JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 5499−5505 **5499**

SPME−GC−Pyrolysis−AFS Determination of Methylmercury in Marine Fish Products by Alkaline Sample Preparation and Aqueous Phase Phenylation Derivatization

ZSUZSA JÓKAI, LÁSZLÓ ABRANKÓ,* AND PÉTER FODOR

Department of Applied Chemistry, Corvinus University of Budapest, Villányi út 29-33, 1118 Budapest, Hungary

Characterization of a cost-efficient analytical method based on alkaline sample digestion with KOH and NaOH, followed by aqueous phase phenylation derivatization with $NaBPh_4$ and solid phase microextraction (SPME) for the determination of methylmercury in typical fish-containing food samples commercially available in Hungary, is reported. The sample preparation procedure along with the applied SPME−GC−pyrolysis−AFS system was validated by measuring certified reference materials (CRM) BCR-464, TORT-2, and a candidate CRM BCR 710. To carry out an estimation of average Hungarian methylmercury exposures via marine fish and/or fish-containing food consumption, 16 commercially available products and 3 pooled representative seafood samples of—according to a previous European survey—the three most consumed fish species in Hungary, herring, sardines, and hake, were analyzed. Methylmercury concentrations of the analyzed samples were in the range 0.016−0.137 μg of MeHg g^{-1} dry weight as Hg.

KEYWORDS: Methylmercury; SPME; phenylation; canned; deep-frozen seafood; fish

1. INTRODUCTION

There is a growing interest in mercury speciation in samples of human food products due to varying toxicity of the different chemical forms of mercury. Organic mercury compounds, of which methylmercury is the most common, are of special concern because of their enhanced toxicity (*1*). The route of human exposure to methylmercury is mainly through the diet, especially via fish and shellfish that bioaccumulate this compound. At the average rates of fish intake (similarly to U.S. consumption, in Hungary it is less than 10 g of fish per day (*2*)), methylmercury exposures are considered to be less than the reference dose (RfD) of 0.1 μg kg^{-1} body weight per day (*3*), when calculating with mercury concentrations averaging between 0.1 and 0.15 μg of MeHg per gram of fish. However, eating fish at above-average intake levels or eating fish that are contaminated at above-average levels might increase the risk of methylmercury intoxication. According to an EPA "fact sheet" (*4*), based on diet surveys, 10% of women of childbearing age eat five times or more (i.e., 40−70 g per day) fish than the average consumer does. Even if the fish consumed have an average methylmercury concentration of 0.1 to 0.15 μg g^{-1}, women's mercury exposures range from near or slightly over the RfD to about twice the RfD. Eating fish with higher mercury concentrations obviously further increases the risk of methylmercury intoxication. Additionally, consumption of food products made from animals fed on fish products can be another contributor to human methylmercury uptake.

In response to growing demand, several analytical techniques have been developed over the past decade to speciate organomercurials in biological samples. The most frequently used techniques for liberating mercury species from solid samples are acidic leaching (*5*, *6*) or alkaline digestion (*7*, *8*) with the option of applying ultrasonic (*9*) or microwave energy (*10*, *11*) to assist in the procedure. Extraction of the liberated analytes was traditionally based on the solvent extraction method first introduced by Westöö (*12*) in the 1960s. In this method, monoalkylmercuric ions in the form of R−Hg−X species (R = alkyl group, X = Cl, Br) maintained at low pH in a medium with high Cl^- or Br^- content were selectively extracted with organic solvents (*9*, *13*). Derivatization of the extracted monoalkylmercuric compounds into nonpolar molecules has been preferred prior to analysis by gas chromatography; however, methods without derivatization have also been developed (*14*).

The most commonly used derivatization procedure is the aqueous phase ethylation reaction with $NaBEt_4$ (*7*, *8*, *15*−*18*). Propylation with $NaBPr_4$ (*19*) and phenylation with $NaBPh_4$ (*6*, *20*−*24*) have also been used.

From the point of view of detection techniques, electron capture detection (ECD) was traditionally used with GC methods. However, one of the drawbacks of this technique was that compounds containing halogens coextracted with organic mercury, causing interference with the determination of organomercurials (*25*). Therefore, this detection technique has generally been replaced by more selective techniques, the most common of which are element-specific detection methods such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), microwave-induced plasma atomic emission spec-

* Author to whom correspondence should be addressed. Tel.: +36 1 482 6363. Fax.: +36 1 466 4272. E-mail: laszlo.abranko@uni-corvinus.hu.

10.1021/jf0501140 CCC: $30.25
Published on Web 06/11/2005

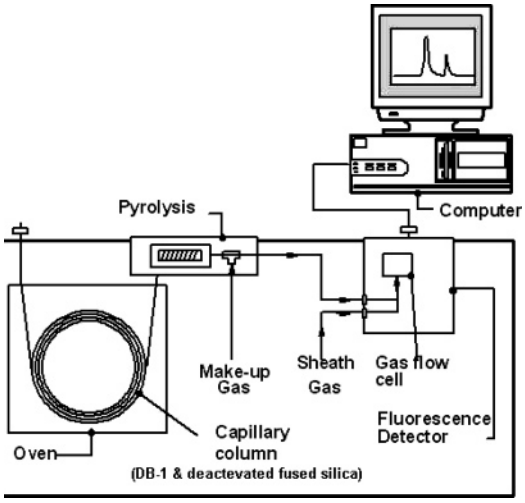

Figure 1. Schematic diagram of the GC–pyrolysis–AFS system.

trometry (MIP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) (*1*). AFS is preferred for mercury analysis due to its relatively low cost and the fact that its sensitivity and selectivity are comparable to those of ICP-MS (*26*).

