196 Fluoride Vol. 33 No. 4 196-204 2000 Research Report

FLUORIDE LEVELS IN HAIR OF EXPOSED AND UNEXPOSED POPULATIONS IN POLAND

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SUMMARY: A microdiffusion procedure was used with a fluoride ion-selective electrode to determine fluoride levels in 548 hair samples collected in 1995-1997 from children and adults living in the low-risk exposure region of Ciechanów, Poland. An additional 71 hair samples were collected from electrolysis workers with high-risk exposure at an aluminium plant in Konin, Poland. The mean hair fluoride values for the low-risk population varied from 1.3 to 2.6 µg/g (range <0.5 to 26 µg/g) and showed only small association with presumed exposure levels. Among the aluminium plant workers, the mean values were 403.4 and 2828.1 µg/g (range <113.7 to 5459.8 µg/g), and they exhibited a strong dependence on the type of work exposure to fluoride. Because hair samples are easily collected, fluoride in hair is a useful indicator of human fluoride exposure.

Keywords: Aluminium plant workers, Ciechanów region, Fluoride exposure, Hair fluoride, Konin Aluminium Works, Obrink diffusion method, Poland.

INTRODUCTION

The most reliable indicator of exposure to fluorides is their level in urine and blood. Estimation of the risk in a whole population requires a large number of samples, which can be difficult to obtain. Hair, on the other hand, can be collected in a non-invasive way and is easy to transport and store. Moreover, the fluoride content of hair provides information on long-term exposure, in contrast to transient information from blood and urine samples.[1-10]

The content of fluorine in hair depends on the concentration of fluorides in the local environment at the place of residence, including daily intake from food and water. Of importance also is the daily intake of tea, fish (especially sea fish) and the use of fluoridated toothpastes.

The aims of the studies reported in this work were:

- determination of the sources of fluorides and levels of their supply in a population of low exposure to fluorides,
- checking the validity of a fluoride analysis method for hair to assess the risk of exposure to fluorides, and
- application of the method to hair samples in populations exposed to low and high levels of fluorides.

MATERIALS AND METHODS

Analytical or better grade chemicals and water doubly distilled from quartz apparatus were used throughout.

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Samples of hair were collected from children and adults living in the Ciechanów region, Poland, and from the workers of the Electrolysis Department of the Konin Aluminium Works in Konin, Poland. The latter group was exposed to high concentrations of fluorides, whereas the former was not. In order to determine the geographical distribution of the fluoride concentrations, the area of the Ciechanów region was divided into five communities: Ciechanów, Działdowo, Mława, Płońsk and Pułtusk (Figure 1).

Figure 1. Map of Poland and places of samples collection

Hair samples (2-3 cm), cut near the scalp – from the occipital, lower back part of the scalp – were collected from individuals who agreed to complete an anonymous questionnaire asking for general data: age, sex, water supply, place of residence, nutrition, and hygienic habits (intake of tea and fish; tooth brushing).

Preparation of hair samples for analysis: Samples of hair were cut into lengths of 3 - 10 mm, placed on filter paper (Filtrak 388, Filtrak GmbH, Germany) and washed three times with acetone, then with 0.5 M H_2SO_4 and twice with distilled water. After drying, portions weighing about 200 mg were prepared.

Fluoride isolation by the Obrink method: The weighed portion of hair was placed in the inner compartment of an Obrink chamber (Figure 2). The intermediate compartment was filled with 2.5 mL of 2.0 M NaOH (JT Baker, "Baker Analyzed" Reagent), used as fluoride absorbing solution.

Figure 2. Obrink chamber

(Note: Round in shape, the chamber is constructed from medical-grade polypropylene. All dimensions are in millimeters)

The solution of 80% H_2SO_4 containing 0.2% sodium dodecyl sulfate was added to the external compartment. About 2.5 mL of 70% $HClO_4$ (JT Baker, "Baker Ultrex-Analyzed" Reagent), saturated with silicon oil DC 200 (Fluka) was added to the inner compartment of the Obrink chamber. The chamber was immediately closed and maintained at 60^0C for 24 hr. The absorbing solution

was then transferred quantitatively in five 1-mL water portions into a 10-mL volumetric flask, neutralised with 2.0 M $HClO_4$ in the presence of phenolophthalein. Immediately after neutralisation 2 mL of acetate buffer (pH 5.3) was added and the mixture diluted to 10 mL. The contents of the volumetric flask were poured into a closed polypropylene container, and samples stored in this way were stable for least one week.[11]

The fluoride concentration in the samples was determined potentiometrically with an ion-selective electrode. The following electrodes were used: an ion-selective fluoride electrode made by Detector, Warsaw (Poland), saturated chlorine-argentine electrode made by Hydromet, Gliwice (Poland), and a glass-complex electrode type ESAgP-301W made by Eurosensor. The other equipment included a digital pH meter N-5170 made by Tel-Eko, Wrocław (Poland), a magnetic stirrer made by Tel-Eko Wrocław, water bath, laboratory drier, and Obrink chambers made of medical-grade polypropylene.

