiometry in dried bone, and the results were expressed per dry weight (dw). The highest F^- concentration was found in men aged <65 years, whereas the lowest was in women aged <65 years (479.4 and 189.5 mg/kg dw, respectively). Bone F^- concentration significantly differed between two age groups of men (<65 and >65 years), with the older men having a 54% higher F^- concentration than the younger men. In addition, the F^- concentration in women aged >65 years was about 51% higher compared to men aged >65 years. Bone F^- concentration in men and women from the north-western Poland showed high diversity, which could have been influenced by sex, age, cessation of water fluoridation in Szczecin, and the modernization of the Police Chemical Plant near Szczecin, which reduced emissions of fluorine compounds to the environment.

Keywords: Arthroplasty; Bioaccumulation; Fluoride; Human bones; Poland.

INTRODUCTION

For many years, mass fluoridation of drinking water was used as an anti-caries measure in a number of countries. It was applied in Europe (Ireland, Spain), South America (Brazil, Chile), Asia (Israel, Hong Kong, Singapore, Malaysia, Japan, South Korea), Africa (Nigeria, Republic of South Africa), Australia, New Zealand and almost all of North America. However, later research showed that the primary mechanism of anti-caries action is local with F^- deposition in the enamel and a reduction in the dental erosion resulting from the production of acids by cariogenic bacteria.[1,2] Accordingly, in the 1990s artificial water fluoridation was abandoned in many countries, including Poland.

Large concentrations of F^- are found in waste from industrial plants producing fertilizers. Their phosphogypsum dumps, often located close to rivers, are the direct source of F^- penetrating surface waters in the area of the plants.[3]

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According to the Food and Nutrition Board, Institute of Medicine, the permissible upper limit of daily intake of F^- is 10 mg per day for adults and children over 8 years old. In areas where the water was fluoridated (≤1.0 mg/L) and in places where the concentration of F^- in the water was less than 0.3 mg/L, the average consumption of F^- by adults was 1.4–3.4 and 0.3–1.0 mg per day, respectively.[4] Most food contains F^- at a concentration of not more than 0.5 mg/kg, with the exception of sea fish, in which the level of F^- can range from 6 to 27 mg/kg.[5]

F^- enters the human body with water (60%), food (35%) and inhaled air (5%). The daily dose of F^- depends on the diet and ranges from 3 to 5 mg.[1,3] It is believed that 80–90% F^- is absorbed in the upper gastrointestinal tract, of which about 40–50% of the ingested F^- is absorbed in the stomach.[5] The respiratory system is the major route of absorption of F^- in the form of gas and dust found in the industrial environment.[5]

Fluoride is stored mainly in hard tissues and to a lesser extent in the soft organs.[6,7] The half-life of F^- in plasma is from 2 to 9 hours, while in the bone it is from a few to up to 20 years.[8] In adults and children, 10 to 50% of the daily ingested F^- is retained in the body.[5,8] About 99% of F^- is accumulated in bones and other mineralized tissues.[5,6,9] The average bone F^- concentration in people living in temperate areas where ground water has low F^- levels, is ≤550 mg/kg dry weight (dw).[8-11] Fluoride ions are easily deposited in the mineralized tissues by being incorporated into the apatite crystals by the process of ion exchange with the hydroxyl ions which are included in the structure of bone-forming apatite. Therefore, bone can be considered a good material for long-term research on F^- concentration.

There are few data available on the F^- levels in the mineralized tissues, collected intraoperatively during arthroplasty, of people environmentally exposed to F^-. Therefore, the aim of this study was to determine F^- levels in the bones of patients subjected to femoral head arthroplasty from the area of north-western Poland.

MATERIAL AND METHODS

The West Pomerania province has two major sources of F^-: the Police Chemical Plant and the Dolna Odra Power Plant.[12,13] The first covers the area of 13 km^2, of which 6.1 km^2 is covered by phosphogypsum dumps.[3] Measurements performed around the Police Chemical Plant showed fluoride contamination of all environmental components of land as much as 40 km away from the emission source. The Dolna Odra Power Plant is located south of the city of Szczecin. In addition, the area of West Pomerania is exposed to pollution coming from the German refinery in Schwedt, situated very close to the Polish-German border; taking into account the prevailing winds in the West Pomerania the refinery has no less impact on the environment than the above-mentioned Polish plants. Figure 1 shows the map of Poland and the counties of the West Pomeranian voivodeship, including the aforementioned plants, the direction of the prevailing winds and the places of residence of patients from whom bone samples were taken for analysis.

