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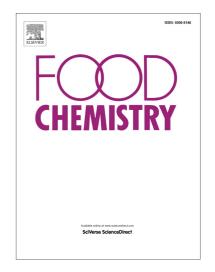
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Methylmercury varies more than one order of magnitude in commercial European rice

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Abstract

Rice is known to accumulate methylmercury (MeHg) in the rice grains. MeHg as a neurotoxin impacts on the human central nervous systems and especially on the developing brain. In this exploratory study, 87 commercial rice products sold in Europe, including nine baby-rice products, were analyzed for total Hg and MeHg content. MeHg concentration in all rice products investigated range from 0.11 to 6.45 μ g kg⁻¹ with an average value of 1.91 \pm 1.07 μ g kg⁻¹ and baby-rice is not significantly different from other rice products. Total Hg ranges from 0.53 to 11.1 μ g kg⁻¹ with an average of 3.04 \pm 2.07 μ g kg⁻¹. MeHg concentrations in all rice products studied in this work would not exceed the provisional tolerable weekly intake (PTWI). 30 % of all commercial market rice products exceeded 10% of the PTWI calculated for toddlers or 13 % of products for adults with rice based diet.

1. Introduction

Mercury is a severe environmental problem with adverse effects on living organisms and ultimately on us as human beings. Studies have shown potential evidence of effects on fetal growth and neurocognitive effects in early childhood through neurotoxic methylmercury (MeHg) transferred through the placenta and food (Karagas et al. 2012). The Minamata convention, a global treaty for the reduction of mercury, emphasizes the awareness and urgency of mercury as a global pollution problem (Minamata Convention 2014). In general the main exposure of humans to methylmercury is through the consumption of marine fish and especially predatory species (Mergler et al. 2007; Fitzgerald et al. 2007).

In addition to seafood, rice was recently identified as a major exposure source to methylmercury (MeHg) in areas where rice is grown on mercury contaminated soil (Li et al. 2012). Rice is a staple food with a production of almost 500 M tons (milled basis, data from 2013) and provides about 20 % of the world's dietary energy supply (FAO 2016). This can be as high as 65-75 % in some Asian countries like Bangladesh and the Laos (FAO 2016), with rice consumption around 400 g per day.

The European rice consumption is approx. 2.6 million tonnes, and ~40 % of rice is imported, with India, Cambodia, Thailand and Pakistan the major exporting countries. (ec.europa.eu factsheet 2013-14).

The Standardization Administration of China has set the limit for total Hg in cereals, including rice, to $20 \mu g \ kg^{-1}$ (Shao et al. 2014). While the maximum level is for total Hg, it is actually the MeHg concentration which is more important: Clarkson and Magus 2006 showed that 95 % of MeHg is absorbed via the human gastrointestinal tract from seafood, while inorganic Hg (iHg) is only

absorbed to 7%. This is the reason why the European Food Safety Authority (EFSA) suggested a provisional tolerable weekly intake (PTWI) of 1.3 μg week⁻¹ kg⁻¹ b.w. for MeHg, not total Hg. Hence, speciation of methylmercury in rice is important.

Extensive research has been carried out in the mercury polluted province of Guizhou, China, over the last decade, especially on the uptake of MeHg by rice in the local paddy fields and the impact on humans (Meng et al. 2011). Li et al. 2012 pointed out that rice is a main MeHg source for people in the Guizhou region and Feng et al. 2008 showed that the elevated MeHg concentration in rice is bioavailable. The exposure from rice consumption in the Guizhou region was comparable with an exposure from a moderate fish diet (Li et al. 2012). Therefore, rice needs to be considered as a provider of MeHg and iHg in general in our diet and should therefore be critically evaluated. However, data on MeHg concentrations in commercial rice from supermarkets in Europe is extremely scarce (Horvat 2003).

Analytically, the main problem in the determination of MeHg speciation in rice is that MeHg is in low ppb levels, and analysis is difficult due to the starch based matrix. No appropriate rice based certified reference material for MeHg is currently available. Therefore, it is pertinent to implement quality controls. So far, the methods used in previous rice surveys (Horvat 2003; Meng et al. 2014) have used seafood based CRMs such as DOLT, DORM and TORT. These however have far higher levels of MeHg (up to several orders of magnitude), and have a different matrix which is easier to digest. Also, the analytical method used has been based on US-EPA method 1630, which is only validated for water samples.

We have previously developed a new method based on solid phase extraction and preconcentration followed by online separation using liquid chromatography with cold vapor atomic fluorescence detection (SPE-HPLC-CV-AFS). The method has been shown to be applicable for low levels of MeHg in water (Brombach, Chen et al. 2015), sewage and sediment (Brombach, Gajdosechova et al. 2015) and more importantly in rice (Brombach, Ezzeldin et al. 2015). The method was validated using standard addition in a series of biological CRMs, and by comparison with species-specific isotope dilution-gas chromatography coupled to ICP-MS (SS-ID-GC-ICPMS). Isotopically enriched Hg species (¹⁹⁹Hg²⁺ and Me²⁰¹Hg) were directly spiked into the sample prior extraction; this approach has been shown to account for possible loss or artifact generation of the analyte (Brombach, Gajdosechova et al. 2015). In order to compare MeHg results between the two methods, a sub-set of samples (n=19) was analysed with both methods for a variety of rice and rice based products as a validation approach for the MeHg results in the rice, showing a linear trendline (Fig.1).

