Mercury in hair as a biomarker of exposure in a coastal Venezuelan population.

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Key words: Mercury, exposure, mercury in hair.

Abstract. To assess mercury exposure and potential risk, total mercury (THg-H) and methylmercury (MeHg-H) in hair were studied in 160 adults. The study group consisted of 60 individuals living in the north central coast of Venezuela. A section of the area was known to be contaminated with mercury from a chlor-alkali plant installed near one of the tributary rivers of the Caribbean Sea. The study group was selected from 4 inclusion criteria points. The control group was composed of 100 individuals selected from Carabobo state with no known exposure to Hg. A questionnaire was designed to collect demographic, health information, work activities and fish consumption habits. Hair samples were analyzed for THg. Samples with THg-H > $5 \mu g/g$ were also analyzed for MeHg. The mean THg-H was 1.88 ± 1.50 and $0.99 \pm 0.87 \,\mu\text{g/g}$ for the study and control groups, respectively. The study group was statistically higher than control individuals, however, no statistical differences of THg-H were found between each of the 4 categories of both groups. Mean MeHg-H value was $3.67 \pm 1.25 \,\mu$ g/g. Associations were made between Hg-H and several variables. No significant relationship was noted between Hg-H levels and clinical symptoms. R analyses and t-tests were used to determine associations between questionnaire variables and THg-H. The main predictors of THg-H levels in the study group were fish consumption and frequency. As both groups presented relatively low values for THg-H and MeHg-H, the results of this study indicate that Hg exposure did not exceed safe levels. However, a more in-depth exposure assessment should be conducted to more accurately assess this exposure, specifically in terms of Hg content in water and fish sampling.

Mercurio en el cabello como bioindicador de exposición en una población costera de Venezuela.

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Palabras clave: Mercurio, exposición, mercurio en cabello.

Resumen. Mercurio total y metil mercurio en cabello (THg-H y MeHg-H) fueron determinados en 160 adultos para caracterizar esta población en términos de exposición a Hg v su potencial riesgo. El grupo Estudio consistió de 60 individuos que viven en la costa Centro-Norte de Venezuela. Parte de esta área se había reportado contaminada, años atrás, con Hg de una planta de cloro-soda instalada junto a uno de los ríos tributarios del mar Caribe. El grupo Estudio se seleccionó con base en 4 criterios de inclusión. El grupo Control estuvo compuesto de 100 individuos del Estado Carabobo, sin exposición conocida a Hg. Se administró un cuestionario para obtener información referida a: demografía, salud, actividades laborales y alimentación con pescado. Se analizaron muestras de cabello para determinar THg. Las que resultaron superiores a $5 \mu g/g$, se analizaron también para MeHg. El promedio de THg-H fue de $1.88 \pm 1.50 \text{ y } 0.99 \pm 0.87 \,\mu\text{g/g}$ para los grupos estudio y control respectivamente. El grupo estudio resultó estadísticamente superior al control, sin embargo, no se hallaron diferencias estadísticamente significantes en el THg-H comparando cada una de las 4 categorías del grupo estudio. La media de MeHg-H fue de 3,67 \pm 1,25 μ g/g. Se calcularon asociaciones entre Hg-H y algunas variables. No se encontró una relación significativa entre los niveles de Hg-H y los síntomas reportados. Se usaron R-análisis y t-test y para examinar las asociaciones individuales entre las variables del cuestionario y el THg-H. Los principales predictores de los niveles de THg-H en el grupo Estudio fueron el consumo de pescado y su frecuencia. Ya que ambos grupos estudiados presentaron valores relativamente bajos de THg-H y MeHg-H, los resultados de este estudio indican que la exposición a Hg no excedió los niveles permisibles. Sin embargo, se requiere una evaluación más profunda para caracterizar, en forma más exacta esta exposición, principalmente en lo que se refiere a determinar concentraciones de Hg en muestras de agua y de pescado en la zona.

