METHYLMERCURY EXPOSURE IN CURRENT JAPANESE: ESTIMATION FROM HAIR ANALYSIS

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SUMMARY

Methylmercury (MeHg) is an environmental pollutant with neurotoxic effects on the central nervous system. The major exposure route of MeHg to humans is via consumption of fish and shellfish which accumulate the chemical through the food web in an aquatic environment. Hair mercury level is an excellent marker for MeHg exposure. We have been conducting a survey on hair mercury contents among general populations from 14 districts to estimate the current Japanese MeHg exposure level. Total mercury levels of all hair samples collected (12923 in total) were analyzed by the oxygen combustion-gold amalgamation method using an atomic absorption mercury detector. Multiple regression analysis revealed that mercury levels were significantly correlated with several covariates, such as sex, age, the amount of daily intake of total fish/shellfish, a preference for certain fish such as tuna or bonito, and artificial waving. The geometric means for the population without artificial waving were 2.47 and 1.65 μg/g for males (n = 5623) and females (n = 3470), respectively. Hair mercury levels varied with age, and the variations were more significant in males. Since the difference between sexes was not evident at younger ages, some hormonal control might also be involved in the mercury uptake by human hair. The average mercury levels in our hair samples varied among the sampling districts. Tuna is a major carnivorous fish with high mercury accumulations that is often consumed in Japan. The amount of fish consumption and the preference rate for tuna would appear to be responsible for the regional variation in hair mercury levels in Japan.

Recently, a provisional tolerable weekly intake (PTWI) of MeHg was revised by 61st JECFA to 1.6 μg/kg/week, which was about half that of the Japanese standard, and corresponded to a hair level of 2.2 ppm. The distribution of hair mercury levels in Japanese populations in the present study indicated that 25% of the Japanese females of child-bearing age were estimated to be exposed to MeHg over the PTWI level. This would reflect the high Japanese consumption of marine products. However, not only mercury contamination, but also the nutritional benefit may have to be considered when discussing the risk involved in the current level of fish and shellfish consumption in Japan.

Keywords: hair mercury; Japanese population; methylmercury exposure; PTWI; fish

consumption

Methylmercury (MeHg) is formed by saprophyte microorganisms from inorganic mercury compounds in the aquatic environment (ATSDR, 1992). It is accumulated in fish and shellfish through the marine food web. Since the MeHg accumulation increases with the food web, carnivorous fish such as tuna, swordfish and shark often exhibit high levels of mercury. Furthermore, due to the long biological half-life of MeHg, the chemical tends to accumulate throughout the life of fish (Clarkson, 1992). Marine mammals such as whales and dolphins also show high concentrations of mercury. Accordingly, the major route of human exposure to MeHg is the ordinary consumption of fish and shellfish. MeHg is readily absorbed from the gastrointestinal tract and distributed among various tissues including the brain. The permeability of the chemical at the blood-brain barrier is responsible for its hazardousness neurotoxic effect.

A WHO report (1990) concluded that the NOAEL (no observed adversary effect level) for adults is 50 μg/g of the hair mercury level based on the analytical data of MeHg pollution in the past. Since the developing nervous system of the fetus has been considered highly susceptible to the effect of MeHg (Cox et al., 1989), the report also mentioned a possible association with an increased risk to the neurodevelopment of the fetus when maternal hair levels rise above 10 μg/g. Accordingly, recent studies on the health effects of MeHg have focused on the exposure risk to pregnant women and the neuropsychological outcomes in newborns.

In Japan, the provisional regulatory standards of mercury and MeHg in fish and shellfish were determined in 1973 to be 0.4 and 0.3 μg/g, respectively, based on the assumption of a safe intake limit of 0.17 mg mercury/person/week (0.48 μg/kg bw/day). On the other hand, the revised reference dose (RfD) of the US Environmental Protection Agency (EPA, 1997) set the safe exposure limit to MeHg of 0.1 μg mercury/kg bw/day in 1997. This RfD is aimed at the protection of the developing fetus from neurological deficits induced by MeHg in utero, and has been calculated as 1/10 of the benchmark dose obtained in a study of the Iraq incident of 1971-1972. However, since the manner of MeHg exposure in that incident was quite different from the ordinary exposure risk incurred through fish consumption, the Committee on Toxicological Effects of Methylmercury convened by the United States National Research Council (NRC, 2000) reevaluated the RfD. Although the committee scientifically verified the EPA's RfD level, it recommended that its calculation should instead be based on the data obtained in a cohort study conducted in the Faroe Islands (Grandjean et al., 1997). On the other hand, a provisional tolerable weekly intake (PTWI) of MeHg was determined to be 1.6 μg mercury/kg/week at the 61st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2003). However, a considerable segment of the Japanese population is thought to be exposed to MeHg in excess of the above levels due to their habitually high consumption of marine products (Yasutake et al., 2003; 2004). Here we reported on a survey of the hair mercury levels in a cross section of representative Japanese sub-populations to estimate the current MeHg exposure levels in Japan.

