

# Comparison of organic and inorganic mercury distribution in suckling rat

Tatjana Orct,\* Maja Blanuša, Maja Lazarus, Veda Marija Varnai and Krista Kostial

Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia

Received 15 February 2006; Revised 15 June 2006; Accepted 15 June 2006

**ABSTRACT:** Thiomersal is used as a preservative in vaccines given to small children. The metabolic product of thiomersal is ethylmercury and its distribution and kinetics are still not known, especially at this early age. The purpose of this study was to compare the body distribution of two forms of mercury: organic (thiomersal) and inorganic (mercury(2+) chloride) in very young, suckling rats. Mercury was applied subcutaneously three times during the suckling period on days 7, 9 and 11 of pups age, imitating the vaccination of infants. A single dose of mercury was equimolar in both exposed groups, i.e.  $0.81 \mu\text{mol Hg kg}^{-1}$ . At 14 days of age the animals were killed and the total mercury analysed in blood and organs (kidney, liver and brain). The analytical method applied was total decomposition, amalgamation, atomic absorption spectrometry. The results showed that the level of mercury was higher in the liver and kidney of the inorganic mercury group than in the thiomersal exposed group. However, the brain and blood concentrations of mercury were higher in the thiomersal exposed group. These results need to be clarified by additional data on the kinetic pathways of ethylmercury compared with inorganic mercury. Copyright © 2006 John Wiley & Sons, Ltd.

**KEY WORDS:** thiomersal; ethylmercury; mercury(2+) chloride; suckling rat; distribution; blood; kidney; liver; brain

## Introduction

Thiomersal (Thiomerosal, Merthiolat) is an organic compound of mercury added as a preservative to the childhood vaccines diphtheria-tetanus-pertussis (DTP) or diphtheria-tetanus (DT). The vaccine is given subcutaneously to infants several times during their first 6 months of life, up to the age of 6 years. The metabolite of thiomersal is ethylmercury and its absorption, distribution and excretion is similar to methylmercury. The similarity of metabolic pathways and actions are due to similar reactions as organic molecules. Both substances increase oxidative stress and are neurotoxic (Ueha-Ishibashi *et al.*, 2004; ATSDR, 1999). Ethylmercury is, however, less toxic than methylmercury due to the lower clearance half-time and the lower possibility to pass the blood–brain barrier (Magos, 2003) than methylmercury. Due to unknown toxicity from low-dose exposures to ethylmercury, there has been concern that this exposure to mercury may be of some detriment to young children. Autistic spectrum disorders and neurodevelopmental disorders have been a controversial topic since 1999

connected with thiomersal-containing vaccines (Parker *et al.*, 2004). Therefore, the American Academy of Pediatrics and the US Public Health Service issued a joint statement calling for the removal of thiomersal from vaccines. However, in our country and probably in many other countries such vaccines are still in use.

Data on the difference between ethylmercury and inorganic mercury distribution in newborns are, however, limited. In this study the distribution of the two chemical forms of mercury, organic (thiomersal) and inorganic (mercury(2+) chloride) was measured and compared in very young, suckling rats. Application of substances was subcutaneous three times during the suckling period, imitating the vaccination of infants.

## Methods

### Animals and Experimental Protocol

Adult animals used in the experiment were Wistar rats raised in the Institute's breeding farm (Institute for Medical Research and Occupational Health, Zagreb, Croatia). They were fed standard rat diet (Mucedola, Milano, Italy) and tap water *ad libitum*. The rats were kept in the animal facility at a constant room temperature of 20–22 °C, constant humidity ( $40 \pm 10\%$ ) and 12 h light/dark cycle. Females were mated with males in the ratio of 3 : 1. Pregnant females were kept in individual

\* Correspondence to: T. Orct, Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, P.O. Box 291, 10001 Zagreb, Croatia.  
E-mail: torct@imi.hr  
Contract/grant sponsor: Ministry of Science, Technology and Sport of the Republic of Croatia; contract/grant number: 0022012.

