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Exposure to mercury in susceptible population groups living in the former mercury mining town of Idrija, Slovenia

Alfred B. Kobal [a], Janja Snoj Tratnik [b,g], Darja Mazej [b], Vesna Fajon [b], Darija Gibičar [b], Ana Miklavčič [b], David Kocman [b], Jože Kotnik [b], Alenka Sešek Briški [b], Joško Osredkar [c], Mladen Krsnik [c], Marija Prezelj [c], Črtomir Knap [d], Bojana Križaj [e], Lian Liang [f], Milena Horvat [b,g,*]

[a] Ex-Department of Occupational Health, Idrija Mercury Mine, Arkova 43, 5280 Idrija, Slovenia
[b] 'Jožef Stefan' Institute, Department of Environmental Sciences, Ljubljana, Slovenia
[c] University Medical Centre Ljubljana, Institute for Clinical Chemistry and Biochemistry, University in Ljubljana, Slovenia
[d] Department of Gynecology, Idrija Health Service, Otona Župančiča 3, 5280 Idrija, Slovenia
[e] Laboratory Department, Idrija Health Service, Otona Župančiča 3, 5280 Idrija, Slovenia
[f] Cebam Analytical, Inc., 18804 North Creek Parkway, Bothell, WA 98011, USA
[g] 'Jožef Stefan' International Postgraduate School, Ljubljana, Slovenia

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ABSTRACT

500 years of mercury (Hg) mining in the town of Idrija has caused severe pollution in Idrija and its surroundings. Following the closure of the mine in 1995, the environment remains contaminated with Hg. Sources of elemental-, inorganic- and methyl Hg exposure were identified, potential environmental level of exposure to Hg was evaluated and actual internal exposure to Hg was assessed in selected susceptible population groups comprising school-age children and pregnant women living in Idrija and in control groups from rural and urban environments. The study of pregnant women ($n=31$) was conducted between 2003 and 2008, and the study of school-age children ($n=176$) in 2008. Potential interaction of Hg with selenium (Se) in plasma was assessed in both study populations, while in pregnant women antioxidative enzyme activity (glutathione peroxidase, superoxide dismutase and catalase) in erythrocytes of maternal and cord blood was also assessed. Actual exposure to Hg as indicated by levels of Hg in children's blood (geometric mean (GM) 0.92 μg/L), mother's blood (GM 1.86 μg/L), children's urine (GM 1.08 μg/g crea.), mother's urine (GM 2.51 μg/L), children's hair (GM 241 ng/g) and mother's hair (GM 251 ng/g) was higher in the two study groups from Idrija than in the control groups from rural areas, but was still at the level of a "normal" population and reflects mainly exposure to elemental Hg (Hg°) from dental amalgam and, to a certain extent atmospheric Hg°. Furthermore, the internal doses of Hg received during pregnancy did not decrease the bioavailability of Se. Based on observation in children, the increase in Se protein expression is suggested to be a consequence of moderately elevated exposure to Hg°. The observed changes in activity of antioxidative enzymes, as biomarkers of oxidative stress, appear to be mainly associated with pregnancy *per se* and not with an increased exposure to Hg. In view of the continuing increased potential for Hg exposure and the low number of pregnant women studied, the results warrant a further longitudinal study of a larger group of pregnant women residing in the area of the former mercury mine.

1. Introduction

Mercury (Hg) is a ubiquitous global pollutant that adversely affects humans and wild life. The general population is exposed to methyl Hg through the diet (mostly from consuming fish) and to elemental Hg (Hg°) from dental amalgam fillings. Populations living in Hg contaminated sites, such as those located in areas near mercury mines and industrially polluted land, may also be exposed to Hg° through inhalation of contaminated ambient air. Over 500 years of mercury mining in the town of Idrija, Slovenia has

Abbreviations: CAT, catalase; CI, confidence interval; CV, coefficient of variation; GM, geometric mean; GPx, glutathione peroxidase; Hb, haemoglobin; HBM I, health-based value, below which no biological and/or adverse health effects are expected; HBM II, health-based value, which could be associated with biological and/or potential adverse health effects; Hg^{2+}, inorganic mercury; Hg°, elemental mercury; Max, maximum; Min, minimum; P10, 10th percentile; P90, 90th percentile; PHIME, Public Health Impact of Long-term Low-level Mixed Element Exposure in Susceptible Population Strata; P-Se, plasma selenium; RfD, reference dose; SD, standard deviation; SOD, superoxide dismutase; THg, total mercury

* Correspondence to: Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia.
E-mail address: milena.horvat@ijs.si (M. Horvat).

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caused severe Hg pollution of the environment in the town and its surroundings (Byrne et al., 1995; Gnamuš et al., 2000; Gosar et al., 1997; Hess, 1991; Kavčič, 1974; Kosta et al., 1974; Lupšina et al., 1992; Palinkaš et al., 1995). In the period after the mine closed in 1995 the Hg concentrations in sediments, soil, vegetation, water, fish, and partly also in air, remained elevated (Gnamuš et al., 2000; Gosar and Šajn, 2003; Gosar et al., 1997; Grönlund et al., 2005; Horvat et al., 2002, 1999; Jereb et al., 2004; Kocman et al., 2011a, 2011b; Kotnik et al., 2005). However, in the period after the mine closed the extent of Hg exposure in the general non-occupationally exposed population living in Idrija has yet to be assessed. An exposure assessment was made possible through the two cross-sectional studies presented in this paper, where school-age children and pregnant women from contaminated and control areas were compared.