Current hair mercury levels in Japanese

To estimate the current hair mercury levels in a cross section of Japanese sub-populations hair samples from 12,923 individuals were collected from 2000 to 2004 at beauty parlors, barbershops, and primary schools in 14 districts of 12 prefectures: Hokkaido (Abashiri and Tomakomai Cities), Miyagi, Chiba, Niigata, Saitama, Nagano, Wakayama, Tottori, Hiroshima, Fukuoka, Kumamoto (Kumamoto and Minamata Cities), and Okinawa (Fig. 1).

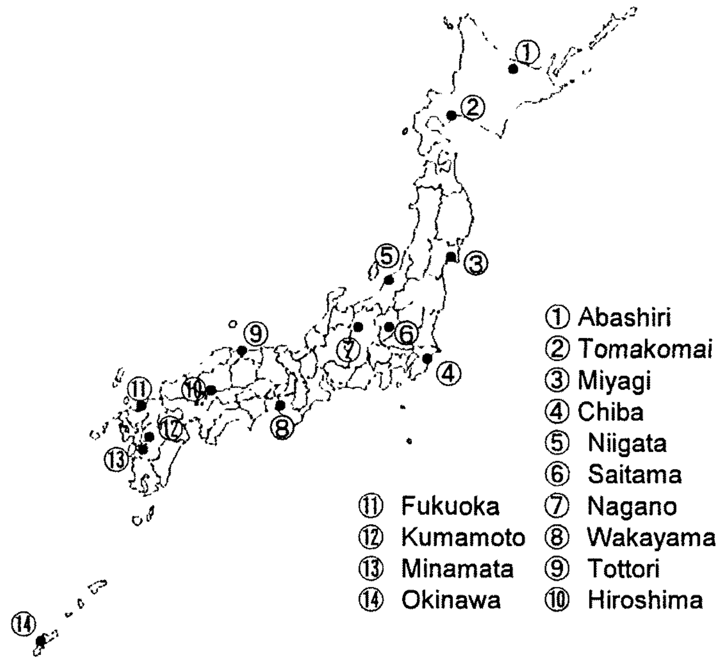

Fig. 1. Hair sampling locations

Using a questionnaire, we gathered information from each individual on age, sex, amount and species of fish consumed, and artificial waving and coloring of hair. Human exposure to MeHg is mostly accounted for by fish consumption, and thioglycolate used in the lotion for artificial waving effectively removed some of the hair mercury (Yamamoto and Suzuki, 1978; Yasutake, et al., 2003). Total mercury levels of all hair samples thus collected were analyzed by the oxygen combustion-gold amalgamation method using an atomic absorption mercury detector. Since the mercury levels analyzed were distributed in a lognormal manner (Fig. 2A, B), a geometric rather than an arithmetic mean was used as representative of hair mercury levels. Multiple regression analysis revealed that mercury levels were significantly correlated with several covariates, such as sex, age, the amount of daily intake of total fish/shellfish, a preference for certain fish such as tuna or bonito, and artificial waving (p<0.001). Some detailed results are given below.