Recently, solid-phase microextraction (SPME) has become a widespread solvent free technique for GC determination of organometallics (*27*). SPME not only offers the possibility of a fast, solvent-free, integrated "sample-extraction−sample-introduction" system but also may provide better analyte preconcentration than liquid−liquid extraction (LLE) techniques (*28*, *29*). Moreover, when applying the headspace (HS) sampling technique, matrix separation based on the volatility differences of the analyte and other compounds present in the sample solution can also be performed. It should be noted, however, that other integrated solvent free extraction−preconcentration methods, i.e., ones applying the purge-and-trap technique or stir bar sorptive extraction (SBSE), are also attractive alternatives to SPME for various organometallic determinations (*30*, *31*).

In the present report, the application of a cost-efficient analytical method (SPME−GC−pyrolysis−AFS system) for the determination of MeHg in typical dietary seafood samples is described. The study focused on investigating the modifications necessary for well-established methods involving alkaline digestion in order to use aqueous phase phenylation and SPME sampling to analyze food samples with complex matrices containing MeHg in the range of 100 ng g^{-1}.

2. MATERIALS AND METHODS

2.1. Instrumentation. A manual SPME device (Supelco, Bellefonte, PA), equipped with a fused silica fiber coated with a 100-μm film of poly(dimethylsiloxane) (PDMS) was used for extraction in all experiments. GC separation was carried out on a HP-5890 gas chromatograph equipped with a 15-m-long, 0.53-mm-ID, 1.5-μm film thickness DB-1 capillary column. The end of the GC column was connected to a 0.5-m-long, 0.32-mm-ID deactivated fused silica capillary. The DB-1 column and the fused silica capillary are both located in the GC oven and are subjected to the same heating conditions. The fused silica capillary was driven through a pyrolyzer tube before being coupled to a PSA 10.750 (PS Analyticals, Orpington, U.K.) atomic fluorescence (AFS) mercury detector. Argon was used as the carrier gas. The split/splitless injector of the GC was fitted with a 0.75-mm-ID (SPME) liner and was used in splitless mode. The schematic diagram of the instrumental setup is presented in **Figure 1**. The operational parameters of the GC−pyrolysis−AFS system are shown in **Table 1**.

For sample preparation, a Realsonic RS 16-F ultrasonic bath (Realsonic, Budapest, Hungary) operated at 50 W $(dm^3)^{-1}$ ultrasonic power was used. The prepared samples were centrifuged with a Hettich Mikro 22R centrifuge (Hettich, Tuttlingen, Germany). An electronically controlled magnetic stirring plate was applied for the agitation of the sample solutions during SPME sampling. Total mercury content of the samples was determined by a Leco AMA 254 direct mercury analyzer (Leco Inc, St. Joseph, MI).

Table 1. Operational Parameters of the Applied GC–Pyrolysis–AFS System

parameter	value
column head pressure	100 kPa
injector temp	190 °C
initial oven temp, hold time	100 °C, 0.6 min
ramp	30 deg min^{-1}
final temp, hold time	250 °C, 1 min
pyrolysis temp	800 °C

2.2. Samples. The seafood samples analyzed in this study can be divided two sets. The first set of samples was collected according to the framework of the EU OT-SAFE project (*32*), which involved a representative sampling of national markets in order to assess the human organotin exposure in 11 European countries. Within this project a national survey was carried out to map Hungarian seafood consumption by determining the consumption pattern of various fish species and the countries of origin. On the basis of the results of the survey for the year 2001, the three most highly consumed fish species, herring (*Clupea harengus*) imported from Poland, sardines (*Clupea pilchardus*) imported from Thailand, and hake (*Merluccius hubbis*) imported from Argentina were selected for this study. The sampling process, which covered 97% of the Hungarian markets, was an adaptation of the procedure described by ICES (*33*). Briefly, the representative samples consisted of at least 25 fish in the case of herring and sardines (in practice, 25 cans of each species in various dressings such as tomato, vinegar, or chili sauce) or at least 25 fillets in the case of hake. The fish samples were prepared by the dissection of the edible parts, which were pooled, homogenized, and freeze-dried. In this study the three, pooled samples were analyzed to determine their methylmercury concentrations, which analyte was originally not involved in the OT-SAFE project. Pooled samples of the OT-SAFE project were hypothesized to be suitable to representatively estimate methylmercury exposures of average consumers via seafood consumption, similarly as they were in the case of the previous organotin study.

Beside the three pooled samples, a second set of samples—containing 16 individual wrapped marine fish products (not the ones blended in the OT-SAFE project) currently available in Hungary—was arbitrarily selected from local supermarkets. The goal of this experiment was to carry out some verification or rebuttal to the assumption of the representativity of pooled samples regarding their methylmercury levels. In the case of some samples, identification of the origin (i.e., country) of fish and the species used in the products could not be carried out, due to incomplete labeling. Sample pretreatment procedure of these samples consisted of the following: about 200 g (wet weight) of each product was subsampled and lyophilized. Freeze-dried samples were homogenized using a Fritsch mill (type 14.702) before analysis. Both types of samples (pooled and individual) were analyzed in the same manner.

