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Methyl mercury in nail clippings in relation to fish consumption analysis with gas chromatography coupled to inductively coupled plasma mass spectrometry: A first orientation

Petra Krystek [a,*], Paulo Favaro [b], Peter Bode [b], Rob Ritsema [c]

[a] Institute for Environmental Studies (IVM), VU University, De Boelelaan 1087, Amsterdam, The Netherlands
[b] Delft University of Technology, Delft, The Netherlands
[c] National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

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ABSTRACT

For the identification of human exposure to one of the most toxic compounds, which is methyl mercury ($MeHg^+$), fingernail clippings were selected as the matrix of interest. Within this pilot study, six samples from different origins and from people with different food consumption patterns were chosen.

Species-analysis of $MeHg^+$ was performed according to the following procedure: dissolution of the sample material in tetramethylammonium hydroxide (TMAH), derivatisation of $MeHg^+$ with sodium tetraethylborate ($NaBEt_4$), extraction into iso-octane and measurement with gas chromatography hyphenated to inductively coupled plasma mass spectrometry (GC-ICPMS) for the quantification $MeHg^+$.

1. Introduction

An independent and graded association between nail clipping mercury levels and the risk of myocardial infarction was reported by Guallar et al., in 2002 [1]. They found that mercury masked an inverse association between docosahexaenoic acid (DHA or C22: 6n–3) levels and the risk of myocardial infarction. Although no conclusive evidence was available on the source and speciation of mercury, Guallar et al. [1] hypothesized that the correlation between mercury (Hg) and DHA suggests fish as the main source of mercury in nail clippings. As such, the Hg levels in nail clippings might also reflect intake of methyl mercury ($MeHg^+$) in humans. Recently, an in vivo study with long Evans Rats was carried out by using nails as a noninvasive indicator of $MeHg^+$ exposures by feed. In this case the Hg/Selenium (Se) ratio was measured in several organs and in nails [2]. Assessment of the chemical speciation of mercury in nail clippings with a focus on $MeHg^+$ attributes to expanding the understanding of this phenomenon.

The abundance of $MeHg^+$ and Hg^{2+} varies substantially in different parts of the biosphere. $MeHg^+$ values less than 1% of Hg as $MeHg^+$ have been reported for natural waters [3] but, especially for unfiltered seawater values as high as 5% [4,5] have been found. This relative contribution increases in some fish species to at least 70% [6], while in fish of prey a maximum of nearly 100% as $MeHg^+$ is possible [7].

The bioaccumulation of $MeHg^+$ in the human body is, given its toxicity, an issue of great interest. Regional case studies were carried out about metals in human hair and nails in Sweden where higher Hg concentrations were found in hair than in nails [8] The abundance of Hg, $MeHg^+$ and Se in scalp hair of inhabitants was investigated from Mediterranean areas [9]. Other special focus groups are pregnant women or the prenatal exposure of Hg and possible related effects [10–12]. Determined $MeHg^+$ in blood and total Hg in hair and showed positive association of both measurands, as well as the positive correlation with increasing fish consumption [13,14]. They also observed an increase in inorganic Hg in blood with increasing number of dental amalgam fillings [15]. Brockman et al. [16] found a good correlation between dietary $MeHg^+$ and nail Hg concentration in a study with rats, and they confirmed the observation of Mozaffarian et al. [17] that this deposition was not significantly disrupted by Se intake. Regarding to toenails, first results are known for using them as biomarkers, e.g. also of $MeHg^+$ exposure [17,18,19].

As these studies do not provide the reverse conclusion that Hg in nail is directly resulting from fish intake since it preserves its chemical speciation as $MeHg^+$, we undertook this pilot study for an identification of the total amount of $MeHg^+$ as well as total

* Corresponding author.
E-mail address: petra.krystek@vu.nl (P. Krystek).

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inorganic Hg^{2+} in nail clippings. The analytical procedure is based on gas chromatography (GC) and includes three steps: (1) dissolution or at least extraction of the species, (2) derivatisation and (3) measurements for quantification. On-line measurements of Hg species can be carried out by GC coupled to inductively coupled plasma mass spectrometry (GC-ICPMS) [20–22]. High performance liquid chromatography (HPLC) coupled to ICPMS [23–25] is also practiced and for faster processing alternative sample preparation procedure like a rapid ultrasound-assisted extraction procedure could be applied before [26,27]. Especially capillary electrophoresis (CE) coupled to ICPMS is used for very fast analysis of less than one minute [28]. For GC-ICPMS, two calibration strategies are common: (a) species-specific isotope dilution technique [29] and (b) advanced external calibration by multiple internal standard correction [22,30] as applied within this study.