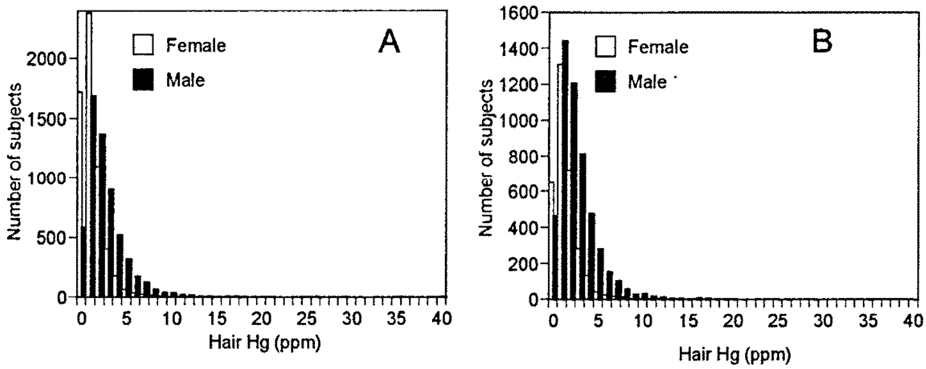

Fig. 2. Distribution of hair mercury content among the total study population (A) and the population without artificial waving (B) Open bar and solid bar indicate female and male populations, respectively.

Sex differences

The mercury levels of 12923 hair samples collected from 14 districts showed a significant sex difference, with females distributed at lower levels than males (Fig. 2A). Geometric means of the levels for males and females in the total population were 2.47 (n = 6,446) and 1.39 µg/g (n = 6,477), respectively. These levels were somewhat higher than those estimated from mercury concentrations in blood or toenails recently reported in western countries (Sanzo, et al., 2001; Guallar, et al., 2002; CDC, 2003). The lower value for females might, at least partly, be due to the high incidence of artificial waving among women. In fact, the frequencies of participants without waving were 52 and 90% for females and males, respectively. Fig. 2B showed the distribution of hair mercury concentrations obtained from the non-waved population. It was evident that female mercury levels were lower than for males. The geometric means for the population without waving were 2.47 and 1.65 µg/g for males (n = 5623) and females (n = 3470), respectively. Even after excluding the contribution from artificial waving, males still showed a higher level than females. Other factors such as the amount of fish consumed might be responsible for the higher male mercury levels. However, the amount of fish consumption shown as per body weight was found to be equal between male and female (Fig. 3). Since the sex difference in the hair mercury levels became evident after the age of puberty, some hormonal control might be one of possible factors as discussed below.

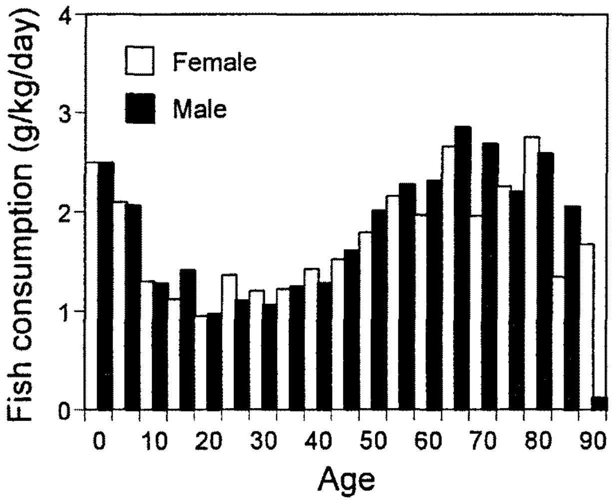

Fig. 3 Age-dependent variation in amount of fish consumption Open bar and solid bar indicate female and male populations, respectively

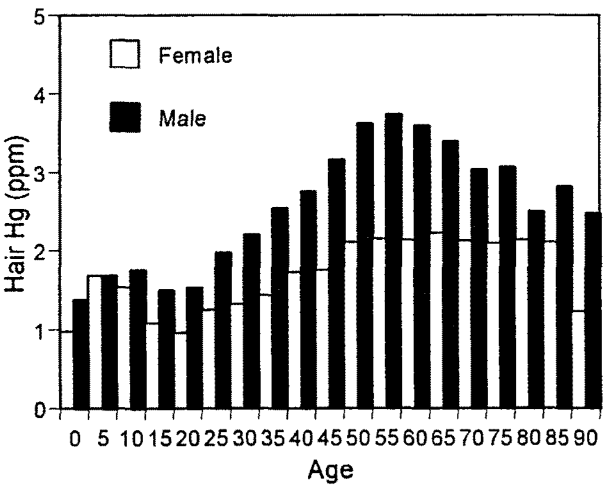

Fig. 4. Age-dependent distribution of the geometric mean of hair mercury content among the population without artificial waving Open bar and solid bar indicate female and male populations, respectively