The aim of this exploratory study is to identify the exposure of the public to MeHg from a diet of commercial rice products bought in Europe. We purchased a variety of rice products (n=87) in supermarkets and from Asian Food suppliers in Europe, including mainly rice grains, but also rice flour (w/o added vitamins and minerals, and pre-cooked baby-food rice products), and processed rice in form of rice noodles and rice crackers. We especially included in our survey processed baby rice products, since rice is a major carbohydrate source for weaned babies up to one year of age (Mennella et al. 2006). Toddlers eat about three times more than adults in relation to their body weight and are at a critical age where the brain is developing, thus the impact of neurotoxins like MeHg is most critical. Finally, MeHg exposure from the consumption of commercial rice products was calculated for toddlers and adults for all 87 rice samples investigated, and compared to the current PTWI.

2. Materials and methods

2.1. Chemicals and standards

A stock solution of methylmercury chloride (Sigma-Aldrich, UK) was prepared in methanol (AnalaR grade; VWR, UK) at 10,000 mg L⁻¹ as Hg. Further working dilutions were prepared in 0.3 M HCl. An Aquatron water still A4000D (Bibby Scientific Limited, Stone, UK) was used for obtaining double-distilled water, and ensures separation of inorganic Hg and MeHg on the column. The mobile phase for HPLC consists of 75% (v/v) methanol (AnalaR grade; VWR, UK) with 1.5 mM ammonium pyrrolidine dithiocarbamate (~99 %; Sigma-Aldrich, UK) in double-distilled water. The oxidant is a solution of 0.01 M Tritrisol® bromide/bromate (Merck, Darmstadt, Germany) and 1.2 M hydrochloric acid (AnalaR grade; VWR, UK) in double-distilled water, and the reductant is a solution of 2 % (m/v) tin(II) chloride (purchased as tin(II)chloride dihydrate (98 %) from Alfa Aesar, UK), and 1.2 M hydrochloric acid (AnalaR grade; VWR, UK) in double-distilled water. The preconcentration material is a mixture of thiol and thiourea bound to silica and can be purchased from PS Analytical, UK (PSA L820K005). Tetramethylammonium hydroxide (TMAH, 99.9999 % (metal basis); Alfa Aesar, UK) was used for alkaline digestion of the rice. 0.5 M acetate-acetic acid buffer was prepared with acetic acid (100 %, HiPerSolv® Chromanorm; VWR, UK), and NaOH (laboratory reagent grade; Fluka Analytical, UK) was used for pH adjustment.

For SS-ID-GC-ICPMS, mercury species were derivatised with sodium tetra(n-propyl)borate (Chemos, Germany) and extracted into 2,2,4-trimethylpentane (Chromasolv® Plus, for HPLC, ≥ 99.5 %; Sigma-Aldrich, UK). Me²⁰¹Hg for SSID-ICP-MS was prepared from ²⁰¹HgO according to literature (Rodriguez Martin-Doimeadios et al. 2002), Nitric acid (69%, AnalaR; VWR, UK) was used for open vessel

digestion of rice for total mercury determination. 0.45 μ m filter discs (Iso-DiscTMFilters, PTFE, 25 mm \times 0.45 μ m; Supelco, USA) were used for filtration.

2.2. Samples

A total amount of 87 samples was purchased in supermarkets in the United Kingdom (samples 1 to 19) and other countries (Germany and Switzerland). Samples 1 to 7 and 20 to 87 represent rice grains of Indian Basmati (n=8), Long Grain rice (n=14), Jasmin rice (n=6), Sweet rice (n=5), wild rice (n=3), Japonic rice (n=24), Risotto rice (n=2), Indica (n=1), Paella rice (n=1), white rice (n=1) and unclassified white rice (n=10). Sample 8 and 9 are rice noodles, sample 10 is rice flour, sample 11 to 17 are precooked milled baby-food rice for the preparation of rice porridge, and sample 18 and 19 are rice cakes for toddlers (Table S1, supporting Information).

All rice in this study used packed rice from supermarkets in the UK for direct consumption; the normal pack size is 1 kg, and we homogenised the rice grains before and after milling; final sample size was approx. 100 g; the samples were homogenised prior aliquoting. Rice samples 1 to 19 were ground into a fine powder (< 0.5 mm) with a Coffee grinder (Krups F203 Grinder, Krups, Germany) and stored dry at room temperature prior to analysis. Rice samples 20 to 87 were ground (< 0.5 mm) with an ultra-centrifugal mill (ZMI, Retsch, Germany).

2.3. Total Hg determination

To 200 mg milled rice, 4.0 mL nitric acid was added, left standing overnight and then digested for 1 h at 120 °C in a hot block. 1.6 mL 0.01 M bromide/bromate was added to the digest and subsequently heated for another 1 h in a hot block. After cooling, the solution was diluted with double-distilled water to 40.0 mL and analyzed with cold vapor-atomic fluorescence spectrometry (CV-AFS; Millennium Merlin, P.S. Analytical Ltd., Orpington, UK), with an LOD of 0.02 μ g/kg and LOQ of 0.06 μ g/kg, respectively, and recovery 95.2 \pm 1.6 %, as HgT in rice.

