

# Determination of Methylmercury, Ethylmercury, and Inorganic Mercury in Mouse Tissues, Following Administration of Thimerosal, by Species-Specific Isotope Dilution GC–Inductively Coupled Plasma-MS

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**Isotopically enriched HgO standards were used to synthesize CH<sub>3</sub><sup>200</sup>Hg<sup>+</sup> and C<sub>2</sub>H<sub>5</sub><sup>199</sup>Hg<sup>+</sup> using Grignard reagents. These species were employed for isotope dilution GC–ICPMS to study uptake and biotransformation of ethylmercury in mice treated with thimerosal, (sodium ethylmercurithiosalicylate) 10 mg L<sup>-1</sup> in drinking water ad libitum for 1, 2.5, 6, or 14 days. Prior to analysis, samples were spiked with aqueous solutions of CH<sub>3</sub><sup>200</sup>Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub><sup>199</sup>Hg<sup>+</sup>, and <sup>201</sup>Hg<sup>2+</sup> and then digested in 20% tetramethylammonium hydroxide and extracted at pH 9 with DDTC/toluene. Extracted mercury species were reacted with butylmagnesium chloride to form butylated derivatives. Absolute detection limits for CH<sub>3</sub>Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>, and Hg<sup>2+</sup> were 0.4, 0.2, and 0.6 pg on the basis of 3σ of five separate blanks. Up to 9% of the C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> was decomposed to Hg<sup>2+</sup> during sample preparation, and it is therefore crucial to use a species-specific internal standard when determining ethylmercury. No demethylation, methylation, or ethylation during sample preparation was detected. The ethylmercury component of thimerosal was rapidly taken up in the organs of the mice (kidney, liver, and mesenteric lymph nodes), and concentrations of C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> as well as Hg<sup>2+</sup> increased over the 14 days of thimerosal treatment. This shows that C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> in mice to a large degree is degraded to Hg<sup>2+</sup>. Increased concentrations of CH<sub>3</sub>Hg<sup>+</sup> were also observed, which was found to be due to impurities in the thimerosal.**

The difference in toxicity of various mercury species<sup>1</sup> makes it important to determine the atomic and molecular forms of

mercury in tissues after acute and chronic exposure. Since organic mercury compounds might be transformed to Hg<sup>2+</sup> in the tissues, it is of interest to study the temporal aspects of transport and transformation of various mercury species.

Organic mercury compounds, primarily CH<sub>3</sub>Hg<sup>+</sup> and C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>, were introduced as agricultural fungicides at the beginning of the 20th century, but after a series of accidental mercury poisonings with fatal outcome<sup>2</sup> and evidence of environmental hazards,<sup>3</sup> alkylmercury compounds were discontinued for agricultural use. Paradoxically, until recently, C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> in the form of thimerosal (sodium ethylmercurithiosalicylate) was added (0.003–0.01%) to several medical preparations for antimicrobial purposes.<sup>4</sup> Then in 1999, the U.S. Public Health Service (USPHS) and the American Academy of Pediatrics (AAP) issued a joint statement<sup>5</sup> in which they identified thimerosal as a widespread source of organic mercury exposure in infants/small children and recommended that it should be reduced or eliminated from childhood vaccines. It has been estimated that an infant might be exposed to ~200 μg of Hg (as C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>) during the first 6 months of life through vaccinations.<sup>4</sup> The effect of childhood C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> exposure has not been systematically studied, but the qualitative effect is thought to be similar to that of methylmercury,<sup>4</sup> which in sufficient doses causes widespread damage to the developing central nervous system.<sup>6</sup> Recently, the Immunization Safety Review Committee of the U.S. Institute of Medicine stated that the hypothesis<sup>7,8</sup> that exposure to thimerosal-containing vaccines is associated with

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neurodevelopmental disorders has not been established but is biologically plausible.<sup>9</sup> The “thimerosal controversy” has initiated a series of class-action lawsuits in the United States seeking compensation from medical and chemical companies that produce vaccines containing thimerosal or vaccine components.<sup>10</sup>

To make possible detailed studies on the toxicology/metabolism of thimerosal, development of sensitive and accurate methods for the determination of  $C_2H_5Hg^+$  and its metabolites in biological samples is a prime concern.