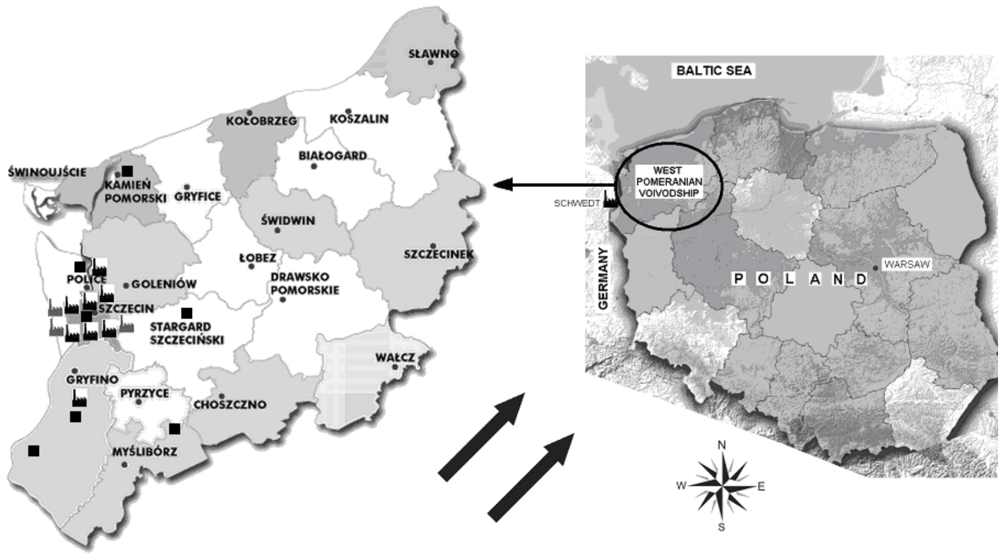

Figure 1. Map of Poland and districts of the province including the West Pomeranian voivodeship plants emitting fluoride, patients' place of residence and direction of predominant winds. Black factory icons: factories with active plants; Grey factory icons: factories with closed plants; Black squares: patients' places of residence; Black arrows: direction of the prevailing wind). (Graphic design by S Komsa).

The research material was collected from 36 patients hospitalized at the Department of Orthopaedics and Traumatology of the Pomeranian Medical University in Szczecin (north-western Poland) in 2009. All the patients underwent arthroplasty of the femoral head. The most common indication for this procedure was degeneration of the left and/or right hip joint (22 women and 11 men) and fracture of the left femoral neck (1 woman and 2 men). In 7 patients (5 women and 2 men) the degeneration of the hip joints was accompanied by osteoporosis. The patients were divided into two groups according to the place of residence: Szczecin (n=26) and other cities in West Pomerania: Barlinek, Chojna, Kamień Pomorski, Nowe Warpno, Pniewo, Stargard Szczecinski (n=10). We did not obtain information on the duration of residence in West Pomeranian voivodeship and the possible occupational exposure to fluoride. According to their age the patients were divided into two categories: <65 yr (<65 years of age) and >65 yr (>65 years of age).

The collected femurs were cleaned from 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. Dried samples were ground, mixed with perchloric acid, and shaken. After cooling, sodium citrate and TISAB II were added to the sample. Fluoride was determined by a potentiometric method with an ion-selective electrode (Thermo Scientific Orion Company, USA) according to Gutowska et al.[14,15] The fluoride content in the samples was calculated based on the difference of potentials measured in each sample, the sample weight and the concentration of the added standard.

Statistical analyses were performed using Stat Soft Statistica 10.0 and Microsoft Excel 2007. The arithmetic means (AM), standard deviations of the means (SD) and medians were calculated. Compliance of distributions of F^- were checked using a Kolmogorov-Smirnov test with Lillefors correction (KS). Means were compared by nonparametric tests: Kruskal-Willis (KW) and Mann-Whitney U test (M-W U). In determining the correlation between F^- levels and age the Spearman's rank correlation coefficient (r_S) was used.