2.4. MeHg determination using SPE-HPLC-CV-AFS

The methodology for MeHg determination in different matrices was published previously by Brombach, Gajdosechova et al. 2015 and for rice Brombach, Ezzeldin et al. 2015. Briefly, approx. 300 mg milled rice is digested with 3 mL of 25 % (m/v) TMAH solution in a laboratory microwave system (Mars 5, CEM, US) using open vessel digestion @ 1600 W for 20 min at 55 °C and 20 min at 60 °C. After this first digestion run, 2 mL hydrochloric acid is added to the TMAH-rice suspension, the mixture is shaken and a second extraction follows in the microwave for 20 min at 55 °C and 20 min at 60 °C. The extract is centrifuged at 13226 g (13000 rpm; Micro Centaur centrifuge, MSE, UK) and

filtered through 0.45 μ m filter discs. The solution is topped up to 40 mL with double-distilled water. 35 mL of this sample was submitted to pre-concentration on the thiol/thiourea-based silica material, and subsequently eluted and online separated using RP-HPLC. The eluted species were oxidized online by bromine/UV irradiation and detected by CV-AFS (Millennium Merlin, P.S. Analytical Ltd., Orpington, UK). A detailed description of the instrumentation, parameters and performance is given in an earlier publication (Brombach, Chen et al. 2015). The method shows no matrix effect for the rice and no Hg species interconversion during digestion. This was confirmed by enriched isotope spiking of 199 Hg²⁺ into the original sample, showing that no artificial methylation or MeHg oxidation to Hg²⁺ took place. Additionally, standard addition into the original sample was used to confirm further that MeHg was stable during sample preparation. (Brombach, Ezzeldin 2015). The instrument was calibrated daily with solutions of 2.5, 5, 10, and 20 ng L⁻¹ MeHg (35 mL preconcentration volume), which resulted in an LOD for MeHg of 0.12 μ g/kg and LOQ of 0.36 μ g/kg, respectively.

2.5. MeHa determination using SS-ID-GC-ICPMS

For SS-ID-GC-ICPMS, the same double digestion/extraction was used, but here for 600 mg rice as described for pre-concentration HPLC-CV-AFS. The only modification was the addition of only 1.38 mL HCl instead of 4 mL HCl for the second extraction, which was necessary to enable buffering to pH 3.9 as needed for the derivatization procedure. The suspension was centrifuged and the supernatant spiked with Me²⁰¹Hg, left standing for equilibration, buffered with 5 mL 0.5 M acetic acid-acetate buffer (pH 3.9) and overlayed with 1 mL 2,2,4-trimethylpentane. 1 mL of 1 % sodium tetra(n-propyl)borate was added and the mixture shaken for 10 min to extract the propylated mercury species into the organic layer. After centrifugation at 1650 g (3500 rpm; ALC 4218 centrifuge, ALC International S.R.L., Italy), the organic layer (approx. 0.5 mL) was removed and preconcentrated by evaporation to ~20 to 50 μ L. 2 μ L of this solution was injected into the GC-ICP-MS system (Agilent 6980 GC coupled to Agilent 7500c ICP-MS via an in-house-built heated transfer line). The exact instrumental setup and conditions are described in an earlier paper (Brombach, Gajdosechova et al. 2015);LOD for MeHg was calculated to 0.12 μ g/kg and LOQ of 0.36 μ g/kg, as MeHg in rice. Recovery for MeHg by SSIDMS was calculated versus SPE-HPLC-CV-AFS (Fig. 1), as no CRM for MeHg in rice exists, and gave a relative recovery of 103.8 \pm 3.84.

3. Quality Control and data analysis

For quality control, a series of biological certified reference materials (NRCC-DOLT-4, NRCC-DORM-3, NRCC-TORT-2, IAEA-085, NIES CRM No. 13, ERM®-CC580) have been analyzed previously and reported recently elsewhere (Brombach, Chem et al. 2015; Brombach, Gajdosechova et al. 2015; Brombach, Ezzeldin et al. 2015). Specifically the SS-ID-GC-ICPMS method has been validated for the rice matrix in our previous paper by using direct spiking into the rice powder (Brombach, Gajdosechova et al. 2015). Here, we use a subset of 19 samples with this validated method and the newly developed SPE-HPLC-CV-AFS. All samples were extracted in duplicates and the variability was recorded to be less than 10%. Data analysis was performed using a one-tailed Student's *t*-test if not mentioned otherwise, and the P-values were reported accordingly.

4. Results and discussion

Total Hg was determined with CV-AFS after open vessel digestion in a hot block, as outlined in 2.3, and rice flour standard reference material 1568a (NIST, USA) was used for quality control. Total Hg recovery in the standard reference material was $95.2 \pm 1.6 \%$ (n=10), and not significantly different from the certified value of $5.8 \pm 0.5 \,\mu g \, kg^{-1}$ (p>0.05).

Total Hg in the rice samples varied from 0.53 to 11,1 μ g kg⁻¹ (Table S1, supporting Information). The average concentration of total Hg in the 87 different samples is 3.04 \pm 2.7 μ g kg⁻¹. This means that the total Hg in commercial market rice products varies by more than a factor of 20.

For quality control of the MeHg analysis, both independent methods were used. For comparison, the two concentrations obtained for MeHg from each rice sample were plotted against each other (Figure 1). A good correlation with an R² of 0.972 was obtained. The slope of 1.013 showed that both results agree well at high and low MeHg levels in rice ((p=0.33), paired two tailed Student's *t*-test). It can been concluded that the MeHg concentration in rice can be deemed to be accurate, since the two orthogonal methods are based on two different analytical method principles, with and without derivatisation. An F-test revealed that the variances of both methods are not significantly different with an average RSD of 9.1 % (p>0.05). Data are shown in Table S2. The analysis with the two orthogonal methods show accurate results for MeHg in the rice matrix, hence all other samples were measured by the simpler SPE-HPLC-CV-AFS method.

Insert Figure 1:

The concentration of MeHg in all 87 rice products varies from 0.11 to 6.45 μ g kg⁻¹ with an average concentration of 1.91 \pm 1.07 μ g kg⁻¹. The variability of the MeHg concentration in the commercial market samples varies by a factor of 58 i.e. a higher variation than for total Hg data.