At present, a large number of methods with different pretreatment procedures and separation/detection techniques are available for mercury speciation in environmental and biological samples.<sup>11</sup> Separation by gas chromatography<sup>12,13</sup> (GC), capillary electrophoresis,<sup>14,15</sup> or high performance liquid chromatography<sup>16</sup> are often applied. When GC is used as a separation technique, most species of interest need to be derivatized in order to become volatile, thermally stable, and nonpolar for optimal chromatographic performance. Ethylation with sodium tetraethyl borate,<sup>17</sup>  $NaBEt_4$ , or butylation with butylmagnesium chloride<sup>12</sup> is commonly used for mercury species separation by GC.  $NaBEt_4$  allows for aqueous phase reaction, which in most cases is very useful. Nevertheless, if the speciation analysis includes  $C_2H_5Hg^+$ ,  $NaBEt_4$  cannot be applied, since  $Hg^{2+}$  forms diethylmercury as well. An alternative to ethylation could be aqueous phase propylation by sodium tetrapropyl borate.<sup>17,18</sup>

One of the most attractive detection systems for speciation analysis is inductively coupled plasma mass spectrometry, ICPMS.<sup>19</sup> It has isotope selectivity, and the detection limits are low for most elements, with a mass above 80 u. For elements having more than one stable isotope, isotope ratios can be measured for species-specific isotope dilution (SSID) calibration.<sup>20–22</sup> With SSID calibration, analyte species of interest, prepared from enriched stable isotopes, are added as internal standards to the sample before analysis. Since the internal standard is of the same chemical form as the analyte species, losses and transformations as well as changes in sensitivity during analysis can then be corrected for.<sup>23</sup> Because such analytical problems are common for speciation analysis,<sup>24,25</sup> SSID calibration has gained increasing popularity.<sup>21,22,26–28</sup> Compared to methods that lack isotopic selectivity, spiking of tracers can be kept at ambient levels as a result of the

high sensitivity and selectivity of the instrumentation.<sup>28</sup> One limitation is that the spiked tracers might not interact with the matrix in the same way as the incipient analyte, thus causing inaccurate results.

The aim with this study was to develop a GC–ICPMS method to determine  $CH_3Hg^+$ ,  $C_2H_5Hg^+$ , and  $Hg^{2+}$  in various mouse tissues using stable enriched isotope standards of each mercury species, produced from different isotopes, for SSID calibration. The method was then applied for determining the mercury species concentrations in various organs from mice treated with thimerosal, and transformations of species during sample preparation was detected and corrected for.

## EXPERIMENTAL SECTION

**Animals.** Female mice of the A.SW strains were obtained under contract breeding from TaconicMB, Ry, Denmark. The mice were housed under 12-h dark/12-h light cycles, kept in steel wire cages and given standard pellets (type R70, Lactamin, Stockholm, Sweden) and drinking water ad libitum. Groups of mice with an approximate weight of 20 g and aged 10–12 weeks at onset of experiment were used.

**Treatment.** Mice were sacrificed after treatment with thimerosal (Fluka Chemie, Buchs, Switzerland), 10 mg  $L^{-1}$  in drinking water ad libitum for 1, 2.5, 6, or 14 days. The liver, mesenteric lymph nodes, and left kidney were excised, transferred to Eppendorf plastic tubes, and stored at  $-18\text{ }^\circ\text{C}$ .

**Instrumentation.** Butylated mercury species were injected in a Varian 3300 GC (Varian, Palo Alto, CA) fitted with a SPB-1, 15 m  $\times$  0.53 mm i.d., 1.5- $\mu\text{m}$  film thickness capillary column (Supelco, Bellefonte, PA). The GC was coupled to an Agilent 7500a ICPMS via a previously described interface.<sup>29</sup> Oxygen was added to the plasma for prevention of carbon deposits on the sampler/skimmer platinum cones. Data for  $m/z$  199, 200, 201, and 202 were collected by peak-hopping. See Table 1 for additional operating parameters of the GC and ICPMS. The chromatographic peak areas were evaluated with the Agilent Chemstation chromatographic software.  $CH_3Hg^+$ ,  $C_2H_5Hg^+$ , and  $Hg^{2+}$  concentrations in the samples corrected for errors due to species transformation were calculated according to a SSID calibration method based on matrix calculations, as previously described.<sup>27</sup> The mercury species concentrations are given as elemental mercury based on wet weight of the sample unless stated otherwise.

