

## FLUORIDE IN HAIR AS AN INDICATOR OF EXPOSURE TO FLUORINE COMPOUNDS

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**SUMMARY:** Adult male Wistar rats were exposed to above-normal fluoride intake for 6 months: a) to HF by inhalation for 2 hr/day at a concentration of  $8.7 \pm 5.7 \text{ mg/m}^3$  and b) to sodium fluoride (NaF) in drinking water containing 20 mg F<sup>-</sup>/L. The fluoride content in dorsal hair, vertebrae L<sub>2</sub> – L<sub>4</sub>, tibia, and incisors was significantly increased in the exposed animals. More fluoride accumulated by the inhalatory than by the oral route. Positive correlations were found between the fluoride content in bone and in washed hair ( $r = 0.696$ ) as well as in unwashed hair ( $r = 0.647$ ). The results of this study indicate that hair may be a useful indicator of long-term exposure to fluorine compounds.

Keywords: Airborne fluoride, Bone fluoride, Fluoride exposure, Hair fluoride, Hydrogen fluoride, Rat exposure.

### INTRODUCTION

During the past four decades, hair has been used to monitor the accumulation of metals and nonmetals in the body in population groups as well as in individual persons. Recently, not only trace elements but also organic xenobiotics (drugs of abuse) are being analyzed in hair.<sup>1</sup>

Hair has certain advantages over other sample sources, since it can be collected quickly and painlessly and it is easy to transport and store. The analysis of hair strands may provide a time-integrated index of exposure for several months back.<sup>2</sup> However, trace element levels in hair may vary with age, sex, anatomic location, and intensity of exposure. Other disadvantages of hair are its ease of external contamination and its often poor correlation between the xenobiotic concentration in hair and target organs.<sup>3</sup>

Trace chemical elements in hair may be of endogenous or exogenous origin. The endogenous elements are those absorbed into the blood and subsequently incorporated into the keratin structure of hair during the growth phase. Exogenous elements derive under a variety of conditions from different contaminants. To distinguish between endogenous and exogenous trace elements, most laboratories include some kind of cleaning procedure in the analytical scheme to remove surface contamination. Such differentiation is necessary when the results of the analysis are used to assess the degree of environmental or occupational exposure. Hair has also been used quite often to evaluate exposure to fluorine compounds.<sup>4-7</sup>

The aim of this study was to determine the accumulation of fluoride in hair, bone, and teeth of rats exposed by oral and inhalatory routes to fluorine compounds and to examine whether a correlation exists between the fluoride levels in hair and in bone and teeth where most of the fluoride accumulates. Such a

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relationship would enable the use of hair in evaluating long-term exposure to fluorine compounds.

### MATERIALS AND METHODS

*Experimental Design:* Eight-week-old male Wistar rats, weighing about 200 g, were divided into three groups of 10 animals each. Group I, controls; Group II, animals exposed to HF by inhalation; Group III, animals given NaF in their drinking water at a concentration of 20 mg F<sup>-</sup>/L. Rats in Groups I and II were given tap water containing 0.3 mg F<sup>-</sup>/L. All animals were fed a standard laboratory pellet diet.

Rats from Group II were put in pairs into a whole-body exposure chamber of about 5 L volume with dynamic exposure conditions to inhale hydrogen fluoride at a concentration of  $8.7 \pm 5.7$  mg/m<sup>3</sup> for 2 hr daily. This concentration was obtained by passage of air at a rate of 50 L/hr through a bubbler tube containing 20 mL of a solution of 48% reagent-grade hydrofluoric acid diluted with water 1:3 (v/v).

Every month, samples of hair were collected from the same area of the dorsal part of the animals and divided into two parts: one sample was analyzed without washing and the other was washed as described below. After six months of treatment, the animals were sacrificed by ether anesthesia and hard-tissue material was collected for fluoride determinations. Vertebrae L<sub>2</sub> – L<sub>4</sub> and femurs were used as bone samples and incisors as tooth samples.

*Preparation of samples:* Specimens of bones and teeth (0.1 g) were cleaned, dried, crushed, and extracted with 6 mL of 2 M perchloric acid for 1 hr at room temperature. Then 2.4 mL of 0.5 M sodium citrate was added and the samples were centrifuged.

Specimens of hair were placed on a fritted glass filter and rinsed with acetone, detergent, and redistilled water. After drying, 100-mg aliquots were treated with 67% sodium hydroxide and heated in boiling water until completely dissolved (about 1 hr). The solution was then cooled and neutralized with 1 M hydrochloric acid and the sample volumes made up with water to 4 mL and diluted with equal volumes of TISAB buffer.

