

# Monitoring fluoride exposure with fingernail clippings

## Summary

The purpose of this review is to discuss new information regarding the relationship between the level of fluoride exposure and the corresponding fluoride concentrations in fingernail clippings. While there are several techniques available to extract fluoride from fingernails prior to analysis with the electrode, the HMDS-facilitated diffusion method is the most popular. Fluoride enters fingernails at the growth end and reaches the distal end approximately three months later. The fluoride concentration in the clipping reflects the average fluoride intake and plasma concentration during the period when the clipping was formed. Therefore, the concentration in the clipping is directly related to the average fluoride exposure that occurred during a 1–2 week period (depending on the length of the clipping) about three months ago and not to recent and possibly variable exposures that occur during the day. Published studies have demonstrated that fingernail fluoride concentrations reflect fluoride exposures from drinking water, toothpaste and the work environment and can be expected to do so for any source of intake including salt.

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## Introduction

Monitoring human exposures to fluoride can be accomplished through the analysis of several biological tissues or fluids although with varying degrees of accuracy. Acute exposures such

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as those that occur in pharmacokinetic studies designed to test, for example, the extent of absorption from the gastrointestinal tract and the time course of body fluid fluoride concentrations following the ingestion of fluoride in infant formula or water, typically involve timed collections of blood plasma or parotid ductal saliva (SPAK et al. 1982; WHITFORD et al. 1999a). The analysis of urinary fluoride concentrations or, what is better, excretion rates in pharmacokinetic studies can also provide useful information (SPAK et al. 1982) although the data are less precise and more difficult to interpret than that derived from the analysis of plasma.

More than 99% of the body burden of fluoride is found in calcified tissues. It is generally agreed, therefore, that the level of chronic exposure extending over a period of years is best assessed based on fluoride concentrations in bone but this tissue is rarely collected for obvious reasons. There is, however, a steady-state relationship between the concentrations of fluoride in the exchangeable pool of bone and extracellular fluid (TAVES & GUY

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1979; WHITFORD 1996). For this reason plasma fluoride concentrations in persons who have had no fluoride intake during the previous several hours are proportional to those in bone. Thus, fasting plasma fluoride concentrations serve as a biomarker for the chronic level of fluoride intake and the total amount of fluoride in the body. Similarly, fasting parotid ductal saliva may also reflect bone fluoride concentrations because its fluoride concentration is relatively constant at about 75% of that in plasma (EKSTRAND et al. 1977; WHITFORD et al. 1999a).

Recent reports have suggested that the fluoride concentration in fingernail clippings reflects the subchronic level of exposure to fluoride. Fingernail concentrations may also reflect the chronic level of exposure provided that an individual's fluoride intake has remained relatively constant. Compared to the fluids mentioned above, fingernails have a distinct advantage in that they can be easily obtained in a noninvasive manner. The purposes of this review are to outline the analytical method used in our laboratory and to discuss the literature concerning the relationship between fingernail fluoride concentrations and fluoride exposure.

## Analytical method

Following the removal of surface contamination from the nail clippings by brushing with an interdental brush and distilled water or by 30-second, low-power sonication in distilled water the nails are dried at  $95 \pm 5^\circ\text{C}$  and weighed. Dry weights in the 10–20 mg range typically contain enough fluoride for accurate analysis. The main task is to quantitatively transfer the fluoride within the nails to a solution which can be placed in contact with the fluoride electrode (Orion, Model 9409) and a miniature calomel reference electrode. The hexamethyldisiloxane (HMDS)-facilitated diffusion method developed by TAVES (1968; modified by WHITFORD 1996) is used for this purpose. Using this method, an experienced analyst can easily prepare and analyze 30–40 samples per day.

Distilled water (3 ml) is placed in non-wettable diffusion dishes (Falcon 1007) and the nail clippings are added. Known quantities of fluoride standards (eg., 9.5, 19 and 95 nanograms in triplicate) are added to the distilled water in other dishes. The alkaline trapping solution (50  $\mu\text{l}$  of 0.05N NaOH) is placed in three drops on the inside of the dish lid. A continuous, unbroken line of Vaseline is then applied to the periphery of the inside of the lid and the lid is sealed to the bottom of the dish. Finally, 3.0N  $\text{H}_2\text{SO}_4$  saturated with HMDS (3 ml) is injected through a hole previously burned through the lid near its periphery with a soldering iron and the hole is immediately covered with Vaseline. The diffusion of fluoride from the nails and standards into the NaOH trap begins at that time.

