Toenail mercury concentration as a biomarker of methylmercury exposure

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The aim of the study was to assess the value of toenail mercury as an alternative biomarker of methylmercury exposure compared with blood and hair. Blood, hair and toenail total mercury concentrations were determined simultaneously in a southern urban sea (n = 35) and an eastern rural lake (n = 37) group and separately in a central Finnish rural lake (n = 39) group. A questionnaire-based index was used for estimating frequency of exposure taking into account high vs low mercury fish. Total mercury concentration was determined by cold-vapour atomic absorption spectrophotometry. The mean Fish Consumption Frequency Index varied from 4.7 to 9.9 on a scale of 1–20. The blood, hair and toenail mercury mean concentrations ranged from 2.9 to 14.6 $\mu g\ l^{-1}$, 0.45 to 1.57 mg kg^{-1} and 0.20 to 0.54 mg kg^{-1}, respectively. In the combined southern and eastern groups (n = 72) sampled simultaneously, the correlations between blood and hair mercury were r = 0.92 and that of blood and toenails r = 0.78 (p < 0.0001). In the central Finnish group the correlations were more moderate. In the three groups all three biomarkers correlated highly with the fish consumption index, r = 0.43–0.76 (p < 0.0001). Men consumed high-mercury fish more frequently than women, 8.6 vs 6.6 (n.s.). The mean mercury levels of blood and hair were two-fold, but toenail mercury levels were only 30% higher in men compared with those of the women. The relative sensitivity (slope) of the sources decreased in the order, blood > hair > toenails. In conclusion, toenails, an easily accessible tissue for the estimation of methylmercury exposure, have been shown to be closely correlated with the well-established samples for biomarkers, viz. blood and hair mercury.

Keywords: *biomarker, mercury, blood, hair, toenails, fish.*

Introduction

In Finland the general population is mainly exposed to mercury through the diet and over 60% of this mercury originates from fish and seafoods (Alfthan *et al.* 1994). Over 90% of the total mercury of fish occurs in the form of methylmercury (WHO 1990). In Finland estimation of (methyl)mercury exposure suffers from uncertainty of the mercury concentration of fish caught both offshore and from the approximately 100 000 lakes with differing ecochemical conditions. The mercury level of fish additionally changes temporally depending on the geographical location and type of lake in question (Louekari *et al.* 1994). To bypass the need for a huge number of fish mercury analyses and estimation of fish intake, biomarkers of methylmercury exposure are to be preferred in population studies.

Methylmercury is metabolically deposited in hair during the growth phase and is firmly bound to sulphur in keratin, which occurs abundantly in hair (Harkey 1993). Nails resemble hair in chemical composition and should in principle be as useful as hair for estimation of methylmercury exposure. So far sampling of nails as a biomarker of methylmercury exposure has only been reported in one previous study which was carried out exclusively on women (Garland *et al.* 1993).

The relationship between exposure to methylmercury vs blood and hair mercury concentrations, as well as the toxicokinetics of methylmercury is well documented in many human studies (WHO 1976, 1990). Important parameters known to modify the distribution of mercury in different organs include age, sex and selenium (Cuvin-Aralar and Furness 1991, Nielsen and Andersen 1992). The ratio between hair and whole blood mercury in the steady-state usually ranges between 200 and 300 (Phelps *et al.* 1980). In epidemiological selenium research toenails have attracted increased interest during the past decade because they are less prone to external contamination compared with hair and fingernails and they reflect the mean selenium intake in time, spanning from several months to a year (Longnecker *et al.* 1993). As hair is not always available or collected in studies (especially those concerning elderly men), but toenails are, this study was undertaken to assess the value of toenails for estimation of fish-derived methylmercury exposure.