2.3. Reagents. Digestion of the samples was carried out with 25% (w/v) potassium hydroxide or 18% (w/v) sodium hydroxide in methanol, which were prepared by dissolving appropriate amounts of KOH or NaOH (Merck, Darmstadt, Germany) in methanol (Carlo Erba Reagents, Milan, Italy).

Freshly prepared 1% (w/v) sodium tetraphenylborate ($NaBPh_4$) solution (Sigma-Aldrich, Budapest, Hungary) was used as a derivatizing reagent. A 1 M sodium acetate buffer solution (NaAc) was prepared by dissolving appropriate amounts of the salt (Reanal, Budapest, Hungary) in deionized water (DIW); the pH was adjusted to 5.0 with acetic acid (Reanal).

Methylmercury stock solution (ca. 1000 mg L^{-1}) was prepared by dissolving appropriate amounts of crystalline (96.2% purity) methylmercury chloride (MeHgCl; Sigma-Aldrich) in methanol. The solution was stored in the dark at 4 °C for a maximum of 2 months. Working

standards of MeHgCl were prepared by serial dilution of the stock solution with DIW. The 10 μg mL^{-1} MeHgCl solution was prepared weekly from the stock solution, while solutions of lower concentrations were prepared daily.

Inorganic mercury (Hg^{2+}) solutions were prepared by serial dilution of 1000 mg L^{-1} $HgCl_2$ stock solution (Merck) with DIW and acidified to 1% (v/v) with 65% HNO_3 (Merck). Deionized water, $R > 10$ MΩ (Elgacan Ultrapure, Vivendi Water Systems Ltd., High Wycombe Bucks, England), was used in all experiments.

Method validation procedures were carried out by analyzing the following CRMs: BCR 464 (Bureau Communautaire de Référence), TORT-2 (National Research Council of Canada, Ottawa, ON, Canada), and T34 (candidate BCR-710) from MULSPOT Certification Exercise (*34*).

2.4. Sample Preparation Procedure for Analysis. Sample preparation was based on the procedure reported by Cai and Bayona (*7*). Briefly, nominally 250 mg of lyophilized and ground sample or CRM was accurately weighed into a 30-mL clean glass vial, and 5−6 mL of 25% (w/v) KOH in methanol or 18% (w/v) NaOH in methanol were added (both were 4.5 M). The vial was then sealed with a PTFE-lined screw cap and placed in an ultrasonic bath for 3 h at 75 °C. After the vial was allowed to cool to ambient temperature, the procedure varied depending on the MeHg content of the sample. In the case of "high-content" samples (containing a few μg g^{-1} MeHg based on dry weight), the entire digest was thoroughly rinsed into a 50-mL volumetric flask. From that solution, 1 mL was pipetted into a 10-mL volumetric flask. If the standard addition method was to be used, 1 mL of aqueous MeHgCl standard was also added to the solution before it was diluted to the nominal volume. Standard addition levels corresponded to result 1×, 2×, or 4× the expected analyte concentration of the sample solution.

In the case of "low-content" samples (containing only around 100 ng g^{-1} MeHg or less), after the vial was allowed to cool to ambient temperature, the digest was shaken for a few seconds, and then 5 mL was pipetted into a 6-mL glass centrifuge vial. The solution was centrifuged for 15 min at 4100*g* and 20 °C. From the supernatant, 1 mL was transferred to a 10-mL volumetric flask, and the standard addition method was carried out as described above.

In both cases, 1 mL from the 10-mL diluted (and spiked) solutions was pipetted into 30-mL glass vials, each containing 10 mL of a 1 M, pH = 5 acetate buffer. At that point, a clean PTFE-coated stirring bar was placed into the vial. Finally, 1 mL of freshly prepared 1% $NaBPh_4$ was added, and the vial was immediately sealed with a screw cap outfitted with a PTFE-coated rubber septum. The vial was placed on a magnetic stirring plate, and during vigorous stirring (700 rpm), headspace SPME extraction was carried out. After extraction, the fiber was introduced into the heated inlet port of the GC for thermal desorption.

The total Hg content of the samples was determined with the direct mercury analyzer according to EPA method 7473, using 100 mg of solid sample and external calibration (*35*).

3. RESULTS AND DISCUSSION

3.1. Optimization of Gas Chromatography and SPME. Since a mercury-selective detection technique was applied, separation involved only mercury-containing compounds. It was therefore possible to use faster temperature programs than those used with methods involving less selective detectors, such as ECD. In addition, due to the solvent-free sample introduction technique, use of a relatively high initial oven temperature was also possible, unlike the case of the solvent injection technique, for which the initial oven temperature is preferred to be below the boiling point of the solvent used.