A general observation is that the rice samples mainly contain mercury in the form of MeHg, with the mean percentage of MeHg in the 87 samples of 71 \pm 26 %. Figure 2a shows that at low total Hg concentrations, the MeHg contribution varies between 6 and 100 % while at total Hg concentrations above 6 μ g kg⁻¹, the maximum MeHg contribution is 60 % (Figure 2b). This means that the MeHg concentration cannot be estimated from the total Hg concentrations and it makes the direct determination of MeHg mandatory to identify the exposure to neurotoxic MeHg. Furthermore, it seems that rice accumulating higher levels of Hg have less methylated mercury, which seems in line with previously published results from polluted Hg regions. The rice from polluted areas (Guizhou region) has generally lower proportions of MeHg vs total Hg, as reviewed recently by Rothenberg et al. 2014. Only a few publications describe MeHg and total Hg in rice from non-contaminated sites with a main focus on China (9 publications) and Cambodia (see review by Rothenberg et al. 2014). Considering only those publications where the MeHg proportion was given, the reported methylation rate ranged from 8.5 to 75 % with an average methylation rate of 34 %. This is variable but is lower than the proportion of MeHg in the commercial market rice found in this study.

Soil chemistry and agricultural technique with flooded and non-flooded periods of the paddy soils have shown to influence the MeHg concentration in rice (Meng et al. 2011; Stubner et al. 1998; King et al. 2000, Wang et al. 2014), and the different rice cultivars with differening phytochelatin amounts may influence the total Hg in rice (Rothenberg et al. 2012; Meng et al. 2010, Krupp et al. 2009). Therefore it is conceivable that commercial market rice of different type and origin may vary significantly in MeHg concentration and proportion to inorganic Hg. Small differences in methylation rates may have a large effect on the MeHg concentration in the rice grain since the bioaccumulation factors for MeHg have been reported to be on average 800 times higher than for iHg (Zhang et al. 2010).

Insert Figure 2 a,b

It is interesting that polished and whole grain rice do not show any significant differences in total Hg (p=0.22) and MeHg (p=0.39) concentration (Figure S1, Supporting Information). Hence, it can be concluded that iHg and MeHg are homogenously distributed and cannot be removed by polishing,

which is in contrast to arsenic and other nutrients such as zinc (Meharg et al. 2008), although this is in contrast with recently reported literature (Zhao et al. 2014).

MeHg and total Hg concentrations in Japonic rice (n=24), Basmati rice (n=8), wild rice (n=3), sweet rice (n=5), Jasmin rice (n=6) and Long grain rice (n=14) were not significantly different (p>0.05). Likewise, no difference could be found between white (n=58), brown (n=12) and black rice (n=5) (Figure S2, S3, Supporting Information). However, all pre-cooked baby rice products (n=9) were significantly lower in total Hg (1.85 \pm 0.71 μ g kg⁻¹) than those of other rice products (3.25 \pm 2.14 μ g kg⁻¹) (p=0.046), while the MeHg concentration of baby rice products (1.71 \pm 0.73 μ g kg⁻¹) was not significantly different to that of all other rice products tested in this study (1.98 \pm 1.10 μ g kg⁻¹, p=0.29) (Tables S1 and S2, Supporting Information). Although, the type and origin of rice was not the same, the data suggests that cooking /processing in preparation for baby food seems not to remove MeHg but removes significant amounts of inorganic mercury. This however needs to be substantiated in a further, more detailed study using the same rice products. It should be noted that both rice noodle samples represent the lowest concentration of MeHg in this study (see Figure S2).

Furthermore, rice products labelled "organic" (n=13) contained the same concentration of total Hg (p=0.19) and MeHg (p=0.62) compared to the other rice products (n=74), however the degree of methylation was significantly lower for organic rice (59 +/- 26 %) than for conventionally grown rice (73 +/- 25 %) (p=0.04) (Figure S4, Supporting Information). Whether this is a result of the agricultural technique needs to be confirmed in a specifically designed study.

Rice of European origin (n=12) is not significantly different from Chinese (n=8), Taiwanese (n=19), Thai (n=6) and USA/Canada rice (n=6) in terms of their total Hg or MeHg (p>0.05), and the variability of total Hg and MeHg of European rice is more than one order of magnitude (max/min = 59 for MeHg and 21 for total Hg) with the highest measured sample to be 6.45 ng/g from a European country. This is in contrast to the rice from other countries of origin which did not show such a variability in MeHg and total Hg (Figure 3 and Figure S5).



According to the results of this exploratory study, the complete data set can be used to evaluate food safety aspects regarding MeHg levels. The European Food Safety Authority (EFSA 2012) has set a new provisional tolerable weekly intake (PTWI) for MeHg of 1.3 µg kg⁻¹ bodyweight (b.w.), which is lower

than the established value from 2004 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of $1.6 \mu g/kg$ bodyweight (FAO 2007).

In a previous study, the daily intake of a consumer group with a rice based diet (Bangladeshi group in the UK) has been determined to be 357 \pm 295 g (range 28-1200 g) daily while in the UK the daily intake is about 17 g (Cascio et al. 2011). The PTWI can then be calculated by using a daily rice consumption of 400 g , the maximum MeHg concentration of 6.45 ng/g, and a body weight of 65 Kg . This would result in a weekly uptake of 0.28 μg MeHg kg^{-1} b.w. which amounts to 21 % of the PTWI. Also, 11 out of the 87 rice samples have concentrations which would reach 10 % of the PTWI for MeHg through rice eating. For the extreme case of rice consumption of 1200 g rice, 64 % of the PTWI would be reached, while the average British citizen just reaches 1% of the PTWI for MeHg.