Age-dependent variations

Hair mercury levels varied with age as shown in Fig. 4. Such variations were more significant in males. Following a transient decline around the 20s, male levels increased into their 50s and 60s, and declined thereafter. The highest levels in the 50s and 60s were mostly twice those in childhood. The age-dependent variation in male hair mercury well fit to fish consumption feature shown in Fig. 3. On the other hand, the age-dependent variations in females were less significant. Although the difference between sexes was not evident at younger ages, the significant increase with age in male mercury levels accounted for a notable sex difference beyond the 20s. Since a marked sex difference subject to modification by hormone treatment has been reported in the tissue uptake and elimination of mercury in MeHg-treated animals (Hirayama et al., 1987), some hormonal control might also be involved in the mercury uptake by human hair.

Regional differences

The average mercury levels in our hair samples varied from 1.72 to 3.81 μg/g for males and from 1.33 to 2.79 μg/g for females among the sampling districts (Fig. 5). Such variations seemed to depend on the total amount of the daily intake of fish/shellfish and on the preference for consuming certain fish. Fish species often consumed in Japan found from the questionnaire in the present study were summarized in Table 2. It should be noted that tuna fish, which has been often consumed by 45% of Japanese, showed extremely high mercury content. Since the mercury content of the second highest fish was less than 1/5 of tuna, tuna consumption supposed to contribute largely to the increase in hair mercury level. Relationships between hair mercury average amounts of the daily intake of fish/shellfish and the rate of preference for tuna consumption. The average consumption of fish/shellfish varied from 50.9 to 115.9 g/day, and the consumption rate of tuna varied from 16.0 to 77.5% (Fig. 6). Tuna is a major carnivorous fish with high mercury accumulations that is often consumed in Japan. The highest rate of tuna consumption was found in Okinawa and Chiba, while Tottori and Minamata had the lowest rate. The highest hair mercury level found in Chiba among all the districts was probably due to the high consumption of tuna there. Although Okinawa also showed a marked tendency to consume tuna, their lower levels of fish/shellfish consumption would tended to depress their hair mercury levels. In contrast, the two districts with the lowest hair mercury levels, Fukuoka and Hiroshima, showed both lower amounts of fish consumption and a lower preference for tuna among all the districts. Thus, the amount of fish consumption and the preference rate for tuna would appear to be responsible for the regional variation in hair mercury levels in Japan.

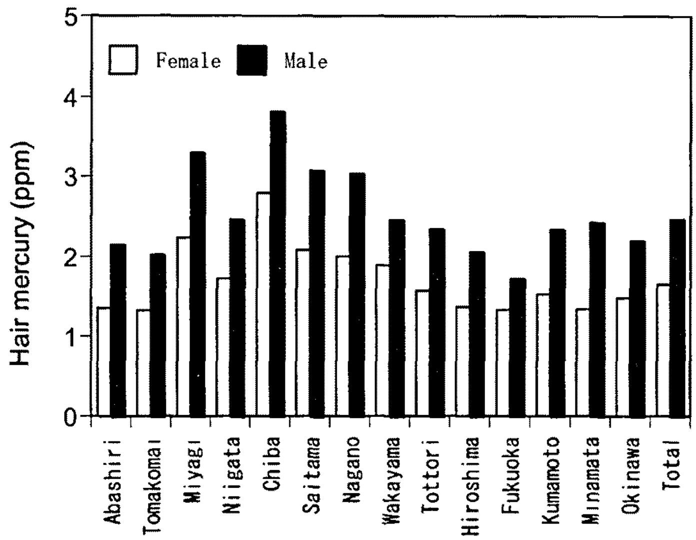

Fig. 5. Geometrical mean of hair mercury levels in 14 districts without artificial waving. Open bar and solid bar indicate female and male populations, respectively.

