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Comparative determination of methyl mercury in whole blood samples using GC–ICP-MS and GC–MS techniques

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ABSTRACT

Two methods for the determination of methyl mercury (MeHg) in whole blood samples based on different mass spectrometric detection techniques are compared. The methods were employed in two studies in which the internal exposure of a group of mercury-exposed workers to total mercury and MeHg was investigated. Blood samples of these workers were analysed for MeHg independently from each other in two laboratories using similar extraction procedures but different detection techniques, *viz.* coupled GC–EI-MS/ICP-MS and GC–MS using D_3-MeHg as internal standard. MeHg was detected in all blood samples in concentrations ranging from 0.3 to 9.0 μg/L. Though different detection techniques were employed, the results obtained by the two laboratories were in relatively good agreement.

1. Introduction

The methylated derivatives of mercury, itself a highly toxic element, are known to be even more hazardous to human health than the metallic and inorganic forms. The high toxicity of MeHg has become obvious in a number of accidents which attracted attention even beyond the scientific community. The best documented case occurred in 1952 at Minamata Bay in Japan where the disposal of mercury containing waste from an acetaldehyde plant led to the poisoning of several thousand people [1,2]. In 1971/1972 more than 6500 Iraqis were poisoned after the consumption of bread unintentionally made of seeds dressed with MeHg as fungicide [3]. Several other cases have been reported [4]. As long as the internal exposure to mercury compounds is assessed, blood and urine samples are analysed, but in most cases only for total mercury and not for organic mercury compounds. If studies are performed to determine exposure to organic mercury compounds as has been done in a survey of 1700 volunteers [5], the concentration of MeHg and other organic mercury species are most often derived from the difference of total and inorganic mercury [6].

In the present paper two selective methods for the analysis of MeHg in whole blood are described and compared. Following quality assessment the methods were applied to the analysis of blood samples obtained in two subsequent studies from 20 and 22 mercury-exposed workers, respectively. In addition, an inter-laboratory comparison was accomplished in a way that the same blood samples were analysed independently from each other in two laboratories using similar extraction procedures but different detection techniques: in one laboratory MeHg was determined by coupled GC–EI-MS/ICP-MS; in the other the samples were analysed by GC–MS using D_3-MeHg as internal standard.

2. Materials and methods/blood sampling

Blood samples were collected from workers of a mercury recycling plant in two subsequent studies. The workers were in part highly exposed to elemental mercury. Twenty workers participated in the first study and 22 in the second one. Some workers took part only in study one or in study two, some participated in both studies. A total of 40 ml of blood was withdrawn from each participant and transferred into four 10 ml tubes containing sodium citrate as anticoagulant (Sarstedt, Germany). Two tubes from each participant were sent immediately after withdrawal to the Medical Laboratory in Bremen (using a cooled DHL transport box for liquid samples) and were analysed within 10 days after storing them at +6 °C in a refrigerator. The remaining two tubes were kept at +6 °C at the Institute of Environmental Analytical Chemistry in Essen until analysis.

3. Analysis

Chemicals, extraction methods and instrumentation are described below separately for the analysis of blood samples in the two laboratories (method A: analysis by GC–EI-MS/ICP-MS; method B: analysis by GC–MS using D_3-MeHg as internal standard).

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3.1. Method A: analysis by GC–EI-MS/ICP-MS

The instrumental set-up of method A, including a combined GC–EI-MS/ICP-MS system, allows the sensitive detection of mercury (ICP-MS), while structure information is obtained simultaneously by EI-MS. To monitor the plasma stability of the ICP-MS an internal standard (rhodium solution) is added to the nebuliser. The mass trace of ^{202}Hg is used for quantification, and if necessary, up to seven mass traces for mercury can be monitored to exclude interferences. To obtain narrow peaks, the analyte is refocused in a programmed temperature vaporisation unit (PTV, UNIS 2000) containing a Supelcoport® packed liner following the derivatisation and purge and trap sampling steps.

3.1.1. Chemicals and reagents

All chemicals used were of analytical grade unless stated otherwise. Aqueous solutions of reagents were prepared with ultrapure water (18.2 MΩ cm) using a Purelab ultra water purification system (Elga, Ransbach-Baumbach, Germany). A 1% (w/w) solution of sodium tetra-*n*-ethylborate (GALAB, Geesthacht, Germany) was prepared in water containing 1% (w/w) potassium hydroxide (Fluka/Sigma–Aldrich, Taufkirchen, Germany). The solution was stored at 6 °C and protected from light. The citrate-buffer solution (pH 5) was purchased from Merck (Darmstadt, Germany). Analytical grade sulphuric acid was purified by subboiled distillation before being used for the preparation of a 2 M/1.5 M H_2SO_4/KBr solution. Potassium bromide was obtained from Riedel-de Häen (Seelze, Germany), and methyl *tert*-butyl ether was purchased from Acros Organics (Geel, Belgium).

