http://dx.doi.org/10.5012/bkcs.2013.34.4.1101

Correlation Between Total Mercury and Methyl Mercury-In Whole Blood of South Korean

Seon A Jung,[†,‡] Dayoung Chung,[†,§] Jiwon On,[†,§] Myeong Hee Moon,[‡] Jeongae Lee,[†] and Heesoo Pyo[†,*]

[†]*Molecular Recognition Research center, Korea Institute of Science and Technology, Seoul 136-791, Korea*
[*]*E-mail: phs3692@kist.re.kr*
[‡]*Department of Chemistry, Yonsei University, Seoul 120-749, Korea*
[§]*Department of Chemistry, Korea University, Seoul 136-701, Korea*
Received October 23, 2012, Accepted January 14, 2013

In this study, total mercury and methyl mercury in whole blood of Korean was analyzed so as to investigate the correlation between total mercury (T-Hg) and methyl mercury (Me-Hg). 4000 whole blood samples were divided in four groups, according to T-Hg concentration in percentile: group I (p25-p50), group II (p50-p75), group III (p75-p95) and group IV (p95-p100). 100 samples were randomly selected from the each group, and Me-Hg concentration was measured. T-Hg concentration in whole blood was analyzed using a Direct Mercury Analyzer-80 and obtained limit of detection (LOD) was 0.2 μgL^{-1}. Me-Hg concentration was analyzed with ethylate derivatization using headspace-gas chromatography-mass spectrometry, and obtained LOD of methyl mercury was 0.5 μgL^{-1}. The geometric means of T-Hg and Me-Hg were 6.35 μgL^{-1} and 4.44 μgL^{-1}, respectively, and 71.91% of T-Hg was presented as Me-Hg.

Key Words : Total mercury, Methyl mercury, Headspace-Gas chromatography-Mass spectrometry, Correlation, Whole blood

Introduction

Mercury is the only metal that exists as a liquid in nature, and can be found in 3 forms on earth: element mercury, inorganic mercury and organic mercury. Element mercury and inorganic mercury are used in manufacturing industry as a thermometer, sphygmomanometer and dental amalgam.[1-3] Some of element mercury and inorganic mercury convert to methyl mercury though microbial action[4,5] when it is released into the aquatic environment.

Methyl mercury was biologically accumulated in human body, and intake of fish is considered as the main route of methyl mercury exposure.[1,6] Accumulation of methyl mercury is more deadly and has serious side effects than inorganic mercury, because longer biological half-life in body (90 day-120 day).[7-9] Biological mechanisms of cytotoxicity of methyl mercury is not completely known, typically the disulfide bond created by sulfhydryl group in protein.[10-16] And methyl mercury encroach the protein synthesis as well as thymidine compound into DNA.[11] In particular, when pregnant women ingest methyl mercury, it has a adverse effect on the growth of fetus.[14,17-20]

The percentage ratio of methyl mercury (Me-Hg) in total mercury (T-Hg) is form 40% up to 60% in fish such as tuna, marlin and sharks.[21] And higher proportion of Me-Hg in T-Hg was reported as 60%-96% in human blood, and positive tendency was observed between fish consumption and fraction of methyl mercury in total mercury.[7,12]

The aim of this study is to investigation remain value of T-Hg and Me-Hg in whole blood, and to understand the correlation between T-Hg and Me-Hg.

T-Hg concentration in whole blood samples of Korean (n = 4000) was analyzed and four groups were categorized according to the percentile of the concentration: group I (25 percentile-50 percentile), group II (50 percentile-75 percentile), group III (75 percentile-95 percentile), group IV (95 percentile-100 percentile). 100 samples were randomly selected from each group and Me-Hg concentration was measured from total number of 400 samples. In case of less than 25 percentile T-Hg concentration range of samples, that are likely to be Me-Hg not detected because the concentration is too low. And most of the low concentration samples are obtained from infants, so that the sample amount is insufficiency for analysis of Me-Hg. Therefore, the range of more than 25 percentile was grouped into the target for Me-Hg analysis.

Blood total mercury analysis was done using the Direct Mercury Analyzer-80 (DMA-80, Milestone, Italy) with gold amalgamation process according to US EPA Method 7473.[22] Generally, Me-Hg analysis has been done using gas chromatography-electron capture detector (GC-ECD),[8] gas chromatography-atomic fluorescence spectrometry (GC-AFS),[23-25] inductively coupled plasma-mass spectrometry (ICP-MS),[26,27] liquid chromatography-cold vapor-atomic absorption spectrometry (LC-CV-AAS)[28] and microwave induced plasma-atomic emission detector (MIP-AED).[29,30] However, in this study, Me-Hg analysis was conducted using derivatization with sodium tetra ethyl borate ($NaBEt_4$) and utilizing headspace-gas chromatography-mass selective detector (HS-GC-MS) for accurate and reproducible analysis.[31]

From results obtained by performing using the above analytical methods, correlation between T-Hg and Me-Hg

was investigated and concentration of T-Hg and Me-Hg were compared in relation to concentration (group I, group II, group III, group IV), age, gender and residential area.