MATERIALS AND METHODS

Subjects

Study A

The subjects participated in a long term selenium follow-up study in May 1995. The first group consisted of the staff of the institute (n = 35) in urban seaside Helsinki who had a mean age of 43 years, range 25–62 (10 men and 25 women). The second group of subjects (n = 37) were engaged in agriculture in rural lake-surrounded Leppävirta (eastern Finland) and had a mean age of 53 years, range 28–71 (17 men and 20 women). They were all apparently healthy adults and none of them used regularly any nutrient supplements which included selenium, or were pregnant.

Study B

The subjects (n = 39) participated in a selenium supplementation trial described previously (Alfthan *et al.* 1991a). They were all healthy male blood donors living in the rural town of Jyväskylä, central Finland, surrounded by lakes. Their mean ± SD age was 51 ± 7 years. The samples for mercury analysis were collected in the autumn of 1987 at baseline before supplementing with selenium.

Informed consent was obtained from all participants and the study protocol was approved by the Ethical Committee of the National Public Health Institute.

Questionnaire

The subjects in both studies were asked to fill in a one-page questionnaire with questions concerning frequency of consumption of fish, species consumed, type (canned, frozen) and origin of fish (sea, lake, river). In addition, occupational exposure to mercury, smoking and health status

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algorithm was developed taking into account the fact that predatory fish from lakes and rivers on average have a two-fold mercury concentration compared with fish from the sea and that the mercury concentration of canned and frozen fish, which are all imported, is low. Thus, from four frequency classes of servings, i.e. less than once a week, once a week, several times per week and daily consumption, an algorithm with a scale of 1–20 was developed: the Fish Consumption Frequency Index (FCFI).

Samples

An overnight fasting blood sample was taken into a heparinized vacuum tube in study A and into a citrated vacuum tube in study B. An aliquot of whole blood in study A and packed red cells in study B were separated and stored at –70°C. Before taking the hair sample the subjects were asked to wash their hair using regular shampoo. A bundle of hair was tied with thread and cut at the scalp from the back of the head. The first 1.5 cm nearest the scalp was used for mercury analysis without further pretreatment. This length corresponds to an integrated intake of the previous 1–1.5 months. Toenail clippings were obtained from each toe and pooled. About half of the clippings in study B were still available for mercury analysis after having been used previously for selenium analyses (Alfthan *et al.* 1991b). They were pretreated by washing with a detergent, sonicated, washed thoroughly with purified water and dried to constant weight at 60°C (Ovaskainen*et al.* 1993). For obtaining data on the variation of mercury between nails, individual nail clippings (5.0–19.4 mg) from all toes were taken on two occasions, 4 weeks apart from one subject. The means ± SD were 0.51 ± 0.08 mg kg^{-1} (range 0.38–0.68 mg kg^{-1}) and 0.50 ± 0.08 mg kg^{-1} (range 0.37–0.64 mg kg^{-1}). On both occasions the intraindividual variation was 17 CV%.

Reagents and equipment

Sulphuric acid, stannous chloride, potassium permanganate, hydroxylammonium chloride were all from Merck and 'mercury-free'. The other reagents were of analytical grade. The ground glass-stoppered test tubes (150 × 12 mm) and tubes (100 mm) ending in a capillary (50 × 1 mm) were borosilicate. The apparatus consisted of an atomic absorption spectrophotometer, Perkin-Elmer Model 400 fitted with a glass tube (240 × 5 mm) in the light path, Technicon AutoAnalyzer flow-through equipment and an aluminium heating block with 60 places.

Determination of total mercury concentration

Aliquots of whole blood or packed red cells (1 ml), hair, or toenail clippings (10–100 mg), were weighed into the pregraded (10 ml) test tubes and the contents dissolved overnight in a 1.5 ml mixture of nitric and sulphuric acids (2/1, v/v). A capillary-ended tube was attached to the tubes and the mixture was then heated from ambient to gentle boiling (30 min) and maintained for 30 min. Sixty tubes were processed in a series together with blanks and reference materials. After cooling in ice, saturated (6%) $KMnO_4$ was added until the colour persisted for 2 min, then 10% hydroxylammonium chloride was added dropwise to reduce excess $KMnO_4$ (colourless). The volume was adjusted to 10 ml with water in the pregraded tubes. The mercuric chloride standards ranged from 0.25 μg l^{-1} to 6.0 μg l^{-1}. The concentration of total mercury was measured by an automated cold-vapour atomic absorption spectrophotometric method (Armstrong and Uthe 1971).