Because mercuric compounds are in their ionic forms in the sample solution after digestion, a derivatization step was inserted into the procedure in order to convert the ionic analytes to nonpolar compounds that are suitable for gas chromatography. The standard solutions were also derivatized during method development. In this study, $NaBPh_4$ was used as the derivatization agent. The authors' preference for this reagent was based on the following: (i) the derivatives of mercury obtained from phenylation have not been found in nature, (ii) $NaBPh_4$ as a derivatizing reagent is more stable (*21*, *29*) than $NaBEt_4$, and (iii) $NaBPh_4$ is cheaper than other derivatizing reagents. Hence, under the optimized operational conditions shown in **Table 1**, MeHgPh (the phenylated derivative of monomethylmercury ion) and PhHgPh (the phenylated derivative of inorganic mercury) could be resolved with elution times of 0.5 and 2.3 min, respectively. (For the optimization of the chromatographic conditions, a 10-min liquid-phase SPME was used for sampling.)

Because the goal of this work was to analyze samples with complex matrices, the headspace SPME technique was intended for use in sequential extractions. The determination of the equilibration time of headspace SPME (i.e., the time required for reaching the maximum concentration of an analyte in the SPME fiber) was carried out with the same standard mixture that was used in the qualitative experiments. Accordingly, 9 min of sampling time (while the solution was stirred at 700 rpm) was sufficient to reach equilibrium conditions for the standard MeHgPh solution. As expected, in the case of Hg^{2+} (i.e., PhHgPh)—due to the much lower volatility of this compound compared to MeHgPh—this sampling time was not sufficient to allow detectable amounts of PhHgPh to be extracted under the described conditions (extraction time, standard concentration, etc.). Since this method was designed for methylmercury determination, this phenomenon did not detract from the method's suitability for this purpose. It should be noted, however, when measuring solutions containing Hg^{2+} in much higher concentrations (i.e., a few ng mL^{-1}), some slight signals for PhHgPh were obtained along with significant Hg^0 peaks, which presumably resulted from the reduction of Hg^{2+} as a possible side reaction during phenylation.

The chromatogram of a standard solution containing 0.1 ng mL^{-1} MeHgCl (as Hg) and 2 ng mL^{-1} Hg^{2+} is shown in **Figure 2**. Even though this concentration distribution of Hg^{2+} and MeHg can hardly be considered natural, the given example nevertheless demonstrates the capability of the chromatographic method to distinguish among these three species despite the applied fast temperature program.

The thermal desorption characteristics of the components from the SPME fiber in the heated inlet port of the GC were also investigated. It was found that satisfactory fiber blanks can be obtained by applying 0.5 min desorption time at 190 °C. Under these conditions, no carryover was observed between acquisitions.

3.2. Optimization of pH. The pH dependence of the phenylation reaction is evident; however, various optimum pH values can be found in the literature. Some authors applied pH = 4.5 for both phenylation and ethylation (*1*). In other publications, a variety of optimum values—determined especially for phenylation—can be found, namely, pH 2, 3, 5, and 7.3 (*20*, *22*−*24*). On the basis of the results of our optimization study, pH = 5 was selected.

3.3. Optimization of Dilution (Effect of Methanol and Potassium). When analyzing samples containing the target analyte(s) in relatively high concentration with an adequately sensitive analytical system, it is possible to dilute the sample digest to reduce matrix effects. With the present method—when SPME is used in combination with alkaline-methanol digestion—dilution of the digest is not only an option to eliminate matrix effects, but an important requirement, considering the effect of methanol on SPME. As a general rule, low-polarity organic solvents would immediately saturate the fiber, inhibiting the

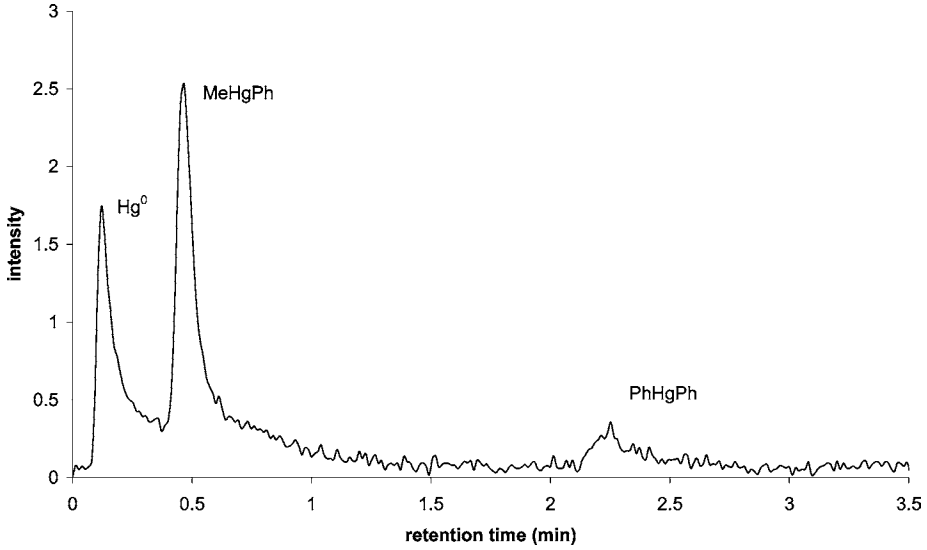

Figure 2. Chromatogram of a standard solution containing 0.1 ng mL^{-1} MeHgCl (as Hg) and 2 ng mL^{-1} Hg^{2+}. The peak eluting with the dead volume is hypothesized to be Hg^0 (see text for details).

extraction of the target compound. According to the literature, the organic solvent concentration of the solution should not exceed 1% when SPME is performed (*36*). Regardless of the matrix effects and methanol content issues, "high-content" samples must be diluted so that the MeHg concentration of the final solution (i.e., which is extracted with SPME) is in the range 0.02−0.6 ng mL^{-1}. Within this concentration range, signals are in the linear range of the AFS detector when the most sensitive 1000× amplification is applied.