Validation of the method for determination of fluoride concentrations: Validation of the method of microdiffusion and determination of fluoride concentration by ion-selective electrode was performed with the same reagents and instruments which were used for hair samples. Instead of the hair sample, the inner compartment of the Obrink chamber contained semicircular bands of filter paper (Filtrak 388, Filtrak GmbH, Germany) covered with a layer of NaF and dried. In the inner compartments of six Obrink chambers were placed bands of filter paper containing 1.0, 2.0, 3.0, 5.0, 10.0 and 20.0 µg of fluoride (as F^-). The procedure was the same as with the hair samples.

Determination of certain parameters of drinking water: General hardness of drinking water was determined by the complexometric method according to PN-71/C-04551 using eriochromic black as an indicator.[12] Chlorides were determined by the argentometric method (PN-75/C-04617) using potassium chromate as an indicator.[13]

RESULTS AND DISCUSSION

During 1995 and 1997, 619 hair samples were collected and analysed, with 71 of them from the workers of the aluminium plants. For the population of low-risk exposure to fluorides, a correlation between the content of fluoride in hair and the season of the year was noted. In spring and summer, the mean concentrations of fluoride in hair were 2.81 and 3.54 µg/g and were considerably higher than those in the samples collected in autumn and winter (1.29 and 1.11 µg/g, (Table 1). These differences can be explained by a greater intake of water and other liquids during spring and summer, resulting in a greater intake of fluoride. The highest determined value of fluorides was 26 µg/g hair and the lowest was less than 0.5 µg/g.

No statistically significant differences were found between the results obtained in different communities of the Ciechanów region. The lowest mean value of fluoride in hair samples was recorded in the Płońsk county - 1.81 µg/g,

and the highest in the Ciechanów county - 2.32 µg/g. These results suggest a similar level of exposure to fluorides in the whole region.

Table 1. Mean content of fluoride (µg/g) in the hair of inhabitants of Ciechanów, Działdowo, Mława, Płońsk and Pułtusk counties

County	Season of the year				
	Spring	Summer	Autumn	Winter	Whole year
Ciechanów	5.92 ± 2.80 n=10	7.26 ± 3.67 n=10	0.82 ± 0.15 n=27	0.87 ± 0.13 n=31	2.32 ± 0.82 n=78
Działdowo	3.01 ± 0.96 n=25	2.97 ± 1.14 n=16	1.20 ± 0.22 n=50	0.98 ± 0.14 n=29	1.76 ± 0.31 n=120
Mława	3.38 ± 0.81 n=35	3.29 ± 0.99 n=28	1.35 ± 0.27 n=47	1.06 ± 0.14 n=39	2.11 ± 0.33 n=149
Płońsk	1.53 ± 0.33 n=22	3.06 ± 1.07 n=26	1.58 ± 1.00 n=20	0.68 ± 0.08 n=19	1.81 ± 0.44 n=87
Pułtusk	1.57 ± 0.18 n=23	3.29 ± 2.12 n=23	1.61 ± 0.53 n=27	1.71 ± 0.18 n=35	1.99 ± 0.49 n=108
Mean	2.81 ± 0.47 n=117	3.54 ± 0.74 n=107	1.29 ± 0.18 n=171	1.11 ± 0.09 n=153	2.00 ± 0.20 n=548

n – number of samples

A comparison of results obtained for city and country residents also revealed no significant differences: the mean values of fluoride concentration in hair samples from the urban and rural areas were 2.0 and 2.1 µg/g.

Table 2 presents mean fluoride values in hair in relation to declared nutrition and hygiene habits. Statistical analysis showed no correlation between the concentration of fluoride in hair and the intake of fish, kind and strength of tea and kind of toothpaste used. No significant differences were found between hair samples from people drinking tea; however, the values obtained for people who declared that they do not drink tea at all were substantially lower.

Analysis of the results in relation to the use of toothpaste showed that the concentration of fluoride was significantly lower in hair samples from people who brushed their teeth less frequently than once a week than in samples from those brushing more frequently.

The annual mean level of fluoride in hair of inhabitants of the Ciechanów region was a little lower (1.3 to 2.6 µg/g) than reported by other authors, which vary from 2.1 to 2.9 µg/g hair.[3]

The main source of fluorides intake by humans is the drinking water. Therefore, the concentration of fluoride was determined in the water supplied by municipal, local, factory water supply systems, and all kinds of wells, randomly selected, over the Ciechanów region.