The study protocol was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0012/115/11).

RESULTS

In the bones taken from patients from the north-western Poland, F^- levels ranged from 54.3 to 1,488.8 mg/kg dw. The highest bone F^- was observed in men aged <65 years, and the lowest in women aged <65 years (respectively 479.4 and 189.5 mg/kg dw) (Table 1).

There were no statistically significant differences in bone F^- between women from the <65 yr and >65 yr groups. A statistically significant difference was found between <65 yr and >65 yr men (U=5, p<0.03); bone F^- in <65 yr men was about 54% higher compared to the older >65 yr men. There was no statistically significant difference in bone F^- between <65 yr men and women, whereas a statistically significant difference was observed between >65 yr men and women (U=12, p<0.02). Bone F^- in women >65 years was about 51% higher compared to men from the same age group. We found no correlation between bone F^- level and the age of patients (r_S=-0.003, p<0.05).

Table 1. Fluoride ion (F^-) concentrations in the bones (mg/kg dw) of patients by sex and age (W: women; M: men; <65 yr: patients aged <65 years; >65 yr: patients aged >65 years; SD: standard deviation)

n	Sex	Age category	F^- concentration in the bones (mg/kg dw)		
			Mean±SD	Median	Range
6	W	< 65 yr	275.1±281.1	189.5	61.7–774.6
17		> 65 yr	525.7±327.5	469.2	54.3–1488.8
23		< 65 yr + > 65 yr	460.3±329.6	443.3	54.3–1488.8
8	M	< 65 yr	551.8±234.1	479.4	245.8–926.2
5		> 65 yr	268.2±123.6	310.0	66.8–385.6
13		< 65 yr + > 65 yr	442.7±240.2	385.6	66.8–926.2
14	W+M	< 65 yr	433.2±283.0	408.8	61.7–926.2
22		> 65 yr	467.2±311.2	442.0	54.3–1488.8
36		< 65 yr + > 65 yr	453.9±296.9	442.0	54.3–1488.8

There were no statistically significant differences in bone F⁻ levels between the inhabitants of Szczecin and patients from other cities in West Pomerania. Median fluoride concentrations in the bones of patients from Szczecin and other cities in the West Pomeranian voivodeship were similar and amounted to 430.3 (mean±SD, 412.8±240.3 mg/kg dw) and 457.0 mg/kg dw (mean±SD, 560.8±405.9 mg/kg dw), respectively.

Kruskal-Wallis test (KW) showed no statistically significant differences of bone F⁻ levels between patients with osteoarthritis, regardless of concomitant osteoporosis, osteoarthritis with concomitant osteoporosis and osteoarthritis without osteoporosis. The bone F⁻ levels in these patients are given in Table 2.

Table 2. Fluoride ion (F⁻) concentrations in the bones (mg/kg dw) of patients by sex and diseases of the osteoarticular system (W: women; M: men; SD: standard deviation)

n	Sex	Osteoarticular disease	F⁻ concentration in the bones (mg/kg dw)		
			Mean±SD	Median	Range
22	W	Hip joint degeneration	462.1±337.2	455.7	54.3-1488.8
11	M		574.1±540.8	436.4	66.8-926.2
33	W+M		449.2±304.8	443.3	54.3-1488.8
5	W	Hip join degeneration with concomitant osteoporosis	574.1±540.8	436.4	54.3-1488.8
2	M		655.9±382.3	655.9	385.6-926.2
7	W+M		597.4±470.0	436.4	54.3-1488.8
17	W	Hip join degeneration without concomitant osteoporosis	429.2±266.6	468.1	58.8-965.7
9	M		371.6±192.0	334.6	66.8-738.7
26	W+M		409.3±240.9	444.6	58.8-965.7
3	W+M	Femoral neck fracture	506.2±227.4	419.9	334.7-764.1

DISCUSSION

Average bone F⁻ levels in humans depend on age, sex, metabolism, concomitant bone and kidney diseases, F⁻ supplementation, and duration of exposure to F⁻. Comparison of various data on bone F⁻ levels in people is difficult because samples come from different types of bones and from people representing different age groups and living in various environments.