Quality assurance

The detection limit of the method was 0.05 μg l^{-1} determined as 3SD of the blank value. Thus the detection limit for blood was 0.5 μg l^{-1} and for a 50 mg hair and toenail sample, 0.01 mg kg^{-1}. The precision between series (5 series, 10 samples) for an in-house hairpool was 0.95 ± 0.02 mg kg^{-1}, 2.1 CV%. The accuracy was established by analysis of certified reference materials in each series: IAEA Copepod, MA-A-1 (n = 7) 0.28 ± 0 .017 mg kg^{-1} (certified value: 0.27 mg kg^{-1}), BCR Pig Kidney 186 (n = 5) 1.86 ± 0.06 mg kg^{-1} mg kg^{-1} (certified value: 1.97 ± 0.04 mg/kg) and NIES Hair (n = 7) 4.25 ± 0.26 mg kg^{-1} (certified value: 4.4 ± 0.4 mg kg^{-1}).

Determination of selenium

Aliquots of whole blood or red cells (0.5 ml) or toenails (10–50 mg) were determined for selenium by acid-digestion fluorimetry (Alfthan 1984). Quality assurance data have been published recently (Aro *et al.* 1994).

Statistical analysis

Spearman correlations, percentile distributions, linear regression and two-sided t-tests were calculated with the SAS program on a VAX computer.

Results

Study A

The mean ± SD frequency of total fish consumption expressed as the Fish Consumption Frequency Index was 7.4 ± 4.6 in all 72 subjects. The FCFI was 4.7 ± 2.5 (range 1—12) in Helsinki and 9.9 ± 4.8 (range 4–20) in Leppävirta. The majority of the participants in Helsinki consumed fish caught offshore, whereas the majority of those living in Leppävirta consumed fish from surrounding lakes.

The mercury concentrations in whole blood, hair and toenails are shown in Table 1. The levels of all three biomarkers were significantly higher in rural Leppävirta compared with urban Helsinki. The highest individual values were: for blood, 33.1 μg l^{-1} for hair, 5.88 mg kg^{-1}; and for toenails, 2.29 mg kg^{-1}, all from different individuals. Only one person, a laboratory technician in Helsinki, was occupationally exposed to mercury, namely, phenylmercuric acetate. Her tissue mercury concentrations (whole blood, 2.3 μg l^{-1}, hair, 0.53 mg kg^{-1}, toenails, 0.22 mg kg^{-1}) were very close to the respective median values of all subjects from Helsinki.

In all subjects in study A the correlation between the mercury concentration of whole blood and hair was r = 0.92 and whole blood and toenails was r = 0.78, $p < 0.0001$. The correlation between hair and toenails was also r = 0.78 ($p < 0.0001$) (figure 1).

The mercury concentrations of whole blood, hair and toenails were all strongly associated with the FCFI: blood, r = 0.76, hair, r = 0.75 and toenails, r = 0.69 ($p < 0.0001$) (Figure 2). All three biomarkers were also significantly associated with

		Whole blood (μg l^{-1})			Hair (mg kg^{-1})			Toenails (mg kg^{-1})		
Area	n	Mean	SD	GM[a]	Mean	SD	GM	Mean	SD	GM
Helsinki (urban)	35	2.9	1.4	2.6	0.45	0.25	0.42	0.20	0.09	0.18
Leppävirta (rural)	37	11.5	9.1	8.0	1.57	1.42	0.95	0.54	0.48	0.36
Overall	72	7.4	7.9	4.7	1.04	1.17	0.64	0.37	0.38	0.26

Table 1. Total mercury concentration of whole blood, hair and toenails in urban and rural adults (study A).

[a]Geometric mean.

