

Mechanisms of Hg species induced toxicity in cultured human astrocytes: genotoxicity and DNA-damage response

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The toxicologically most relevant mercury (Hg) species for human exposure is methylmercury (MeHg). Thiomersal is a common preservative used in some vaccine formulations. The aim of this study is to get further mechanistic insight into the yet not fully understood neurotoxic modes of action of organic Hg species. Mercury species investigated include MeHgCl and thiomersal. Additionally HgCl₂ was studied, since in the brain mercuric Hg can be formed by dealkylation of the organic species. As a cellular system astrocytes were used. *In vivo* astrocytes provide the environment necessary for neuronal function. In the present study, cytotoxic effects of the respective mercuricals increased with rising alkylation level and correlated with their cellular bioavailability. Further experiments revealed for all species at subcytotoxic concentrations no induction of DNA strand breaks, whereas all species massively increased H₂O₂-induced DNA strand breaks. This co-genotoxic effect is likely due to a disturbance of the cellular DNA damage response. Thus, at nanomolar, sub-cytotoxic concentrations, all three mercury species strongly disturbed poly(ADP-ribosyl)ation, a signalling reaction induced by DNA strand breaks. Interestingly, the molecular mechanism behind this inhibition seems to be different for the species. Since chronic PARP-1 inhibition is also discussed to sacrifice neurogenesis and learning abilities, further experiments on neurons and *in vivo* studies could be helpful to clarify whether the inhibition of poly(ADP-ribosyl)ation contributes to organic Hg induced neurotoxicity.

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Introduction

The three most exposure relevant chemical forms of mercury (Hg) are elemental Hg, mercuric Hg and organometallic compounds. Among these methylmercury is by far the most common in the environment and in the aquatic food-chain. In addition to the consumption of inorganic Hg contaminated food, inorganic Hg exposure might occur through medicinal products.¹ Inhaled elemental Hg vapour from dental amalgam is another source that is likely to increase internal Hg exposure.² Methylmercury exposure occurs nearly exclusively *via* fish and seafood, with generally 80–100% of total fish Hg being

methylmercury.² In Hg polluted areas in China, methylmercury contaminated rice is a further possible contributor.³ Non-dietary exposure to organic Hg might result from the application of thiomersal (sodium 2-ethylmercurothio-salicylate). Thiomersal is used as a preservative in multidose vials of some vaccines,⁴ as well as in several cosmetic products and cleaning solutions for contact lenses.⁵ Its antimicrobial effect is based on its decomposition in aqueous medium to thiosalicylic acid and the ethylmercury cation. Rapid hydrolysis of thiomersal in aqueous biological solution has been demonstrated before.⁶

In 2003, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) revised the PTWI for methylmercury to 1.6 µg kg⁻¹ body weight (b.w.); developmental neurotoxicity was identified as the most sensitive toxicological endpoint. In 2006 JECFA confirmed this PTWI.⁷ Based on the information that beneficial nutrients in fish may have confounded previous adverse outcomes in some of these studies, the European Food Safety Authority (EFSA) Scientific Panel on Contaminants in the Food Chain established in December 2012 a TWI for methylmercury of 1.3 µg kg⁻¹ b.w. Moreover, the Panel concluded that high fish consumers, which might include pregnant women,

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