A weaned baby at the age of one year typically eats a portion of 20 to 25 g rice in a single serving. Considering three servings are given per day, the corresponding MeHg consumption of baby-food rice varies between 0.5-1.88 μ g per day. A child at the age of one year weighs 9.25 kg (i.e., the average of the median weight for a boy (9.6 kg) and a girl (8.9 kg) at the age of 1 year) (WHO 2006) and the weekly intake would therefore calculate to 0.05-0.20 μ g kg⁻¹ b.w. Taking into account the highest MeHg concentration in a rice sample in this study, it increases to 0.38 μ g kg⁻¹ b.w. week⁻¹.

Although MeHg in baby-food rice is below the recommended maximum level (up to 28 % of PTWI), the established tolerable weekly intake defined by EFSA, it should be taken into account that toddlers have up to three times the intake of adults. This is not considered in the calculations, and at this age, small children are especially vulnerable to the neurotoxin MeHg since the brain is just developing. Overall 30 % (26 out of 87) of the tested rice reached more than 10 % of the PTWI for toddlers, which is concerning in particular when the large variability of the MeHg concentration amongst the sample cohort of 87 samples is considered (Figure 4).

Insert Figure 4

5. Conclusions

87 rice samples were analyzed for MeHg and total Hg. The MeHg concentration in baby food is not significantly different from other rice products, which is of concern because MeHg is a neurotoxin which is most effective in the developmental stages of the brain. Although the maximum level of MeHg in our study does not reach the PTWI, it was evidenced that the MeHg concentration in the rice samples vary by a factor of 58. Especially the %age variation of MeHg from 6 to 100 % shows

that no assumption on the MeHg level of rice products can be made on the basis of total Hg. Therefore there is an urgent need to further survey commercial market rice-based products, with a focus on rice-products for baby and toddlers, and must determine MeHg concentration, not total Hg only.

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References

Brombach, C.-C., Chen, B., Corns, W. T., Feldmann, J., & Krupp, E. (2015). Methylmercury in water samples at the pg/L level by online preconcentration liquid chromatography cold vapour-atomic fluorescence spectrometry. *Spectrochimica Acta B* 105, 103-108.

Brombach, C.-C., Gajdosechova, Z., Chen, B., Brownlow, A., Corns, W., Feldmann, J., & Krupp, E. (2015). Direct online HPLC-CV-AFS method for traces of methylmercury without derivatisation: a matrix-independent method for urine, sediment and biological tissue samples. *Analytical & Bioanalytical Chemistry 407* (3), 973-981.

Brombach, C.-C., Ezzeldin, M. F., Chen, B., Corns, W. T., Feldmann, J., & Krupp, E. M. (2015). Quick and robust method for trace determination of MeHg in rice and rice products without derivatisation. *Analytical Methods*, 7(20), 8584-8589.

Cascio, C., Raab, A., Jenkins, R. O., Feldmann, J., Meharg, A. A., & Haris, P. I. (2011). The impact of a rice based diet on urinary arsenic. *Journal of Environmental Monitoring*, 13 (2), 257-265.

Clarkson, T. W., & Magos, L. (2006). The toxicology of mercury and its chemical compounds. *Critical-Reviews in Toxicology, 36* (8), 609-662.

EFSA (2012). EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985 [241 pages]. doi:10.2903/j.efsa.2012.2985; Available online: www.efsa.europa.eu/efsajournal.

http://ec.europa.eu/agriculture/cereals/trade/rice/economic-fact-sheet_en.pdf; (accessed 23.06.2016) (accessed

FAO (2007), Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives: Evaluation of certain food additives and contaminants, 2007. http://www.who.int/ipcs/publications/jecfa/reports/trs940.pdf (accessed 11.04.2015).

FAO (2016). Food and Agriculture Organization of the United Nations (FAO). Rice and human nutrition, http://www.fao.org/rice2004/en/f-sheet/factsheet3.pdf (accessed 18.02.2016).

Feng, X. B., Li, P., Qiu, G. L., Wang, S., Li, G. H., Shang, L. H., Meng, B., Jiang, H. M., Bai, W. Y., Li, Z. G., & Fu, X. W. (2008). Human exposure to methylmercury through rice intake in mercury mining areas, guizhou province, china. *Environmental Science & Technology*, 42 (1), 326-332.

Fitzgerald, W. F., Lamborg, C. H., & Hammerschmidt, C. R. (2007). Marine biogeochemical cycling of mercury. *Chemical Reviews*, *107* (2), 641-662.

Horvat, M., Nolde, N., Fajon, V., Jereb, V., Logar, M., Lojen, S., Jacimovic, R., Falnoga, I., Liya, Q., Faganeli, J., & Drobne, D. (2003). Total mercury, methylmercury and selenium in mercury polluted areas in the province Guizhou, China. *Science of the Total Environment*, 304 (1–3), 231-256.

Karagas, M. R., Choi, A. L., Oken, E., Horvat, M., Schoeny, R., Kamai, E., Cowell, W., Grandjean, P., & Korrick, S. (2012) Evidence on the Human Health Effects of Low-Level Methylmercury Exposure. *Environmenatl Health Perspective*, *120* (6), 799-806.

King, J. K., Kostka, J. E., Frischer, M. E., & Saunders, F. M. (2000). Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments. *Applied Environmental Microbiology*, 66 (6), 2430-2437.

Krupp, E. M., Mestrot, A., Wielgus, J., Meharg, A. A., & Feldmann, J., The molecular form of mercury in biota: identification of novel mercury peptide complexes in plants. *Chemical Communications*. **2009**, (28), 4257-4259.