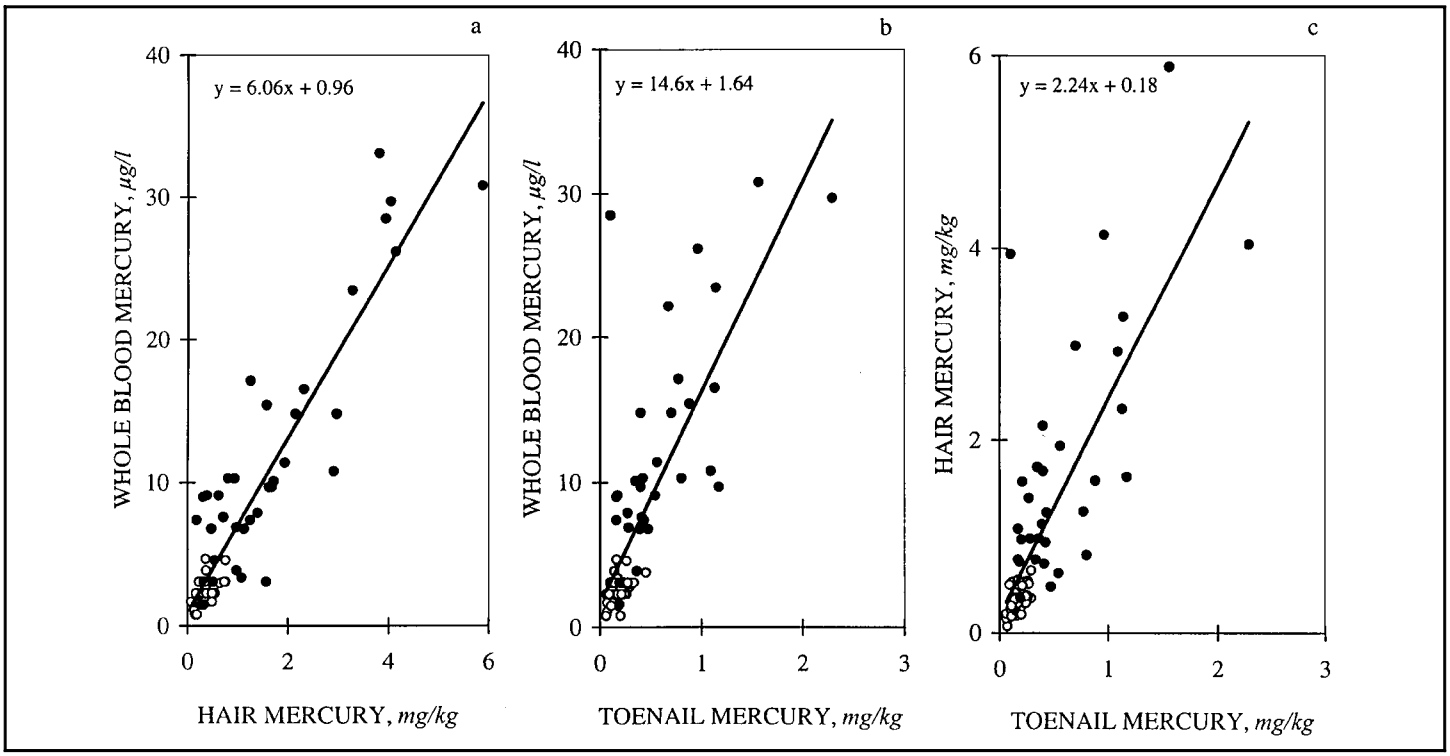

Figure 1. Correlation between mercury concentration of whole blood and hair (a), whole blood and toenails (b) and hair and toenails (c) in adult Finns living in urban seaside Helsinki (open circles) and rural lake-surrounded Leppävirta (filled circles), study A.