The diffusion process is allowed to continue overnight. The lid is removed and 20  $\mu\text{l}$  of 0.20N acetic acid is added and combined with the NaOH into a single drop. This forms a buffered sodium acetate/acetic acid solution with a pH of approximately 5. The solution is drawn into the tip of a Finnpiptette and the final volume of the solution is adjusted to 75  $\mu\text{l}$  with distilled water. Other final volumes such as 50 or 100  $\mu\text{l}$  can be used provided that the volume is the same for the standards and fingernail samples. [Note: If the lowest standard is 9.5 nanograms and the final volume is 75  $\mu\text{l}$ , the concentration is 0.13 mg/l, a value well above the electrode's theoretical limit of sensitivity of 0.019 mg/l.] The electrodes are placed in contact with the solution with gentle mixing movements of the dish or electrodes every 15–20 seconds until a stable mV reading is obtained which typically occurs within 2–4 minutes. A stable mV reading is defined as one which

does not change more than 0.1 or 0.2 mV following three episodes of gentle movement.

The relationship between the mV readings and the concentrations (actually activity) of fluoride in the solution which is analyzed is logarithmic, so semilogarithmic graph paper is used to construct the standard curve with mV on the linear x-axis and nanograms of fluoride on the logarithmic y-axis. Alternatively computer generated linear regression analysis can be used.

The amount of fluoride captured from the nails is determined by comparison with the standard curve which should have a slope of 57–59 mV (depending mainly on the temperature of the solution) for a 10-fold change in fluoride concentration. For example, if the mV reading for the 9.5 nanogram standard is 100 mV, then the reading for the 95 nanogram standard should be 41–43 mV. The amount of fluoride captured is divided by the weight of the nail clippings to obtain the fluoride concentration, which is expressed as mg F/kg (ppm).

## Preliminary studies

It appears that fluoride enters the nail mainly, perhaps exclusively, via the growth end and that the concentration in the nail clipping is determined by the average plasma fluoride concentration that existed while the clipping was forming. This is based on the results of two studies. An individual increased his fluoride intake from approximately 3 to 6 mg/day for one month and continued clipping his nails every two weeks. A 1-month increase in his fingernail fluoride concentrations began 3.5 months after starting the increased intake (WHITFORD et al. 1999b). In the other study, peak fingernail and toenail fluoride concentrations of ten 2–3-year-old children occurred 16 weeks after starting the use of a 1,570-ppm toothpaste (RODRIGUES et al. 2004).

It was not known, however, if fluoride could be gained or lost by exposure to external factors such as fluoridated water or fluoride-free water. To answer these questions nails clipped on three different days were cut in half. One half of each clipping was placed in deionized water for 0.5, 1.0 or 6.0 h, while the other half was not exposed to water. There were no significant differences between the test and control results. Half of the nail clippings collected on a different date were placed in water containing 1.0 ppm fluoride while the other half was not exposed to water. Again there was no difference between the test and control results. These data suggest that fluoride is not readily diffusible from nails into fluoride-free water nor is it readily diffusible into nails from fluoridated water (WHITFORD et al. 1999b).

To test whether the fluoride within nail clippings was completely extracted during the HMDS-facilitated diffusion process, the following analyses were done. Half of the nails from four different collections were dry ashed at  $600^\circ\text{C}$  to remove all organic material while the other half of each collection served as the non-ashed control. The ashed residues were transferred to diffusion dishes with two 1.0-ml rinses of deionized water. The average fluoride concentrations ( $\pm$  SE) of the control and ashed samples were  $2.36 \pm 0.13$  and  $2.39 \pm 0.19$  mg/kg (WHITFORD et al. 1999b).

One investigation of fluoride concentrations in nail clippings from rats found that the HMDS-extractable amount of fluoride decreased markedly with time after the nails had been clipped (FURLANI et al. 2001). Nails from a group of 24 rats were clipped on a single day, pooled and then divided into three portions. One portion was prepared for analysis immediately and the others after two or three months had passed. The average concentrations were 37.4, 2.7 and 1.4 mg F/kg, respectively. No explanation

for the markedly lower concentrations at two and three months could be given.