**Reagents.** All chemicals used were of analytical reagent grade unless stated otherwise. All acids used were purified in-house by subboiling distillation (Heraeus Quartzschmelze, Hanau, Germany). A solution of 20% (w/w) tetramethylammonium hydroxide, TMAH, (Sigma, Sweden) was prepared by dissolving the salt in Milli-Q water (Millipore Milli-Q water system, Bedford, MA) followed by purification over Chelate S resin (Serva, Heidelberg Germany). A 0.5 mol  $\text{dm}^{-3}$  sodium diethyldithiocarbamate solu-

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Table 1. Operating Conditions for the GC and ICPMS

GC parameters	
injection volume	1–2 $\mu\text{L}$
carrier helium gas flow	22 mL/min
injector temp	180 °C
oven temp	50 °C $\rightarrow$ 50 °C min <sup>-1</sup> $\rightarrow$ 180 °C, hold 180 °C 1.5 min
transfer line temp	200 °C
ICPMS parameters	
ICP rf power	1050 W
plasma argon gas flow	15 L min <sup>-1</sup>
nebulizer argon gas flow	1.2 L min <sup>-1</sup>
auxiliary argon gas flow	0.9 L min <sup>-1</sup>
auxiliary oxygen gas flow	3 mL min <sup>-1</sup> (0.3% of total auxiliary flow)
sampler/Skimmer cones	platinum
dwell time	50 ms for <sup>199</sup> Hg <sup>+</sup> , <sup>200</sup> Hg <sup>+</sup> , <sup>201</sup> Hg <sup>+</sup> , and <sup>202</sup> Hg <sup>+</sup>

tion, DDTC, was prepared daily by dissolving the salt (Merck) in Milli-Q water. Borate buffer pH 9 was obtained from Merck, Darmstadt, Germany, Toluene was from Fluka, Buchs, Germany, and Grignard reagents 2.0 M ethylmagnesium chloride in tetrahydrofuran (THF) and 2.0 M butylmagnesium chloride in THF were obtained from Aldrich, Steinheim, Germany. All gases used were from AGA, Sundbyberg, Sweden, with purity 2.1 A. A 12.2  $\mu\text{g g}^{-1}$  aqueous standard of enriched <sup>201</sup>Hg(NO<sub>3</sub>)<sub>2</sub> (obtained from Prof. Dr. Klaus G. Heumann, Johannes Gutenberg University, Mainz, Germany) was stepwise diluted to suitable concentrations. Enriched CH<sub>3</sub><sup>200</sup>Hg<sup>+</sup> was synthesized in toluene from <sup>200</sup>HgO (Oak Ridge National Laboratory) and was thereafter extracted into the aqueous phase. Enriched C<sub>2</sub>H<sub>5</sub><sup>199</sup>Hg<sup>+</sup> was prepared in-house in a similar way as the CH<sub>3</sub><sup>200</sup>Hg<sup>+</sup> standard, (see below). Diluted standards of CH<sub>3</sub><sup>200</sup>Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub><sup>199</sup>Hg<sup>+</sup>, and <sup>201</sup>Hg<sup>2+</sup> were prepared on a daily basis.

**Sample Preparation.** In a 10-mL glass centrifuge tube with a plastic cap, thawed mouse tissue, 6–140 mg was mixed with 50–200  $\mu\text{L}$  of each CH<sub>3</sub><sup>200</sup>Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub><sup>199</sup>Hg<sup>+</sup>, and <sup>201</sup>Hg<sup>2+</sup> diluted aqueous standards (11–580 ng mL<sup>-1</sup>) and 2 mL of TMAH.<sup>27</sup> This was agitated in a Falc, F 205 (Lurano, Italy) agitator until the solution was homogeneous (~3.5 h). This solution was adjusted to pH ~9 with the borate buffer and dropwise addition of 4 M HCl (~0.3 mL). To the solution, 1.0 mL of DDTC and 1.0 mL of saturated NaCl solution were added. After vigorous shaking, 2.5 mL of toluene was added. The tube was agitated for 5–10 min, and after 5 min centrifuging at 5800 rpm, the organic phase was transferred to a new centrifuge tube standing on ice, 0.3 mL butylmagnesium chloride was added, and the tube was allowed to stand for 5 min reaction at room temperature. The Grignard reagent was quenched with 0.5 mL of 0.6 M HCl (glass centrifuge tube was then standing on ice). After centrifugation (5 min at 5800 rpm), the toluene phase was transferred to a 2-mL screw-capped GC glass vial.