Experimental

Chemicals and Reagents. Mercury standard (1000 mgL^{-1}) was purchased from Wako chemical (Osaka, Japan) and is dissolved in 5% (v/v) nitric acid to make 100 mgL^{-1} mercury stock solution. The nitric acid was obtained from Dong Woo Fine-Chem (Iksan, Korea). Me-Hg standard stock solution (1000 mgL^{-1}) was prepared by dissolving 10 mg Me-Hg chloride that was purchased from Sigma Aldrich (St. Louis, MO, U.S.A.) in 10 mL methanol. Ethyl mercury stock solution (1000 mgL^{-1}) was prepared as an internal standard by dissolving ethyl mercury chloride (10 mg) that was purchased from Wako chemical (Osaka, Japan) in 10 mL methanol. A derivative reagent, 2% (w/v) sodium tetra ethyl borate (St. Louis, MO, U.S.A.), was dissolved in distilled water and stored in dark at –4 °C. Potassium bromide (KBr) and sulfuric acid (H_2SO_4) were purchased from Kanto chemical co Inc (Tokyo, Japan) and J.T. Baker (Phillipsburg, NJ, U.S.A.), respectively, and 2 M KBr /1 M H_2SO_4 solution was prepared in distilled water. Sodium acetate and acetic acid were obtained from Sigma Aldrich (St. Louis, MO, U.S.A.) and used to prepare acetate buffer solution (pH 5.2) was prepared by mixing 0.2 M sodium acetate and 0.2 M acetic acid. A glass headspace vial (10 mL) that purchased from the Interface Engineering (Seoul, Korea) was used for the analysis. All other chemicals were of pesticide residue analysis grade.

Instrumentation. The concentration of total mercury (T-Hg) in whole blood was determined by utilizing gold-amalgam method using Direct Mercury Analyzer-80 (DMA-80, Milestone, Italy). DMA-80 is a analyzer for the analysis of T-Hg from solid or aqueous samples without chemical pre-treatment. Heat treatment chemically decomposes the samples and then the following products are carried by oxygen flow to an amalgam. Total mercury is selectively trapped at gold amalgam, afterwards, quantitative information can be measured by UV absorbance of trapped mercury.

The concentration of methyl mercury (Me-Hg) in the sample was measured by utilizing headspace-gas chromatography-mass spectrometry (HS-GC-MS). The analyzing system was constructed with the autosampler (Combi-PAL, CTC Analytics, AG, Zwingen, Switzerland) along with 6890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) which was interfaced with 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer was equipped with electron impact ionization source and quadruple mass analyzer. The operating parameters of this system are listed in Table 1.

The mass spectrometer was operated in selected ion monitoring (SIM) mode. Total ion chromatograms were acquired and conducted using G1701DA D.01.02 standalone data analysis software (Agilent Technologies) that was also used to control the whole system.

Table 1. GC-MS parameters for methyl mercury chloride analysis

GC condition			
· Column	Ultra-2: 5% phenyl-methylpolysiloxane (25 m × 0.20 mm I.D. × 0.33 μm film thickness)		
· Injector temp.	250 °C		
· Column flow rate	1.0 mL/min, He with constant flow		
· Injection mode	Split ratio (1:10), Headspace, 2 mL		
· Oven temp. program	50 °C to 300 °C (20 °C/min, 1 min hold)		
· Running time	14.50 min		
MS condition			
· Detector temp.	280 °C	· Quadruple temp.	150 °C
· Ion source temp.	230 °C	· Ionization mode	EI
· Solvent delay	2.00 min		
· SIM measured ion (*m/z*)	242, 244, 246, 248, 256, 258, 260, 262		

Sample Collection and Classification. The aim of this study was to investigate total mercury (T-Hg) and methyl mercury (Me-Hg) concentration and the correlation between T-Hg and Me-Hg according to the concentration group, age, gender and residential area.

T-Hg concentration in whole blood samples of Korean (n = 4000) was analyzed and four groups were categorized according to the percentile of the concentration: group I (25 percentile-50 percentile), group II (50 percentile-75 percentile), group III (75 percentile-95 percentile), group IV (95 percentile-100 percentile). Each of the four groups, we randomly selected 100 samples and analyzed Me-Hg concentration. The collected 400 samples information was represented in Table 2.

Korea Food and Drug Administration (KFDA) collected blood sample between 2010 and 2011 in Korea. Collected whole blood sample was bottled in anticoagulant treated EDTA tube and stored at –20 °C. The study protocol was approved by the ethical committee of KRDA, Cheongwon-gun, Chungcheongbuk-do, Republic of Korea, and informed consent was obtained from each subject.

Determination of Total Mercury in Whole Blood. 100 μg of blood sample put on the nickel boat, and the boat were injected into DMA-80 (Milestone, Italy) for analysis of T-Hg. The calibration curve was evaluated in the range of 0.05 μgL^{-1}-50.0 μgL^{-1} by using standard solution, which dissolved in 5% (v/v) nitric acid. Quartz boat was used for calibration experiment to prevent chemical alteration of the boat by using nitric acid.