To determine whether there was a similar change in the detectable amount of fluoride in human nail clippings, the following analyses were done (WHITFORD, unpublished). Nail clippings from each of three subjects were cut into four parts. One part was prepared for analysis immediately and the other parts after 3, 6 or 12 months of storage at room temperature. The average concentrations ( $\pm$  SE) were  $2.12 \pm 0.57$ ,  $2.25 \pm 0.57$ ,  $2.06 \pm 0.50$  and  $2.24 \pm 0.33$  mg/kg, respectively, which indicated that the amount of detectable fluoride in human nail clippings does not change with time for at least one year.

### Fingernail fluoride concentrations

Table I shows the results of a study which determined the fluoride concentrations in fingernail clippings obtained from 6-to-7-year-old Brazilian schoolchildren on two occasions, once in December 1997 and again in February 1998 (WHITFORD et al. 1999b). They were residents of three small communities with different fluoride concentrations in the drinking water: 0.1, 1.6 and 2.3 ppm. The water in each community came from a single well. Fluoridated salt, milk and dental products were not available, so the water and other dietary components were the only known sources of fluoride intake. The nail clippings were cleaned and analyzed as described above.

There was a direct relationship between the concentrations of fluoride in the water and the fingernail clippings ( $p < 0.0001$ ). The combined fingernail concentrations for both collections in the 1.6-ppm area (5.28 mg/kg) and the 2.3-ppm area (7.52 mg/kg) were higher than in the 0.1-ppm area ( $p < 0.0001$ ) and the concentration for the 2.3-ppm area was higher than in the 1.6-ppm area ( $p < 0.01$ ). The 95% confidence intervals showed no overlap for the 0.1-ppm and 1.6- or 2.3-ppm areas and only a small overlap for the 1.6- and 2.3-ppm areas. Considering individual data, however, it can be seen that the maximum concentration in the 0.1-ppm area was greater than the minimum concentration in the 1.6-ppm area, and the same was true for the 1.6-ppm and 2.3-ppm areas. The causes of these overlaps were not determined.

Table II shows a summary of the average concentrations in fingernails or toenails reported by other investigators. Several preparative methods and analytical techniques have been used which may partly explain the rather wide range in the reported concentrations. Assuming that the nails were cleaned to remove surface contamination, some of the values are clearly of doubtful accuracy particularly those reported by CZARNOWSKI & KRECHNIAK

(1990). In general, however, it can be seen that the fluoride concentrations in the nail clippings were positively correlated with the level of fluoride exposure in each report.

### Advantages and disadvantages

There are several real or potential disadvantages of using nail clippings to monitor fluoride exposure. (1) Unlike urine which can be analyzed by the "direct" method which requires only the addition of a suitable buffer to the samples, the fluoride in fingernails must be extracted in some way. This increases both time and cost. It should be noted that the fluoride in plasma and ductal saliva should also be extracted because the concentrations are often near or even below the limit of sensitivity of the analytical method. (2) Some nails are not, or may not be, suitable for analysis. For example, nails covered with polish should not be analyzed because polish adds weight which would lower the calculated fluoride concentration. Further, some polishes contain fluoride. Further studies are needed to determine whether polish can be removed without altering the endogenous fluoride concentration. (3) The possible effects of several variables on the rate of fluoride incorporation into fingernails remain to be determined. These include diseases of nails, the rate of nail growth, and the age and sex of the donor.

A major advantage of using fingernail clippings is that they are easily obtained noninvasively and without objection from virtually all donors. Other advantages include: (1) The concentration of fluoride reflects the average level of intake and plasma concentration over a protracted period, usually 1–2 weeks depending on how often the nails are clipped. This is in contrast to the analysis of urine, plasma or ductal saliva whose fluoride concentrations are more like 'snapshots' subject to change due to recent fluoride intake and certain physiological variables. (2) Unlike urinary fluoride concentrations, fingernail concentrations are not affected by variables such as fluoride intake within the last few hours or differences in glomerular filtration rate, urinary pH or urinary flow rate. These advantageous features make the analysis of fingernail clippings an attractive alternative to other body fluids or tissues for the purpose of monitoring fluoride exposure.

### Zusammenfassung

Ziel dieser Arbeit ist eine Diskussion neuer Befunde über das Verhältnis zwischen Fluoridexposition und entsprechenden Fluoridkonzentrationen in Fingernagelproben. Verschiedene Techniken zur Fluoridextraktion aus Fingernägeln sind aus Zeiten vor

Tab. I Fluoride concentrations (mg/kg) in fingernail clippings from 6-to-7-year-old Brazilian children residing in communities with different water fluoride concentrations.