Table 2. Mean content of fluoride in hair samples according to different criteria of fluoride exposure

Option	Description	Number of samples	Mean F content [μg/g]	SD
Tea consumption				
Type	Leaves	85	1.8	± 0.6
	Granulated	331	1.8	± 0.2
Strength	Weak	113	1.8	± 0.5
	Medium	261	1.8	± 0.3
	Strong	47	1.9	± 0.7
Volume	None	9	1.1	± 0.4
	1-2 glasses/day	183	1.8	± 0.4
	3-4 glasses/day	184	1.9	± 0.4
	above 4 glasses/day	54	1.6	± 0.3
Fish consumption	Very small	166	2.0	± 0.4
	Up to 0,5 kg	165	1.7	± 0.4
	0,5-1 kg	65	1.6	± 0.4
	1-2 kg	23	1.3	± 0.4
	Above 2 kg	12	2.0	± 0.8
Toothpaste	With fluoride	396	1.8	± 0.2
	Without fluoride	26	1.6	± 0.7
Tooth brushing	Twice or more a day	286	1.7	± 0.2
	Once a day	115	1.7	± 0.4
	2-3 times a week	22	2.5	± 2.2
	Once a week	4	2.6	± 2.0
	Less than once a week	4	1.0	± 2.1
Residence	Town	372	2.0	± 0.3
	Village	176	2.1	± 0.3
Education	Elementary school	61	2.3	± 0.9
	High school	211	2.1	± 0.3
	University	52	1.7	± 0.4

As seen in Table 3, over 50% of the water sources have a fluoride concentration around 0.3 mg/L. This is the lowest value of fluoride concentration recommended by the Health Ministry regulations of May 1990. Almost 99% of the population drink water containing fluorides at a concentration below 1 mg/L, and only 1% use water with fluoride concentration above 1 mg/L, but no higher than the highest permissible 1.5 mg/L. Water was also analysed for the concentrations of chlorides, pH value and general hardness. A significant positive correlation was observed between general hardness and chloride concentration, with an inverse one between the concentration of fluoride and pH.

Table 3. Number and fluoride content of water supplies in the Ciechanów, Działdowo, Mława, Płońsk and Pułtusk counties

F content (mg/L)	Number of water supplies					Total	
	Ciechanów	Działdowo	Mława	Płońsk	Pułtusk	Number	%
< 0.1	1	3	17	-	-	21	9.0
0.1-0.2	14	9	4	4	-	31	13.3
0.2-0.3	27	16	10	1	-	54	23.2
0.3-0.5	45	16	15	8	2	86	36.9
0.5-1.0	9	11	18	-	-	38	16.3
> 1.0	-	1	2	-	-	3	1.3

The workers in the aluminium plant, on the other hand, are a population at high risk of exposure to high concentrations of fluorides. The concentrations of fluoride in hair samples from this population were 2 or even 3 orders of magnitude higher than in the other samples. The mean daily intake of fluoride based on the specific character of work was estimated as 17.3 mg/day. The hair samples collected from the workers of the aluminium plant in Konin contained very high levels of fluoride – from 113.7 to 5459.8 μg/g. Estimations of the exposure to fluorides at the work stands revealed concentrations higher than the Highest Admissible Concentration specified in the Polish Standards, which for fluorides in the ash form is 1 mg/m^3, while for the gas form is 0.5 mg/m^3.

Table 4. Mean content of fluoride in hair samples of workers in the Electrolysis Department of the Konin Aluminium Works

Position	Mean age of workers (years)	Mean yrs. of work	Mean F content of hair (μg/g)	Confidence level
Electrolysis workers, n=27	39.9	11.7	2828.1	± 427.9
Interchangers of anodic kingpins, n=13	48.3	21.1	1351.2	± 17.5
Metal collectors, n=12	43.3	19.4	188.9	± 11.8
Workers at the anodic gas cleaning station, n=11	38.5	15.7	403.4	± 12.4
Electricians, n=8	39.0	15.3	501.5	± 7.0

n – number of samples

The analytical method was validated by checking its selectivity, specificity, range of the electrode responses, linearity and range of the calibration curve, sensitivity, precision, reproducibility and fluoride recovery.[14]

Table 5 presents results of the studies on recovery of fluoride from standard samples prepared by soaking the paper filter with a solution of sodium fluorine. For a standard sample containing 5 micrograms of fluoride the recovery was ~95%.

Table 5. Data on determination of fluoride in control samples

Sample No.	F^- (μg) taken	F^- (μg) determined (series) I	II	III	IV	V	Mean F^- (μg)	SD	Conf. level of mean*
1	1.0	0.7	0.6	1.1	0.9	1.1	0.88	0.23	± 0.20
2	2.0	1.7	1.8	1.7	1.7	1.8	1.74	0.05	± 0.05
3	3.0	2.5	2.7	2.6	3.2	2.9	2.78	0.28	± 0.24
4	5.0	5.6	4.5	4.5	4.6	4.6	4.76	0.47	± 0.41
5	10.0	8.4	9.1	9.0	8.5	9.9	8.98	0.69	± 0.68
6	20.0	15.3	17.8	22.0	24.1	19.4	19.72	3.45	± 3.03

*α = 0.05

The influence of the presence of some other metal ions on the results of determinations was also checked. However, the results indicate that under the conditions of the analysis, the effect of metals forming complexes with fluorides is insignificant.

The coefficients of variability for the two series of standard samples with concentrations of 0.5 and 1.0 mg F/L, were 2.3 and 3.1%, so the method can be considered as quite accurate.

Thus the results of the validation procedure showed that the method is suitable for determination of fluoride in hair samples over a relatively wide concentration range.

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