Bull. Environ. Contam. Toxicol. (1996) 57:315-320

Environmental Contamination and Toxicology

Methylmercury in Hair of Fishermen from Turkish Coasts

N. Vural,[1] H. Ünlü[2]

[1]Department of Toxicology, Pharmacy Faculty, Ankara University, 06100 Tandogan Ankara, Turkey
[2]Poison Information Centre, RSMHE, Ministry of Health, 06100 Ankara, Turkey

Received: 20 October 1995/Accepted: 22 March 1996

Environmental methylmercury mainly arises from the methylation of inorganic mercury. The change in speciation of mercury from inorganic to methylated forms is the first step in the aquatic bioaccumulation process. The bioconcentration factor of methylmercury in fish tissue to that in water is usually between 10^4 and 10^5 (WHO, 1990). Among seafood, fish products are the main source of methylmercury absorbed by men from the environment. Since Minimata epidemic health injuries and deaths in relation to mercury pollution, environmental and biological monitoring of inorganic and organic mercury species has gained importance through out the world and many reports have been published on the health effects and biological monitoring of mercury compounds including some Mediterranean countries (Westöö 1966; Takizawa 1970; Aiery 1983; Dermelj et.al. 1967; WHO/FAO/UNEP 1989).

In risk evaluation, scalp hair analysis of toxic elements has some advantages. As mercury (organic and inorganic) is incorporated into hair and is no longer in equilibrium with the body, determination of mercury and methylmercury is considered to be a good biological indicator for environmental, occupational and forensic medical purposes (Suzuki 1988; Ashraf et.al. 1994).

This study is focused on the determination of methylmercury in hair of fishermen living in different geographical Turkish coasts (Southern, western and northern regions) and relation to eating fish habit. A gas chromatographic method (equipped with Ni-63 electron capture detector) was used to determine methylmercury in hair after cleanup and extraction.

MATERIALS AND METHODS

Hair samples were taken from the fishermen (n:68) living in different geographical Turkish coasts, Mediterranean Sea Coast, Black Sea Coast and Aegean Sea Coast. Subjects were asked to answer a questionnarie form including sex, age, smoking habit and to estimate their fish eating habit. Fishermen were grouped as: Group 1, Few fish eaters (1-2 times fish meal per month); Group 2, Moderate fish eaters (1-3 times fish meal per week); Group 3, Heavy fish eaters (4-7 times fish meal per week).

Control group (n:12) was selected among the people living in Ankara, who do not eat fish or their fish consumption is negligible. Heavy fish eaters are also grouped to their living area as: Group 3a,

Correspondence to: N. Vural

living in Mediterranean Coast (town called Mersin); Group 3b, living in Aegean Sea Coast (village called Edremit) and Group 3c, living in Black Sea Coast (town called Trabzon).

Hair samples were cut with stainless steel scissors from several sites of the scalp. Samples were taken in l-2 cm segments as close as possible to the scalp. 100-300 mg of samples is sufficient for analysis. Hair samples were kept in plastic bags at room temperature after weighing.

Hair samples were washed with 4 times non-ionic detergent and twice with distilled de-ionized water. Then they were cleaned with acetone (Suzuki 1966) and dried at room temperature. Isolation of MeHg from the hair samples was carried out following the procedure of Westöö (1968) partly modified by Kamps and Mc Mahon (1972) and then by Dermelj et.al. (1987). 0.100 g of dry hair samples were digested with 50 ml 7.5 M NaOH for 30 minutes at 90°C. After filtration of digestion solution through cotton wool, methylmercury was extracted into 30 ml toluene. Organic phase was stripped twice with 15 ml 0.1 M $Na_2S_20_3$ solution and reextracted into 20 ml benzene in the presence of 5 ml 2 M H_2SO_4 and 10 ml 4 M KBr solution for derivatization. After the evaporation of organic phase to 2 ml, the concentrated extract was used for the determination of methylmercury by gas chromatography (GLC).

MeHg (as bromide derivative) in the hair extract was determined by Gas Chromatograph (Tracor 565) equipped with Ni 63 ECD and 2mxl/8 inch i.d glass column. Column packing was a mixture of 1.5 % OV-17 and 1.95 % OV-210 on 80-100 mesh Chromosorb WAW-DMCS. Other parameters of the GLC were: carrier gas_a nitrogen, column, injection port and detector temperatures were 170°C, 220°C and 240°C respectively. Varian 4270 integrator was used for quantitation of MeHg.