3.1.2. Extraction and derivatisation

Apart from a few details, the extraction procedure of method A basically follows the procedure outlined in Section 3.2 which had been published previously [7]. Briefly, 2 ml of blood were transferred into a 15 ml PP-tube (Greiner bio-one) and 2 ml of a 1.5 M KBr/2 M H_2SO_4 solution were added. The tube was sealed and vigorously shaken for 1 min. Following the addition of 5 ml of methyl *tert*-butyl ether (MTBE) the tube was shaken in a horizontal position for 60 min (Gerhardt-Schüttler) and subsequently centrifuged at 4000 rpm (Sigma 4-10, SIGMA Laborzentrifugen GmbH, Germany) for 3 min. The organic phase (4.5 ml) was transferred into a 20 ml headspace vial filled with 1 ml of citrate buffer. The vial was placed into a heating block and MTBE was removed by evaporation at 70 °C for 30 min. Accordingly, the vial was closed, and 100 μl of the sodium tetra-*n*-ethylborate solution was injected through the septum. After a reaction time of 15 min the sample was ready for analysis. As a minimum, each sample was extracted and analysed three times.

3.1.3. Instrumentation and MS analysis

The samples were measured using a coupled GC–EI-MS/ICP-MS system. This system was composed of a gas chromatograph 6890 N (Agilent Technologies, Waldbronn, Germany) equipped with a UNIS 2000 Inlet-System (Joint Analytical Systems, Moers, Germany), simultaneously connected both to a 5973N EI-mass spectrometer and to a 7500a ICP-MS (both instruments from Agilent Technologies, Waldbronn, Germany). The parallel detection was realised by a post-column split inside the GC oven connecting the column with two capillary lines which lead to the particular detectors as described elsewhere [8]. The operating conditions of this system are listed in Table 1.

The signals obtained by EI-MS were used for the identification of ethylated MeHg (MeHgEt), whereas the ion trace of *m*/*z* 202 (^{202}Hg) was used for quantification by ICP-MS (retention time: 14.7 min; including the derivatisation as well as the purge and trap time of 10 min.). A typical EI-mass spectrum of MeHgEt is shown in Fig. 1.

Table 1
Operating conditions of the GC–EI-MS/ICP-MS system.

GC	
Inlet (PTV)	Adsorbent: Supelcoport® SP-2100 phase Initial temperature: −100 °C for 5 min Heating rate: 800 °C min^{-1} (max.) Final temperature: 230 °C for 5 min
Injection	Split 1:5
Column	HP 5-MS; 30 m, 0.25 mm I.D., 25 μm (Agilent)
Oven	Initial temperature 35 °C for 14 min Heating rate 1: 15 °C min^{-1} Final temperature 1: 100 °C Heating rate 2: 100 °C min^{-1} Final temperature 2: 250 °C for 2 min
Carrier gas	He; head pressure: 3.89 bar
EI-MS	
Transferline temperature	280 °C
Mode	TIC (195–350 *m*/*z*)
Source temperature	230 °C
Ionisation energy	70 eV
ICP-MS	
Masses	^{200}Hg; ^{202}Hg
Argon flow	15 L min^{-1}
Carrier gas	0.53 L min^{-1}
Make-up gas	0.56 L min^{-1}
RF power	1580 W
Sampling depth	4.6 mm

Calibration was performed by subsequent addition of MeHg to a blood sample with very low mercury content. The sample was obtained from a volunteer who is on a special diet containing no fish or other animal proteins.

3.2. Method B: analysis by GC–MS using D_3-MeHg as internal standard

Method B involves the use of a simple commercially available headspace-GC–MS equipped with a coolable injection system. To prevent peak broadening the analyte is focussed prior to the GC separation. Tenax is used as adsorbent, which allows an effective focussing at moderate temperatures (5 °C). The detection system is a quadrupole MSD (HP5971) operating in the SIM mode. This system has been used for the routine determination of a multitude of different organic volatile compounds since many years.

3.2.1. Chemicals and reagents

All chemicals used were of analytical grade unless stated otherwise. Aqueous solutions of reagents were prepared with ultra-pure water (18.6 MΩ cm) using a Purelab ultra water purification system (SG, Barsbüttel, Germany). A 1% (w/w) solution of sodium tetra-*n*-ethylborate (LGC Standards GmbH, Wesel, Germany) was prepared in water. The solution was stored at −18 °C and protected from light. The acetate buffer solution (pH 5) was prepared by adding approx. 20 ml of concentrated hydrochloric acid to a solution of 100 g sodium acetate trihydrate in 180 ml water. Hydrochloric acid (37%), sulphuric acid (98%), potassium bromide, sodium acetate trihydrate, MTBE, methylmercury(II) chloride (>98%) and mercury(II) chloride were obtained from Merck (Darmstadt, Germany). D_3-Methyl magnesium iodide in diethylether (1 M) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

3.2.2. Preparation of D_3-MeHgCl

D_3-MeHgCl used as reference compound was prepared by conversion of D_3-methylmagnesium iodide in a Grignard reaction with an excess of $HgCl_2$ according to the synthesis described by Breitlinger and Herrmann [9]. The structure of D_3-$MeHgH^+$ was