

# 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  $\text{CH}_3^{200}\text{Hg}^+$  and  $\text{C}_2\text{H}_5^{199}\text{Hg}^+$  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)  $10 \text{ mg L}^{-1}$  in drinking water ad libitum for 1, 2.5, 6, or 14 days. Prior to analysis, samples were spiked with aqueous solutions of  $\text{CH}_3^{200}\text{Hg}^+$ ,  $\text{C}_2\text{H}_5^{199}\text{Hg}^+$ , and  $^{201}\text{Hg}^{2+}$  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  $\text{CH}_3\text{Hg}^+$ ,  $\text{C}_2\text{H}_5\text{Hg}^+$ , and  $\text{Hg}^{2+}$  were 0.4, 0.2, and 0.6 pg on the basis of  $3\sigma$  of five separate blanks. Up to 9% of the  $\text{C}_2\text{H}_5\text{Hg}^+$  was decomposed to  $\text{Hg}^{2+}$  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  $\text{C}_2\text{H}_5\text{Hg}^+$  as well as  $\text{Hg}^{2+}$  increased over the 14 days of thimerosal treatment. This shows that  $\text{C}_2\text{H}_5\text{Hg}^+$  in mice to a large degree is degraded to  $\text{Hg}^{2+}$ . Increased concentrations of  $\text{CH}_3\text{Hg}^+$  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  $\text{Hg}^{2+}$  in the tissues, it is of interest to study the temporal aspects of transport and transformation of various mercury species.

Organic mercury compounds, primarily  $\text{CH}_3\text{Hg}^+$  and  $\text{C}_2\text{H}_5\text{Hg}^+$ , 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,  $\text{C}_2\text{H}_5\text{Hg}^+$  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  $\sim 200 \mu\text{g}$  of Hg (as  $\text{C}_2\text{H}_5\text{Hg}^+$ ) during the first 6 months of life through vaccinations.<sup>4</sup> The effect of childhood  $\text{C}_2\text{H}_5\text{Hg}^+$  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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