Methylmercury bromide (Merck, p.a.) was used as a standard substance. Stock solution (10 ppm), prepared in benzene, was diluted appropriately to obtain 0.5; 1,2 and 5 and 10 ppm working standard solutions. Working standards were prepared weekly and stored at 0°C in dark bottles. Standard solutions were treated beginning from wet digestion to extraction procedures as desribed above for hair sample preparation and determination by GLC. Calibration curve was prepared plotting concentrations versus peak area. Recovery of extraction was calculated using double working standard solutions.

Statistical analysis of methylmercury results was performed using one way variance analysis and comparison of groups was carried by student (t) test.

RESULTS AND DISCUSSION

After cleanup, wet digestion and extraction of methylmercury from hair, determination was carried out by gas chromatography. The conditions and parameters of gas chromatography we used (Aygün et al 1985) gave best results as seen in figure 1. The recovery of the method was found 87.6 % + 0.21 with the 1 ppb (ng/ml) level sensitivity

Methylmercury levels in scalp hair of fishermen (n:68) are shown in Table I. When fishermen were grouped to their fish eating habit, mean methylmercury level of the fishermen who ate fish 4-7 times per week (group 3:10.22 + 1.50 ppm with a range 1.52 - 26.70 ppm) was

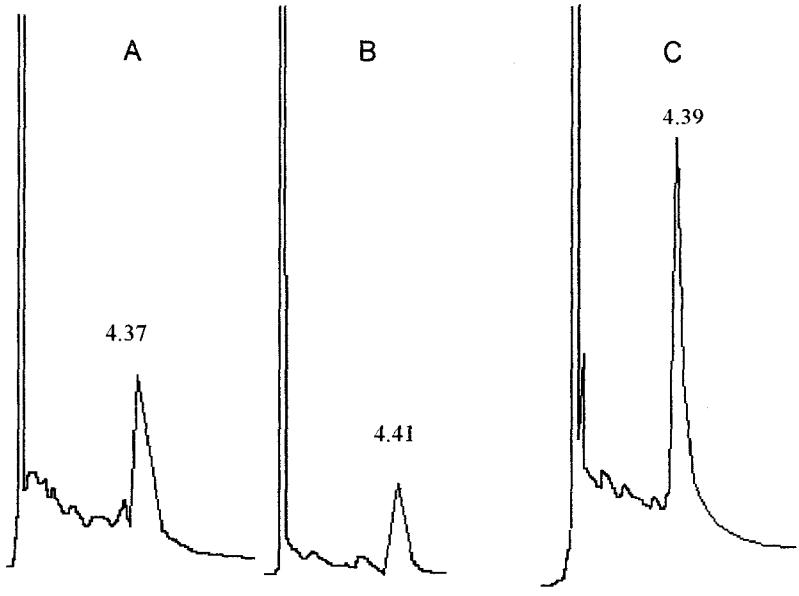

Figure 1. Chromatograms of derivatized standard and isolated methylmercury from hair sample. a: Sample b: Standard (μg/ml) c: Standard + Sample (mixture)

found significantly higher than those who ate fish l-3 times per week (mean: 6.06 ± 2.70 ppm; range: 0.70 - 12.33 ppm) and 1-2 times per month (mean: 2.46 ± 1.52 ppm, range: 0.8 - 8.92 ppm). Mean methylmercury level of subjects who do not eat fish was 1.15 ± 0.34ppm with a range 0.5 - 1.8 ppm and no significant difference was found as compared with fishermen who ate fish 1-2 times per month. Statistical results of the groups compared were shown in Table 2.

Seafood is the main source of methylmercury absorbed by man from the environment. Among the sea food fish consumption is more frequent and intake of methylmercury varies depending on the amount of fish and level of methylmercury in the fish. Many surveys carried out indicating methylmercury content of scalphair in relation to fish eating habit (Kyle and Nasreen 1982; Airey 1963; Dermelj et al 1987; Foo et al 1988; WHO 1989). Our results confirm that methylmercury accumulation in hair increases with the increase of fish consumption. Fishermen eating 3-4 times fish per week (n:25) were also studied in subgroups (3a, 3b, 3c) according to their location namely living in Mediterranean coast, Aegean Coast and Black Sea Coast. Fishermen living in Black Sea Coast have the lowest mean hair methylmercury level. As the results compared statistically, there is a difference between those living in Black Sea Coast and those living in western and southern sea coasts ($p < 0.01$, Table 2).