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INTRODUCTION

Natural degassing of the earth's crust is the major source of environmental mercury; however, industrial activities can be an additional source of toxic levels, either directly or indirectly through the use and misuse of mercurial compounds (1). Several studies have indicated an association between Hg exposure and a diet rich in fish (2-4). This work was performed at the Puerto Cabello-Morón coastal area of Venezuela, an ideal location for industries that require large land areas and water transportation. Part of the area was known to be contaminated with mercury due to

the installation of a chlor-alkali plant in a petrochemical complex close to one of the tributary rivers ("Caño Alpargatón"), that empties into the Caribbean Sea (5, 6). Mercury spills from these plants between 1957-1976 produced concern in the 70 km coastal zone from Puerto Cabello to Chichiriviche and in the National Park area. The government of Venezuela requested an impact evaluation of major industrial discharges in that location. This evaluation identified high Hg levels (> 3 ppm) in Caño Alpargaton sediments (6). During the following years, several potential polluting industries were built close to areas where the main source of food intake was fish. The main contamination pathway of MeHg to humans is through the consumption of fish (7). In some of the villages studied, fish is generally the only source of animal protein.

Mercury exposure was evaluated by measuring the Hg content of hair. Total and methylmercury in the hair (THg-H and MeHg-H) are useful bioindicators of long-term exposure to Hg. MeHg-H is specific for MeHg contamination, particularly from ingestion of Hg-contaminated fish, where MeHg is easily bio-accumulated and biomagnified thus becoming concentrated in fish, particularly, carnivorous fish (8).

Once incorporated into the hair root, Hg remains stable. Hair grows at the rate of approximately 1 cm per month. Hair represents recent and historical exposures, while blood Hg content reflects only recent Hg exposure (9). One of the advantages of using hair as a biological sample is that the segmental analysis of strands provides the opportunity to assess past exposure history based on growth rate (9-11). MeHg builds up during hair formation and shows a correlation with blood Hg levels. There is evidence that TH_g-H is 250 to 300 times higher than Hg in blood (7, 8). Therefore a conversion factor of 1:250 has been used to convert THg-H to Hg in whole blood (12).

The authors have updated part of the above-mentioned surveillance and investigated the current potential Hg-contamination in a selected population of villagers in the region of Puerto Cabello-Morón coastal area of Venezuela.

METHOD

Analytical, case-control study. The study group included 60 individuals living on the north central coast of Venezuela (Falcón-Carabobo States) and was selected using the following criteria:

- Retired individuals that worked in the petrochemical industry at the time of the contamination (n = 20);
- Current petrochemical workers (n=13);
- Residents of a fishing village located 10 km from the petrochemical industry (n = 14) with high intake of fish eaught locally).
- People living in Puerto Cabello, a Carabobo State port, located 20 km from the petrochemical industry and whose fish intake is considered high (n = 13).

The control group consisted of 100 individuals (50 male; 50 female) from Valencia, Carabobo State with no known Hg-exposure. Valencia is located 94 km from the fishing village; 64 km from Puerto Cabello and 84 km from the petrochemical industry. This city was chosen as the control group region for the following reasons:

- Location (same State and region)
- It was difficult to assure, with confidence, that we could find people "with no known exposure" to Hg in the exact area of the study group. This includes the area between Puerto Cabello and the fishing village; an industrial area with many contaminants present.

A questionnaire, including socio-demographic information, health history, Hg-related signs/symptoms presented during the past 2 years, work activities (potential Hg

exposure) and life styles was given to both groups by way of interviews. Respondents were also questioned about their fish consumption (yes/no), estimated average number of times per week they consumed fish in the past 6 months, and type of fish consumed. The respondents were categorized according to frequency of fish intake (1 = Daily; 2 = 2-5x/week; 3 = 1-3x/week; 4 = No fish consumed). The questionnaire did not include information such as quantity of fish consumed (grams) nor the relative size (length and weight) of fish consumed. The study was carried out during the dry season.