Determination of Methyl Mercury in Whole Blood. Blood samples of experimental methods were followed.[31] 1 mL of blood sample was injected into the 15 mL conical centrifuge tube, and ethyl mercury (100 mgL^{-1}, 50 μL) was spiked. 1 mL of 2 M KBr/1 M H_2SO_4 was added to separate organic mercury from protein. After the denaturation procedure, liquid-liquid-extraction was conducted with 5 mL methyl *tert*-butyl ether (MTBE) for 30 min. followed by centrifugation for 5 min. at 2800 rpm so as to separate of organic and aqueous layer. Upper layer of organic solvent

Table 2. Classification of collected sample for methyl mercury analysis

	Concentration Group				Total (N)
	Group I	Group II	Group III	Group IV	
Range	p 25-p 50	p 50-p 75	p 75-p 95	p 95-p 100	
T-Hg conc.[a]	1.51-4.04	4.05-6.26	6.28-9.66	9.67-41.95	
N	100	100	100	100	400
Age					
19	-	3	12	31	46
20-29	3	7	11	15	36
30-39	17	27	21	23	88
40-49	23	16	23	14	76
50-59	31	31	17	9	88
60 ≥	26	16	16	8	66
Gender					
Male	68	45	42	34	189
Female	32	55	58	66	211
Region					
Coast	69	62	45	33	209
Inland	31	38	55	67	191

[a]Total mercury concentration range (μgL^{-1})

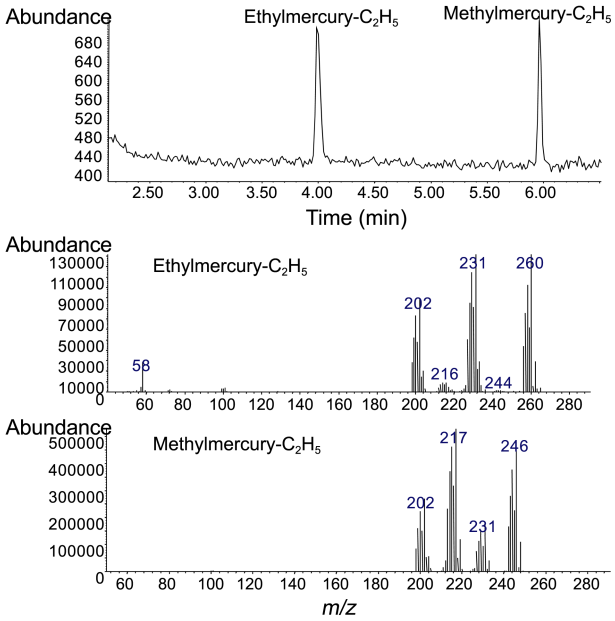

Figure 1. Total ion chromatogram and mass spectra of diethyl mercury (ISTD) and ethyl methyl mercury spiked in blank water (5 μgL^{-1}).

was transferred into a glass test tube and 1 mL acetate buffer (pH 5.2) was added. After vortex for 1 min. and evaporate the organic solvent by using a nitrogen evaporator at room temperature. Remained 1 mL of acetate buffer layer was transferred to 10 mL headspace vial and then 50 μL of 2% sodium tetra ethyl borate ($NaBEt_4$) solution was added. Derivatization and extraction process was conducted automatically agitating as 500 rpm for 30 min. at 80 °C with headspace auto sampler system (Combi-PAL, CTC Analytics, AG, Zwingen, Switzerland) followed by taken 2 mL supernatant gas was injected into the GC-MS.

To create the calibration curve, addition of standard (methyl mercury chloride) and internal standard (ethyl mercury chloride) in 1 mL distilled water: methyl mercury chloride (0.5 μgL^{-1}-50 μgL^{-1}), constant concentration of ethyl mercury chloride (5 μgL^{-1}). And experiments were conducted for calibration in the same way as above.

Statistical Analysis. Statistical analysis was conducted with SPSS ver 18.0 for Windows (SPSS Inc, Korea). The *t*-test was performed to determine the significant difference of total mercury and methyl mercury concentration in whole blood between characteristic groups, and also tested on the methyl mercury portion in total mercury between each groups. Correlation coefficient (r) was obtained between total mercury and methyl mercury concentration in whole blood.

Results and Discussion

Method Validation. Determination of T-Hg in whole blood samples is conducted by using two calibration curves, which were drawn in full concentration range (0, 0.5, 1, 2, 5, 10, 50, 50 μgL^{-1}) and low concentration range (0.5, 1, 2, 5 μgL^{-1}). Full range calibration curve was applied to analyzed concentration greater than 5 μgL^{-1} and low range calibration curve was used to the concentration less than 5 μgL^{-1}. Linearity (r^2) of all calibration curve was 0.999 and obtained limit of detection (LOD) was 0.2 μgL^{-1}, that calculated by equation: LOD = 3.14*σ (σ = the standard deviation of control blood sample analysis, N = 7).

Me-Hg had to be convert into non polar compound before injection into GC-MS, thus sodium tetra ethyl borate was used as derivatization reagent. After the derivatization, ethylated Me-Hg was detected by HS-GC-MS and its resulting molecular ion (*m/z*) was confirmed (*m/z* 242, 244, 246, 248) through the mass spectrum. Similarly, ethyl mercury used to internal standard was confirmed as diethyl mercury compound (*m/z* 256, 258, 260, 262). The mass spectra of the compounds (ethyl methyl mercury, diethyl mercury) and total ion chromatogram are illustrated in Figure 1.

Quantification of Me-Hg is based on a calibration procedure using internal standard (ethyl mercury). And divide the calibration curve by concentration, which were the low concentration range (0.5 μgL^{-1}-5 μgL^{-1}) and high concentration range (5 μgL^{-1}-50 μgL^{-1}), and applied depending on the concentration of the sample. As a result, 0.99 or more linearity (r^2) was identified, and the limit of detection was 0.5 μgL^{-1} (S/N > 3).