As methylmercury absorption depends on the extend of environmental exposure, the higher results of those living in the southern and western sea coasts of Turkey can be explained with the higher mercury

Table 1. Hair methylmercury levels (ppm) in different groups as related to fish consumption

Group	S.No	Sex	Age	MeHg
Control	1	F	15	0.52
	2	F	40	1.02
	3	F	18	1.05
	4	F	36	0.90
	5	F	31	1.20
	6	F	25	1.50
	7	F	19	0.09
	8	F	25	1.30
	9	M	29	1.00
	10	M	48	1.45
	11	M	38	0.95
	12	M	12	1.25
range			12-45	0.50-1.80
Mean±Sd			28	1.50±0.34
Group 1	1	F	50	1.65
	2	F	64	8.92
	3	F	18	0.80
	4	F	14	1.40
	5	F	11	1.32
	6	F	5	2.10
	7	F	17	6.35
	8	M	42	1.46
	9	M	49	0.90
	10	M	47	2.50
	11	M	43	1.20
	12	M	13	0.92
range			8-64	0.80-8.92
Mean±Sd			44.20	2.46±1.52
Group 2	1	F	20	0.70
	2	F	21	3.53
	3	F	65	0.95
	4	F	35	9.16
	5	F	25	9.00
	6	F	31	7.21
	7	F	23	8.29
	8	M	48	8.00
	9	M	60	6.20
	10	M	30	2.72
	11	M	75	6.22
	12	M	35	7.16
	13	M	43	8.90
	14	M	4	6.79
	15	M	22	4.35
	16	M	21	12.33
	17	M	46	5.65
	16	M	34	5.20
	19	M	33	5.75
	20	M	37	5.85
	21	M	30	7.40
range			20-75	0.70-12.33
Mean±Sd			36.85	6.06+2.70

Group	S.No	Sex	Age	MeHg
Group 3a	1	M	66	4.10
	2	M	51	9.70
	3	M	17	1.90
	4	M	40	6.90
	5	M	75	7.10
	6	F	47	5.44
	7	F	45	1.55
	8	F	40	1.72
Group 3b	9	M	36	8.33
	10	M	22	7.48
	11	M	40	7.50
	12	M	32	7.12
	13	M	22	11.00
	14	M	42	18.20
	15	M	44	16.01
	16	M	32	20.15
	17	M	35	10.00
	10	M	41	8.41
	19	M	15	8.04
	20	M	15	9.14
	21	M	38	13.14
	22	M	25	14.40
Group 3c	23	M	26	17.16
	24	M	28	7.00
	25	M	40	17.13
	26	M	23	12.82
	27	M	23	10.65
	28	M	18	13.72
	29	M	50	26.70
	30	M	41	23.91
	31	M	22	9.11
	32	M	28	1.42
	33	M	26	3.24
	34	M	23	10.38
	35	M	28	5.68
range(tota1)			15-75	1.52±26.7Q
Mean±Sd			34.17	10.22 ± 6.15

Table 2. Statistical results of methylmercury levels

Groups(n) (Fish consumption)	MeHg (ppm) range	Mean±Sd	Student t test(p)
Control(C:12)(any or very rare fish consumption)	0.50-1.80	1.15±0.34	$p > 0.05$ (Control-Group 1) $p < 0.001$ (Control-Group 2)
1(12) (1-2 times/month)	0.80-8.92	2.46±1.52	$p < 0.001$ (Control-Group 3) $p < 0.001$ (Group 1-2)
2(21) (1-3 times/week)	0.70-12.33	6.06±2.70	$p < 0.001$ (Group 1-3) $p < 0.01$ (Group 2-3)
3(35) (4-7 times/week)	1.52-26.70	10.22±6.15	
3a(8) (Black Sea Coast)	1.55-9.70	4.80±3.00	$p < 0.01$ (Group 3a-3c)
3b(14) (Aegean Sea Coast)	7.12-20.15	11.44±4.30	$p < 0.01$ (Group 3a-3b)
3c(13) (Mediterranean Sea Coast)	1.42-26.70	12.28±7.4	$p > 0.02$ (Group 3b-3c)