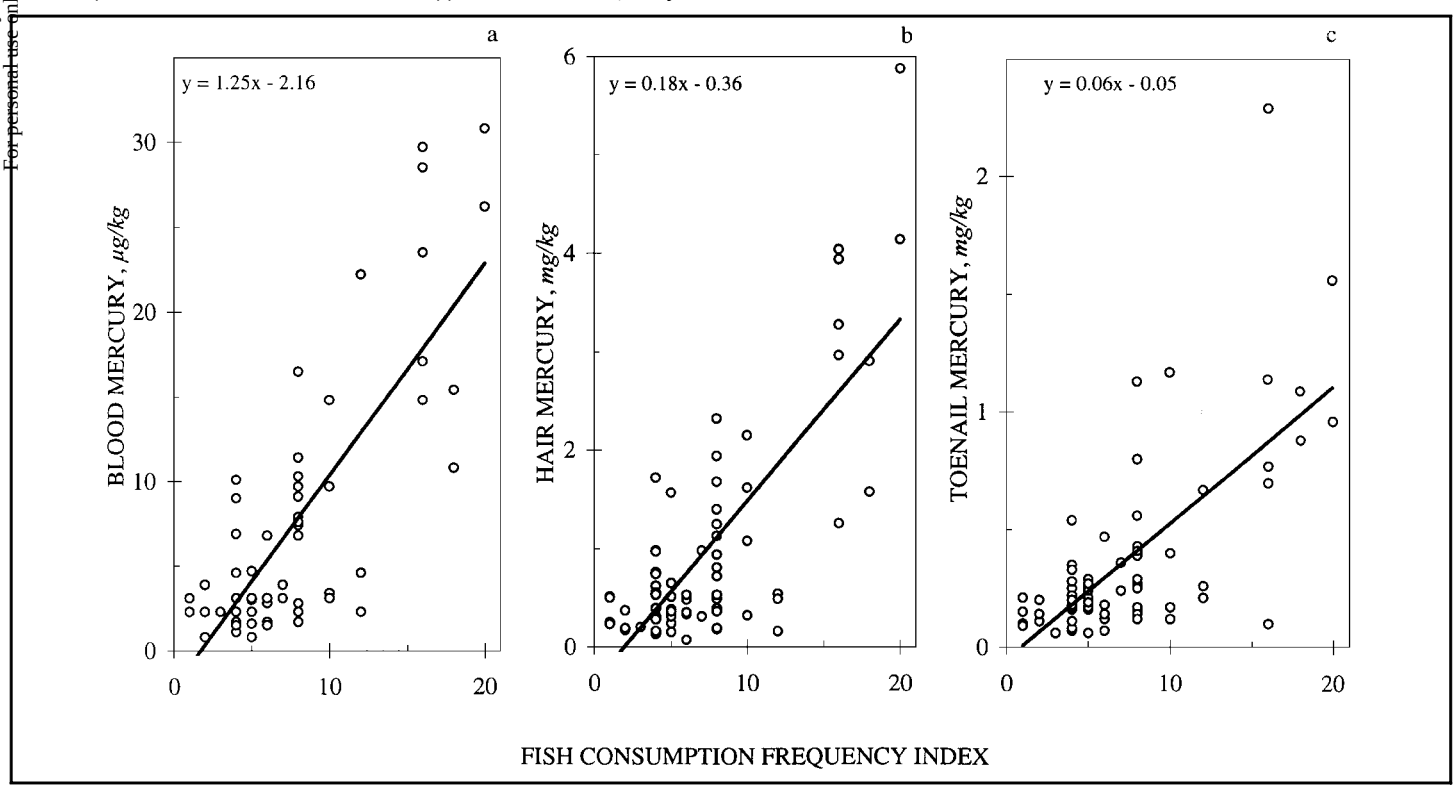

Figure 2. Relationship between Fish Consumption Frequency Index and mercury concentration of whole blood, hair and toenails in adult Finns living in urban seaside Helsinki and rural lake-surrounded Leppävirta, study A.

age, $r = 0.34$, $r = 0.28$, and $r = 0.28$ ($p < 0.02$–0.006), respectively.

