Positive Patch Test for Mercury Possibly from Exposure to Amalgam

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Abstract

Objectives: Mercury allergy is a serious health problem. We investigated the relationship between positive patch test for mercury and sources of mercury exposure, indicated by concentrations in biological samples from healthy medical students.

Methods: Patch tests for mercury (Hg-PT) were performed on 580 students. For a group of 55 students with a positive Hg-PT result (Hg-PT(+)) and a reference group of 79 students with a negative Hg-PT result (Hg-PT(-)), mercury concentrations in urine (Hg-u) and hair (Hg-h) were measured. In our search for environmental indicators of mercury exposure, the level of fish intake and mercurochrome usage were determined using a self-administered questionnaire. The oral cavity was investigated and the numbers of decayed teeth filled with amalgam (NA) were counted by dentists.

Results: For the male Hg-PT(+) group, Hg-u and Hg-h were higher than those of a male reference Hg-PT(-) group; Hg-u values obtained in the early morning and after supper were significantly different. Multiple regression analysis with Hg-u as the objective variable among all students showed that increases in the level of fish intake, mercurochrome usage, and the NA independently increased Hg-u measured in the early morning for both gender groups. NA significantly affected Hg-u.

Conclusions: We showed that a higher NA was related to a higher Hg-u measured in the early morning. Therefore, exposure to amalgam may increase Hg-u. It was suggested that Hg-PT(+) might be related to a high Hg-u, and possibly to a high NA.

Key words: mercury, patch test, amalgam, allergy

Introduction

The toxicity of mercury has been known for a long time. Many cases of mercury poisoning have been reported. The central nervous system is particularly susceptible and other organs due to a large intake of fish contaminated with methylmercury, and mercury poisoning is a social problem in many countries (1–3). Occupational exposure to mercury in the chemical industry (4–6) and in dentistry (7, 8) has been reported.

For ordinary persons who are not occupationally but are environmentally exposed to low levels of mercury, allergy caused by a very small amount of mercury including inorganic

mercury is another problem. Mercury has been recognized as a potent allergen ranked more highly than nickel and chrome (9, 10). Contact dermatitis, stomatitis caused by dental amalgam, and sequentially autonomic imbalance syndrome has been reported as ‘amalgam disease’ (11–22). Cases of allergy caused by mercurochrome have been reported (23), and thiomersal, an additive in vaccine acting as a microbicides, has been highlighted as a health risk (24). In spite of these problems, dental amalgam and mercury containing microbicide, such as mercurochrome and thiomersal, are still used widely in daily life because of their relative benefits.

We previously reported on the relationship of a positive patch test for mercury indicating mercury sensitization and environmental factors as sources of mercury exposure among students (25, 26). The students were in good health, without obvious signs of mercury poisoning, and absorbed small amounts of mercury in their normal daily life. We performed a patch test (PT) as an indicator of skin sensitization with mercury, measured mercury concentrations in urine (Hg-u) and

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hair (Hg-h) as indicators of mercury exposure, and then investigated the relevant factors affecting PT results and the relationship between mercury concentrations and exposure factors, that is, dental amalgam, fish consumption, and mercurochrome usage. Information on fish consumption was obtained using a nonvalidated self-administered questionnaire. Preceding studies obtained information on fish intake in terms of preference of fish (27), daily amount of fish intake (28), or estimated mercury intake from fish (29). To avoid using much time and manpower, we used a simplified questionnaire that can be completed within three days. The Hg-h values among students with a positive Hg-PT (Hg-PT(+)) group were significantly higher than those among students with a negative Hg-PT (Hg-PT(-)) group; the number of decayed teeth filled with metals among the Hg-PT(+) group was higher than that among the Hg-PT(-) group.

In the above studies of the authors, the rate of Hg-PT(+) students was around 0.1, that is, approximately 10 students out of 100 in each grade. Since the rate was not high, we applied the case-control study to investigate the relevant factors affecting Hg-PT(+). Hg-PT(+) students were the case group, and the Hg-PT(-) students were the control group (reference group).

We recruited subjects in addition to those investigated in the previous reports. We examined the same relationships. Moreover, we performed multiregression analysis of Hg-u with dental amalgam number, fish consumption, and mercurochrome usage. It was suggested that Hg-PT(+) might be related to the number of amalgam.