levels of fish in those locations. We have no data on the environmental and biological methylmercury levels before this study in Turkey. But there are some publications, on the total mercury levels of the seafood including fish, in the several coastal areas. Mercury levels were found generally below the "ppm" level and the mean levels were 0.345 ppm in the Mediterranean Sea fish, 0.338 ± 0.0184 ppm in the Aegean Sea fish and 0.278 ± 0.134 ppm in the Black Sea fish (Sanli and Ceylan 1980; Ceylan et al 1980). Those results indicate a common mercury pollution of fish species in Turkish coasts mainly in the western and southern parts and seem not have a potential alimentary risk for people for those years (1980-1981). But we have no other results reflecting the mercury pollution of sea food in the last two decades. There is one publication (Kunç 1987) showing the total mercury level of hair from those subjects (n:10) living in Adana (south part of Turkey) which average concentration was reported 0.06 ppm (ranged from 0.01 to 1.2 ppm).

Our methylmercury results show a significant difference in relation to fish eating habit and geographical location. Turkish population did not appear to be at risk. The results fall between the world mean levels methylmercury reported in WHO publication (1990) but higher than other Mediterrenean countries (Dermelj et al 1987; WHO 1989). However, one subject in this study had hair methylmercury level close to 30 ppm (Table I). Generally in adults with a minumum 30 ppm methylmercury

concentration hair can produce clinically detectable effects (WHO 1969). Therefore we beleive that this survey must be extended in number of subjects and other personal parameters (age, sex occupational and nonoccupational exposure to mercury etc) and from the point of mercury speciation in the environment and the biological media in several geographical locations of Turkey.

REFERENCES

Aierey D (1983) Total mercury concentration in human hair from 13 countries in relation to fish consumption and location. Sci Total Environ 31: 157-180

Ashraf W, Jaffer M, Mohammed D (1994) Trace metal contamination study on scalp hair of occupationally exposed workers. Bull Environ Contam Toxicol 53: 516-523

Aygün S, Seçkin MA, Ataman OY (1985) Evaluation of standardization techniques for mercury speciation by gas liquid chromatography. Microchim Acta III: 307-317

Ceylan S, Sanli Y, Kaya S (1980) Mercurial pollution in various fish species from the Bays of Aegean Sea A.Ü.Vet Fak Mec, XXVII (3-4): 674-692

Dermelj M, Horvat M, Byrne AR, Stegnar P (1987) Mercury, methylmercury and selenium in scalp hair of inhabitants for Mediterranean areas. Chemosphere, 16: 877-886

Foo SG, Ngim CH, Phoon WO, Lee J (1988) Mercury in scalp hair of healthy Singapore residents. Sci Total Environ 72: 113-122

Kamps LR, McMahon B (1972) Utilization of Westöö Procedure for the determination of methylmercury in fish by gas liquid chromatography. J AOAC 55(3): 590-593

Kunç S (1987) The Determination of certain trace elements in human serum, urine and hair. J Firat Univ., 2(1): l-9

Kyle JH, Nasreen G (1962) Methylmercury in human hair: A study of a Papua New Guinean population exposed to methylmercury through fish consumption. Arch Environ Health 37: 266-277

Suzuki T (1988) Hair and nails. Advantages and piffalls when used in biological monitoring. Biological Monitoring of Toxic Metals Plenum Press 623-635

Sanli Y, Ceylan S (1980) Determination of the mercurial pollution level in the various fish species of the Turkish coast of Black Sea. A.Ü Vet Fak Derg, XXVII, (l-2): 11-23

Takizawa Y (1970) Studies on the Niigata episode of Minamata disease outbreak; investigation of causative agents of organic mercury poisoning in the district along the River Agano. Acta Med biol (Niigate), 17(4): 293-297

Westöö G (1966) Determination methylmercury compounds in foodstuffs. Acta Chem Scand. 20: 2131-2137

Westöö G (1968) Determination of methylmercury salts in various kinds of biological material. Acta Chem Scand 22: 2277-2280

WHO/FAO/UNEP report (1984) Biological monitoring of methylmercury in Mediterranean populations (MED POL phase II). ICP/CEH/mO_2, WHO Copenhagen

WHO/FAO/UNEP report (1989) Mediterranean Health-Related Environmental Quality Criteria (MED POL Phase II) EUR/ICP/CEH 059, 0242 W

WHO (1990) Methylmercury, Environmental Health Criteria 101