2. Experimental

2.1. Reagents, standards and reference materials

Deionised water was purified by a Millipore system (Milli-Q, 18 M Ω cm). All chemicals were of analytical grade or of higher purity. Acetic acid (HAc) and sodium acetate (NaAc) were purchased from Merck, Darmstadt, Germany. Tetramethylammonium hydroxide (TMAH) was supplied by Fluka, Buchs, Switzerland. Sodium tetraethylborate ($NaBEt_4$) was purchased from ABCR, Karlsruhe, Germany. Iso-octane was obtained from Baker, Deventer, The Netherlands. $MeHg^+$ standards were prepared out of solid methyl mercury chloride (MeHgCl) from Riedel de Haan, Seelze, Germany. (Safety note: Organic mercury compounds are extremely toxic. Direct contact with skin can lead to death. During handling precautions are absolutely necessary, e.g. inhalation must be avoided and protective clothes must be worn [20,23]).

The internal standard for the sample pre-treatment was dibutyl-dipentyltin (DBT-pe), synthesized at the IVM laboratory of the VU University, Amsterdam, The Netherlands. The certified reference material "Lyophilized tuna fish", CRM 463 supplied by IRMM, Geel, Belgium, was used for verification of the degree of trueness of the results obtained by the procedure applied.

2.2. Instrumentation

A GC was on-line coupled via a heated interface to a quadrupole based inductively coupled plasma mass spectrometer (ICPMS) type HP 4500. All parts of GC-ICPMS were purchased from Agilent Technologies former Hewlett Packard, Amstelveen, The Netherlands.

Instrumental details about the GC and the interface are given in Table 1 and the temperature program of the GC is summarized in Table 2. Both the operating conditions and the methodical set-up for the ICPMS are given in Table 3. Sensitivity and stability are tuned by optimizing the continuous signal of $^{126}Xe^+$ which is in the GC carrier gas; see also Tables 1 and 3. The signal intensity must be $>150\,000$ counts per second and the relative standard deviation of this signal must be $<2\%$. Additionally, no significant background signal should be abundant on $^{200}Hg^+$ and $^{202}Hg^+$. The "ICP-MS Chromatographic Software C.01.00" from Agilent Technologies, former Hewlett Packard, Amstelveen, The Netherlands was used for data analysis with the following steps:

(1) Manual integration of the obtained chromatographic peaks of Hg and Sn while an interval of 0.1 min was used;
(2) Correction of the integrated peaks with the peak of Xe at the same point of time;
(3) Update of the internal standard (IS) file (DBT-pe);

Table 1
Instrumental details of GC and GC-ICPMS interface.

GC instrumentation	HP6890
V(injection)	1 μL; splitless
T(injection)	250 °C
Carrier gas	Helium (He) with 0.1% Xenon (Xe) (which is used as instrumental internal standard) supplied by Hoek Loos BV, Amsterdam, The Netherlands
Flow rate	6.5 mL/min with constant flow
Column	HP-1 (polydimethylsiloxane)
GC-ICPMS interface	Transfer line
Temperature control of interface via GC	
T(port 1)	280 °C
T(port 2)	280 °C

Table 2
GC temperature program.

T rate (°C/min)	*T* (°C)	*t* (min)
	50	1
10	60	
80	140	
40	170	
120	270	1.5
	280	

Table 3
Operating conditions and methodical set-up of ICPMS.

RF power	1220 W
Cool gas flow	15 L/min Ar
Auxiliary gas flow	1 L/min Ar
Sample gas flow	0.9 L/min Ar
Additional gas flow	25 mL/min air
Operation mode	With shield torch
Measured isotope	Integration time
$^{200}Hg^+$	70 ms
$^{202}Hg^+$	70 ms
as internal standards	
$^{120}Sn^+$	70 ms
$^{126}Xe^+$	50 ms
Total run time of method	8 min

(4) For all calibration points: Determination of the ratio Hg/IS(DBT-pe);
(5) Recalculation (update) the actual concentration of the standards with respect to the iso-octane phase;
(6) Calculation and display of the calibration curve;
(7) Integration and quantification of samples by following (1)–(3) and using the obtained calibration curve (6).

2.3. Samples of interest

Fingernail clippings were collected from persons of different geographical location (Brazil and The Netherlands) and hence wit different food consumption patterns. In addition, nail clippings were taken from two "reference" persons, i.e. persons with no special food consumption pattern. A total of six samples were analyzed (See Table 4). The fingernail clipping was collected from all ten fingers. The sample material was cut to a length of around 3 mm and pooled.