Men ($n = 27$) had significantly higher blood and hair mercury levels than women ($n = 45$), 10.3 ± 9.1 vs 5.3 ± 5.4 µg l^{-1} ($p < 0.01$) and 1.45 ± 1.48 vs 0.75 ± 0.77 mg kg^{-1} ($p < 0.05$), respectively, but only slightly higher mean toenail mercury concentrations, 0.43 ± 0.37 mg kg^{-1} vs 0.33 ± 0.40 mg kg^{-1} (n.s.). Men also consumed (high-mercury) fish somewhat more frequently than women, FCFI 8.6 ± 5.0 vs 6.6 ± 4.3 (n.s.). The mean age of men and women was si

Relative sensitivity

Study A

A means to illustrate the relative sensitivity of hair and toenails to classify subjects according to blood mercury was done in study A by dividing the subjects into quartiles, see Table 2. All four blood mercury quartiles were significantly different from each other as was the case for hair and toenails. The relative sensitivity of the biomarkers measured as quartile means per lowest quartile decreased in the order blood > hair > toenails.

Study B

The mean Fish Consumption Frequency Index was 5.8 ± 3.7 (range 1–16). The mercury concentration of the three biomarkers, red blood cells, hair and toenails are shown in Table 3. The correlation between red blood cell and hair mercury was $r = 0.45$ ($p < 0.004$) and between toenails, $r = 0.55$ ($p < 0.0006$). The correlation between hair and toenail mercury was $r = 0.72$ ($p < 0.0001$) (Figure 3). The frequency of fish consumption was significantly ($p < 0.001$) related to all the three sources of biomarkers: hair, $r = 0.57$, red cells, $r = 0.43$ and toenails, $r = 0.72$ ($p < 0.001$). The biomarkers were not associated with age in this group of middle-aged men.

Relationship between tissue mercury and selenium

In study A the mean ± SD whole blood and toenail selenium concentrations were 139 ± 14 μg l^{-1} and 0.72 ± 0.11 mg kg^{-1}, respectively. In study B the previously determined (Alfthan *et al.* 1991a) values for red cell selenium were 186 ± 19 μg l^{-1} and for toenail selenium (Alfthan *et al.* 1991b) 0.69 ± 0.10 mg kg^{-1}. Among the mercury biomarkers in study A only hair mercury was weakly associated with toenail selenium ($r = 0.26$, $p < 0.03$), none with blood selenium and in study B there were no associations between tissue mercury and selenium.

Discussion

The most important finding of this report is that toenail total mercury concentration is as good a biomarker of exposure to (methyl)mercury as is hair. This was shown both by the close relationship between the FCFI and the three biomarkers and indirectly by the high correlations between toenail mercury and the well-established biomarkers of blood and hair mercury concentrations in two groups of fish consumers. Previously Garland *et al.* (1993) had shown an association between the questionnaire-based frequency of consumption of fish and toenail mercury in a group of 127 women. They showed that toenail mercury was also highly reproducible over time on two occasions. Apart from the study of Garland *et al.* (1993) toenails have so far not been used for this purpose in human studies.

Most of the total mercury of hair has been shown to be present as methylmercury (Kyle and Ghani 1982, Suzuki 1993). Exposure to methylmercury results in deposition in hair at a hair:blood ratio of 200–300 (Phelps *et al.* 1980). In study A the ratio was 166, suggesting that these subjects were not in a steady-state or that the low ratio results from exposure to inorganic mercury from dental amalgams. Previous exposure to mercury can be followed retrospectively by analysing lengths of hair corresponding to, for example, one month, *c.* 12 mm. Toenail clippings on the other hand should preferably be cut from all toes and pooled to obtain a representative sample because each toenail grows at an individual rate (Dykyj 1989). Another alternative could be to analyse the clipping from only a certain toe, e.g. big toe (van Noord *et al.* 1993). Then, however, the interindividual variation in the length of the clipping would introduce a temporal bias. This was exemplified by analysing the mercury concentration of single toenail clippings from one individual on two occasions showing an intraindividual variation of 17 CV% between toenails, which exceeds by far the precision of the method (see 'Samples' section).