Me-Hg concentration was converted to concentration as Hg by calculated using the ratio of molecular weight of mercury and methyl mercury chloride, for comparative analysis between T-Hg and Me-Hg. Reduced concentration of Me-Hg was marked as Hg in tables and figures.

In order to verify the validity of the analytical method, conducted analysis of standard reference material (SRM) 955c Caprine blood level 2 for T-Hg and that of level 3 for

Table 3. SRM results of total mercury and methyl mercury

	N	SRM 955c Caprine Blood	Reference values (μgL^{-1})	Measured values (μgL^{-1})	Accuracy (%)	Precision (%)
T-Hg[a]	15	Level 2	4.95 ± 0.76	5.17 ± 0.35	104.44	6.79
Me-Hg[b]	3	Level 3	4.50 ± 1.00	4.49 ± 0.69	99.79	15.42

[a]total mercury. [b]methyl mercury.

Me-Hg. The SRM were obtained from NIST (New York, U.S.A.) and results confirmed the accuracy (99.79% at T-Hg, 104.44% at Me-Hg) and precision (6.79% at T-Hg, 15.42% at Me-Hg), presented in Table 3.

Analysis of Total Mercury and Methyl Mercury in Whole Blood. The whole blood samples were collected over two years from the 4000 people, who live in the Korea. Collected whole blood samples were analyzed total mercury (T-Hg) concentration. The geometric mean (GM) concentration of total mercury (T-Hg) of the adults (3.90 μgL^{-1}) was higher than children (2.06 μgL^{-1}), that of male (3.11 μgL^{-1}) was higher than female (2.76 μgL^{-1}). Based on the above results, whole blood samples of 4000 people divided into four groups according to the T-Hg concentration. 100 whole blood samples were selected randomly from each group and the selected total of 400 samples were analyzed for Me-Hg, analysis results are shown in Table 4.

T-Hg and Me-Hg GM concentration in randomly selected in four concentration group were 6.35 μgL^{-1} and 4.44 μgL^{-1}, respectively. Me-Hg was detected in all of samples (N = 400), ranged 1.34 μgL^{-1}-26.32 μgL^{-1}. Study performed in Sweden,[32] the AM (SD) methyl mercury concentration was 1.32 (1.10) μgL^{-1} in 30 women, with a range of 0.15 μgL^{-1}-4.48 μgL^{-1}. From Canada,[7] T-Hg and Me-Hg AM (SD) concentration were 1.10 (0.85) μgL^{-1} and 0.70 (0.70) μgL^{-1} among the 288 persons, respectively.

Research 400 people divided according to age: under 19 years, twenties, thirties, forties, fifties, and older than 60 years. Increasing with age showed a positive tendency to increased T-Hg and Me-Hg concentration. The study from Denmark[33] showed that Me-Hg concentration is difference between 7 years and 14 years of whole blood sample. Me-Hg concentration of 14 years (3.81 μgL^{-1}) is higher than 7 years (1.93 μgL^{-1}). But, the study performed in Canada[7] represented that the there is no significant effects for age ($p = 0.27$).

Compared GM concentration of T-Hg and Me-Hg between male and female, female values showed higher than male. The *t*-test was performed, and the results exhibited significant difference of T-Hg and Me-Hg concentration in gender ($p < 0.05$). According to study conducted in Canada,[7] female subject's blood T-Hg and Me-Hg are lower than male subject's. And there is significant effect for gender ($p < 0.05$).

In addition, T-Hg and Me-Hg concentration of coastal area residents were higher than that of inland areas residents with

Table 4. Total mercury and methyl mercury concentration in whole blood of South Korean

	N	T-Hg[a] conc. (μgL^{-1})						Me-Hg[b] conc. (as Hg, μgL^{-1})					
		AM[c]	GM[d]	MIN[e]	MAX[f]	Percentiles		AM[c]	GM[d]	MIN[e]	MAX[f]	Percentiles	
						75th	95th					75th	95th
Total	400	7.72	6.35	1.51	41.95	9.67	18.93	5.50	4.44	1.37	26.32	6.88	13.98
Conc. group													
I[g]	100	3.15	3.12	1.51	4.04	3.49	3.97	2.36	2.29	1.37	4.03	2.79	3.39
II[h]	100	4.67	4.63	4.05	6.26	4.88	6.00	3.37	3.24	1.90	5.93	4.09	5.24
III[i]	100	7.83	7.79	6.28	9.66	8.36	9.53	5.36	5.16	2.08	9.45	6.53	7.87
IV[j]	100	15.23	14.43	9.67	41.95	17.23	26.79	10.92	10.17	3.85	26.32	13.06	19.67
Age													
≤ 19	46	3.72	3.56	1.51	7.41	4.30	6.99	2.55	2.41	1.37	5.56	3.09	4.72
20-29	36	5.04	4.52	1.99	12.57	7.07	11.64	3.50	3.12	1.48	11.60	3.87	10.29
30-39	88	7.28	6.12	1.98	27.55	8.43	20.40	4.99	4.20	1.64	20.95	5.85	12.53
40-49	76	8.76	6.99	2.87	41.95	11.34	24.91	5.91	4.75	1.64	20.19	7.31	15.47
50-59	88	9.22	7.96	2.40	29.53	12.13	19.34	6.75	5.69	1.52	26.32	8.54	14.35
≥ 60	66	9.35	7.93	2.86	25.97	12.74	22.79	7.19	5.93	1.91	22.93	10.00	17.79
***Gender**													
Males	189	9.39	7.59	2.45	41.95	12.26	23.78	6.48	5.13	1.56	26.32	8.36	16.60
Females	211	6.23	5.40	1.51	20.31	7.86	13.68	4.63	3.91	1.37	18.03	5.61	11.56
****Region**													
Coast	209	9.02	7.53	1.51	32.17	11.30	23.53	6.60	5.40	1.37	26.32	8.49	16.51
Inland	191	6.30	5.26	1.98	41.95	7.89	15.43	4.30	3.59	1.48	20.95	5.24	10.53