polycarbonate cages (26 × 20 × 14 cm) with stainless steel lids (Macrolon cages, Ehret, Germany) 2 weeks before delivery. The cages were cleaned and sterile pine shaving bedding changed daily. Three litters delivered the same day were taken for the experiment. The number of pups was reduced to eight male pups. At the age of 7 days, subcutaneous application of mercury in a volume of 0.05 ml was started. Two pups from each litter received saline solution (control group); three pups received mercury chloride (HgCl<sub>2</sub> group), (HgCl<sub>2</sub> p.a., Kemika, Croatia) and three pups received thiomersal solution (thiomersal group), (ethylmercury thiosalicylic acid sodium salt, SERVA Electrophoresis, Germany). The total number of pups was six pups in the control group and nine pups in each experimental group. The subcutaneous application was repeated three times, at the age of 7, 9 and 11 days. The dose of mercury used in these experiments was based on findings from previous LD<sub>50</sub> studies in suckling rats of 2.2 mg HgCl<sub>2</sub> kg<sup>-1</sup> (Maljković, 1983). Ten percent of this dose was used, which is known to have no adverse health effects in sucklings (no observed difference in body and organ weights and general appearance and behavior). The single dose of mercury in the two experimental groups was equimolar, i.e. 0.81 μmol kg<sup>-1</sup> body weight, each. At the age of 14 days the animals were killed by bleeding from the abdominal aorta under i.p. Narketan and Xylapan anaesthesia (0.8 ml kg<sup>-1</sup> + 0.6 ml kg<sup>-1</sup>, Vetoquinol AG, Switzerland).

The body weights of the pups were measured each day from 7 to 14 days throughout the experiment. After dissection and before digestion the wet weight of each organ was recorded.

All procedures carried out with laboratory animals were approved by the Croatian Ministry of Agriculture and Forestry.

### Sampling and Analysis

After dissection all organs (kidneys, liver and brain) were frozen at -20 °C until mercury analysis. During anaesthesia, blood was sampled directly from the heart under heparin. Digestion of organs was carried out in closed tubes in a Digestion System (DS-40, Tecator, Sweden). Each organ was mixed with 2 ml of concentrated nitric acid. After standing overnight the system was heated stepwise up to 80 °C during 6 h. After digestion the samples were adjusted to 10 ml with deionised water. In an aliquot of 20–100 μl, depending on the concentration of mercury in the sample, total mercury was analysed by total decomposition, amalgamation, atomic absorption spectrometry (TDA-AAS) (Orct *et al.*, 2005; Boylan *et al.*, 2001) in a mercury analyser (AMA 254, LECO, USA). Blood was analysed for mercury directly in 50–100 μl without prior digestion. For internal quality control of the analytical method certified standard samples of

whole blood (Seronorm TM Elements, Sero AS, Norway) and horse kidney (H8, IAEA, Vienna, Austria) were treated identically as samples from the experiment. The concentration of mercury obtained in whole blood was 7.93 ± 0.43 μg Hg l<sup>-1</sup> and in horse kidney 0.894 ± 0.046 μg Hg g<sup>-1</sup> (mean ± SD). These data were within the range of certified values: 8.2 ± 0.9 μg Hg l<sup>-1</sup> in whole blood and 0.910 ± 0.070 μg Hg g<sup>-1</sup> in horse kidney dry weight, respectively.

### Statistical Analysis

Statistical evaluation of the data was performed by using the program 'Statistica for Windows' (StatSoft, Inc. 2001, release 6.0). The results are presented as arithmetic means and standard deviations of mercury concentrations expressed as μg Hg g<sup>-1</sup> of wet tissue weight in organs, or as μg Hg l<sup>-1</sup> in blood. When variances were not homogeneous logarithmic transformation was performed. The results were analysed by one-way analysis of variance (one-way ANOVA) and compared by *post hoc* Duncan test. Where variances were not homogeneous even after logarithmic transformation, Kruskal-Wallis ANOVA was applied followed by Mann-Whitney *U* tests with Bonferroni correction. When ANOVA all effects were significant (at a level of *P* < 0.05) three comparisons were tested: HgCl<sub>2</sub> and thiomersal group versus control and HgCl<sub>2</sub> versus thiomersal group.

### Results and Discussion

The body weights of pups were about 18.5 g at the beginning and 35.5 g at the end of the experiment. No difference was noticed among groups in the body weight gain of about 17 g or organ weights at the end of the experiment.