A 1-g hair sample was obtained from the subjects, trimming close to the scalp. Hair samples were identified and stored in plastic bags prior to being analyzed. Samples were analyzed at the Laboratory of the Sciences Institute for Minamata Disease in Japan. THg-H was determined in study and control groups by a flameless atomic absorption spectrometer with a mercury analysis vaporizer (Rigaku Mercury SP-1; Nippon Instruments Co. Ltd, Tokyo). Samples with THg-H > $5 \mu g/g$ were also analyzed for MeHg-methods of Nakamura et al, 1999 (13) and Nakano and Miyamaoto, 2003 (14), by atomic absorption spectrophotometry. Analytical quality control was ensured with standard hair samples, provided by the Japanese Institute for Environmental Studies, and used as a control.

It was determined that THg-H levels below 5 μ g/g did not represent significant concentrations of Me-Hg. THg is the sum of inorganic and organic Hg. Me-Hg accounts for approximately 80% of THg-H (15), therefore very small amounts of THg-H will represent even lower amounts of Me-Hg. Then we determined the Me-Hg contents in the hair containing high concentration of THg-H (over 5 ppm). As shown in Table I, the control group had no THg-H levels higher than 5 μ g/g.

Volunteers were informed of the study objectives and confidentiality issues. Written consent was obtained from the volunteers and was provided with the results.

Statistics

Descriptive statistical analyses were used to illustrate socio-demographic variables and concentrations of Hg-H and to characterize the fish diet. Mean comparisons were carried out using the Student's t test. R package was used for the one-way analysis of variance (15). This was the most suitable model for the treatment of data with independent categorical variables. The proposed model used to estimate the relationship between the dependent variable THg-H and the independent variables sex, group classification, smoking and drinking habits, fish intake and frequency of fish intake, is the following: THg-H = $\mu + \beta_1 \text{sex}$ + β_2 group classif. + β_3 smoking habit + β_4 alcohol intake + β_5 fish intake + β_6 frequency fish intake $+ \varepsilon$, where each independent variable is measured in two or more factor levels.

Initially the idea was to compare the study group with the control group. However, the results showed there was a gradient of exposure that could be compared. It is a fact that the study group sample may not be representative; however, it allowed us to show that there is a tendency toward the dose-response relationship.

RESULTS

The distribution of the study population according to group, sex, age and Hg-H values is represented in Table I. The study group had statistically higher THg-H values (p < 0.01) than the control group. Four individuals (3 male, 1 female) of the study group had THg-H > 5 μ g/g. Mean MeHg-H concentration was 3.67 \pm 1.25 μ g/g. Table II shows the distribution of the study

DISTRIBUTION OF THE STUDY POPULATION ACCORDING TO GROUP, SEX, AGE AND THG-H VALUES

Group	Age (years)	Sex	THg (\(\mathbe{g}/\g\))			ТНg-H (µg/g)		MeHg-H (μ g/g)
				n	% 1	$X \pm SD$	Range	$X \pm SD(R)$
			۸ م	33	58.92	1.53 ± 1.05	0.21-4.38	
	53.08 ± 13.67 (R = 27.74)	Male	VI ro	3	75	5.78 ± 1.05	5.16-7.00	3.85 ± 1.47 $(2.17-4.90)$
			Total male	36	09	1.88 ± 1.57	0.21-7.00	
			۸ م	23	41.08	1.72 ± 1.21	0.31-4.70	
Study	39.17 ± 12.13 (R = 21.72)	Female	VI N	1	25	5.61	5.61	3.14
			Total female	24	40	1.88 ± 1.42	0.31-5.61	
			A N	56	100	1.61 ± 1.11	0.21-4.70	,
	47.47 ± 14.66 (R = 21.74)	Total Study Group	۷۱ ص	4	100	5.74 ± 0.86	5.16-7.00	3.67 ± 1.25 $(2.17-4.90)$
			Total	09	100	1.88 ± 1.50 *	0.21-7.00	
		Male	۸ م	50	50	1.08 ± 0.84	0.13-4.37	•
Control	36.86 12.35	Female	A N	50	50	0.90 ± 0.89	0.09-4.31	•
	(R = 18 - 74)	Total Control	A rv	100	100	0.99 ± 0.87	0.09-4.37	
		Group						