**Synthesis of Methyl- and Ethylmercury Chloride.** The synthesis of isotopically labeled CH<sub>3</sub>Hg<sup>+</sup> has been described earlier by Snell et al.<sup>29</sup> C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> was prepared as follows: In a borosilicate glass tube, 5 mg of HgO enriched in the 199 isotope (Oak Ridge National Laboratory) was dissolved in 1 mL of concentrated hydrochloric acid. The solution was then evaporated at 90 °C, leaving a dry mercury chloride powder, which was

Table 2. Isotopic Abundances (%) for All Enriched Isotope Standards Used, IUPAC or Mass Bias Compensated Values

standard	196	198	199	200	201	202	204
CH <sub>3</sub> <sup>200</sup> Hg <sup>+</sup>	<0.01	0.13	0.99	96.41	1.46	0.91	0.10
C <sub>2</sub> H <sub>5</sub> <sup>199</sup> Hg <sup>+</sup>	<0.01	1.63	91.95	4.92	0.66	0.73	0.11
<sup>201</sup> Hg <sup>2+</sup>	<0.01	0.10	0.17	0.53	97.79	1.31	0.11
Hg natural (IUPAC)	0.15	9.97	16.87	23.10	13.18	29.86	6.87

Table 3. Concentrations<sup>a</sup> of Species in the Enriched Isotope Standards

standard	species <sup>b</sup>				
	CH <sub>3</sub> Hg <sup>+</sup>	C <sub>2</sub> H <sub>5</sub> Hg <sup>+</sup>	Hg <sup>2+</sup>	(CH <sub>3</sub> ) <sub>2</sub> Hg	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> Hg
CH <sub>3</sub> <sup>200</sup> Hg <sup>+</sup>	33.0	<DL	2.30	<DL	<DL
C <sub>2</sub> H <sub>5</sub> <sup>199</sup> Hg <sup>+</sup>	<DL	191	14.9	<DL	<DL
<sup>201</sup> Hg <sup>2+</sup>	0.02	<DL	12.2	<DL	<DL

<sup>a</sup>  $\mu\text{g g}^{-1}$ . <sup>b</sup> Detection limits, DL, are 0.0003, 0.0001, 0.0007, and 0.0003  $\mu\text{g g}^{-1}$  for CH<sub>3</sub>Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>, (CH<sub>3</sub>)<sub>2</sub>Hg, and (C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>Hg, respectively. Before analysis, the CH<sub>3</sub><sup>200</sup>Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub><sup>199</sup>Hg<sup>+</sup>, and <sup>201</sup>Hg<sup>2+</sup> standards were diluted 30, 200, and 10 times, respectively.

subsequently dissolved in 10 g of toluene. A 5-g portion of this solution was transferred to another glass tube and reacted with 1.6 g of 2 M ethylmagnesium chloride in tetrahydrofuran at 0 °C during mixing and then at room temperature for 0.5 h. Excess Grignard reagent was quenched by adding 1.6 g of 0.6 M hydrochloric acid to the vessel at 0 °C. The mixture was then centrifuged, and the organic phase was retained and weighed. The resulting diethylmercury in toluene was mixed with an equimolar amount of mercury chloride in toluene and then refluxed overnight at 120 °C to yield C<sub>2</sub>H<sub>5</sub>HgCl in toluene. The C<sub>2</sub>H<sub>5</sub>HgCl toluene solution was evaporated to dryness under a mild stream of nitrogen at 70 °C, and the dry C<sub>2</sub>H<sub>5</sub>HgCl was dissolved in 10 mL of 10% ethanol. (**Safety note:** Organic mercury compounds are extremely toxic and can be absorbed through the skin. They must be handled in a glovebox with severe precaution wearing protective clothing. Samples and standards containing elevated levels of mercury should be handled and stored in a ventilated fume hood for protection against volatile mercury species.)

Final concentration of C<sub>2</sub>H<sub>5</sub><sup>199</sup>HgCl and CH<sub>3</sub><sup>200</sup>HgCl in the aqueous stock solutions were determined using reversed isotope dilution (see Table 3).