[a]total mercury. [b]methyl mercury. [c]arithmetical mean. [d]geometric mean. [e]minimum. [f]maximum. [g]p 25-p 50. [h]p 50-p 75. [i]p 75-p 95. [j]p 95-p 100. *: $p < 0.05$, total mercury and methyl mercury concentration by gender. **: $p < 0.05$, total mercury and methyl mercury concentration by region.

Table 5. The fraction of methyl mercury concentration in total mercury concentration

	N	Me-Hg[a] conc. (μg/L)/T-Hg[b] conc. (μg/L), %							
		AM[c]	GM[d]	MIN[e]	MAX[f]	Percentiles			
						25th	50th	75th	95th
Total	400	71.91 ± 16.27	70.02	30.68	100.0	59.20	71.50	83.78	100.0
Conc. group									
I[g]	100	75.33 ± 16.57	73.50	45.31	100.0	61.13	73.14	89.86	100.0
II[h]	100	71.85 ± 16.28	70.05	44.01	100.0	60.76	68.42	82.07	100.0
III[i]	100	68.22 ± 16.08	66.23	30.68	100.0	54.54	68.83	80.18	98.28
IV[j]	100	72.23 ± 15.61	70.50	39.36	100.0	58.98	71.72	84.78	98.74
Age									
≤ 19	46	69.57 ± 16.32	67.71	41.57	100.0	57.70	69.56	77.64	100.0
20-29	36	70.71 ± 16.18	68.98	49.53	100.0	56.74	68.68	80.86	100.0
30-39	88	70.73 ± 16.79	68.66	30.68	100.0	58.47	71.24	82.73	100.0
40-49	76	69.80 ± 16.14	67.98	37.11	100.0	58.09	66.63	84.34	100.0
50-59	88	73.45 ± 17.02	71.44	42.08	100.0	59.26	73.33	86.61	100.0
≥ 60	66	76.12 ± 14.21	74.73	44.01	100.0	68.01	74.75	86.65	99.82
***Gender**									
Males	189	68.85 ± 13.67	67.51	41.57	100.0	58.36	68.19	77.55	94.88
Females	211	74.64 ± 17.89	72.35	30.68	100.0	61.54	73.50	89.32	100.0
Region									
Coast	209	73.42 ± 15.89	71.64	41.04	100.0	60.63	73.26	84.34	100.0
Inland	191	70.25 ± 16.57	68.29	30.68	100.0	58.25	68.69	83.00	100.0

[a]total mercury. [b]methyl mercury. [c]arithmetical mean. [d]geometric mean. [e]minimum. [f]maximum. [g]p 25-p 50. [h]p 50-p 75. [i]p 75-p 95. [j]p 95-p 100. [*]: $p < 0.05$, the fraction of methyl mercury in total mercury by gender.

statistical significance ($p < 0.05$). A study from Japan,[36] compared the T-Hg and Me-Hg concentration in blood according to the local area: a city, a fishing villages and two isolated fishing village in a gold mining area. T-Hg concentration was (12.2 ± 8.2) μgL^{-1} in the city, (90.4 ± 71.5) μgL^{-1} in the fishing villages and (130.7 ± 78.5) μgL^{-1} and (149.9 ± 49.5) μgL^{-1} in gold mining area. And Me-Hg concentration was (9.0 ± 6.7) μgL^{-1}, (90.0 ± 76.6) μgL^{-1}, (149.2 ± 52.5) μgL^{-1} and (131.9 ± 84.2) μgL^{-1}, respectively.

Though the above results, the T-Hg concentration in whole blood was consistent with the general tendency of the monitoring results, and Me-Hg concentration also representing the same tendency was confirmed.

The Fraction of Methyl Mercury Concentration in Total Mercury Concentration. The present study was performed to confirm the fraction of methyl mercury (Me-Hg) concentration in total mercury (T-Hg) concentration and shown in Table 5. The arithmetic mean (AM) fraction of T-Hg present as Me-Hg was 71.91%, range 30.68%-100.00%. As shown above, most of the T-Hg in the body that exists in the form of Me-Hg.