Mercury concentrations in all measured organs and blood of two exposed groups of suckling animals were significantly higher than in the corresponding tissues of the control group (Table 1). The results of mercury levels in two exposed groups are also presented as the percent of administered dose of mercury. Animals exposed to inorganic mercury had slightly but significantly higher mercury concentration in liver than animals exposed to thiomersal. This may indicate a higher excretion of inorganic mercury by the endogenous faecal route although it is considered that the process of biliary secretion of mercury does not occur in suckling animals (Clarkson, 2002). The kidney mercury concentration was four times higher in the HgCl<sub>2</sub> exposed group than in the thiomersal group. It could be hypothesized that a higher kidney retention of inorganic mercury could indicate a higher urinary excretion of inorganic compared with organic mercury. However, for any firm conclusion regarding the route of

**Table 1.** Mercury distribution 72 h after last subcutaneous application of HgCl<sub>2</sub> or thiomersal at postnatal day 14

Group	Whole blood		Brain		Kidneys		Liver	
	µg Hg l <sup>-1</sup>	% Dose	µg Hg g <sup>-1</sup> tissue	% Dose	µg Hg g <sup>-1</sup> tissue	% Dose	µg Hg g <sup>-1</sup> tissue	% Dose
Control	0.635 ± 0.213		0.033 ± 0.004		0.076 ± 0.033		0.026 ± 0.015	
HgCl <sub>2</sub>	36.3 ± 3.19 <sup>a</sup>	0.8 ± 0.1	0.073 ± 0.007 <sup>a</sup>	27.3 ± 0.7	8.24 ± 1.15 <sup>a</sup>	27.3 ± 0.7	1.83 ± 0.19 <sup>a</sup>	15.2 ± 1.3
Thiomersal	849 ± 113 <sup>ab</sup>	1.2 ± 0.2	0.108 ± 0.016 <sup>ab</sup>	6.2 ± 0.5	2.01 ± 0.27 <sup>ab</sup>	6.2 ± 0.5	1.37 ± 0.21 <sup>ab</sup>	10.8 ± 1.2

Values represent mean ± SD ( $n = 6$  for control group and  $n = 9$  for exposed groups).

Percent of dose is calculated on the basis of total mercury in tissue/total mercury administered × 100.

Thiomersal and HgCl<sub>2</sub> were applied subcutaneously on days 7, 9 and 11 of pups age at a dose of 0.81 µmol Hg kg<sup>-1</sup> body weight, each (11.52 µg Hg, each).

Mercury concentrations in brain and kidneys were analysed after logarithmical transformation with one-way ANOVA followed by *post hoc* Duncan test.

Whole blood and liver mercury concentrations were analysed (due to heterogeneous variances) by Kruskal-Wallis ANOVA followed by Mann-Whitney *U* test with Bonferroni correction.

<sup>a</sup> Significantly different from control ( $P < 0.002$ ).

<sup>b</sup> Significant difference between HgCl<sub>2</sub> and thiomersal group ( $P < 0.001$ ).

mercury excretion an appropriate toxicokinetic experimental design should be applied.

The brain mercury concentration was, however, 1.5 times higher in the thiomersal exposed pups. This confirms the fact that ethylmercury, which is a metabolic product of thiomersal, more easily passes the blood–brain barrier. The mercury concentration in the blood of thiomersal exposed pups was even 23 times higher than in the HgCl<sub>2</sub> exposed pups. This high difference in mercury blood concentration may indicate a longer biological half-time of ethylmercury excretion compared with inorganic mercury excretion at such an early age. In this experiment ethylmercury was compared with inorganic mercury because its toxicity lies between methylmercury and inorganic mercury. In some studies ethylmercury metabolic pathways are compared with the methylmercury form, which is logical reasoning since both forms are organic (Burbacher *et al.*, 2005; Magos *et al.*, 1985). However, the high tissue levels of inorganic mercury seen in both humans and animals indicate that ethylmercury breaks down to inorganic mercury more rapidly than methylmercury (Clarkson, 2002). It has never been compared with inorganic mercury and above all not in the suckling period, which is the most interesting period when infants are vaccinated with thiomersal as a preservative. The other reports are concentrated on each studied form separately without comparison with other forms of mercury (Castoldy *et al.*, 2001; Pfab *et al.*, 1996; Tan and Parkin, 2000; Blair *et al.*, 1975; Magos, 2001). Recently, Burbacher and co-workers (2005) studied the comparative toxicokinetics of methylmercury and thiomersal in newborn and infant monkeys. Thiomersal exposed monkeys had 3-fold lower concentrations of mercury in the brain when compared with methylmercury exposed infants. Also a high percentage of total mercury in the brain was in the form of inorganic mercury for the thiomersal exposed animals (34% compared with 7%). They concluded that methylmercury is not a suitable reference for assessment from exposure to thiomersal-derived mercury. In the present study the brain retention of total mercury was only 1.5 times higher in the thiomersal exposed sucklings than in the inorganic mercury exposed pups. The toxicokinetics of ethylmercury, however, remain unknown, especially in newborns and during the suckling period.