Li, P., Feng, X., Yuan, X., Chan, H. M., Qiu, G., Sun, G.-X., & Zhu, Y.-G. (2012). Rice consumption contributes to low level methylmercury exposure in southern China. *Environmental International*, 49, 18-23.

Meharg, A. A., Lombi, E., Williams, P. N., Scheckel, K. G., Feldmann, J., Raab, A., Zhu, Y., & Islam, R. (2008). Speciation and Localization of Arsenic in White and Brown Rice Grains. *Environmental Science & Technology*, 42 (4), 1051-1057.

Meng, B., Feng, X., Qiu, G., Cai, Y., Wang, D., Li, P., Shang, L., & Sommar, J. (2010). Distribution Patterns of Inorganic Mercury and Methylmercury in Tissues of Rice (Oryza sativa L.) Plants and Possible Bioaccumulation Pathways. *Journal of Agriculture and Food Chemistry*, *58* (8), 4951-4958.

Meng, B., Feng, X., Qiu, G., Liang, P., Li, P., Chen, C., & Shang, L. (2011) The Process of Methylmercury Accumulation in Rice (Oryza sativa L.). *Environmental Science & Technology, 45* (7), 2711-2717.

Meng, M., Li, B., Shao, J.-J., Wang, T., He, B., Shi, J.-B., Ye, Z.-H., & Jiang, G.-B. (2014). Accumulation of total mercury and methylmercury in rice plants collected from differ mining areas in China. *Environmental Pollution*, *184*, 179-186.

Mennella, J. A., Ziegler, P., Briefel, R., & Novak, T. (2006). Feeding Infants and Toddlers Study: The Types of Foods Fed to Hispanic Infants and Toddlers. *Journal of American. Dietary. Association, 106* (1), 96-106.

Mergler, D., Anderson, H. A., Chan, L. H. M., Mahaffey, K. R., Murray, M., Sakamoto, M., & Stern, A. H., (2007). Methylmercury exposure and health effects in humans: A worldwide concern. *Ambio, 36* (1), 3-1.

Minamata Convention (2014). Minamata Convention on Mercury - Text and Annexes. http://www.mercuryconvention.org/Portals/11/documents/Booklets/Minamata%20Convention%20 on%20Mercury booklet English.pdf (accessed 28.08.2014).

Rodríguez Martín-Doimeadios, R. C., Stoichev, T., Krupp, E., Amouroux, D., Holeman, M., & Donard, O. F. X. (2002). Working methods paper: Micro-scale preparation and characterization of isotopically enriched monomethylmercury. *Applied Organometallic Chemistry*, *16* (10), 610-615.

Rothenberg, S. E., Feng, X., Zhou, W., Tu, M., Jin, B., & You, J. (2012). Environment and genotype controls on mercury accumulation in rice (Oryza sativa L.) cultivated along a contamination gradient in Guizhou, China. *Science of the Total Environment, 426* (0), 272-280.

Rothenberg, S. E., Windham-Myers, L., & Creswell, J. E. (2014). Rice methylmercury exposure and mitigation: A comprehensive review. *Environmental Research*, 133, 407-423.

Shao, Y., Wang, J., Chen, X., & Wu, Y. N. (2014). The consolidation of food contaminants standards in China. *Food Control*, 43, 213-216.

Stubner, S., Wind, T., & Conrad, R. (1998). Sulfur oxidation in rice field soil: Activity, enumeration, isolation and characterization of thiosulfate-oxidizing bacteria. *System. Appled Microbiology*, *21* (4), 569-578.

Wang, X., Ye, Z. H., Li, B., Huang, L. N., Meng, M., Shi, J. B., & Jiang, G. B. (2014), Growing Rice Aerobically Markedly Decreases Mercury Accumulation by Reducing Both Hg Bioavailability and the Production of MeHg. *Environmenatl Science & Technology*, 48 (3), 1878-1885.

WHO (2006) WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass indexfor-age: Methods and development. Geneva: World Health Organization (312 pages).

Zhang, H.; Feng, X. B.; Larssen, T.; Shang, L.; Li, P. (2010) Bioaccumulation of Methylmercury versus Inorganic Mercury in Rice (Oryza sativa L.) Grain. *Environmental Science & Technology*, 44 (12), 4499-4504.

Zhao, J., Li, Y. F., Li, Y. Y., Gao, Y., Zhao, Y., & Chai Z.F. (2014) Selenium modulates mercury uptake and distribution in rice (Oryza sativa L.) in correlation with mercury species and exposure level. *Metallomics*, 6, 1951-1957.