(1): % based on each total according to THg values: Study: $< 5 \,\mu g/g$ (n = 56) and = $5 \,\mu g/g$ (n = 4). Control: $< 5 \,\mu g/g$ (n = 100). (*): Significant difference with respect to Control group (t-Student for independent samples).

TABLE II "STUDY" GROUP ACCORDING TO CLASSIFICATION AND MEAN VALUES OF Hg-H $\,$

	SICDI CIN	OU ACCONDING	I O CIMODIFICATION	ONO OI ACCONDING TO CLEADING MAIN MAIN VALOES OF 118-11	ED OF 118-11	
Classification	(g/gω) H-gHT		THg-	ТНg-Н (µg/g)		MeHg-H (μ g/g)
		n	%1	$X \pm SD^*$ (R)	Range	$X \pm SD(R)$
	۸ ر	18	32.1	1.07 ± 0.75	0.21-2.97	
Retired workers	VI R	2	50	6.09 ± 1.27	5.19-7.00	3.33 ± 1.64 (2.17-4.49)
	Total	20	33.3	1.57 ± 1.72	0.21-7.00	
	۸ ر	13	23.2	2.04 ± 1.07	0.60-4.70	•
Current workers	VI ro	0				
	Total	13	23.2	2.04 ± 1.07	0.60-4.70	
	۸ ر	13	23.2	2.29 ± 1.42	0.31-4.38	•
Fishing Village	π ε	1	25	5.16	5.16	4.90
	Total	14	23.3	2.50 ± 1.56	0.31-5.16	
	A N	12	21.4	1.20 ± 0.63	0.40-2.70	•
Puerto Cabello	VI rv	1	25	5.61	5.61	3.14
	Total	13	21.6	1.54 ± 1.36	0.40-5.61	1
	۸ ۳	56	100	1.61 ± 1.11	0.21-4.70	,
Total	۷۱ ت	4	100	5.74 ± 0.86	5.16-7.00	3.67 ± 1.25 $(2.17-4.90)$
	Total	09	100	1.88 ± 1.50	0.21-7.00	1
D. D 4						

(1): % based on each frequency according to THg values: $< 5 \,\mu g/g$ (n = 56) and = $5 \,\mu g/g$ (n = 4). (*): No statistical differences between studied groups (one-way ANOVA).

group according to classification and mean values of Hg-H.

Percentile distributions with mean THg-H levels for the 4 classifications in the study group are presented in Fig 1. Fig. 2 describes box-plots showing concentrations of THg-H in the study group based on "frequency of fish intake." Each box shows the median, 25th and 75th percentiles.

Fifty-seven individuals in the study group (95%), preferred to eat fish from nearby bodies of water. Table III shows the frequency of fish intake and mean values of THg-H (μ g/g). Twenty-nine fish species were reported. From these, the sea species "Lisa" (Mugil sp.) was most frequently reported, followed by "Pargo" (Lutjanus sp.), Carite (Scomberomorus sp.) and Tuna (Thunnus sp.). Twenty-five species were sea fish and four were river fish.

Table IV shows the results obtained from the study group using the R statistical package for one-way variance analysis. Only variables that could influence the THg-H values were "fish intake" and "frequency of fish intake". When this analysis was made for the control group, it did not reveal any contributing factors to THg-H.

Twenty-three Hg-related signs and/or symptoms were investigated. In the study group, 28/60 individuals (46.7%) reported some type of sign and/or symptom (26 with THg < 5 and 2 with \geq 5 μ g/g). The signs/symptoms reported most in that group were: sleepiness (13/21.7%), hearing disturbance (10/16.7%), anxiety and sadness (7/11.7%) and tremor (6/10%). No significant relationship was noted between hair mercury levels and clinical symptoms in the current study. The symptom reported most frequently was headache (8/13.3%), followed by joint pain (6/10%) (Table V).