## RESULTS AND DISCUSSION

**Mercury Species Preparation.** The use of a Grignard reagent for the synthesis of isotopically enriched CH<sub>3</sub>HgCl has proven very convenient in previous work.<sup>28,29</sup> In this work, the Grignard reagent-based method was found to be equally well applicable for the preparation of C<sub>2</sub>H<sub>5</sub>HgCl. Yields above 92% were achieved, and any measurable traces of diethylmercury were removed by evaporating the toluene solvent and redissolving the dry C<sub>2</sub>H<sub>5</sub><sup>199</sup>-HgCl salt in an aqueous phase. Both stock solutions (in water) of C<sub>2</sub>H<sub>5</sub><sup>199</sup>HgCl and CH<sub>3</sub><sup>200</sup>HgCl were found to be stable over at least a 3-month period. The isotopic composition and species concentrations in the standards, determined by GC–ICPMS, are shown in Tables 2 and 3.

Table 4. Determined and Certified Concentrations<sup>a</sup> for 2 Reference Materials, the  $\pm$  Values Represent 95% Confidence Interval

sample <sup>c</sup>	determined <sup>b</sup>			cert	
	CH <sub>3</sub> Hg <sup>+</sup>	Hg <sup>2+</sup>	CH <sub>3</sub> Hg <sup>+</sup> +Hg <sup>2+</sup>	CH <sub>3</sub> Hg <sup>+</sup>	Hg <sub>total</sub>
BCR-710 <sup>d</sup>	0.110 $\pm$ 0.005	not determined		0.115 $\pm$ 0.009	
TORT-2	0.155 $\pm$ 0.003	0.142 $\pm$ 0.017	0.297 $\pm$ 0.017	0.152 $\pm$ 0.013	0.27 $\pm$ 0.06

<sup>a</sup>  $\mu\text{g g}^{-1}$  (based on dry weight). <sup>b</sup> Values based on three replicate sample preparations. <sup>c</sup> BCR-710 (oyster tissue) pending certification, Community Bureau of Reference of the Commission of the European Communities and TORT-2 (lobster hepatopancreas), National Research Council of Canada. <sup>d</sup> Concentrations expressed as CH<sub>3</sub>Hg<sup>+</sup>.

**Analytical Figures of Merit.** The method was applied for CH<sub>3</sub>-Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>, and Hg<sup>2+</sup> determinations in liver, kidney, and mesenteric lymph nodes from mice. The accuracy of the CH<sub>3</sub>-Hg<sup>+</sup> determination was verified by analyzing certified reference materials (see Table 4). Although the concentrations of Hg<sup>2+</sup> are not certified in these materials, the Hg<sup>2+</sup> concentrations determined by this method are in agreement with the difference between the certified concentration of total mercury and CH<sub>3</sub>Hg<sup>+</sup>. Because there are no materials certified for the C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> amount, the recovery of C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> from an C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>-spiked reference material (BCR 710) was determined. Enriched stable isotope species were added for SSID calibration. To 0.15-g portions of the reference material, 5 additions of aqueous C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> solution (15–124 ng as Hg) were made. The calculated increase in the amount of C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> was 99.6  $\pm$  0.8% of the expected increase, where the  $\pm$  value is the standard deviation of the regression line.

Absolute detection limits were 0.4, 0.2, and 0.6 pg for CH<sub>3</sub>-Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>, and Hg<sup>2+</sup> on the basis of three times the standard deviation of the determined species amount in five separately prepared blanks analyzed on three different days. In this work, the samples were usually smaller than 0.1 g (wet weight), and for this sample amount, the detection limits were 7, 3, and 10 ng g<sup>-1</sup> for CH<sub>3</sub>Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>, and Hg<sup>2+</sup>, respectively. A chromatogram showing the peaks of butylated Hg<sup>2+</sup>, CH<sub>3</sub>Hg<sup>+</sup>, and C<sub>2</sub>H<sub>5</sub>-Hg<sup>+</sup> is depicted in Figure 1. At the retention time for dimethyl- and elemental mercury, 26 and 53 s, minor signals at or below the detection limit are visible in the chromatograms.