Materials and Methods

Subjects

About 100 students attended classes during their fourth year each year during the study period from 1993 to 2002, resulting in a total student number of nearly 1,000. PTs were performed for 530 students (65.6%) who gave written informed consent (323 males and 207 females). Students who had dermatitis for various reasons were excluded from the study. PT(+) students were considered as the case. The control group was initially sampled from PT(-) students by matching the genders and ages at a control-to-case ratio of 2 : 1. Some students actually refused. Finally, we considered the remaining PT(-) students as the reference group. Those who consented to be references also provided us with urine and hair samples.

PT

PTs were performed using the method of the Japanese Contact Dermatitis Society: a patch sink with 0.05% mercury chloride solution was attached to the medial aspect of the arm (30). We observed the skin after 48 hours and evaluated the PT to be positive if skin findings were (+) or more extensive than (+) according to the International Contact Dermatitis Research Group (ICDRG) standard (31).

Analysis of environmental factors

Hair and urine were sampled in the early morning and after supper among the Hg-PT(+) and Hg-PT(-) groups. At the same time, the level of fish intake 3 days before sampling urine and the extent of mercurochrome usage during their childhood were

assessed using a self-administered questionnaire (Annex). We defined the number of meals including fish consumed in a total of 9 meals for 3 days to be the level of fish intake (FI). As for the answer on mercurochrome usage during their childhood, we classified their answers, 'I always used' and 'I sometimes used' as positive and 'I did not use' as negative. Moreover, the number of decayed teeth filled with amalgam (NA) and the condition of the oral cavity as regards stomatitis were monitored by a dentist.

Measurement of mercury concentration

Urine (25–100 ml) was assessed for its specific gravity and creatinine concentration. It was frozen, stored, and refrozen at the time of mercury concentration measurement. Creatinine was measured by an enzyme method (32) using the kit manufactured by Dai-ichi Kagaku Yakuhin Inc. About 1 g of hair was cut from the head and washed by exposure to ultrasonic waves in 1% neutral cleanser for 30 minutes, dried at 40°C for 2 hours, and stored in a desiccator.

Mercury concentrations in 500 µl of urine and in 1–3 mg of hair were measured by the gold amalgam method (33) using the mercury analyzer SP-3D (Nippon Instrument Co., Japan).

The concentration of urine markedly changes over time. We corrected such concentration changes in urine using the creatinine method (34, 35), whereby measured concentrations (W/V) were divided by creatinine concentrations.

Statistical analysis

Since Hg-u and Hg-h in this study were almost logarithmically distributed, we transformed Hg-u and Hg-h using common logarithms, and performed the following statistical analysis on the log-transformed data separately for males and females.

We performed t-tests for the differences in Hg-u and Hg-h, respectively, between the Hg-PT(+) and Hg-PT(-) groups. We performed t-tests for FI and NA, respectively, between the Hg-PT(+) and Hg-PT(-) groups, and χ^2 -tests for the extent of mercurochrome usage between the Hg-PT(+) and Hg-PT(-) groups. Since the mercury concentration in urine of the Hg-PT(+) group was significantly higher than that of the Hg-PT(-) group, NA, FI, mercurochrome usage, and age were entered into the multiple regression model using Hg-u as a dependent variable.

From the multiple regression analysis result, it was found that NA significantly increased Hg-u among the male students. Accordingly, we aimed at quantitatively studying the relationship between NA and the rate of PT(+). The case and reference groups were pooled as a whole and divided by gender. The whole group was trisected according to NA. Spearman's regression test was used among these groups.

In all analyses, differences were considered significant when the p-value was less than 0.05. These analyses were performed using SPSS (ver. 8).

Results

Characteristics of subjects

Fifty-five subjects (36 males, 19 females) were Hg-PT(+) out of a total of 530 (10.4%) subjects (Table 1). Seventy-nine

(52 males, 27 females) Hg-PT(−) subjects participated in this study as a reference group.

The number and age of the Hg-PT(+) and Hg-PT(−) group subjects are shown in Table 2, showing no significant differences.

Mercury concentration and environmental exposure to mercury

The results of Hg-u and Hg-h measurements among the Hg-PT(+) and Hg-PT(−) groups are shown in Figs. 1 to 3. Hg-u values in the early morning (A) and after supper (B), and Hg-h (C) for the male Hg-PT(+) group were all higher than those of the male Hg-PT(−) group. Out of the three mercury concentrations (A, B, C) of the male Hg-PT(+) group, two (A, B) were significantly higher than those of the male Hg-PT(−) group.

Factors affecting Hg-u

There were no significant differences in FI, NA, and mercurochrome usage in either male or female subjects between the Hg-PT(+) and Hg-PT(−) groups (Tables 3 and 4). Dental analysis did not reveal stomatitis caused by mercury in any of the subjects.