2.4. Sample preparation and measurements with GC-ICPMS

An aliquot of approximately 50 mg of dried fingernail clipping was cut in small pieces and weighed into a 50 mL tube and 5 mL TMAH was added. The sample material was totally dissolved after shaking the samples for 12 h (overnight).

The solution was further diluted with water to a total volume of 50 mL. An aliquot of 25 mL was transferred into a 50 mL tube and 8 mL buffer solution of 2 M HAc/ 2 M NaAc was added. DBT-pe, used as internal standard for possible evaporation of the extract during the sample pre-treatment, was dissolved in the organic solvent iso-octane with a concentration of 2%. Hereof 3 mL was added to the sample mixture. For derivatisation 3 mL of a freshly prepared solution of 1% $NaBEt_4$ in water was added and the mix was shaken for 30 min. To achieve complete derivatisation 1 mL of 1% $NaBEt_4$ in water was added and shaken again for 30 min. After centrifugation both phases were separated and an aliquot of the upper layer (organic phase) was transferred into a GC-vial. The measurements were carried out with the GC-ICPMS system according to the described procedure. Raw data were corrected with both used standards: DBT-pe for the derivatisation and extraction process and Xe (from the GC carrier gas) for the measurements.

A standard stock solution was prepared by dissolving MeHgCl in 0.04% HCl with a concentration of 100 µg/L Hg as $MeHg^+$. For the calibration of $MeHg^+$ different dilutions were prepared from the stock solution. In addition a blank was prepared. These solutions were pre-treated (ethylated and extracted) according to the same described procedure of samples.

Table 4
Qualitative results of two Hg-species in fingernails.

Sample name	Significance of $MeHg^+$	Significance of Hg^{2+}
(1) Brazilian fisherman A	Yes	Yes
(2) Brazilian fisherman B	Yes	Yes
(3) Brazilian reference person	Little	Little
(4) Dutch vegetarian with fish consumption	Yes	Yes but less than (1)+(2)
(5) Dutch vegetarian without fish consumption	No	Little
(6) Dutch reference person	No	Yes but less than (1)+(2)

2.5. Accuracy, quality control aspects and other methodical aspects

Reference materials, certified for the amount of $MeHg^+$ in nail do not exist, and hence another biological matrix (lyophilized tuna fish; IRMM CRM 463) was used for quality control, in particular for assessing the degree of trueness. For the speciation by GC-ICPMS, 100 mg of CRM 463 was taken and treated according to the described procedure.

In addition, a known amount of $MeHg^+$ was spiked to a duplicate sample of fingernails for the calculation of the recovery. Other methodical details are also described in [22].

3. Results and discussion

3.1. Nail clippings

The chromatograms from the six samples of nail clippings were firstly qualitatively interpreted. An example is given in Fig. 1. The achieved results of all samples are summarized in Table 4. Both $MeHg^+$ and Hg^{2+} are detectable in all samples analyzed. $MeHg^+$ is significantly abundant in fingernails from persons who eat fish regularly.

Within this study the quantification of Hg^{2+} is not aimed to be of interest. As described above, the aim is the identification of $MeHg^+$. The different results are given in Fig. 2. On-going investigations about the determination of the total concentration of Hg in nails clippings by instrumental neutron activation analysis (INAA) shows that the total concentration from thirty different samples is in the range between <30 and 700 ng Hg/g nails [31]. In correlation with the obtained results for $MeHg^+$ (see also Fig. 2), it can be concluded that a maximum of around one fifth of the total Hg can be abundant in the form of $MeHg^+$. These first results should be controlled by e.g. a large exposure study with significantly more participants.

3.2. Quality control materials

All results of quality control experiments are summarized in Table 5. All recoveries of $MeHg^+$ were in the range of 95%–103% of the certified value (3.04 µg/g $MeHg^+$).

Spike experiments of a known amount of $MeHg^+$ to the matrix of fingernails were performed several times and led to a recovery of 75%–95%. In addition, one sample was analyzed as independent duplicate and these results show a good agreement on a practice relevant concentration level (Fig. 2).