From measured changes in the isotope ratios, it was found that up to 9% of the added C<sub>2</sub>H<sub>5</sub><sup>199</sup>Hg<sup>+</sup> had been transformed to Hg<sup>2+</sup> during sample pretreatment and analysis. Without SSID calibration, it would be difficult to account for such species transformations. As has been discussed elsewhere,<sup>24,25,27</sup> no other mercury species transformations, such as demethylation, methylation, or ethylation, were detected during sample workup of these mouse samples. Because the degree of transformation of mercury in this case is different for different mercury species, the use of a non-species-specific internal standard would lead to analytical errors.

**Study on the Mercury Species Distribution in Various Organs of Ethylmercury (Thimerosal)-Treated Mice.** In a pilot study, the concentrations of C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>, CH<sub>3</sub>Hg<sup>+</sup>, and Hg<sup>2+</sup> were determined in kidney, liver, and mesenteric lymph nodes from mice treated with thimerosal for 1–14 days (using 2 mice per treatment).

The concentrations of C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> were below the detection limit (0.003–0.03  $\mu\text{g g}^{-1}$ ) in all three organs from control mice. The

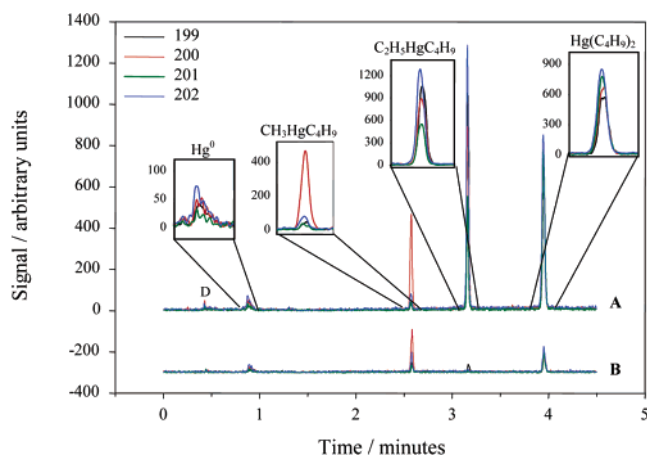


Figure 1. Isotopic chromatogram for mercury species in mesenteric lymph node from (A) a mouse treated with thimerosal for 6 days and (B) a mouse not treated with thimerosal. The lower traces, B, have been displaced for clarity. The samples have been spiked with CH<sub>3</sub><sup>200</sup>-Hg<sup>+</sup>, C<sub>2</sub>H<sub>5</sub><sup>199</sup>Hg<sup>+</sup>, and <sup>201</sup>Hg<sup>2+</sup>. The peak marked as D has been identified as dimethylmercury.

C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> concentration in kidney was increased after 1–2.5 days treatment with thimerosal and with only slight variations between the animals (Figure 2a). After 6 and 14 days, the concentration continued to increase, but with a larger variation between the animals. The mean Hg<sup>2+</sup> concentration in kidney was increased after 2.5 days, and increased continuously up to 14 days' treatment.

Both the C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> and Hg<sup>2+</sup> concentrations increased continuously in the liver during the 14 days' treatment, but the increase was much stronger for C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> (Figure 2b). The results were similar in the mesenteric lymph nodes, with a steady increase in C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>, while the Hg<sup>2+</sup> concentration reached a plateau after 6 days (Figure 2c).

The C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> and Hg<sup>2+</sup> concentrations in liver were ~50% of that in kidney. In the mesenteric lymph nodes, the C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> concentration was only 9% of that in the kidney, and the Hg<sup>2+</sup> concentration was ~20% of that in kidney, but with a high variation.