Among the mercury and selenium biomarkers measured in the present study, only hair mercury and toenail selenium were correlated. This finding is to be expected as fish contributes only 10% of the mean selenium intake (Ekholm *et al.* 1994) and 60% of the mercury intake in Finland (Alfthan *et al.* 1994).

The consumption of fish in Finland is typically seasonal with respect to both amount and species of fish. The very high correlation between blood and hair and toenails is therefore surprising taking into account the different timespans they reflect, i.e. blood and hair (1.5 cm) reflect weeks and months respectively and, based on human selenium supplementation trials, toenails reflect exposure times ranging from 6 to 12 months (Longnecker *et al.* 1993). The reason for this may be the large range (1–20 FCFI) in exposure to high mercury fish.

One must be aware of potential sources of error in using either hair or toenail mercury as biomarkers of methylmercury exposure. Externally deposited mercury cannot be removed from either hair or toenails without c

	Mean	SD	Geometric mean
Red blood cells (μg l^{-1})	14.6	8.6	12.8
Hair (mg kg^{-1})	1.19	1.19	0.76
Toenails (mg kg^{-1})	0.28	0.21	0.22

Table 3. Red blood cell, hair and toenail total mercury concentrations of middle-aged men from the lake-surrounded town of Jyväskylä (study B).

	Blood		Hair		Toenails	
n	(μg l^{-1})	*Q/Q1*	(mg kg^{-1})	*Q/Q1*	(mg kg^{-1})	*Q/Q1*
18	1.8 ± 0.5	1.0	0.29 ± 0.15	1.0	0.14 ± 0.06	1.0
18	2.8 ± 0.4	1.6***	0.51 ± 0.31	1.7*	0.19 ± 0.07	1.4*
18	6.5 ± 2.1	3.6***	0.84 ± 0.47	2.7**	0.29 ± 0.13	2.1**
18	18.6 ± 8.1	10.3***	2.67 ± 1.37	9.1***	0.88 ± 0.51	6.2***

Table 2. Classification of subjects in study A by quartiles, mean ± SD.
Q/Q1 = ratio of quartile mean per lowest quartile mean. Significance compared with lowest quartile, * $p < 0.05$, ** < 0.01, *** < 0.001.