*Determination of fluoride:* Fluoride concentrations were measured by a fluoride-ion specific electrode and an Ag/AgCl reference electrode with a double jacket. Calculations were based on a response factor from a standard curve prepared daily. Recovery of F from analyzed materials amounted to 100 ± 8%. The coefficient of variation in samples of hair was 12% but in bones and teeth only 2.3%. Significance was determined by Student's t-test.

### RESULTS

No differences in appearance, behavior, and weight gain between both groups of exposed animals and controls were noticed in this experiment.

The fluoride content in hair, bone, and teeth of the exposed animals was significantly increased when compared to controls. More fluoride accumulated in animals exposed by the inhalatory than by the oral route (Tables 1 and 2).

**Table 1.** Fluoride content in hair

Months exposed	Hair	Group I (controls)	Group II (HF inhalation exposure)	Group III (NaF in water at 20 mgF <sup>-</sup> /L)
<i>Mean of 8-10 rats (<math>\mu\text{g F}^-/\text{g}</math>) <math>\pm</math> SD</i>				
0	U	4.73 $\pm$ 0.51	4.39 $\pm$ 0.91	5.08 $\pm$ 0.61
	W	4.5 $\pm$ 0.42	4.99 $\pm$ 1.29	4.77 $\pm$ 0.58
1	U	7.64 $\pm$ 3.22	334.24 $\pm$ 69.49	7.37 $\pm$ 1.41
	W	5.46 $\pm$ 1.18	9.14 $\pm$ 3.36	6.13 $\pm$ 1.63
2	U	6.77 $\pm$ 2.23	385.11 $\pm$ 101.11	17.46 $\pm$ 2.02
	W	5.8 $\pm$ 1.68	17.2 $\pm$ 6.26	9.31 $\pm$ 2.82
3	U	8.11 $\pm$ 1.59	1160.1 $\pm$ 262.62	31.61 $\pm$ 6.61
	W	6.62 $\pm$ 0.95	62.61 $\pm$ 12.61	10.37 $\pm$ 1.77
4	U	9.98 $\pm$ 1.64	1862.4 $\pm$ 661.33	25.79 $\pm$ 3.01
	W	6.74 $\pm$ 1.78	102.28 $\pm$ 62.22	8.7 $\pm$ 1.69
5	U	9.77 $\pm$ 1.30	1651.15 $\pm$ 587.57	27.57 $\pm$ 6.93
	W	8.03 $\pm$ 0.75	73.1 $\pm$ 20.48	13.09 $\pm$ 2.49
6	U	7.78 $\pm$ 1.59	1382.0 $\pm$ 319.98	32.96 $\pm$ 9.88
	W	6.09 $\pm$ 1.49	90.96 $\pm$ 36.89	15.67 $\pm$ 2.85

U - unwashed hair. W - washed hair.

**Table 2.** Fluoride content in bone and teeth after 6 months of treatment

Group		Vertebrae L <sub>2</sub> – L <sub>4</sub>	Femur	Incisors
I Controls (N=9)	X	485.4	567.5	223.2
	SD	$\pm$ 89.15	$\pm$ 50.2	$\pm$ 45.7
II HF (N=8)	X	1356.7	1426.7	694.6
	SD	$\pm$ 232.5	$\pm$ 133.0	$\pm$ 44.4
II NaF (N=10)	X	1048.6	1034.6	324.4
	SD	$\pm$ 224.4	$\pm$ 121.0	$\pm$ 70.9

N - number of samples. X - Mean of 8-10 rats ( $\mu\text{g F}^-/\text{g}$ ).

In animals exposed to hydrogen fluoride the fluoride level in hair was distinctly higher than in controls during the whole treatment period, and in unwashed hair it was more than a hundred times the level in control animals. During the first months of exposure the fluoride concentration in hair rose, but after saturation even a decline in hair fluoride occurred. However, due to the dispersion of data the decline was not statistically significant (Figures 1 and 2).