Since there was a significant difference in Hg-u in the early morning between the Hg-PT(+) and Hg-PT(−) groups, we carried out multiple regression analysis to clarify the relationship between exposure to Hg, as measured by Hg-u and NA, FI, mercurochrome usage, and age. NA, FI, mercurochrome usage, and age were explanatory variables and Hg-u in the early morning was the objective variable. The results of multiple regression analysis are shown in Table 5. In both male and female subjects, NA, FI, and mercurochrome usage all affected Hg-u in the early morning. In males, NA significantly increased Hg-u.

Relationship between amalgam and PT

The rates of PT(+) are shown in Fig. 4 among the trisected group for the male and female subjects. There was no significant difference.

Table 1 Numbers of medical students by gender positive or negative for patch test for mercury surveyed from 1993 to 2002

Patch test for mercury	Male	Female	Total
+	36 (65.5 [#])	19 (34.5 [#])	55 (100.0 [#])
−	287 (60.4 [#])	188 (39.6 [#])	475 (100.0 [#])
Total	323	207	530

[#] percentages of students by gender according to patch test results.

Table 2 Numbers of biological samples of urine and hair taken from students by gender positive or negative for patch test for mercury

Patch test for mercury		Urine taken after supper		Urine taken in early morning		Hair	
		male	female	male	female	male	female
+	No.	34	19	34	19	29	18
	Mean age (SD)	23.9 (3.7)	22.2 (0.9)	23.9 (3.7)	22.2 (0.9)	24.1 (4.0)	22.2 (0.9)
−	No.	52	27	52	27	36	22
	Mean age (SD)	23.2 (2.1)	22.4 (2.3)	23.2 (2.1)	22.5 (2.3)	23.3 (2.0)	22.1 (1.2)

Fig. 1 Mercury concentrations in urine taken after supper among students by gender positive or negative for patch test for mercury. Bars represent standard deviations.

Fig. 2 Mercury concentrations in urine taken in early morning among students by gender positive or negative for patch test for mercury. Bars represent standard deviations.

Fig. 3 Mercury concentrations in hair among students by gender positive or negative for patch test for mercury. Bars represent standard deviations.

Table 3 Relationship of patch test for mercury and numbers of teeth filled with amalgam or fish intake among students by gender

Patch test for mercury	Male		Female	
	NA ^a	NF ^b	NA ^a	NF ^b
+	2.16 (2.38) [#]	1.03 (1.19) [#]	1.65 (2.57) [#]	1.00 (1.12) [#]
–	1.85 (2.91) [#]	1.13 (1.18) [#]	1.67 (1.96) [#]	1.00 (0.84) [#]

^a NA: number of decayed teeth filled with amalgam.^b NF: total number of fish dishes consumed for 3 days.[#] Average (standard deviation).**Table 4** Relationship of patch test for mercury and mercurochrome usage among students by gender

Patch test for mercury	Male			Female		
	Mercurochrome		Total	Mercurochrome		Total
	Usage	No usage		Usage	No usage	
+	20	8	28	7	9	16
–	19	12	31	11	10	21
Total	39	20	59	18	19	37

Table 5 Results of multiple regression analysis of urinary mercury with environmental factors as sources of mercury exposure

Variable	Male	Female
	SRC ^a	SRC ^a
NA ^b	0.289*	0.274
FI ^c	0.243	0.273
mercurochrome usage	0.106	0.305
Age	–0.005	0.055
Coefficient of determination	0.175*	0.281*

* $p < 0.05$.^a SRC: standardized regression coefficient.^b NA: number of decayed teeth filled with amalgam.^c FI: total number of fish dishes consumed for 3 days. **Fig. 4** Rates of positive patch test for mercury in groups trisected according to number of decayed teeth filled with amalgam grouped by gender.

Discussion

The number of PT(+) students was small. The measurement of mercury concentration among all students entails money, time, and effort and the need to meet ethical requirements. Accordingly, we classified the PT(+) students as the case group and the PT(–) students as the reference group. A case-control (reference) study can be used when the number of the case is small and when it is difficult to take sufficient number to study the dose-response or exposure-response relationships. A case-control (reference) study is considered to be sufficient for clarifying relevant factors.

We tried to sample the control from students with PT(–) by matching with gender and age at the ratio of two to one. However some denied. We could not have the sufficient number of the reference. Therefore the result from the present study may be limited. Nevertheless the significant difference in Hg-u among the groups has an impact.