Many studies have investigated the proportion of Me-Hg in T-Hg. Study performed in Sweden observed that percentages (Mean ± SD) of T-Hg presented as Me-Hg was (60 ± 27)% from 37 whole blood samples, ranged 6%-100%.[32] From Canada, T-Hg and Me-Hg in whole blood and cord blood of Inuit mother (n = 37) were analyzed, 80% Me-Hg fraction in whole blood and 98% Me-Hg portion in cord blood.[34] Similarly the data from Denmark indicate that the fraction of Me-Hg in T-Hg was 83%.[35] And in the north America, 1127 men of blood sample collected and analyzed, the results of organic mercury proportion was overall 75%.[36]

The AM percentage proportion of Me-Hg in T-Hg of each group did not show significant difference between them: group I (75.33%), group II (71.85%), group III (68.22%), group IV (72.23%). However, in the study done in Southwest Quebec showed increase in curvilinearity as the proportion organic mercury percentage in total mercury increase.[7]

The results from the above were divided in relation to gender. As a result, male of the T-Hg and Me-Hg concentration were higher than female, but the portion of Me-Hg concentration in T-Hg concentration is higher in female (74.64% > 68.85%, arithmetic mean). The difference (5.79%, arithmetic mean) is within the standard deviation, but the *t*-test performed using SPSS that there is significant difference between male and female ($p < 0.05$). Me-Hg fraction of female was higher than male, and the differences were more pronounced than by region. Female appeared larger ratio of the Me-Hg than male represented in Figure 2. So that toxicity from mercury is more affects in female by metabolism of mercury in human body.

The results of T-Hg and Me-Hg concentration were divided by age and compared. Fraction of Me-Hg in T-Hg of 60s (76.12%, arithmetic mean) is higher than 10s (69.57%, arithmetic mean), but according to the results of *t*-test that there is no significant difference ($p = 0.41$).

The T-Hg and Me-Hg concentration results of people who live in coastal area and inland area were divided and the

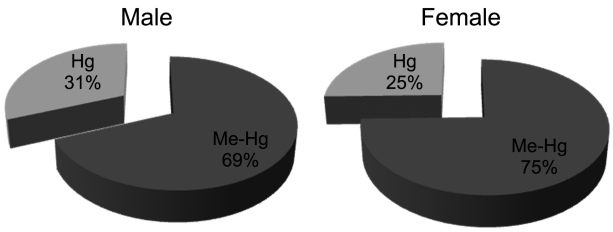

Figure 2. Gender effects on total mercury and methyl mercury concentration fraction in whole blood.

fraction of Me-Hg in T-Hg was compared. There is no significant difference in *t*-test results although the residents of the coastal area of Me-Hg content in T-Hg is higher than of the inland area (73.42% > 70.25%, arithmetic mean). That results were indicates that intake of fish and Me-Hg concentration were related and the same result was shown in many researches. A study from Japan,[37] compared the T-Hg and Me-Hg concentration in blood according to the local area: a city, a fishing villages and two isolated fishing village in a gold mining area. From the analysis results of Japan, approximately the Me-Hg fraction of T-Hg in residents of fishing village was shown the highest values (98%). According to data from Canada,[7] increase in consumption of fish as increase in percentage of organic mercury: among nonconsumers of local fish (51.0 ± 28.5 %, n = 175) < total population (56.6 ± 27.2%, n = 288) < among persons who consumed local fish in one season (61.4 ± 21.0%, n = 47) < among persons who consumed local fish in two season (68.2 ± 23.5%, n = 66).

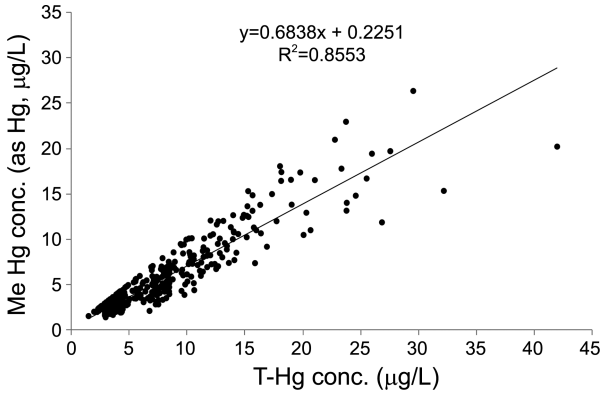

Figure 3. Correlation between total mercury and methyl mercury concentration.

Correlation between Total Mercury and Methyl Mercury Concentrations. The correlation between total mercury (T-Hg) and methyl mercury (Me-Hg) in 400 people was represented at Figure 3. As shown in the figure, the T-Hg and Me-Hg have a high correlation ($r = 0.92$, $p < 0.05$).

When correlation between T-Hg and Me-Hg concentrations of each group was studied, group IV showed the strongest correlation while the other groups showed low correlation between the concentration: group I (r = 0.53), group II (r = 0.59), group III (r = 0.52), group IV (r = 0.80).