Literature reports show that bone F⁻ levels substantially depend on the duration of exposure to F⁻ and its dose. A single case of skeletal fluorosis caused by occupational exposure was described by Wolff and Kerr[16] and Weidmann et al.[17] It concerned a man exposed to F⁻ containing dust for 18 yr (3.88% F⁻ in phosphorus rocks). In his long bones the F⁻ levels ranged from 1,800 to 2,900 mg/

kg dw, while in ribs they reached 5,600 mg/kg dw. Several cases of severe industrial fluorosis were reported in the residents of the city of Saxon (Germany), located near a fluoride-producing plant. Average bone F^- levels in those individuals exceeded 5,000 mg/kg dw (Table 3a),[18] ~11.0 times higher than the average concentration of this element in the bones of patients from the north-western Poland (442 mg/kg dw).

Hać et al. studied the concentration of F^- in the ribs of people (aged 17–87 years) from the city of Gdańsk and the surrounding area.[19] Until recently, phosphorus plants situated near the city at the mouth of the Vistula River have emitted significant amounts of F^-. The average rib F^- level was 626 mg/kg dw, being ~1.5 times higher compared to the average femur F^- level (413 mg/kg dw) determined in the inhabitants of Szczecin investigated in this work.

At the end of the 1970s in Finland, Alhava et al.[20] conducted a comparison between hip bone F^- of people living in areas with different concentrations of F^- in drinking water. In the city of Kuopio, water fluoridation was conducted from the 1950s to the 1990s (~1 mg/L), but, in the vicinity of the city, the water was not fluoridated and accordingly contained not more than 0.32 mg/L. The highest bone F^- (399–2,360 mg/L bone ash, which corresponds to 235–1,388 mg/kg dw) was found in women from Kuopio (Table 3b). That range is different from the one we found for the women in Szczecin (54.3–1,488.8 mg/kg dw). Much greater femoral F^- levels (4,130 mg/kg dw) were detected in the residents of Lubbock, USA, with high F^- levels in tap water (4 mg/L, Table 3c).[21] That level is about ~2.7 times higher than the highest femoral F^- level in patients from north-western Poland (1,489 mg/kg dw).

Bone F^- levels of patients coming from Szczecin,[22] where F^- level in tap water is ~0.3 mg/L, are similar to bone F^- levels in people, who consumed water containing <1.0 mg/L as described by Zipkin et al.[21] and Alhava et al.[20]

Many studies demonstrate a relationship between age and F^- accumulation in human bones.[19,23] The lowest F^- levels are reported in the bones of foetuses and young children, not exceeding 30 mg/kg dw.[24] Suzuki examined the bone F^- levels of Japanese from different age groups living in F-contaminated areas.[23] The lowest average hip F^- was found in men and women aged 20 to 39 years (365 and 222 mg/kg dw, respectively), while the highest were found in men and women aged 60-79 years (520 and 418 mg/kg dw, respectively). At the same time, Suzuki pointed out that, in people aged 70–79 years, bone F^- decreases compared to people aged 60-69 years (Table 3b).[23] This is probably related to the progressive physiological loss of bone mineral density and bone mass.

Table 3a. Fluoride (F^-) concentrations (mg/kg) in highly mineralized human tissues based on our own results and the papers of other authors (F: female; M: male; dw: dry weight; SD: standard deviation; Suppl: supplementation; Ref: reference)

Place	Age (years unless specified)	Sex	n	Group	F^- concentration (mg/kg dw or ash) (mean±SD or mean)	Suppl	Ref
Femur							
Poland, West Pomerania province	46–65	M	8		551±234 dw	-	This study
	65–78	M	5		268±124 dw		This study
	32–65	F	6		275±281 dw		This study
	65–82	F	17		526±328 dw		This study
	36–76	F + M	21	Control	536±327 dw		30
	36–76	F + M	8	Osteosclerosis	818±408 dw		30
Poland, West Pomerania, Zone I (0.4 µg F^-/dm^3)	-	F + M	40		430 dw		11
Poland, West Pomerania, Zone II (1.6 µg F^-/dm^3)	-	F + M	40		590 dw		11
Japan, Morioka	0–79	F + M	101		115±18 dw		23
Israel, Tel-Aviv	Fetuses 6–9 months	F + M	4		41 ash (24 dw)	~0.1 mg F^-/L in drinking water	31
Israel, Jerusalem	Fetuses 6–9 months	F + M	4		75 ash (44 dw)	~0.55 mg F^-/L in drinking water	31