	Drinking Water Fluoride Concentration								
	12/97	0.1 ppm 2/98	Both	12/97	1.6 ppm 2/98	Both	12/97	2.3 ppm 2/98	Both
Mean	2.09	1.59	1.85	4.77	5.80	5.28	7.57	7.44	7.52
SE	$\pm 0.29$	$\pm 0.21$	$\pm 0.19$	$\pm 0.67$	$\pm 0.73$	$\pm 0.50$	$\pm 0.80$	$\pm 1.49$	$\pm 0.73$
N	10	9	19	6	6	12	9	6	15
Min	1.07	0.75	0.75	2.76	2.28	2.28	4.13	4.00	4.00
Max	3.53	2.32	3.53	7.53	7.06	7.53	11.70	13.18	13.18
CI 95%	1.43–2.75	1.11–2.07	1.46–2.25	3.05–6.48	3.92–7.68	4.19–6.38	5.73–9.41	3.62–11.27	5.95–9.09

The data for the fingernail collections made in Dec. 1997 and in Feb. 1998 are shown separately and in combination. Data from WHITFORD et al. (1999b).

Tab. II Summary of literature reports of fluoride concentrations in fingernails or toenails.

Reference	Preparation of Nails	Analytical Method	Source of F Exposure	Nail [F], ppm
SCHAMSCHULA et al. (1985)	Digestion in strong alkali	Electrode	Water: <0.1 ppm	0.79
			0.5–1.1 ppm	1.31
			1.6–3.1 ppm	2.31
MACHOY (1989)	Digestion in strong alkali	Gas chromatography	Air pollution; Low-F water	12.5
			Low-F water in 3 communities	2.5
				6.4
				7.6
	Water: 0.64 ppm	8.6		
SCHMIDT & LEUSCHKE (1990)	Ashed	Electrode	Air pollution: Yes	0.46
			No	0.79
CZARNOWSKI & KRECHNIAK (1990)	Digestion in strong alkali	Electrode	Work exposure: Yes	19.2–624
			No	8.4
SPATE et al. (1994)	None	Neutron activation	Water: 0.1 ppm	4.2*
			1.0 ppm	6.4*
LEVY et al. (2004)	HMDS diffusion	Electrode	Water: 0.1 ppm	2.3
				1.6*
			0.8 ppm	3.6
				2.8*
RODRIGUES et al. 2004	HMDS diffusion	Electrode	Toothpaste: F-free	2.7
			1,579 ppm	5.0

Values marked with an asterisk are for toenails.

der Erfindung der Fluoridelektrode vorhanden, doch ist die HDMS-Diffusionsmethode die gebräuchlichste. Fluorid wird am Wachstumsende des Nagels eingelagert und erreicht das distale Ende etwa drei Monate später. Der Fluoridgehalt in abgeschnittenen Nagelproben widerspiegelt die Fluorideinnahmen und -plasmakonzentration während einer Periode von 1 bis 2 Wochen (abhängig von der Länge der Nagelprobe) etwa drei Monate vor Abschneiden der Nagelprobe, aber nicht unterschiedliche Einnahmen im Verlaufe eines Tages. Die veröffentlichten Studien widerspiegeln die «Fluoridexposition» aus Trinkwasser, Zahnpasten und industrieller Exposition und lassen erwarten, dass dies für jede Art von Fluorideinnahme gilt, so auch für Fluoride im Kochsalz.

## Résumé

L'objet de cet exposé est de discuter des informations nouvelles concernant la relation entre le niveau d'exposition au fluorure et la concentration de fluorure constatée dans des rognures d'ongles. Il y a plusieurs techniques servant à extraire le fluorure des ongles préalablement à l'analyse avec l'électrode; la méthode de diffusion facilitée par HMDS est la plus répandue. Le fluorure pénètre les ongles à leur racine et en atteint l'extrémité distale au bout de trois mois environ. La concentration de fluorure dans les rognures d'ongle reflète la moyenne de l'ingestion de fluorure pendant la formation de ces rognures. La concentration que l'on y constate est donc en rapport direct avec l'exposition moyenne au fluorure durant une période d'une à deux semaines – ceci dépend de la longueur des rognures – trois mois auparavant. Par contre cette concentration ne reflète pas de plus récentes expositions possiblement variables. Des études ont été publiées démontrant que la concentration de fluorure dans les ongles reflète l'exposition au fluorure due à l'eau potable, la pâte dentifrice, au

lieu de travail; on peut s'attendre à des effets similaires résultant d'autres sources d'ingestion, y compris le sel.

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