A "high content" tuna fish certified reference material (CRM), BCR 464, was the first to be analyzed by the present method. In this case, a 250-mg solid sample was leached to 5 mL of a KOH−methanol solution. Afterward, the 5-mL digest was diluted 10-fold. Use of the standard addition method described above resulted in another 10-fold dilution of each sample. Further dilution resulted from transferring 1 mL from the 10-mL spiked solution to the final 12-mL volume. In the case of BCR 464, the 5-mL digest was diluted by a factor of 1200. Thus, the final solution contained only about 0.08% MeOH. With such high dilution of the digest, practically no matrix effects were observed as both external calibration and the standard addition method provided satisfactory recovery results of 92% and 95% on average ($n = 3$), respectively.

In the case of the TORT-2 lobster hepatopancreas CRM and oyster tissue T34 (candidate BCR-710), the same extent of dilution could not be carried out because of their approximately 40-fold lower MeHg concentrations. Therefore, the first 10-fold dilution step (i.e., rinsing the 5-mL digest into a 50-mL volumetric flask) was eliminated, and the following procedure was applied instead: the 5-mL digest (in a KOH−methanol solution) was centrifuged for 15 min at 4100*g* and 20 °C; afterward, 1 mL of the supernatant was transferred to a 10-mL volumetric flask for standard addition. The remaining steps were similar to those in the previous experiment. With this new protocol, the MeOH content of the final solution intended for SPME was still below 1%. Surprisingly, no methylmercury signal could be obtained for any of the samples—either TORT-2 or T34—prepared by this means. Even the samples intended for the standard addition method—containing severalfold higher methylmercury concentrations compared to the original sample due to spiking—did not yield any signal either. Similar experiences have been reported in the literature where alkaline digestion was thought to be the reason for the phenomenon (*37*); however, the authors attributed this observation to sediment samples, not biological tissues. On the other hand, Grinberg and co-workers (*19*) analyzed TORT-2 without any difficulty, using the same alkaline digestion procedure that was applied in this study. The only difference between the present and Grinberg's method was the derivatizing reagent used. In the present work phenylation was used instead of ethylation.

As a result, signal loss has been attributed to the potential precipitation reaction between potassium and sodium tetraphenylborate (*35*, *38*). It should be noted that the same methodology was used successfully to analyze BCR-464 CRM (see above), where, due to the dilution of the digest, the potassium content of the final solution was approximately 10 times lower. In other words, $NaBPh_4$ remained in excess after the precipitation of potassium. KOH was replaced with NaOH in the sample preparation procedure in order to avoid the above-described undesired reaction. Although severe matrix effects could be observed due to the lack of satisfactory dilution (i.e., the slopes of calibration curves obtained from external calibration and standard addition were significantly different), average standard addition quantification recovery values of 99.7% and 99.5% ($n = 3$) were obtained for TORT-2 and T34, respectively.

3.4. Validation of the Method. Two different CRMs and a candidate CRM were used for validation purposes, namely, BCR 464 tuna fish, TORT-2 lobster hepatopancreas, and T34 oyster tissue (candidate BCR 710). As it was discussed earlier, two methodologies had to be developed for the involved CRMs—one for the "high-content" sample and one for the remaining two "low-content" samples—in order to obtain accurate results. A summary of the validation results is given in **Table 2**.

BCR 464 (tuna fish) is considered a matrix-matching CRM and is thus suitable for the validation of the present method intended for use in determining MeHg in seafood samples. On the other hand—as it is demonstrated in our study—the matching of a CRM with a market sample in terms of the analyte concentration is also a basic requirement. In this special application, the latter requirement turned out to be more important, because the methodology suitable for a "high-content" sample was invalid for "low-content" samples. On the other hand, when the procedure was adequately tuned to "low-content" samples, it was suitable for both TORT-2 and T34 even though consisting of different matrices.

Table 2. Results of CRM Measurements[a]

	measd MeHg (as Hg) mg kg^{-1} dry wt (n = 3, 1 SD)	certified MeHg (as Hg) mg kg^{-1} dry wt
BCR-464 (tuna fish)	4.85 ± 0.20	5.12 ± 0.16
TORT-2 (lobster hepatopancreas)	0.151 ± 0.018	0.152 ± 0.013
T34 (oyster tissue)	0.105 ± 0.017	0.106 ± 0.013

[a] Each sample was digested in triplicate, and standard addition was used for quantification. Sample preparation procedures differed depending on MeHg content (see text for details).

3.5. Measurement of Seafood Samples. The seafood samples from the OT-SAFE project had already been lyophilized, ground, and homogenized on receipt while the other 16 samples were lyophilized and milled in our laboratory. According to the results of total Hg measurements, their MeHg concentrations were expected to be closer to TORT-2 and T34 than to BCR 464. Therefore, the "low-content" sample preparation methodology was used for these samples. Results of analysis are shown in **Table 3**.