DISCUSSION

Since Me-Hg accounts for more than 80% of THg in hair (16), THg could be used

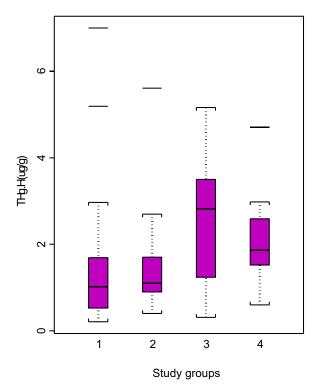


Fig. 1. Percentile distributions of mean THg-H levels (1 = retired workers; 2 = current workers; 3 = fishing villagers; 4 = Puerto Cabello individuals).

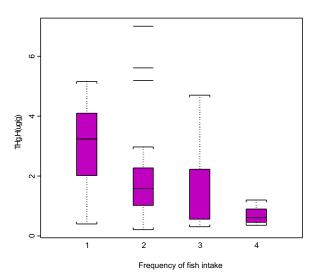


Fig. 2. Concentrations of THg-H of the Study Group based on "frequency of fish intake" (1 = daily; 2 = 2-5x/week; 3 = 1-3x/week; 4 = Do not eat fish).

TABLE III FREQUENCY OF FISH INTAKE IN THE STUDY AND CONTROL GROUPS ACCORDING TO GROUP CLASSIFICATION AND MEAN VALUES OF THg-H ($\mu g/g$)

Study Group	Frequency	n	%1	THg-H X ± SD	Range
	Daily	2	10	0.6 ± 0.3	0.4-0.8
Dectard of the	2-5 times/week	16	80	1.8 ± 1.8	0.21-7
Retired workers	1-3 times/week	2	10	0.4 ± 0.2	0.3-0.6
	Total	20	100	1.5 ± 1.7 *	0.2-7
	Daily	8	57	3.5 ± 0.9	2.2-5.16
	2-5 times/week	3	21	1.8 ± 0.9	1.2-2.8
Fishing Village	1-3 times/week	2	15	0.4 ± 0.1	0.3-0.5
_	Don't eat fish	1	7	0.3	-
	Total	14	100	2.5 ± 1.5 *	0.3-5.1
	2-5 times/week	6	46	1.8 ± 0.4	1.2-2.5
0 1	1-3 times/week	6	46	2.4 ± 1.3	0.6-4.7
Current workers	Don't eat fish	1	8	0.6	-
	Total	13	100	2 ± 1*	0.6-4.7
Puerto Cabello —	2-5 times/week	8	57	1.8 ± 1.6	0.4-5.6
	1-3 times/week	4	28.5	1.0 ± 0.4	0.4-1.4
	Don't eat fish	1	7	0.9	-
	Total	13	100	$1.5 \pm 1.3*$	0.4-5.6
	Daily	2	2	0.79 ± 0.57	0.38-1.20
	2-5 times/week	9	9	1.02 ± 0.98	0.13-3.38
Control Group	1-3 times/week	25	25	1.20 ± 1.09	0.27-4.37
-	Don't eat fish	64	64	0.91 ± 0.76	0.09-4.31
	Total	100	100	0.99 ± 0.87 *	0.09 -4.37

^{(*):} No statistical differences on THg-H between groups studied. (1): % based on total individuals in each group.