Table 3b. Fluoride (F^-) concentrations (mg/kg) in highly mineralized human tissues based on our own results and the papers of other authors (W: women; M: men; dw: dry weight; SD: standard deviation; Suppl: supplementation; Ref: reference)

Place	Age (years)	Sex	n	Group	F^- concentration (mg/kg dw or ash) (mean±SD or mean)	Suppl	Ref
				Femur and iliac crest			
Germany, Erfurt		W + M	8	Control	0.042± 0.015 dw % (420± 150 dw)	-	18
			7	Subtle symptoms of fluorosis	0.20± 0.03 dw % (2000± 300 dw)		
			8	Stage 0–I of radiological fluorosis	0.27± 0.08 dw % (2700± 800 dw)		
			10	Stage I of radiological fluorosis	0.33± 0.09 dw % (3300± 900 dw)		
			9	Stage II of radiological fluorosis	0.44± 0.05 dw % (4400± 500 dw)		
			4	Stadium III of radiological fluorosis	0.57± 0.11 dw % (5700± 1100 dw)		
Finland, Kuopio	24–86	M	14		347–1980 ash (204–1165 dw)	0.97 mg F^-/L in drinking water	20
	44–85	W	24		399–2360 ash (235–1388 dw)		
Finland, areas near Kuopio	20–86	M	17		106-539 ash (62-317 dw)	0.02–0.32 mg F^-/L in drinking water	
	31–92	W	23		144-790 ash (85-465 dw)		
Japan, Morioka	0–9	M	5		148±52 dw	-	23
	40–49	M	12		459±168 dw		
	50–59	M	14		492±141 dw		
	60–69	M	20		529±123 dw		
	70–79	M	10		511±145 dw		
	0–9	W	4		91±28 dw		
	30–39	W	1		212 dw		
	40–49	W	3		408±70 dw		
	50–59	W	8		424±92 dw		
	60–69	W	13		446±158 dw		
	70–79	W	9		378±204 dw		

Table 3c. Fluoride (F^-) concentrations (mg/kg) in highly mineralized human tissues based on our own results and the papers of other authors (W: women; M: men; dw: dry weight; SD: standard deviation; Suppl: supplementation; Ref: reference)

Place	Age (years)	Sex	n	Group	F^- concentration (mg/kg dw or ash) (mean±SD or mean)	Suppl	Ref
Femur and iliac crest							
USA, Boston, MA	25–70	W + M	16	Osteoporosis	541-7019 ash (318-4129 dw)	-	27
			5	Other bone diseases	815-3046 ash (479-1792 dw)		
			9	Control	0.087 ± 0.053 ash % (511± 311 dw)		
			15		0.358±0.157 ash % (2100±923 dw)	Treatment with high doses of NaF for less than 2 years	
USA, New York City, NY, and San Antonio, TX	27–87	W + M	33		0.041±0.003 dw % (410±30 dw)	<1 mg F^-/L in drinking water	21
USA, Grand Rapids, MI	64–85	W + M	5		0.138±0.016 dw % (1380±160 dw)	1 mg F^-/L in drinking water	
USA, Colorado Springs, CO, and Amarillo, TX	36–90	W + M	27		0.267±023 dw % (2670±2300 dw)	2.6 mg F^-/L in drinking water	
USA, Lubbock, TX	26–74	W + M	4		0.413±0.53 dw % (4130±5300 dw)	4 mg F^-/L in drinking water	

In this paper, analysis included both men and women, and we found no significant difference in the femoral F^- between patients younger than 65 years (433 mg/kg dw) and older than 65 years (467 mg/kg dw). However, there was a statistically significant difference in bone F^- between men <65 and >65 yr of age (552 and 268 mg/kg dw, respectively). It should also be emphasized that in men F^- was significantly higher in the group of younger patients than in the older ones, which to some extent is consistent with the observations of Suzuki.[23] This may be related to the hormonally conditioned differences in the processes occurring in the bones of aging men and age-related bone loss.[25] Although Suzuki[23] reported a decrease in the bone F^- levels of women aged >69 yr, we found no such regularity. Probably this was related to the modulating effect of osteoporosis found in up to one third of the women aged over 65 yr in our study. At the same time, taking into account that at the current stage of research the number of this population was not large, further studies should analyse a larger group to verify this hypothesis.