As it was described earlier, pooled samples analyzed in this study were originally produced to provide samples for a representative assessment of organotin levels in Hungarian marine fish containing products; however, on the basis of the same concept they were used similarly in this study to estimate methylmercury levels. According to the data in **Table 3**, MeHg concentrations of the 16 arbitrarily selected samples scatter around the ones of the three pooled samples comparing them by dry weight based values. These results provide an additional contribution to the verification of the approach of using pooled samples for estimating average MeHg levels in fish and/or fish-containing food products. On the other hand, if the appropriate estimation of the average MeHg intake of a typical consumer is aimed, concentrations calculated on fresh weight should be concerned. On the basis of wet weight data, pooled samples result in approximately 3-fold smaller concentration values compared to the average (0.063 μg g^{-1} MeHg (as Hg)) of the independent individual samples. This discrepancy is originating from the different water content of the two sets of samples. The arbitrarily selected products were unexceptionally ready-to-fry fish fillets, while, in the case of pooled samples, several other, succulent fish-containing products (e.g., canned fish with dressings such as tomato, vinegar, or chili sauce) were also blended. Since food consumption statistics distinguishing between the consumption of "pure" fish and fish-containing food are generally not available, estimation of average methylmercury exposures might be misleading when the above-mentioned differences are not taken into account.

As a conclusion, it should be mentioned, however, that obtained methylmercury levels (methylmercury concentrations calculated as either MeHg or Hg) both in the pooled and in the individual samples are to some degree less than typical values found in the literature (*4*, *39*). In this study, no attempt was made to unravel the reason behind that fact; however, the age and size of fish used in the investigated products may be one of the possible explanations. Accordingly, it can be calculated that the MeHg reference dose of 0.1 μg kg^{-1} body weight per day is reached only when approximately 100−300 g (fresh weight) of marine fish containing food product is consumed per day (based on a 70-kg average body weight), or equally 25−70 g when calculations are based on a 14.5-kg body weight for children. Considering the average Hungarian consumption (in 2002) of these products, namely, 3.1 kg of fish (in general) a year per person (*2*), the risk of methylmercury intoxication of an average consumer via fish consumption is negligible.

SAFETY

Organomercurials are very toxic; therefore, inhalation or skin contact must be avoided. These compounds must be handled with the use of appropriate personal protection equipment and in an adequately ventilated environment.

ACKNOWLEDGMENT

This work was financially supported by OTKA 37215 and 35216 projects, which are gratefully acknowledged. Special thanks to M. Dernovics and K. E. Paquette (U.S. FDA) for their useful notes on the manuscript.

Table 3. Results of MeHg and Total Hg Analyses of Fish-Containing Seafood Products

samples	measd MeHg (as Hg) mg kg^{-1} dry wt (n = 3, 1 SD)	measd total Hg mg kg^{-1} dry wt (n = 3, 1 SD)	av dry matter content m/m%	av MeHg content (as Hg) μg g^{-1} (fresh wt)
Argentine hake (pooled I)	0.122 ± 0.006	0.153 ± 0.002	19.1	0.023
Polish herring (pooled II)	0.070 ± 0.001	0.101 ± 0.002	30.0	0.021
Thai sardines (pooled III)	0.073 ± 0.005	0.090 ± 0.001	28.5	0.021
crumbed cod fillets, Alaska	0.066 ± 0.007	0.081 ± 0.002	83.7	0.055
crumbed fish fillets, France	0.036 ± 0.004	0.045 ± 0.001	85.1	0.031
crumbed fish fillets, Estonia	0.060 ± 0.006	0.078 ± 0.002	82.4	0.049
crumbed fish bits[a]	0.137 ± 0.008	0.159 ± 0.001	81.8	0.112
crumbed fish bits, Germany	0.119 ± 0.008	0.138 ± 0.002	83.7	0.100
fish fillets (vendor I), Argentina	0.118 ± 0.007	0.145 ± 0.001	77.0	0.091
fish fillets (vendor II), Argentina	0.035 ± 0.004	0.043 ± 0.001	83.9	0.029
fish fillets (vendor II), Alaska	0.107 ± 0.009	0.125 ± 0.003	84.5	0.090
fish fillets (vendor III)[a]	0.089 ± 0.008	0.110 ± 0.001	83.0	0.074
fish fillets (vendor IV)[a]	0.016 ± 0.002	0.021 ± 0.001	74.0	0.012
fish fillets (vendor V, brand I)[a]	0.047 ± 0.005	0.060 ± 0.001	84.0	0.040
fish fillets (vendor V, brand II)[a]	0.124 ± 0.010	0.140 ± 0.002	78.9	0.098
hake fillets[a]	0.063 ± 0.006	0.080 ± 0.001	79.9	0.050
hake fillets, Argentina	0.108 ± 0.010	0.130 ± 0.002	84.9	0.092
hake fillets, South America[a]	0.068 ± 0.007	0.085 ± 0.002	79.4	0.054
salmon fillets, Argentina	0.029 ± 0.003	0.037 ± 0.001	84.4	0.024

[a] Country of origin is not indicated on label.

LITERATURE CITED

(1) Cai, Y.; Monsalud, S.; Jaffe, R.; Jones, R. D. Gas chromatographic determination of organomercury following aqueous derivatization with sodium tetraethylborate and sodium tetraphenylborate—Comparative study of gas chromatography coupled with atomic fluorescence spectrometry, atomic emission spectrometry and mass spectrometry. *J. Chromatogr. A* **2000**, *876*, 147−155.