Hg-u was in a range similar to that reported among the general public with no occupational exposure (36). In spite of this, Hg-u among the male Hg-PT(+) group in our present study was significantly higher than that in the corresponding Hg-PT(–) group. While Hg-h in the male Hg-PT(+) group was higher than that in the male Hg-PT(–) group, the difference was not significant. In our previous report (26) Hg-h of the Hg-PT(+) group was significantly higher than that of the Hg-PT(–) group, while Hg-u of the Hg-PT(+) group was higher than that of the Hg-PT(–) group, but not significantly. A tendency for Hg-h and Hg-u amongst the Hg-PT(+) group to be higher than in the Hg-PT(–) group was found both in our previous and current studies. Significances for biological samples, i.e., urine or hair differently appeared between our two studies. In the previous study, the number of Hg-PT(+) subjects might not have been high enough to generate a significance.

It is said that methyl mercury tends to accumulate readily in hair (37). On the other hand, inorganic mercury, e.g., from amalgam absorbed in the oral cavity, is excreted rapidly in urine and not stored in the body. William et al. (38) assumed that inorganic mercury from amalgam transform in oral cavity to methyl mercury to a very small amount. The absorption content of methylated mercury in oral cavity is quite far less than that of absorbed methyl mercury from fish. Even though, methyl mercury through the oral cavity from amalgam may move to hair. Therefore, the finding in our previous report that Hg-h of the Hg-PT(+) group was significantly higher than that of the Hg-PT(–) group may reflect this metabolism.

Our present results on the relation of PT and Hg revealing no significant difference in Hg-u of female students coincides with the previous report (39). Zimmer et al. (39) could not find any differences in amalgam fillings or mercury levels in biological samples between the sensitive subjects and the non-sensitive controls for amalgam. As shown in Table 3, amalgam might not have been present as many in females as in males.

Multiple regression analysis showed that NA was significantly related to Hg-u in the male students. Therefore, exposure to amalgam might increase Hg-u. Since Hg-u in the male Hg-PT(+) group was sometimes higher than in the male Hg-PT(–) group, exposure to amalgam reflected by mercury excreted in

urine might be related to Hg-PT(+).

NA was counted by the dentist at our University. On the other hand, FI and mercurochrome usage were researched by the not-validated self-administered questionnaire. The measurement of NA contributes to a significant coefficient of the mercury level. On the other hand, FI did not have a significant coefficient with mercury level in urine. This may be from semi-quantitative measurement for FI.

Hg-u in the male Hg-PT(+) group was significantly higher than in the male Hg-PT(-) group. Multiple regression analysis showed that NA was significantly related to Hg-u in the male students. It was suggested that Hg-PT(+) might be related to NA. To prove this suggestion, we should primarily have done the exposure-response analysis between the rate of PT(+) and NA. Therefore as a part of analysis we pooled the case and the reference and made regression analysis of the rate of PT(+) with NA among them. We could not find any significance. This might be because we did not include all the students patch-tested.

In the general population, sources of mercury exposure may include fish consumption, dental amalgam, and mercuro-

chrome usage. The literature on mercury in hair with or without fisherman job reported the following: the fishermen in Malaysia had 12.08 mg/kg (40); inhabitants in the metropolitan Tokyo and in area surrounding Tokyo in Japan had 2.98 ppm in the male and 2.02 ppm in the female (27); inhabitants in five districts in Japan had 2.55 µg/g (29); the female including inhabitants living near the fishing harbor in Akita in Japan had 1.73 µg/g (28). The average value of 1.54 µg/g Hg-h in the present study is less, comparing to those values. The intake of mercury from fish may be very low among the students. Our analysis showed that mercurochrome usage did not affect the mercury concentration in biological samples, especially in hair. Long term exposure to dental amalgam as one of the sources of exposure substantially affected the levels of mercury in the body.

It is said that dental amalgam clearly causes many health problems such as dermatitis and Hg sensitization. This epidemiological study suggests that, amongst people with no clinical symptoms, Hg-PT(+) might be related to a high Hg-u, and possibly to a high NA.