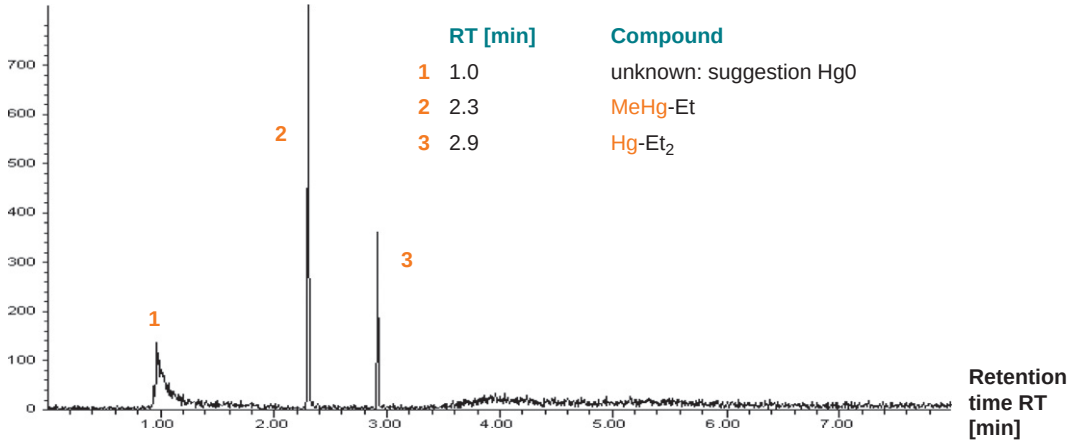

Fig. 1. Chromatogram of sample 4) Dutch vegetarian with fish consumption. Monitored isotope: ^{202}Hg.

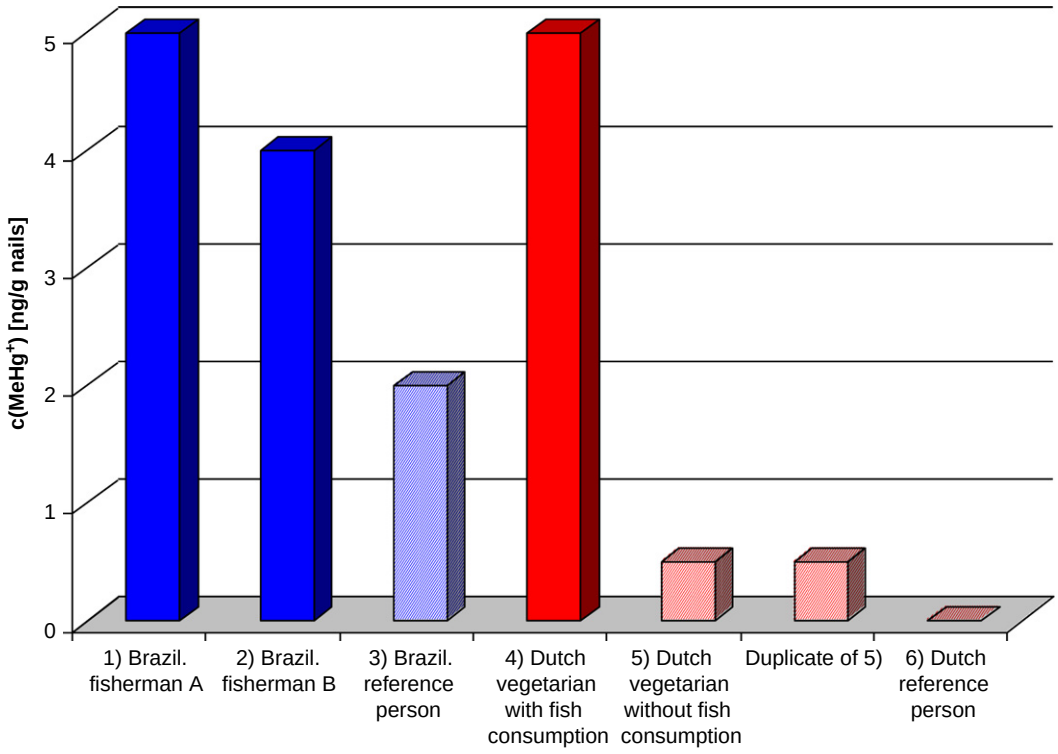

Fig. 2. First quantitative results of $MeHg^+$ in fingernails.

Table 5
Results of quality control experiments regarding to justness.

Experiment	Results
Addition of a known amount of $MeHg^+$ to determine recovery percentages in the matrix	$(n=4):(85 \pm 10)\%$
Analysis of a certified reference material (CRM 463 tuna fish)	$(n=4):(99 \pm 4)\%$

4. Conclusions

The presented results are very promising to focus more closely on the correlation of $MeHg^+$ in fingernails and the frequent fish consumption. Both experiments of quality control show good results and the developed procedure should be applied for an extended monitoring study about human exposure to $MeHg^+$ by fish consumption. Within this study, also the total amount of Hg will be determined. It will complete the existing list of biomarkers for Hg and $MeHg^+$, which are up to now, blood, hair and toenails, by the easily accessible matrix of fingernails.

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