(2) *Food balances and food-stuff consumption*; Hungarian Central Statistical Office, Agricultural Statistics Department, Production Statistics Section; 2004.

(3) *Mercury Study Report to Congress, Volume V: Health Effects of Mercury and Mercury Compounds*; EPA-452/R-97-007; U.S. EPA: Washington, DC, 1997.

(4) *Mercury Update: Impact on Fish Advisories*; EPA-823-F-01-11; U.S. EPA: Washington, DC, 2001.

(5) Ortiz, A. I. C.; Albarran, Y. M.; Rica, C. C. Evaluation of different sample pre-treatment and extraction procedures for mercury speciation in fish samples. *J. Anal. At. Spectrom.* **2002**, *17*, 1595−1601.

(6) Rodil, R.; Carro, A. M.; Lorenzo, R. A.; Abuin, M.; Cela, R. Methylmercury determination in biological samples by derivatization, solid-phase microextraction and gas chromatography with microwave-induced plasma atomic emission spectrometry. *J. Chromatogr. A* **2002**, *963*, 313−323.

(7) Cai, Y.; Bayona, J. M. Determination of Methylmercury in Fish and River Water Samples Using in-Situ Sodium Tetraethylborate Derivatization Following by Solid-Phase Microextraction and Gas-Chromatography Mass-Spectrometry. *J. Chromatogr. A* **1995**, *696*, 113−122.

(8) Cai, Y.; Monsalud, S.; Furton, K. G.; Jaffe, R.; Jones, R. D. Determination of methylmercury in fish and aqueous samples using solid-phase microextraction followed by gas chromatography atomic fluorescence spectrometry. *Appl. Organomet. Chem.* **1998**, *12*, 565−569.

(9) Rio-Segade, S.; Bendicho, C. Ultrasound-assisted extraction for mercury speciation by the flow injection cold vapor technique. *J. Anal. At. Spectrom.* **1999**, *14*, 263−268.

(10) Tseng, C. M.; DeDiego, A.; Martin, F. M.; Amouroux, D.; Donard, O. F. X. Rapid determination of inorganic mercury and methylmercury in biological reference materials by hydride generation, cryofocusing, atomic absorption spectrometry after open focused microwave-assisted alkaline digestion. *J. Anal. At. Spectrom.* **1997**, *12*, 743−750.

(11) Gerbersmann, C.; Heisterkamp, M.; Adams, F. C.; Broekaert, J. A. C. Two methods for the speciation analysis of mercury in fish involving microwave-assisted digestion and gas chromatography atomic emission spectrometry. *Anal. Chim. Acta* **1997**, *350*, 273−285.

(12) Westöö, G. Determination of methylmercury salts in various kinds of biological material. *Acta Chem. Scand.* **1968**, *22*, 2277−2280.

(13) Pereiro, I. R.; Wasik, A.; Lobinski, R. Determination of mercury species in fish reference materials by isothermal multicapillary gas chromatography with atomic emission detection after microwave-assisted solubilization and solvent extraction. *J. Anal. At. Spectrom.* **1998**, *13*, 743−747.

(14) Caricchia, A. M.; Minervini, G.; Soldati, P.; Chiavarini, S.; Ubaldi, C.; Morabito, R. GC-ECD determination of methylmercury in sediment samples using a SPB-608 capillary column after alkaline digestion. *Microchem. J.* **1997**, *55*, 44−55.

(15) Bloom, N. Determination of Picogram Levels of Methylmercury by Aqueous Phase Ethylation, Followed by Cryogenic Gas-Chromatography with Cold Vapor Atomic Fluorescence Detection. *Can. J. Fish. Aquat. Sci.* **1989**, *46*, 1131−1140.

(16) Fernandez, R. G.; Bayon, M. M.; Alonso, J. I. G.; Sanz-Medel, A. Comparison of different derivatization approaches for mercury speciation in biological tissues by gas chromatography/inductively coupled plasma mass spectrometry. *J. Mass Spectrom.* **2000**, *35*, 639−646.

(17) Leenaers, J.; Van Mol, W.; Infante, H. G.; Adams, F. C. Gas chromatography-inductively coupled plasma-time-of-flight mass spectrometry as a tool for speciation analysis of organomercury compounds in environmental and biological samples. *J. Anal. At. Spectrom.* **2002**, *17*, 1492−1497.

(18) Logar, M.; Horvat, M.; Falnoga, I.; Stibilj, V. A methodological study of mercury speciation using Dogfish liver CRM (DOLT-2). *Fresenius' J. Anal. Chem.* **2000**, *366*, 453−460.

(19) Grinberg, P.; Campos, R. C.; Mester, Z.; Sturgeon, R. E. A comparison of alkyl derivatization methods for speciation of mercury based on solid phase microextraction gas chromatography with furnace atomization plasma emission spectrometry detection. *J. Anal. At. Spectrom.* **2003**, *18*, 902−909.