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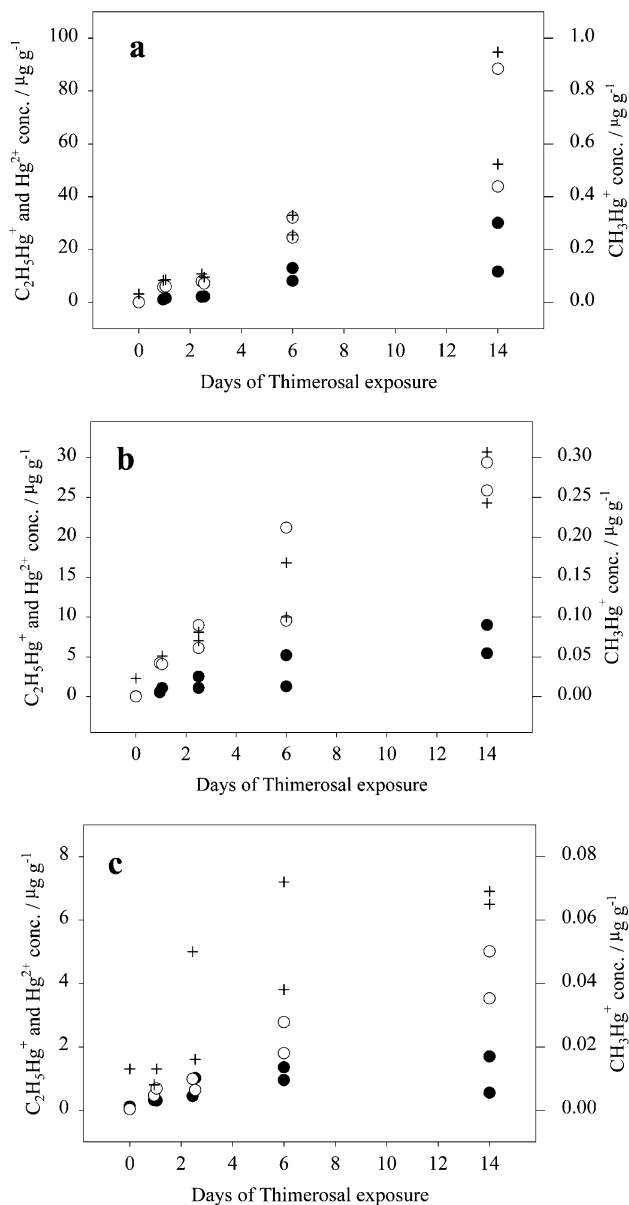


Figure 2. Determined (+) CH<sub>3</sub>Hg<sup>+</sup>, (O) C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>, and (●) Hg<sup>2+</sup> concentrations in (a) kidney, (b) liver, and (c) mesenteric lymph nodes as a function of days of thimerosal exposure.

We conclude first that thimerosal is rapidly taken up in the organs as C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> after oral treatment. Second, C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> has a

limited stability, although with large interindividual variations. In general, C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> is considered to be converted to Hg<sup>2+</sup> more rapidly than CH<sub>3</sub>Hg<sup>+</sup>.<sup>30</sup> The fact that the mercury–carbon bond in C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> is less stable<sup>30,31</sup> than that of CH<sub>3</sub>Hg<sup>+</sup> also favors a faster degradation of C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>. The bond lengths between mercury and carbon in CH<sub>3</sub>HgH and C<sub>2</sub>H<sub>5</sub>HgH have been calculated by Kaupp et al. to be 2.115 and 2.150 Å, respectively.<sup>31</sup> Systems producing reactive oxygen species<sup>32</sup> as well as several microbial systems<sup>33</sup> are able to dealkylate ethylmercury; however, the mechanisms responsible for dealkylation of methyl- and ethylmercury species are largely unknown.<sup>34</sup>

Our data show that the variation in conversion between organs and among animals is large. The large variations in the kidney might be related to different excretion of Hg<sup>2+</sup>, depending on the damage caused by Hg<sup>2+</sup> in the tubules.<sup>35</sup>

CH<sub>3</sub>Hg<sup>+</sup> concentrations in the samples were interestingly also higher in mice treated with thimerosal compared to the controls, ~1 to 2% of the C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> concentrations. Impurity of methylmercurithiosalicylate in the thimerosal was found to be 1.5%, which would explain the increased CH<sub>3</sub>Hg<sup>+</sup> concentrations. The CH<sub>3</sub>Hg<sup>+</sup> and Hg<sup>2+</sup> concentrations in the control mice represent normal background levels. In addition, the mouse food was analyzed in the same way as the mouse organs, and it was found to contain 4 ng g<sup>-1</sup> of CH<sub>3</sub>Hg<sup>+</sup> and 23 ng g<sup>-1</sup> of Hg<sup>2+</sup>. The other mercury species were below the detection limits.

## CONCLUSIONS

Because the carbon–mercury bond in C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> is less stable than that in methylmercury, it calls for more attention to the stability of the C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup> during sample preparation. The use of species-specific isotope dilution calibration is, therefore, in this case important to obtain accurate results.

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