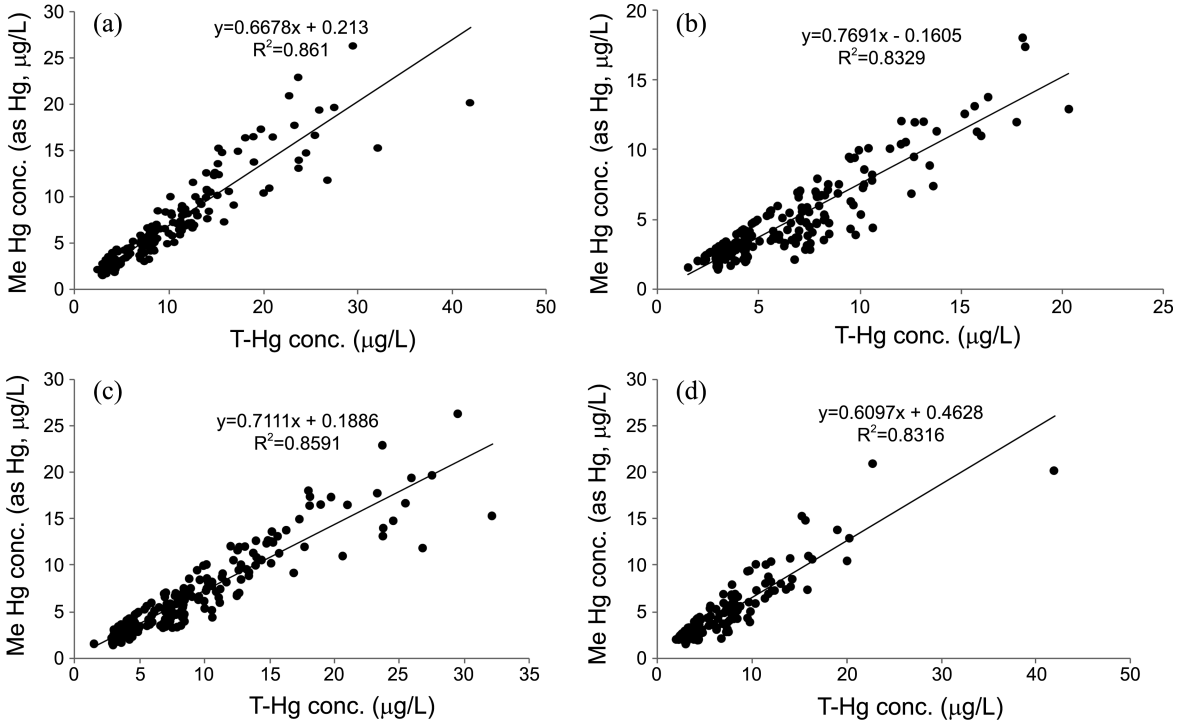

Figure 4. Correlation between total mercury and methyl mercury in gender and region. (a) Correlation between total mercury (T-Hg) and methyl mercury (Me-Hg) concentration in male blood samples. (b) Correlation between total mercury (T-Hg) and methyl mercury (Me-Hg) concentration in female blood samples. (c) Correlation between total mercury (T-Hg) and methyl mercury (Me-Hg) concentration in coastal resident blood samples. (d) Correlation between total mercury (T-Hg) and methyl mercury (Me-Hg) concentration in inland resident blood samples.

Concentration range of group IV is wide and varied, that bring about high correlation coefficient.

Compared the correlation between T-Hg and Me-Hg, in male and female, and in people who live in the coastal area and who live in inland area, shown in Figure 4. The correlation showed a high correlate of 0.9 of greater and no difference was observed.

Conclusion

In this study to investigate the correlation between T-Hg and Me-Hg, analysis of T-Hg conducted in whole blood sample from 4000 Korean. Based on the concentration of T-Hg, divided the four concentration group as shown in Table 2. 100 samples randomly selected from the each group. T-Hg was analyzed by utilizing Direct Mercury Analyzer and the limit of detection was 0.2 μgL^{-1}. Me-Hg analysis progressed with ethylation by using sodium tetra ethyl borate ($NaBEt_4$) and Headspace-GC-MS. The limit of detection of Me-Hg was 0.5 μgL^{-1}. In order to verify the validity of the analytical method, SRM Carprin blood level 2 and 3 (NIST, New York, U.S.A.) was analyzed. The analysis results from 400 sample showed a positive trend, with increasing age, an increase in the concentration of T-Hg and Me-Hg. T-Hg and Me-Hg concentration in male was higher than female. In addition, samples of resident in coastal area were observed high concentration of T-Hg and Me-Hg. Comparative analysis of samples in T-Hg and Me-Hg concentration, Me-Hg proportion of T-Hg was 71.91% (arithmetic mean), and showed a high correlation ($r = 0.9248$). The fraction of Me-Hg in T-Hg is generally similar, but female were significantly higher than male ($p < 0.05$). That represents the toxicity effect of Me-Hg is stronger in female than male.

Acknowledgments. This research was supported by a grant (10162KFDA994) from Korea Food & Drug Administration in 2011, Eco Innovation Technology Development Program of Ministry of Environment and a grant from the Intramural Research Program at the Korea Institute of Science and Technology.