Bohatyrewicz found a relationship between F^- level in femurs derived from 51 patients (aged from 54 to 92 yr, after femoral arthroplasty following femoral fractures) and their age.[26] The material for the research came primarily from female residents of Szczecin and its surroundings. He found a positive correlation between F^- level in compact bone and the age of patients ($r=0.71$, $p<0.0001$). In the bone samples obtained from osteoporotic women from the same region of Poland, Bohatyrewicz et al. found no significant correlation between the concentration of fluoride in different bone structures and the age of patients, but confirmed a positive correlation between its concentration in entire bone samples and the age of subjects ($r=0.87$; $p<0.001$).[27] In another study, Bohatyrewicz found that the content of fluoride in compact and cancellous bone in patients with hip degeneration did not depend on their age.[28] In this paper, we observed no statistically significant differences in femoral F^- levels between women aged <65 and >65 yr of age which is consistent with the observations of Bohatyrewicz.[27,28]

In people suffering from various diseases osteoarticular F^- levels can vary from ~50 to over 4,100 mg/kg dw. One of the highest F^- levels was reported by Gron et al. in a sample of bone ash taken from a patient diagnosed with osteoporosis (4,129 mg/kg dw), coming from Boston, USA (Table 3c).[29] In contrast, one of the lowest F^- levels were found in patients from the north-western Poland, in a sample of the patient's femur diagnosed with osteoarthritis, where F^- level was slightly higher than 54 mg/kg dw. Bohatyrewicz, who conducted a study on the concentration of F^- in 32 patients subjected to an osteoarthritis-related arthroplasty in Szczecin in 1994–1997, found that the mean compact bone F^- level was 760 mg/kg dw.[28] Compared with those results, in our studies concerning 22 compact bone samples obtained from the femoral head of patients who also underwent osteoarthritis-related arthroplasty in 2009, the cited mean F^- level was more than 60% higher.

In comparison with the aforementioned data on femoral F^- levels in the residents of north-western Poland hospitalized in the 1990s, we can conclude that patients of the orthopedic ward at a hospital in Szczecin in 2009 had from 57% to 60% lower bone F^- levels. The observed differences could have been caused by the cessation of artificial water fluoridation in Szczecin in 1997 and the modernization of the

Police Chemical Plant which was launched in the mid-1990s with the aim of reducing F^- emissions to the environment.