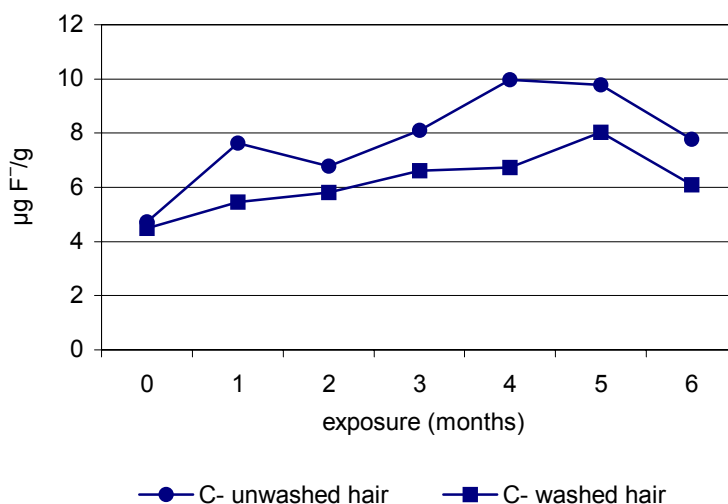
In rats ingesting sodium fluoride in drinking water the hair fluoride increased significantly after the second month of the experiment and then surpassed the level in controls by about two to four times (Figures 1 and 3).

Washing the hair showed that a large part of the fluoride in it is of exogenic origin. In controls and in animals treated orally with sodium fluoride about 20% and 50%, respectively, of the fluoride was removed by washing. In animals exposed by the inhalatory route as much as 95% was removed by this procedure, yet the remaining 5% (of endogenic origin) was markedly higher than in orally treated rats (Figure 4).

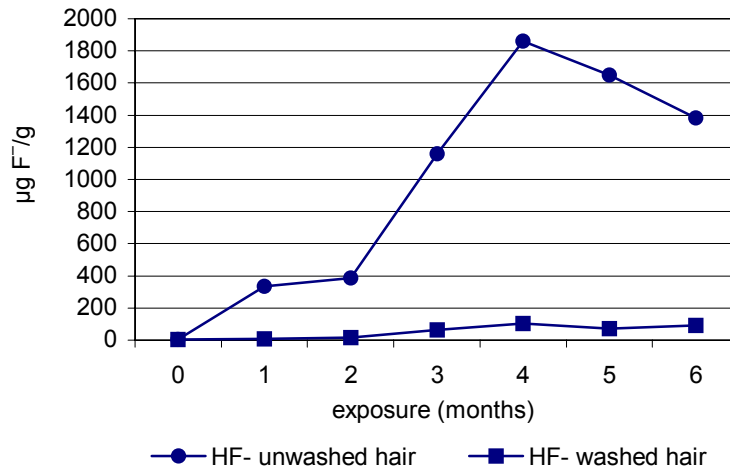
The fluoride content in bone and teeth of the animals after 6 months of treatment was approximately twice as high in rats treated orally as in controls. In rats that inhaled hydrogen fluoride, it was about three times higher than in controls (Table 2).

Positive correlations were found between fluoride levels in bone and washed as well as unwashed hair of the animals (Figures 5 and 6). It seems therefore that even unwashed hair may serve as a passive indicator of exposure, especially to gaseous fluorine compounds.

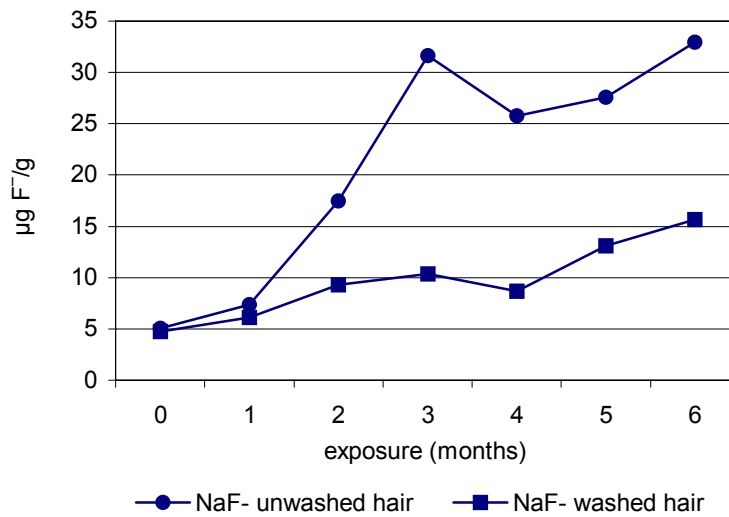
**Figure 1.** Fluoride content in hair of control animals