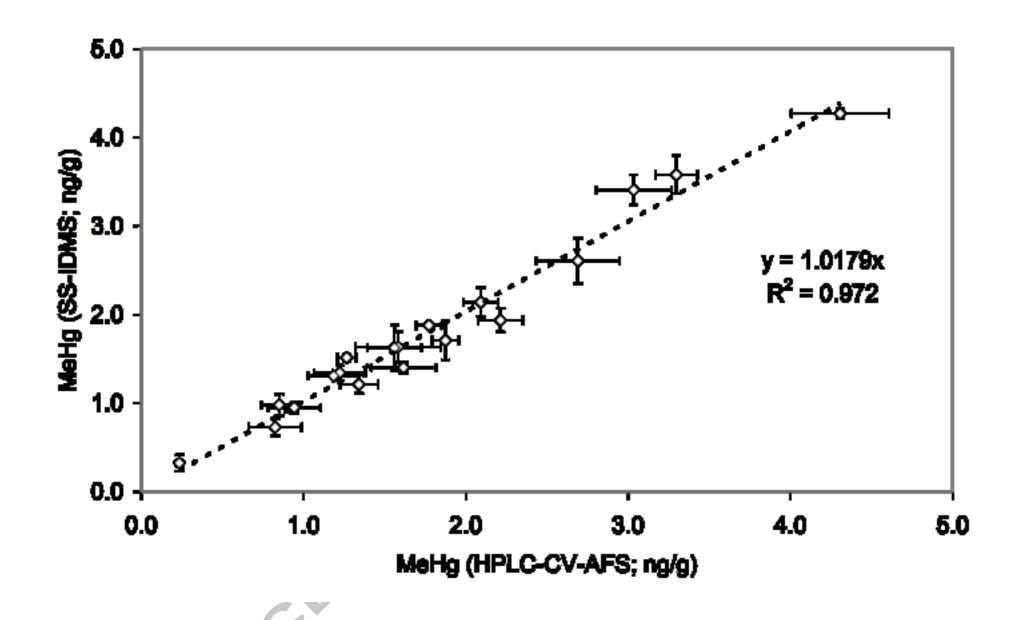
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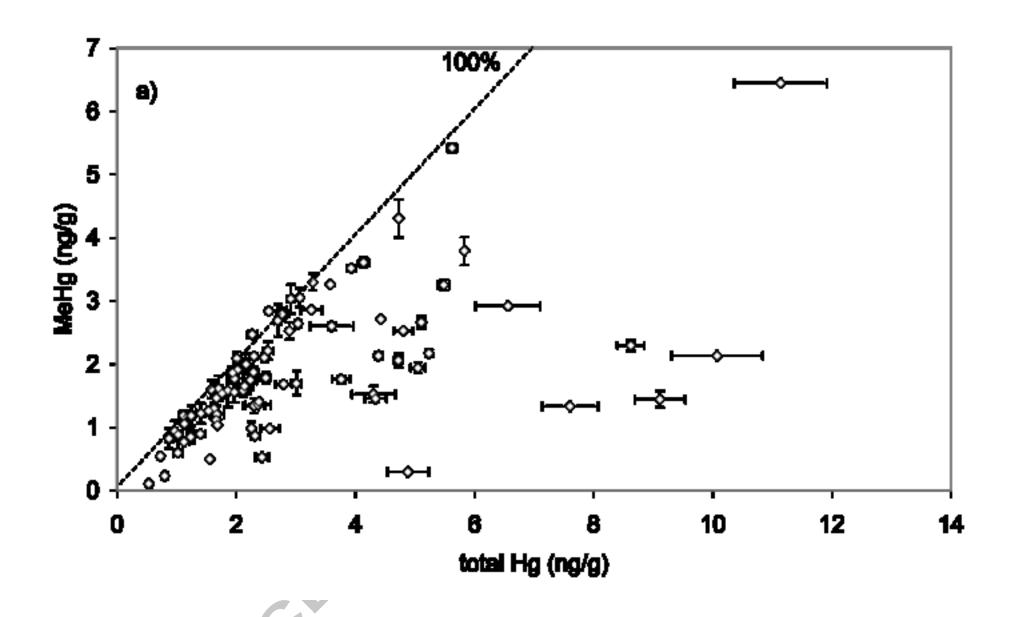
Figure 1: Methylmercury concentration in rice samples 1 to 19 determined by both HPLC-CV-AFS and SSID-GC-ICP-MS. Error bars 1 standard deviation (n = 3).

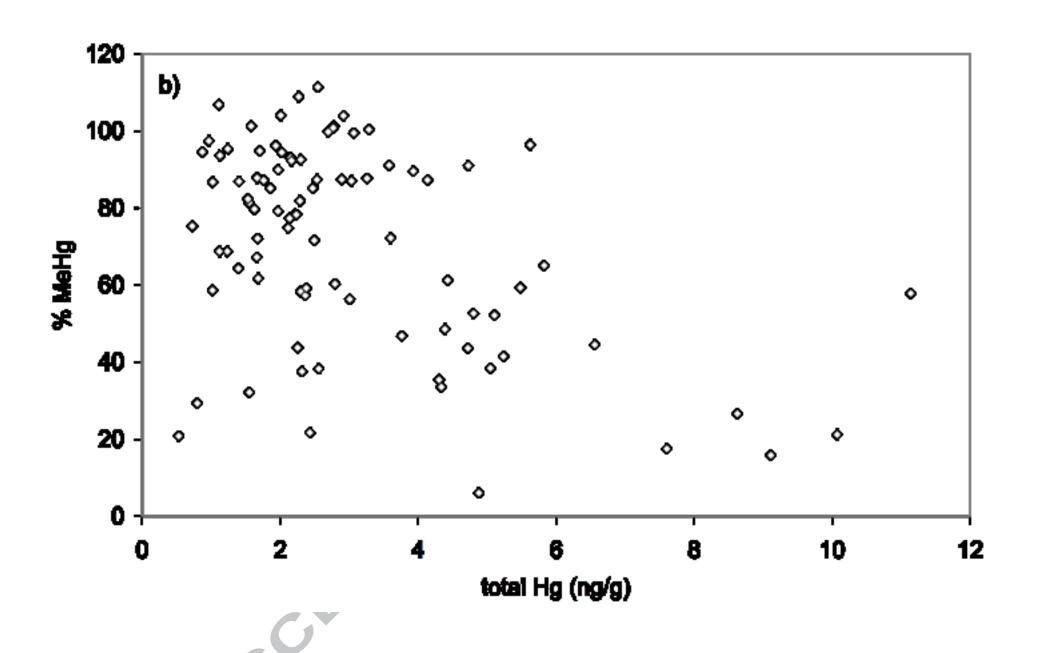
Figure 2: Correlation of MeHg and total Hg in the rice products. (a) MeHg versus total Hg concentrations. Error bars indicate one standard deviation of the analysis and the dotted line indicates the 100 % line. (b) Relative proportion of MeHg in % versus total Hg concentrations; error bars not displayed for clarity.