(20) Mena, M. L.; McLeod, C. W.; Jones, P.; Withers, A.; Minganti, V.; Capelli, R.; Quevauviller, P. Microcolumn Preconcentration and Gas-Chromatography Microwave-Induced Plasma-Atomic Emission-Spectrometry (Gc-Mip-Aes) for Mercury Speciation in Waters. *Fresenius' J. Anal. Chem.* **1995**, *351*, 456−460.

(21) Minganti, V.; Capelli, R.; Depellegrini, R. Evaluation of Different Derivatization Methods for the Multielement Detection of Hg, Pb and Sn Compounds by Gas-Chromatography Microwave-Induced Plasma-Atomic Emission-Spectrometry in Environmental-Samples. *Fresenius' J. Anal. Chem.* **1995**, *351*, 471−477.

(22) Abuín, M.; Carro, A. M.; Lorenzo, R. A. Experimental design of a microwave-assisted extraction-derivatization method for the analysis of methylmercury. *J. Chromatogr. A* **2000**, *889*, 185−193.

(23) Carro, A. M.; Neira, I.; Rodil, R.; Lorenzo, R. A. Speciation of mercury compounds by gas chromatography with atomic emission detection. Simultaneous optimization of a headspace solid-phase microextraction and derivatization procedure by use of chemometric techniques. *Chromatographia* **2002**, *56*, 733−738.

(24) Ipolyi, I.; Massanisso, P.; Sposato, S.; Fodor, P.; Morabito, R. Concentration levels of total and methylmercury in mussel samples collected along the coasts of Sardinia Island (Italy). *Anal. Chim. Acta* **2004**, *505*, 145−151.

(25) Cai, Y.; Jaffe, R.; Alli, A.; Jones, R. D. Determination of organomercury compounds in aqueous samples by capillary gas chromatography atomic fluorescence spectrometry following solid-phase extraction. *Anal. Chim. Acta* **1996**, *334*, 251−259.

(26) Armstrong, H. L.; Corns, W. T.; Stockwell, P. B.; O'Connor, G.; Ebdon, L.; Evans, E. H. Comparison of AFS and ICP-MS detection coupled with gas chromatography for the determination of methylmercury in marine samples. *Anal. Chim. Acta* **1999**, *390*, 245−253.

(27) Mester, Z.; Sturgeon, R.; Pawliszyn, J. Solid phase microextraction as a tool for trace element speciation. *Spectrochim. Acta B* **2001**, *56*, 233−260.

(28) Abrankó, L.; Yang, L.; Sturgeon, R. E.; Fodor, P.; Mester, Z. Solid phase microextraction for the determination of chromium in sea-water. *J. Anal. At. Spectrom.* **2004**, *19*, 1098−1103.

(29) Tutschku, S.; Schantz, M. M.; Wise, S. A. Determination of methylmercury and butyltin compounds in marine biota and sediments using microwave-assisted acid extraction, solid-phase microextraction, and gas chromatography with microwave-induced plasma atomic emission spectrometric detection. *Anal. Chem.* **2002**, *74*, 4694−4701.

(30) Feldhaus, R.; Buscher, W.; Kleine-Benne, E.; Quevauviller, P. Development of a new automated speciation analyser—result of an international interdisciplinary co-operation between scientific and industrial partners. *TrAC, Trends Anal. Chem.* **2002**, *21*, 356−365.

(31) Vercauteren, J.; Peres, C.; Devos, C.; Sandra, P.; Vanhaecke, F.; Moens, L. Stir bar sorptive extraction for the determination of ppq-level traces of organotin compounds in environmental samples with thermal desorption-capillary gas Chromatography−ICP mass spectrometry. *Anal. Chem.* **2001**, *73*, 1509−1514.

(32) *OT-SAFE: Sources, consumer exposure and risks of organotin contamination in seafood*; EU project QLK1-CT-2001-01437; Institute for Environmental Studies: Amsterdam, 2004.
(33) ICES, 2004: http://www.ices.dk/env/refcodes/guidelines.asp-?topic=sample (accessed on 4/27/2005)
(34) *MULSPOT certification exercise on species of As, Hg and Sn, and on Se in oyster tissue, sample T34 (candidate BCR-710)*; EC Contract SM&T CT98-2232; European Commission: Brussels, 2004.
(35) *EPA method 7473: Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry*; Environmental Protection Agency: Washington, DC, 1998.
(36) Pawliszyn, J. Theory of Solid Phase Microextraction. In *Solid Phase Microextraction: Theory and Practice*; Wiley-VCH: New York, 1997; p 51.
(37) Lorenzo, R. A.; Vazquez, M. J.; Carro, A. M.; Cela, R. Methylmercury extraction from aquatic sediments—A comparison between manual, supercritical fluid and microwave-assisted techniques. *TrAC, Trends Anal. Chem.* **1999**, *18*, 410−416.
(38) Schall, E. D. Volumetric Determination of Potassium. *Anal. Chem.* **1957**, *29*, 1044−1046.
(39) Storelli, M. M.; Storelli, A.; Giacominelli-Stuffler, R.; Marcotrigiano, G. O. Mercury speciation in the muscle of two commercially important fish, hake (Merluccius merluccius) and striped mullet (Mullus barbatus) from the Mediterranean sea: estimated weekly intake. *Food Chem.* **2005**, *89*, 295−300.

Received for review January 18, 2005. Revised manuscript received May 9, 2005. Accepted May 16, 2005.

JF0501140