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REFERENCES

1 Fordyce FM, Vrana K, Zhovinsky E, Povoroznuk V, Toth G, Hope BC, Iljinsky U, Baker J. A health risk assessment for fluoride in Central Europe. Environ Geochem Health 2007;29:83-102.
2 Kaczmarek U. The cariostatic mechanisms of fluoride. Czas Stomatol 2005;68:404-13.
3 Kłódka D, Misik D, Wójcik K, Telesiński A. Fluorine content in selected vegetables grown within the area affected by emission of that element from the “Police” chemical plant. Bromat Chem Toksykol 2008;41:964-9.
4 Pollick HF. Water fluoridation and the environment. Int J Occup Environ Health 2004;10:343-50.
5 Whitword GM. Intake and metabolism of fluoride. Adv Dent Res 1994;8:5-14.
6 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.
7 Tsunoda M, Aizawa Y, Nakano K, Liu Y, Horiuchi T, Itai K, Tsunoda H. Changes in fluoride levels in the liver, kidney, and brain and in neurotransmitters of mice after subacute administration of fluoride. Fluoride 2005;38:284-92.
8 Kaminsky LS, Mahoney MC, Leach J, Melius J, Miller MJ. Fluoride: benefits and risks of exposure. Crit Rev Oral Biol Med 1990;1:261-281.
9 Vieira AP, 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.
10 Reddy DR. Neurology of endemic skeletal fluorosis. Neurol India 2009;57:7-12.
11 Zawierta J, Chlubek D, Dąbkowska E, Bohatyrewicz A, Wieczorek P, Walat S, Machoy Z. Comparison of fluoride concentration in bones of humans and animals inhabiting the region of Western Pomerania and exposed to emissions of fluorine compounds from industrial sources. Environ Sci 2002;9:283-8.
12 Gutowska I, Machoy Z, Machaliński B, Chlubek D. Living conditions of deer in provinces of Western Pomerania and Lubuskie as revealed by mandibular content of fluoride, calcium, and magnesium. I. Inter-relations between fluoride, calcium, and magnesium content in mandible. Ann Acad Med Stetin 2004;50:42-6.
13 Straszko J, Fidecka M, Paulo LA. Assessment of air quality in industrial areas. Ochr Srodow 1996;4:45-8.
14 Gutowska I, Baranowska-Bosiacka I, Rybicka M, Noceń I, Dudzińska W, Marchlewicz M, Wiszniewska B, Chlubek D. 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.
15 Gutowska I, Baranowska-Bosiacka I, Noceń I, Piotrowska K, Marchlewicz M, Wiernicki I, Chlubek D, Wiszniewska B. Soy isoflavones administered pre- and postnatally may affect the ERα and ERβ expression and elements' content in bones of mature male rats. Hum Exp Toxicol 2012;31:346-54.
16 Wolff WA, Kerr EG. The composition of human bone in chronic fluoride poisoning. Am J Med Sci 1938;195:493-7.
17 Weidmann SM, Weatherell JA, Jackson D. The effect of fluoride on bone. Proc Nutr Soc 1963;22:105-10.
18 Franke J. Fluoride and ash content of bone in various stages of human fluorosis. Fluoride 1989;22:195-203.
19 Hac E, Czarnowski W, Gos T, Krechniak J. Lead and fluoride content in human bone and hair in the Gdańsk region. Sci Total Environ 1997;206:249-54.

20 Alhava EM, Olkkonen H, Kauranen P, Kari T. The effect of drinking water fluoridation on the fluoride content, strength and mineral density of human bone. Acta Orthop Scand 1980;51:413-20.
21 Zipkin I, McClure FJ, Leone NC, Lee WA. Fluoride deposition in human bones after prolonged ingestion of fluoride in drinking water. Public Health Rep 1958;73:732-40.
22 Lubkowska A. Assessment of the safety of drinking water in the West-Pomeranian Voivodeship, Poland. FEB 2009;18:1086-89.
23 Suzuki Y. The normal levels of fluorine in the bone tissue of Japanese subjects. Tohoku J Exp Med 1979;129:327-36.
24 Hellström I. Studies on fluoride distribution in infants and small children. Scand J Dent Res 1976;84:119-36.
25 Rabijewski M, Papierska L, Kozakowski J, Zgliczyński W. Osteoporosis in men: pathogenesis, prevention and treatment. Gerontol Pol 2007;15:54-60.
26 Bohatyrewicz A. Bone fluoride in proximal femur fractures. Fluoride 2001;34:227-35.
27 Bohatyrewicz A, Ogoński T, Dąbkowska E, Machoy Z. Mineral composition of trabecular and cortical bone in female patients with osteoporotic femoral neck fracture. Met Fluor 1998;8:70-4.
28 Bohatyrewicz A. Effects of fluoride on bone metabolism and mechanical competence: clinical observations and experimental studies in rats. Ann Acad Med Stetin 2002;73:1-85.
29 Gron P, McCann HG, Bernstein D. Effect of fluoride on human osteoporotic bone mineral. J Bone Joint Surg Am 1966;48:892-8.
30 Dąbkowska E, Bohatyrewicz A, Wieczorek P, Rzeuski R, Mikołajek W, Noceń I, Ciechanowski K. Estimation of fluoride and calcium contents in bones with osteosclerosis. Met Fluor 1998;8:75-8.
31 Adler P, Armstrong WD, Bell ME, Bhussry BR, Büttner W, Cremer H-D, et al. Fluorides and human health. World Health Organization Monograph Series No. 59. Geneva: WHO; 1970.