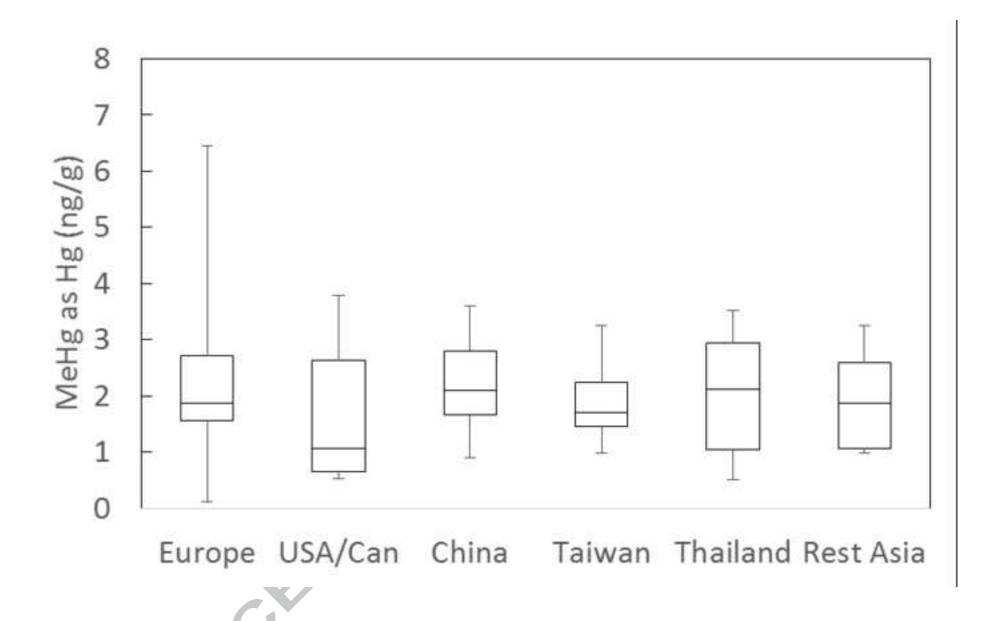
Figure 3: Box Whisker Plot from rice products sold in European of which the country of origin was known. Other Asia (n=9), China (n=8), Europe (Italy, France, Spain Switzerland); (n=12), Taiwan (n=19), Thailand (n=6), USA/Canada (n=6).

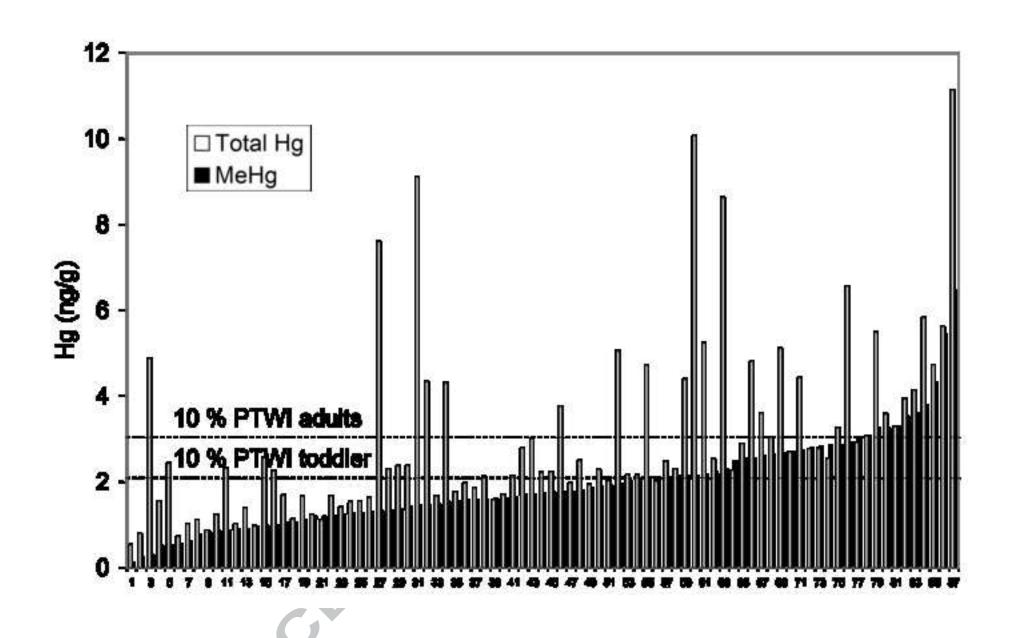
Figure 4: Total Hg and MeHg concentrations in commercially available market rice in relation to the 10 % PTWI value when a rice based diet for a toddler and an adult is assumed.













Highlights:

- Methylmercury and total mercury were measured in 87 rice and rice products bought in Europe as an exploratory study.
- A subset of 19 samples which include 9 pre-cooked baby rice products were measured by two orthogonal validated methods for the first time to enhance quality assurance.
- Precooked baby rice products have similar methylmercury content compared to non-processed rice, however a higher proportion of total mercury is methylmercury.
- European rice has similar mean value than rice from other regions but a significantly higher variability (factor 58).
- Considering the average rice consumption with respect to Bangladeshi Communities in the
 UK (average of 400g/day), all rice samples were below PTWI with a significant number of rice
 samples above 10% of the PTWI.