



Applied methodology

In vitro study of thimerosal reactions in human whole blood and plasma surrogate samplesStefan Trümpler^a, Björn Meermann^{a,c}, Sascha Nowak^a, Wolfgang Buscher^a, Uwe Karst^a, Michael Sperling^{a,b,*}^a University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr. 30, Münster 48149, Germany^b European Virtual Institute for Speciation Analysis, Mendelstr. 11, Münster 48149, Germany^c Federal Institute of Hydrology, Department G2 - Aquatic Chemistry, Am Mainzer Tor 1, 56068 Koblenz, Germany

ARTICLE INFO

Article history:

Received 24 July 2013

Accepted 29 January 2014

Keywords:

Thimerosal
Ethylmercury
Speciation
analysis
Hyphenated
techniques
Human whole
blood

ABSTRACT

Because of its bactericidal and fungicidal properties, thimerosal is used as a preservative in drugs and vaccines and is thus deliberately injected into the human body. In aqueous environment, it decomposes into thiosalicylic acid and the ethylmercury cation. This organomercury fragment is a potent neurotoxin and is suspected to have similar toxicity and bioavailability like the methylmercury cation. In this work human whole blood and physiological simulation solutions were incubated with thimerosal to investigate its behaviour and binding partners in the blood stream. Inductively coupled plasma with optical emission spectrometry (ICP-OES) was used for total mercury determination in different blood fractions, while liquid chromatography (LC) coupled to electrospray ionisation time-of-flight (ESI-TOF) and inductively coupled plasma-mass spectrometry (ICP-MS) provided information on the individual mercury species in plasma surrogate samples. Analogous behaviour of methylmercury and ethylmercury species in human blood was shown and an ethylmercury-glutathione adduct was identified.

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1. Introduction

In 1931, ethylmercury thiosalicylate (thimerosal, THI) was introduced into the market as a bactericidal and fungicidal additive to drugs that are distributed in multi-dose ampullae. Since its market introduction for many decades this mercury-containing agent has been used with little or no attention to possible dangerous effects which might occur upon its intramuscular or intravenous injection into the human body. Although the methylmercury cation ("methylmercury", MeHg) is a strong toxin that is to be avoided even at small levels when consumed in foods such as seafood and rice (in Asia), the World Health Organization considers small doses of thimerosal safe regardless of multiple/repetitive exposures to vaccines that are predominantly taken during pregnancy or infancy. Anyhow, an ongoing discussion about suspected neurotoxic effects [1–3] in patients treated with THI-preserved drugs lead to the recommendation of government organizations towards the

pharmaceutical industry to phase out thimerosal as an adjuvant in vaccines in 2001. Temporarily withdrawn from routine childhood vaccination schedules in Europe and the US, thimerosal is still in use in the United States of America and in developing countries, and was present in most anti-flu vaccines against the H1N1 virus (swine influenza) in 2009. In aqueous media, THI undergoes a hydrolysis equilibrium reaction, dissolving into thiosalicylic acid (TSA) and the ethylmercury cation ("ethylmercury", EtHg) [4,5]. Recently Dorea et al. reviewed the toxicity of thimerosal and ethylmercury in comparison to methylmercury [6] and concluded that the *in vitro* toxicity of ethylmercury and thimerosal is comparable with the toxicity of methylmercury, but the different pharmacokinetics leading to a shorter residence time of ethylmercury in the blood warrants special attention for studying the *in vivo* toxicity. However, since the target organ for the toxicity of organic mercury compounds is the brain, the shorter residence time in blood is not necessarily a risk reducing factor.

Due to the strong affinity of mercury towards sulphur and the almost universal presence of this chalcogen in the human body in the form of thiols and disulphides in peptides, proteins and DNA, sulphur is the major binding partner of mercury compounds under physiological conditions. Mercury unfolds its neurotoxic effects by binding to thiols or disulphides in the nervous system thus inhibiting enzyme activities, distorting protein structure or

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