References

- (1) Kitamura S. Epidemiology of the Minamata disease. *Saishin-igaku*. 1971;26:1966–1972. (Article in Japanese)
- (2) Ohi G, Yamamoto S, Fujino T, Itai Y, Ueno K, Saito H, et al. Urinary beta-2-microglobulin dose not serve as diagnostic tool for Minamata disease. *Arch Environmental Health*. 1982; 37:336–341.
- (3) Tokuomi H, Uchino M, Imamura S, Yamanaga H, Nakanishi R, Ideta T. Minamata disease (organic mercury poisoning): neuroradiologic and electrophysiologic studies. *Neurology*. 1982;32:1369–1375.
- (4) Kishi R, Doi R, Fukuchi Y, Satoh H, Satoh T, Ono A, et al.; The Mercury Workers Study Group. Subjective symptoms and neurobehavioral performances of ex-mercury miners at an average of 18 years after the cessation of chronic exposure to mercury vapor. *Environ Res*. 1993;62:289–302.
- (5) Ellingsen DG, Thomassen Y, Langard S, Kjuus H. Urinary mercury excretion in chloralkali workers after the cessation of exposure. *Scand J Work Environ Health*. 1993;19:334–341.
- (6) Cardenas A, Roels H, Bernard AM, Barbon R, Buchet JP, Lauwerys RR, et al. Markers of early renal changes induced by industrial pollutants. I Application to workers exposed to mercury vapour. *Br J Ind Med*. 1993;50:17–27.
- (7) Nilsson B, Gerhardsson L, Nordberg GF. Urine mercury levels and associated symptoms in dental personnel. *Sci Total Environ*. 1990;94:179–185.
- (8) Jokstad A. Mercury excretion and occupational exposure of dental personnel. *Community Dent Oral Epidemiol*. 1990; 18:143–148.
- (9) Friberg L, Enestrom S. Toxicology of inorganic mercury. In: Dayan AD, Hertel RF, Heseltine E, Kazan-tzis G, Smith EM, Van der Venne MT editors. *Immunotoxicity of Metals and Immunotoxicology*. New York: Plenum Press; 1990. p. 163–173.
- (10) Friberg L. Inorganic mercury. *Environmental Health Criteria*. 1991;118:84–146.
- (11) White RR, Brandt RL. Development of mercury hypersensitivity among dental students. *J Am Dent Assoc*. 1976;92: 1204–1207.
- (12) Thomas W, Clarkson TW, Friberg L, Hursh JB, Nylander M. The prediction of intake of mercury vapor from amalgams. In: Clarkson TW, Friberg L, Nordberg GF, Sager DR editors. *Biological Monitoring of Toxic Metals*. New York: Plenum; 1988. p. 247–260.
- (13) Vimy MJ, Lorscheider FL. Dental amalgam mercury daily dose estimated from intra-oral vapor measurements: a predictor of mercury accumulation in human tissues. *J Trace Elem in Exp Med*. 1990;3:111–123.
- (14) Aposhian HV, Bruce DC, Alter W, Dart RC, Hurlbut KM, Aposhian MM. Urinary mercury after administration of 2,3-dimercaptopropane-1-sulfonic acid: correlation with dental amalgam score. *FASEB J*. 1992;6:2472–2476.
- (15) Handley J, Todd D, Burrows D. Mercury allergy in a contact dermatitis clinic in Northern Ireland. *Contact Dermatitis*. 1993;29:258–261.
- (16) Skare I, Engqvist A. Human exposure to mercury and silver released from dental amalgam restorations. *Arch Environ Health*. 1994;49:384–394.
- (17) Lorscheider FL, Vimy MJ, Summers AO. Mercury exposure from “silver” tooth fillings: emerging evidence questions a traditional dental paradigm. *FASEB J*. 1995;9:504–508.
- (18) Barregard L, Stallsten G, Jarvholm B. People with high mercury uptake from their own dental amalgam fillings. *Occup Environ Med*. 1995;52:124–128.
- (19) Koch P, Bahmer FA. Oral lesions and symptoms related to metals used in dental restorations: a clinical, allergological, and histological study. *J Am Acad Dermatol*. 1999;41:422–430.
- (20) Gottowald B, Traenckner I, Kupfer J, Ganss C, Eis D, Schill WB, et al. “Amalgam disease”-poisoning, allergy, or psychic disorder? *Int J Hyg Environ Health*. 2001;204:223–229.