The much higher concentration of mercury in the blood and the lower retention in the kidney of thiomersal exposed pups in the present experiment need to be clarified by more data on the kinetic pathway of mercury retention in the organs and blood and excretion in urine with time during the same period of suckling.

**Acknowledgement**—The authors thank Assistant Professor Anamarija Jazbec, Faculty of Forestry, University of Zagreb, for statistical suggestions and Marija Ciganović for technical assistance. This work was supported by Ministry of Science, Technology and Sport of the Republic of Croatia (Project No. 0022012).

## References

- ATSDR Website. <http://www.atsdr.cdc.gov/toxprofiles/tp46.html> [1 February 2006].
- Blair AMJN, Clark B, Clarke AJ, Wood P. 1975. Tissue concentrations of mercury after chronic dosing of squirrel monkeys with thiomersal. *Toxicology* **3**: 171–176.
- Boylan HM, Richter RC, Kingston HM, Ricotta AC. 2001. Rapid analysis for the field: method development and application to natural gas utility sites. *Water Air Soil Poll.* **127**: 255–270.
- Burbacher TM, Shen DD, Liberato N, Grant KS, Cernichiari E, Clarkson T. 2005. Comparison of blood and brain mercury levels in infant monkeys exposed to methylmercury or vaccines containing thiomersal. *Environ. Health Perspect.* **113**: 1015–1021.
- Castoldi AF, Coccini T, Ceccatelli S, Manzo L. 2001. Neurotoxicity and molecular effects of methylmercury. *Brain Res. Bull.* **55**: 197–203.
- Clarkson TW. 2002. The three modern faces of mercury. *Environ. Health Perspect.* **110**(Suppl 1): 11–23.
- Magos L. 2001. Review on the toxicity of ethylmercury, including its presence as a preservative in biological and pharmaceutical products. *J. Appl. Toxicol.* **21**: 1–5.
- Magos L. 2003. Neurotoxic character of Thiomersal and the allometric extrapolation of adult clearance half-time to infants. *J. Appl. Toxicol.* **23**: 263–269.
- Magos L, Brown AW, Sparrow S, Bailey E, Snowden RT, Skipp WR. 1985. The comparative toxicology of ethyl- and methylmercury. *Arch. Toxicol.* **57**: 260–267.
- Maljković T. 1983. *The Effect of Slag from Coal Gasification on the Toxicokinetics of Some Metals*. Ph.D. Thesis, Faculty of Science, University of Zagreb, Croatia (in Croatian).
- Orct T, Blanuša M, Ciganović M. 2005. Comparison of methods for mercury determination in biological material. (in Croatian). In *Proceedings of the XIX. Croatian Meeting of Chemists and Chemical Engineers, Opatija 2005*, Rapić V, Rogošić M (eds). Croatian Society of Chemical Engineers: Croatian Chemical Society: Zagreb; 241.
- Parker SK, Schwartz B, Todd J, Pickering LK. 2004. Thiomersal-containing vaccines and autistic spectrum disorder: A critical review of published original data (Review). *Pediatrics* **114**: 793–804.
- Pfab R, Muckter H, Roider G, Zilker T. 1996. Clinical course of severe poisoning with thiomersal. *J. Toxicol. Clin. Toxicol.* **34**: 453–460.
- Tan M, Parkin JE. 2000. Route of decomposition of thiomersal (thiomersal). *Int. J. Pharm.* **208**: 23–34.
- Ueha-Ishibashi T, Oyama Y, Nakao H, Umebayashi C, Nishizaki Y, Tatsuishi T, Iwase K, Murao K, Seo H. 2004. Effect of thiomersal, a preservative in vaccines, on intracellular Ca<sup>2+</sup> concentration of rat cerebellar neurons. *Toxicology* **195**: 77–84.