References

1. Susan, E. S. *et al. The Journal of the American Medical Association* **2003**, *289*, 1667.
2. US Environmental Protection Agency, *Mercury Study Report to Congress, 1*; Executive Summary, Washington, DC; Environmental Protection Agency; 1997; Publication EPA -452/R-97-003.
3. National Academy of Sciences, *Toxicological Effects of Methyl Mercury*, Washington, DC, National Research Council, 2000.
4. Mason, R. P.; Lawson, N. N.; Lawrence, A. L.; Leaner, J. J.; Lee, J. G.; Sheu, G.-R. *Marine Chemistry* **1999**, *65*, 77.
5. Park, J.-S.; Lee, J.-S.; Kim, G.-B.; Cha, J.-S.; Shin, S. K.; Kang, H.-G.; Hong, E.-J.; Chung, G.-T.; Kim, Y.-H. *Water Air Soil Pollut* **2010**, *207*, 391.
6. Toffleson, L.; Cordle, F. *Environ Health Perspect* **1986**, *86*, 203.
7. Kathryn, R.; Mahaffey, D. M. *Environment Research*, Section A **1998**, *77*, 104.
8. Takeuki, A.; Takeuki, N.; Gen, J. *Physiol.* **1962**, *45*, 1181.
9. Bakir, F.; Damluji, S. F.; Amin-Zaki, L.; Murtadha, M.; Kahalidi, A.; Al-Rawi, N. Y.; Tikriti, S.; Dhahir, H. I.; Clarkson, T. W.; Smith, J. C.; Doherty, R. A. *Science* **1973**, *181*, 230.
10. Silbergeld, E. K.; Devine, P. J. *Fuel Process Technol.* **2000**, *65-66*, 35.
11. Sanchez Uria, J. E.; Sanz-Model, A. *Talanta* **1998**, 509.
12. Ipolyi, Massanisso, P.; Sposato, S.; Fodor, P.; Morabito, T. *Analytical Chimica Acta* **2004**, *505*, 145.
13. Jurng, J. S.; Shim, S. G. *Journal of Korean Society for Atmospheric Environment* **2009**, *25*(2), 99.
14. Björnberg, K. A.; Vahter, M.; Berglund, B.; Niklasson, B.; Bennow, M.; Sandborgh-Englund, G. *Environmental Health Perspectives* **2005**, *113*(10), 1381.
15. Hughes, W. L. A. *Annals of the New York Academy of Sciences* **1957**, *65*, 454.
16. Lee, K. E.; Hong, Y. S.; Kim, D. S.; Han, M. S.; Yu, B. C.; Kim, Y. W.; Rho, M. S.; Lee, H. J.; Lee, J. W.; Kwak, J. Y.; Kim, J. Y. *Korean Journal of Occupational and Environmental Medicine* **2007**, *19*, 268.
17. Lawery, R.; Buchlet, J. P.; Roels, H.; Hubermont, G. *Environmental Research* **1978**, *5*, 278.
18. Gray, D. G. *Toxicology and Applied Pharmacology* **1995**, *132*, 91.
19. Tsuchiya, H.; Mitani, K.; Kodama, K.; Nakata, T. *Archives of Occupational and Environmental Health* **1984**, *39*, 11.
20. Steuerwald, U.; Weibe, P.; Jorgensen, P. J.; Bjerve, K.; Brock, J.; Heinzow, B.; Budtz-Jorgenen, E.; Grandjean, P. *Journal of Pediatric* **2000**, *136*, 599.
21. Kim, H.-Y. *et al*. *Korean J. Food. Scl. Technol.* **2005**, *37*(6), 882.
22. US EPA Method 7473 (CAS No. 7439-97-6), 2005, Mercury solids and solutions by thermal decomposition, gold amalgamation, and atomic absorption spectrophotometry for mercury analysis, Obtained 20 January 2005 www.epa.gov/epaoswer/hazwaste/mercury/pdf/7473.pdf.
23. Leemakers, M.; Nguyen, H. L.; Kurunczi, S.; Vanneste, B.; Galletti, S.; Baeyens, W. *Anal. Bioanal. Chem*. **2003**, *377*, 327.
24. Abranko, L.; Jokai, Z.; Fodor, P. *Anal. Bioanal. Chem*. **2005**, *383*, 448.
25. Logar, M.; Horvat, M.; Akagi, H.; Pihlar, B. *Anal. Bioanal. Chem*. **2002**, *374*, 1015.
26. De Smaele, T.; Moens, L.; Sandra, P.; Dams, R. *Microchim. Acta* **1999**, *130*, 241.
27. Bouyssiere, B.; Bako, F.; Savary, L.; Lobinski, R. *J. Chromatogr. A* **2002**, *976*, 431.
28. Quevauviller, P.; Filippelli, M.; Hovat, M. *Trends Anal. Chem*. **2000**, *19*, 157.
29. Tu, Q.; Qian, J.; French, W. *J. Anal. At. Sepectr.* **2000**, *15*, 1583.
30. Mothes, S.; Wennrich, R. *J. High Resolut. Chromatogr*. **1999**, *22*, 181.
31. Hippler, J.; Hoppe, H. W.; Mosel, F.; Rettenmeier, A. W.; Hirner, A. V. *J. Chromatogr. B* **2009**, *877*, 2465.
32. Douglas C. Baxterm, Ilia Rodushkin, Emma Engstrm, Dennis Klockare, Hans Waara, *Clinical Chemistry* **2007**, 111.
33. Budtz-Jørgensen, E.; Grandjean, P.; Jørgensen, P. J.; Weihe, P.; Keiding, N. *Environmental Research* **2004**, 385.
34. Hansen, J. C.; Tarp, U.; Bohm, J. *Arch. Environ. Health*. **1990**, *45*, 355.
35. Grandjean, P.; Weihe, P.; Jorgensen, P. J. *Arch. Environ. Health*. **1992**, *47*, 185.
36. Kingman, Albertini, T.; Brown, L. J. *JDR* **1998**, *77*(3), 461.
37. Akagi, H.; Malm, O.; Branches, F. J. P.; Kinjo, Y.; Kashima, Y.; Guimaraes, J. R. D.; Oliveria, R. B.; Karaguchi, K.; Pfeffer, C.; Takizawa, Y.; Kato, H.; *Water Air Soil Pollut.* **1995**, *80*, 85.