- (21) Scalf LA, Fowler JF Jr, Morgan KW, Looney SW. Dental metal allergy in patients with oral, cutaneous, and genital lichenoid reactions. *Am J Contact Dermat.* 2001;12:146–150.
- (22) Stromberg R, Langworth S, Soderman E. Mercury inductions in persons with subjective symptoms alleged to dental amalgam fillings. *Eur J Oral Sci.* 1999;107:208–214.
- (23) Galindo PA, Feo F, Garcia R, Gomez W, Borja J, Fernandez F. Mercurochrom allergy. Immediate and delayed hypersensitivity. *Allergy.* 1997;52:1138–1141.
- (24) van't Veen AJ. Vaccines without thiomersal: why so necessary, why so long coming? *Drugs.* 2001;61:565–572.
- (25) Sato K, Kusaka Y, Yanagihara M, Ueda K, Mori T, Miyakoshi S. An epidemiological study of factors relating to mercury sensitization. *Allergy.* 1995;44:86–92. (Article in Japanese with abstract in English)
- (26) Mori T, Hirai T, Tomiyama T, Iida K, Miyakoshi S, Sato K, et al. Mercury sensitization induced by environmental exposure. *Nippon Eiseigaku Zasshi.* 1998;52:661–666. (Article in Japanese with abstract in English)
- (27) Nakagawa R. Concentration of mercury in hair of Japanese people. *Chemosphere.* 1995;30:127–133.
- (28) Iwasaki Y, Sakamoto M, Nakai K, Oka T, Dakeishi M, Iwata T, et al. Estimation of daily mercury intake from seafood in Japanese women: Akita cross-sectional study. *Tohoku J Exp Med.* 2003;199:67–73.
- (29) Yasutake A, Matsumoto M, Yamaguchi M, Hachiya N. Current hair mercury levels in Japanese: survey in five districts. *Tohoku J Exp Med.* 2003;199:161–169.
- (30) Matsunaga K. Practice and problems of patch testing. *Skin Research.* 1990;32 Suppl.8:15–22. (Article in Japanese with abstract in English)
- (31) Fisher AA. *Contact Dermatitis.* 2nd ed. Philadelphia: Lea and Febiger; 1973. p. 25–38.
- (32) Kageyama N. Quantitative analysis of chemical components, urea nitrogen, uric acid, creatine, creatinine in urine. *Medical Technology.* 1987;4:127–137. (Article in Japanese)
- (33) Pharmaceutical Society of Japan. *Standard Methods of Analysis for Hygienic Chemists with Commentary.* Tokyo: Kinbara Shuppan; 1990. p. 57–58. (Article in Japanese)
- (34) Barber TE, Wallis G. Correction of urinary mercury concentration by specific gravity, osmolality, and creatinine. *J Occup Med.* 1986;28:354–359.
- (35) Lee E, Park HK, Kim HJ. Adjustment of urinary mercury in health risk assessment of mercury. *J Korean Med Sci.* 1996; 11:319–325.
- (36) Yamamura Y, Yoshinaga Y, Arai F, Kishimoto T. Background levels of total mercury concentrations in blood and urine. *Jpn J Ind Health.* 1994;36:66–69.
- (37) Pharmaceutical Society of Japan. *Methods of Analysis in Health Science.* Tokyo: Kinbara Shuppan; 2005. p. 430–431. (Article in Japanese)
- (38) William A, Sellars MD, Sellars R, Liang L, Hefley J. Methyl mercury in dental amalgams in the human mouth. *J Nutr Environ Medicine.* 1996;6:33–36.
- (39) Zimmer H, Ludwig H, Bader M, Bailer J, Eickholz P, Staehle HJ, et al. Determination of mercury in blood, urine and saliva for the biological monitoring of an exposure from amalgam fillings in a group with self-reported adverse health effects. *Int J Hyg Environ Health.* 2002;205:205–211.
- (40) Sarmani SB, Kiprawi AZ, Ismail RB. Mercury determination in hair of Malaysian fishermen by neutron activation analysis. *Biol Trace Elem Res.* 1994;43–45:435–441.

Annex Questionnaire concerning environmental factors related to biological mercury concentrations

1. Question relating to fish consumption

Have you eaten fish on the following days? Please fill in the following completely: kind of fish, (red or white if unknown), cooking method (sashimi, baking, or boiling), and volume (head, no. of pieces or units in half-size)

		Kind of fish	Cooking method	Volume
Month Day 1 day before sampling urine in early morning on day of sampling urine after supper	breakfast			
	lunch			
	supper			
Month Day 2 days before sampling urine in early morning	breakfast			
	lunch			
	supper			
Month Day 3 days before sampling urine in early morning	breakfast			
	lunch			
	supper			

2. How often did you apply mercurochrome on a wound during your childhood. Please put open circle as the number if appropriate.

- (1) Always
- (2) Sometimes
- (3) Never