TABLE IV ANALYSES OF VARIANCE OF THE USED MODEL

Variables	Df	Sum of Sq	Mean Sq	F	Pr (F)
Sex	1	0.118	0.118	0.058	0.811
Group classification	1	5.402	5.402	2.657	0.109
Smoking habit	1	1.662	1.662	0.818	0.370
Alcohol intake	1	0.038	0.038	0.018	0.892
Fish intake	1	9.506	9.506	4.676	0.035
Frequency of fish intake	1	9.633	9.633	4.738	0.034

TABLE V
DISTRIBUTION OF THE SYMPTOMS OF THE STUDY GROUP WHOSE PRESENCE
WAS "VERY FREQUENTLY", IN THE LAST 2 YEARS

Symptoms	Retired	Fishing Village	Current Workers	Pto. Cabello	Total	%/60
Gums Inflammation	0	1	0	0	1	1.7
Writing impairment	1	0	0	0	1	1.7
Anxiety	1	0	0	0	1	1.7
Somnolence	0	0	0	1	1	1.7
Fear	0	1	0	0	1	1.7
Appetite decrease	1	1	0	0	2	3.3
Bronchitis	0	0	1	1	2	3.3
Paresthesia	0	1	0	1	2	3.3
Hearing impairment	1	0	1	0	2	3.3
Doubtfulness	1	1	0	0	2	3.3
Sweating	2	1	0	0	3	5.0
Sadness	1	2	0	0	3	5.0
Tremor	2	1	0	0	3	5.0
Nasal irritation	0	1	1	2	4	6.7
Sleepiness	1	2	1	0	4	6.7
Museular pain	2	2	0	1	5	8.3
Joints pain	2	3	0	1	6	10.0
Headache	0	5	2	1	8	13.3

to estimate exposure to Me-Hg (17). Mean levels of THg-H were below the safety limit reported by WHO (10 μ g/g) and values of MeHg-H were not significantly different from the permissible limit of 2 μ g/g (18). None of the women studied had THg-H over 10 μ g/g, the upper limit guideline for pregnant women (19). A MeHg-H level of 50 μ g/g is associated with a 5% risk of neurological damage to adults (8). Participant's levels were lower than those associated with such effects among adults (50 μ g/g hair) and among children exposed in uterus (10 μ g/g hair) (18).

The THg-H values are consistent with those obtained by Nilson et al, 2001 (20),

although the MeHg-H levels were lower in that study. Both mean values obtained were lower than the ones reported in studies from other countries such as Brazil (21, 22) and French Guiana (23). However, the statistically higher THg-H values in the study group may indicate the potential for Hg contamination in the area.

When evaluating the different fish species reported, with the exception of tuna, no one fish was found to be more susceptible to Hg uptake as reported by other researchers (7, 22). Given the variety of fish species available and reported, it is difficult to determine the typical fish diet in this region. Further epidemiological studies are recommended.

Levels of THg-H in the fishing village group were higher, although not statistically different from those in the other groups. This may be due to the higher fish consumption. As expected, THg-H concentrations decreased in the following order: Fishing village > Current workers > Retired workers > Puerto Cabello.

Table IV shows that the only variables that can influence the THg-H values in the study group were fish intake and frequency of fish intake, as the probability of rejection of the F test is 0.035 and 0.034 respectively, which implies they are significant at 4%. Other factors studied did not contribute significantly to THg-H levels.

As expected, Fig. 2 shows an increase in THg-H concentrations in the study group individuals whose frequency of fish intake was noted as "daily". The fact that mean THg-H value of the fishing village residents was the highest in the study group (2.5 \pm $1.56 \mu g/g$) and 57% of this population reported a daily frequency of fish intake (mean THg-H 3.55 \pm 0.97 μ g/g), indicates that diet factors contributed substantially to the Hg-H levels. These findings suggest that fish from the coastal areas may be contaminated with permissible Hg levels. This potential contamination should be further monitored with methods including the Hg determination in fish samples.

Since all Hg-H concentrations in this population were below $10 \,\mu\text{g/g}$ (considered the limit below which clinical signs are not apparent) (24), signs and/or symptoms reported could not be considered specific to mercury intoxication and thus we cannot derive definitive conclusions. However, a more in-depth exposure assessment is recommended to assess this exposure specifically in terms of water and fish sampling for Hg content.

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