School-age children and pregnant women were chosen for the study since both are recognised as susceptible population groups. Exposure to methyl Hg, particularly in the prenatal period, has neurotoxic effects on the development of a child's central nervous system. Even low levels of exposure in pregnant women appear to have subtle persistent effects on children's mental development (Davidson et al., 2000; Grandjean and Landrigan, 2006; Grandjean et al., 1997; NRC, 2000; WHO, 1990). In pregnant women the transfer of inhaled Hg° to the developing foetus has also been observed (Clarkson, 1989, 2002), but in spite of it having similar mechanisms of toxicity *e.g.*, oxidative stress from the stimulation of free radical generation and reducing the amount of bioavailable selenium (Ganther, 1980; Kosta et al., 1974; Lund et al., 1993; Rana and Boora, 1992; WHO, 1991, 1990), no definitive data on its effects on children's mental development has been documented (Davidson et al., 2004; WHO, 2003).

On a biochemical level, Hg can promote lipid peroxidation by stimulating free radical generation as a catalyst in a Fenton-type reaction and through other mechanisms by interacting with antioxidant enzymes that could modify its activity (Björkman et al., 1993; Lund et al., 1993). The main antioxidant enzymes in human cells are superoxide dismutase (SOD), catalase (CAT) and the selenoenzyme glutathione peroxidase (GPx), which, among other functions, transform the superoxide radical into hydrogen peroxide and hydrogen peroxide into oxygen and water. Normal pregnancy is also associated with increased oxidative stress, resulting in the induction of antioxidant enzymes to protect embryonic development against free radical attack (Carone et al., 1993; Mistry et al., 2008; Sugino et al., 2000).

The aims of this study were to evaluate the environmental distribution and concentrations of Hg in the area of the town of Idrija, and to estimate the actual exposure of school-age children and pregnant women to Hg°, methyl Hg and inorganic Hg through inhalation of ambient air, ingestion of drinking water and locally grown seasonal vegetables, as well the intake of methyl Hg from the consumption of fish from the river Idrijca and from the fish market. Possible interactions of Hg with selenium (Se) and antioxidative enzymes were also examined in a subgroup of the studied population. This study is important in terms of identifying if the most susceptible population groups living in the contaminated area of the town of Idrija are still at risk.

2. Methods

2.1. Study design and subjects

First, we evaluated the data from environmental studies performed in the town of Idrija since the mine closed in 1995. From these data, we estimated the concentration of Hg° in air, inorganic and methyl Hg in local seasonal vegetables, drinking water and in fish from the river Idrijca. Second, we evaluated Hg absorption by determining the total Hg concentrations in urine, blood and hair of 6–11 years old school children, pregnant women and in the cord blood of newborns, with these tissues serving as biological markers of exposure to Hg (UNEP Chemicals, 2002; UNEP/WHO, 2008; WHO, 2003, 1990). Third, we determined the concentrations of Se in plasma (P-Se) in school-age children, pregnant women and, in a subgroup of pregnant women, the activity of antioxidative enzymes in erythrocytes during pregnancy and in cord blood. These act as peripheral markers of antioxidative activity associated with pro-oxidative effects of Hg (Kobal et al., 2008, 2004; Lund et al., 1993), and/or with the pregnancy itself (Agarwal et al., 2005; Sugino et al., 2000). Among these antioxidative enzymes, we focused on the activity of selenoenzyme GPx, CAT and SOD in erythrocytes.

The study included the following: 1) inhabitants of the former mercury mining area of the town of Idrija: 63 children 6–11 years of age (all together there were 289 children of the same age in the town) and 20 pregnant women aged 20–35 years (on average, 41 women gave birth per year in the study period in the town of Idrija), and 2) a control group of age-matched children from a rural uncontaminated area (Žužemberk, $n=66$) and an urban area (Ljubljana, $n=47$) and 15 age-matched pregnant women from a rural uncontaminated area.

The *study population of school-age children* was recruited from schools in each study area in the years 2008 – contact was established first with the headmaster and then with parents and their children. Written consent was obtained from a parent of each child, while oral consent was obtained from the child prior to sampling. Only healthy children, with no evidence of chronic diseases were recruited.

The *study population of pregnant women* was conducted in years between 2003 and 2008. The selection of pregnant women was based on medical examinations performed by a gynaecologist and a specialist in occupational medicine, who among other standard data also evaluated the number of dental amalgam fillings and calculated the dental amalgam score using the method of Aposhian and co-workers (Aposhian et al., 1992). The following selection criteria for pregnant women were applied: informed consent from the pregnant women to participate in the study; the study and control groups were neither currently nor previously occupationally exposed to Hg, lead, cadmium or halogenated hydrocarbons; the medical history and medical examinations did not reveal any hypertension, alcoholism, lung diseases (asthma, chronic obstructive pulmonary diseases, pulmonary fibrosis), metabolic disorders, auto-immune diseases (rheumatoid arthritis, lupus erythematodes); and hepatic and renal diseases (proteinuria) which can influence the absorption or excretion of Hg and antioxidative enzyme activity.

The National Medical Ethics Committee of Republic of Slovenia approved the study.

2.2. Biological sampling

Venous *blood* from school-age children was collected by venipuncture in 4 mL Lithium Heparin plastic tubes (Greiner-Bio One GmbH, Frickenhausen, Germany) for analysis of total Hg and plasma Se. Venous blood from pregnant women was collected during the first and last trimester of pregnancy and in cord blood of infants for analysing total Hg, Se in plasma and antioxidative enzymes CAT, CuZn-SOD and GPx in erythrocytes. Blood was collected by venipuncture in 3 mL and 7 mL BD Vacutainer® tubes (Benton Dickinson) containing the anticoagulant K3-EDTA and Lithium Heparin, respectively. The samples were transported to the laboratory within 2 h at 2–8 °C, where they were divided into aliquots and stored at −20 to −30 °C prior to analysis.

To determine *urinary Hg*, morning urine samples of school-age

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