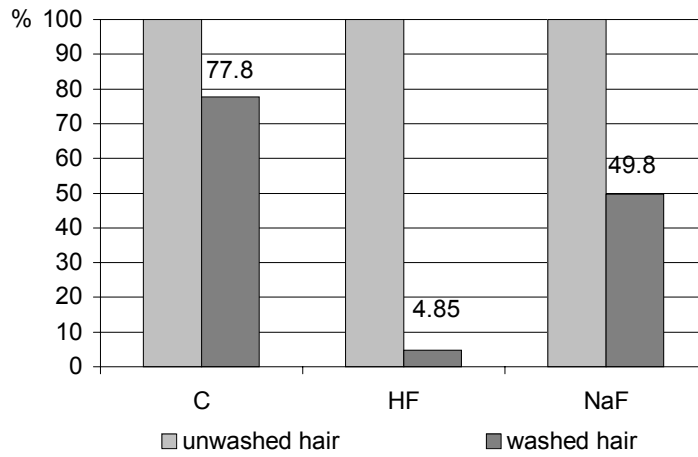
**Figure 2.** Fluoride content in hair of rats exposed to HF (8.7 mg/m<sup>3</sup>)



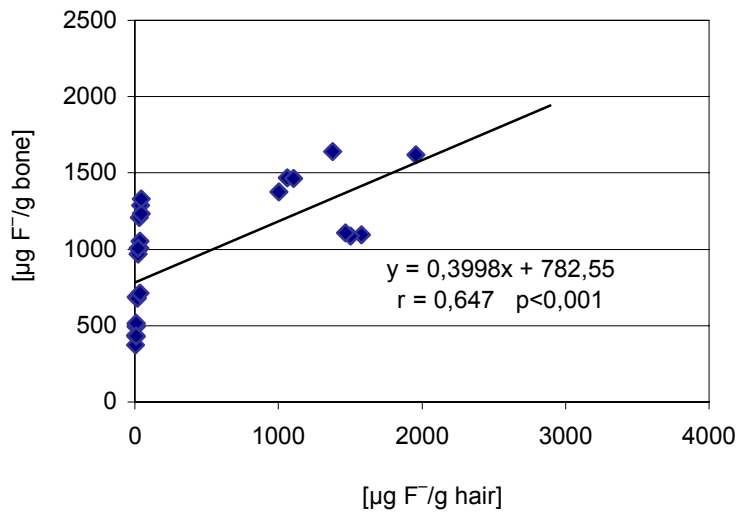
**Figure 3.** Fluoride content in hair of rats exposed to NaF in drinking water (20 mg F<sup>-</sup>/L)



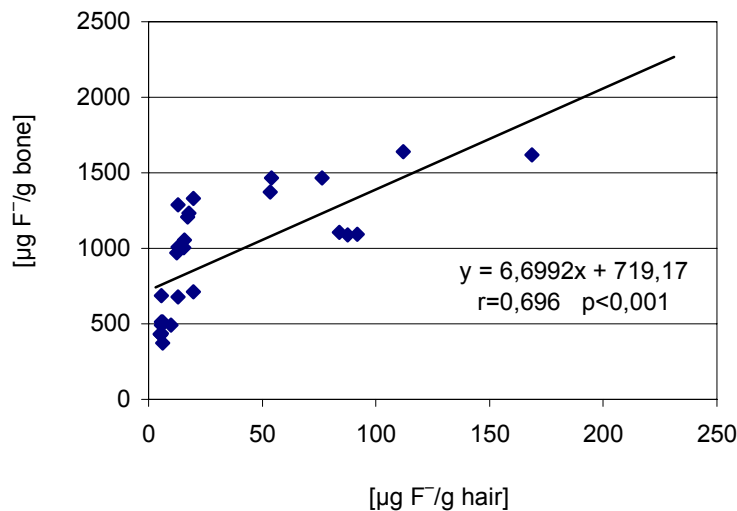
**Figure 4.** Relative amount of fluoride in washed hair in relation to unwashed hair



**Figure 5.** Correlation between fluoride content in vertebrae and unwashed hair of all rats after 6 months



**Figure 6.** Correlation between fluoride content in vertebrae and washed hair of all rats after 6 months



#### DISCUSSION

Hair may be useful in assessing variations in exposure to fluoride over the long-term, while blood and urine better reflect recent exposure. Analyses may be performed on segments of the hair so that the fluoride content of the newest growth can be compared with past exposure. The results of this study therefore indicate that hair may be useful in evaluation of environmental or occupational exposure to fluoride.

This paper was presented at the XXIIIrd Conference of the International Society for Fluoride Research, Szczecin, Poland, June 11-14, 2000.

#### ACKNOWLEDGMENT

This study was supported by grants W-40 and W-53 from the Medical University in Gdańsk.

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