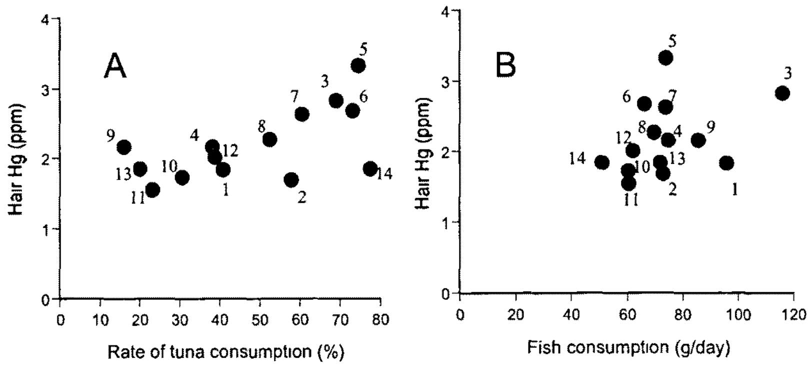

Fig. 6. Relation of hair mercury levels to tuna consumption rates (A) and total amount of fish consumption (B) in 14 districts.

Artificial waving

The lower levels in the adult females might be partly due to artificial hair waving. A frequency of the hair waving above 20's in each district was about five folds higher in females (44.6 to 68.7%) than males (8.4 to 17.1%). Yamamoto and Suzuki (1978) demonstrated that thioglycolate in the artificial waving lotion effectively removed hair mercury. To make sure we treated hair samples from non-artificial hair waved women up to 3 times with waving lotions that were commonly used in Japanese beauty saloons. More than 30% of the hair mercury was removed by a single treatment of the lotions (Fig. 7). Repeated treatments further removed the hair mercury. Removal of a portion of hair mercury was evident also from the longitudinal hair analysis of whole hair samples from females. The typical features for waved and non-waved hairs were shown in Fig. 8. The levels at the root of the artificially waved hair were significantly higher than those at the tip ($p<0.001$) (Table 1). On the other hand, the difference was not significant between the two sides of the non-waved hairs.

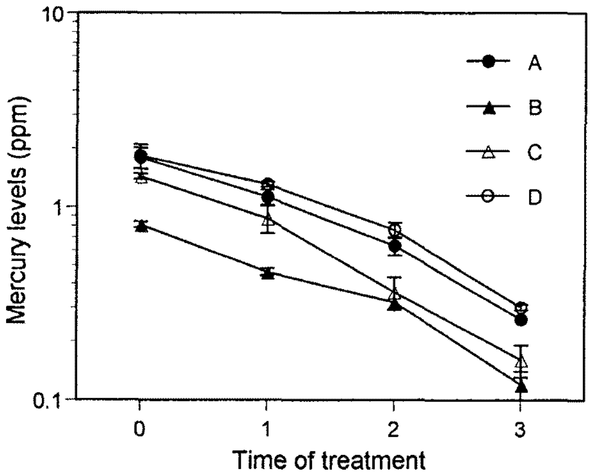

Fig. 7 Effect of artificial waving on hair mercury levels. Non-artificial permanent waved hair samples from 4 women (A to D) were treated with waving lotion up to 3 times Hair mercury levels were determined after each treatment. Each value represent the mean ± SD of 3 measurements.

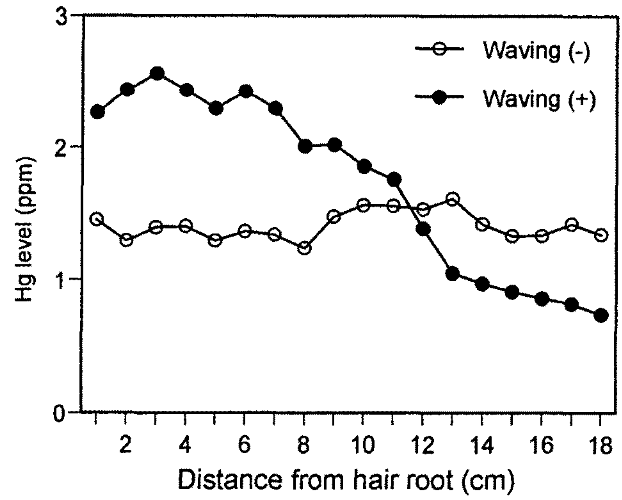

Fig 8 Whole length analysis of hair mercury levels Mercury levels of whole hair strands from two women (with and without artificial permanent waving) were analyzed at 1-cm sections.