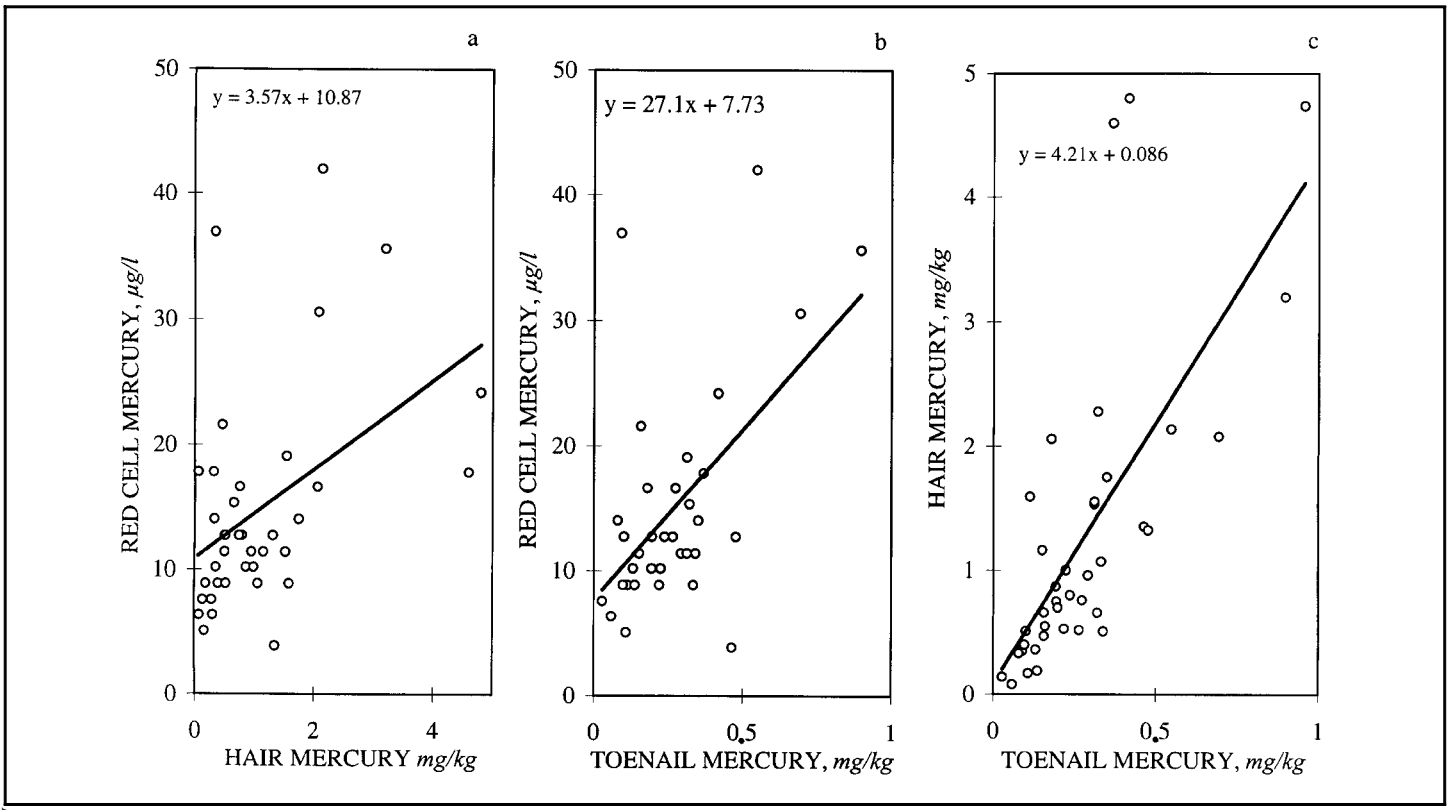

Figure 3. Correlation between mercury concentration of red blood cell and hair (a), red blood cell and toenails (b) and hair and toenails (c) in adult Finns living in lake-surrounded Jyväskylä, study B.

structure. Previous *in vitro* experiments have shown that endogenous (methyl)mercury could not be removed by washing with several types of chemicals (Sky-Peck *et al.* 1990). Likewise, in an *in vitro* experiment, exposure of a pool of toenail clippings either to mercuric mercury or methylmercury at two concentration levels in solution showed that the externally deposited mercury could not be removed by either neutral or alkaline detergents or dilute EDTA or hydrochloric acid (unpublished data).

Although the mean mercury concentrations of the three sources, blood, hair and toenails, were 3.9-, 3.4- and 2.7-fold in lake-surrounded Leppävirta compared with seaside Helsinki, the mercury levels are still quite moderate. The toenail mercury levels can at one extreme be compared with the hair mercury concentrations of 89 Finnish teenagers who suffered from fish allergy (confirmed by a positive reaction in a serological test) showing a mean ± SD value of 0.16 ± 0.01 mg kg^{-1}, representing an unexposed population (unpublished data). The other extreme comes from a recent survey among high fish consumers in eight different areas of Finland where the fish is known to contain elevated mercury levels (Alfthan 1995). The area mean hair mercury concentrations of 123 subjects ranged from 2.3 mg kg^{-1} to 5.0 mg kg^{-1}. These values are moderate compared with the WHO recommended upper level for hair mercury of 50—125 mg kg^{-1} at which the earliest signs of methylmercury poisoning may occur (WHO 1990).

In conclusion, toenail mercury, an easily accessible tissue for the estimation of methylmercury exposure, has been shown to be closely correlated with the well-established biomarkers, viz. mercury in whole blood and hair.

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