Table 1. Ratio of hair mercury levels at the hair tip to the root in female Minamata citizens

Artificial waving	No (38)	Yes (98)
Hg Ratio: Tip/Root	0.92 ± 0.21	0.56 ± 0.22

Numbers of hair samples are shown in parentheses

Safe MeHg exposure levels

The exposure level to MeHg can be estimated from hair mercury levels using the following formula (NRC, 2000):

$$d = \frac{C \times b \times V}{A \times f \times bw}$$

where

C = mercury concentration in blood (μg/L) = hair level (μg/g) x 1000/250

b = elimination rate constant (0.014/day)

V = blood volume (9% of body weight)

A = fraction of the dose absorbed (0.95)

f = absorbed fraction distributed to the blood (0.05)

bw = body weight (kg)

d = dose (μg/kg bw/day)

Various levels has been recommended as a safe exposure limit to MeHg in several countries and by international committees. In Japan 0.17 mg mercury/person/week (3.4 μg mercury/kg bw/week) was suggested as a safe exposure limit in 1973. This is almost equal to the former PTWI (3.3 μg mercury/kg bw/week) reaffirmed at the 53rd JECFA meeting (JECFA, 1999), and corresponds to a hair mercury level of about 5 μg/g. On the other hand, 0.1 μg mercury/kg bw/day, which was suggested as an RfD by the EPA (1997) and reevaluated by the NRC (2000), is the lowest level, and corresponds to a hair level of 1.0 μg/g. However, EPA's fish advisories announced that their RfD level had been applied exclusively to recreationally caught fish. In 2003, considering the fetal effect, the 61st JECFA has revised the PTWI to 1.6 μg mercury/kg bw/week (JECFA 2003). They calculated the PTWI based on the NOEL/BMD obtained from Faroes and Seychelles studies using an uncertainty factor 6.4. The new PTWI corresponds to a hair mercury level of 2.2 μg/g. Recently, Japanese Food Safety Commission (MHLW 2005) recommended for pregnant women to reduce the previous PTWI by 60% to 2.0 μg mercury/kg bw/week, corresponding to a hair mercury level of 2.75 μg/g. They also used NOEL/BMD of Faroes and Seychelles studies, but employed an uncertainty factor 4.

Table 2 Frequencies (%) of sub-populations exceeding certain levels in current Japanese

Hair mercury (μg/g)	0≤	1<	2<	2.2<	2.75<	5<	10<
		US EPA (1997)		JECFA (2003)	Japan (2005)	Japan (1973)	
Male (total)	100	90 1	60 9	55.7	42 9	14 5	2.1
Female (total)	100	70 4	29.8	24.8	15.8	2.4	0.2
15-49 years female							
(total)	100	63.4	22.2	18.9	10.7	1.3	0.2
(without waving)	100	73.7	29.2	24.9	14.7	1.7	0.1
Total	100	80.2	45.1	40.0	29.3	8 4	1.1

The cumulative frequency of hair mercury levels in our survey was shown in Table 2. The districts that exceeded the 5 μg/g which was recommended in Japan and by the former PTWI (JECFA, 1999) were less than 10% of the total population surveyed. When restricted to females of child-bearing age, 1.7% of the sub-population had hair mercury concentrations exceeding that level. However, the majority (87% of the total, 80% of females, 74% of females child-bearing age from 15 to 49 years, and 91% of males) exceeded 1 μg/g. On the other hand, the average hair mercury levels of all Japanese females (1.65 μg/g, without waving) and females of child-bearing age (1.43 μg/g, without waving) were lower than the new PTWI level for pregnant women (JECFA, 2003). However, considerable population segments (25% of females of child-bearing age) exceeded the PTWI level recommended by the 61st JECFA (2003). Similarly, 15% of females of child-bearing age exceeded the Japanese new PTWI (MHLW 2005). Although it is difficult to assess the risk level for females of child-bearing age, they may not be urgently at risk, since none of them in our survey exceeded the NOEL/BMD levels obtained in the Faroes and Seychelles studies.

For pregnant women and those who may become pregnant, The Ministry of Health, Labor and Welfare, Japan (2005) recently announced a program to regulate the consumption of several kinds of fishes and whales that showed high concentrations of mercury. Such a program may be sufficiently effective to bring about some reduction in fish consumption in Japan. However, not only the risk of mercury contamination, but also food habits and nutritional benefits may have to be considered when determining a regulatory standard of fish and shellfish. Sufficient and accurate information must be provided to reach an appropriate decision on fish consumption. Hair analysis may, at least in part, contribute to such decisions by providing information on the MeHg exposure levels of each individual.

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