Supporting Information

Carbon stable isotope analysis of methylmercury toxin in biological materials by gas chromatography isotope ratio mass spectrometry

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Table S1. Instrumental conditions for $\delta^{13}C_{CH3Hg}$ analysis by GC-C-IRMS

GC parameters		
	Derivatization	**Halogenation**
Column	Agilent DB-5 (30 m, 0.25 mm I.D., 0.25 µm)	Restek RTX-5 (30 m, 0.25 mm I.D., 0.1 µm)
GC program	Initial temp.: 40°C Hold time: 2 min. Ramp: 5°C.min^{-1} - 100°C Hold time: 2 min. Ramp: 50°C.min^{-1} - 250°C Hold time: 1 min.	Initial temp.: 40°C Hold time: 2min. Ramp: 10°C.min^{-1} - 120°C Hold time: 0 min. Ramp: 35°C.min^{-1} - 250°C Hold time: 1 min.
Carrier gaz (He)	1.4 ml.min^{-1}	1.4 ml.min^{-1}
Injector set up	250°C Splitless (SSL), 1µl	250°C Splitless (SSL), 3µl
IRMS parameters		
	Derivatization	**Halogenation**
GC-C-IRMS	Delta V Advantage IRMS (Thermo Scientific, Bremen, Germany) GC-Isolink system (Thermo Scientific, Bremen, Germany) Combustion furnace: 1000°C	
Peak detection methods	ISODAT "Individual Background" method Start slope:0.4mV.s^{-1} End slope:0.2mV.s^{-1} Background time=5s	ISODAT "CalcMean background" method Start slope:2mV.s^{-1} End slope:2mV.s^{-1} Background time=1s

Table S2: Quantitative extraction and halogenation efficiency of CH_3Hg from biological material processed by SEM and determined by GC-SF-ICP-MS.

Mass of ERM-CE464 tuna fish extracted (g)	Derivatization/ Halogenation	Quantification	Recovery ±SD (%)	Ref.
0.25	Propylation	Isotope dilution	98±3	[1]
0.25	Halogenation	Isotope dilution	96±3	This study
1	Halogenation	External calibration*	105±16	This study
4	Halogenation	External calibration*,**	97±12	This study

*Determined by external calibration using an independent CH_3HgI standard calibration curve (calibration curve standards not processed by SEM, and not evaporated)

** Independent measurement performed on the same solution analyzed in Table 1 by GC-C-IR-MS, and diluted by volume in hexane to account for the difference of sensitivity between GC-C-IR-MS and GC-SF-ICP-MS. The slight but larger uncertainty on the recovery value measured by GC-SF-ICP-MS (Table S2) relative to GC-C-IR-MS (Table1) is primarily due to the dilution uncertainties caused by the need to work at the level of a few µL of solvent solutions taken originally from the limited hexane solution available for GC-C-IR-MS analysis.

Mercury speciation analysis of SEM final CH_3Hg-thiosulfate extracts quantified by isotope dilution and external calibration conditions are summarized in the table above. All samples were prepared under the conditions used for GC-C-IR-MS analysis, which consisted of applying the SEM procedure, the halogenation of CH_3Hg in hexane, and its

final preconcentration under a N2 stream. These solutions were then diluted back into hexane to account for the significantly higher sensitivity of GC-SF-ICP-MS. Measurements were performed by isotope dilution ID-GC-SF-ICPMS analysis (see Table S3) using an enriched $CH_3{}^{201}Hg$ standard (ISC, Spain) and experimental conditions detailed elsewhere[1]. Complementary measurements by GC-SF-ICPMS obtained by external calibration using CH_3HgI standards prepared from the CH_3Hg stock solution were also performed for comparison. Results show that similar and quantitative CH_3Hg recovery results were obtained when processing approximately 0.25g of ERM-CE464 Tuna fish material by species-specific isotope dilution quantification using either propylation and halogenation conditions applied to the same final CH_3Hg thiosulfate solutions. This suggests that CH_3Hg is efficiently and quantitatively extracted from the biological tissue matrix, confirming earlier results[1]. This also indicates that both derivatization and halogenation method forming $CH_3HgC_3H_7$ and CH_3HgI compounds respectively lead to accurate determinations when isotope dilution quantification approaches are used. Similar quantitative extraction efficiencies and accurate determination without matrix effects could also be achieved using the halogenation method while extracting larger quantities of ERM-CE464 material and determining CH_3Hg concentrations by external calibration using an independent CH_3HgI standard calibration curve. Both approaches confirm the robustness and efficiency of the SEM, leading to a quantitative extraction of CH_3Hg from biological materials when processing different sample mass (0.25-4g), the further quantitative formation of the CH_3HgI complex, and the absence of loss during the N_2 preconcentration step. Note that a

complementary test consisting of performing a second CH_3Hg extraction from the acidic solution of the SEM with an additional 10 ml of toluene was performed. Results indicated that CH_3Hg values were similar to blank levels, confirming that CH_3Hg was quantitatively extracted within the first 10mL of Toluene used in the SEM procedure.

Table S3: Instrumental conditions for ID-GC-SF-ICP-MS analysis

	GC parameters	
	Derivatization	**Halogenation**
Column	Agilent DB-5 (30 m, 0.25 mm I.D., 0.25 µm)	Restek RTX-5 (30 m, 0.25 mm I.D., 0.1 µm)
GC program	Initial temp: 40°C Hold time: 1 min Ramp: 35°C.min^{-1} - 250°C Hold time: 2 min	Initial temp: 60°C Hold time: 1min Ramp: 30°C.min^{-1} - 250°C Hold time: 2 min
Carrier gaz (He) Make-up gas (Ar)	0.7 ml.min^{-1} 0.7 L.min^{-1}	1.0 ml.min^{-1} 0.7 L.min^{-1}
Injector Set Up	250°C PTV splitless (1µl)	250°C PTV splitless (1µl)
Transfer Line (T°C):	250°C	
	SF-ICP-MS parameters	
Set Up Rf Power Cool gas Auxiliary gas Nebulizer gas Isotopes/ dwell times	Element XR SF-ICP-MS (Thermo Scientific, Bremen, Germany) 1200W 16 L.min^{-1} 0.8 L.min^{-1} 0.45 L.min^{-1} Hg : 196, 198, 199, 200, 201, 202, 204 (40ms) Tl : 203, 205 (30ms)	

Table S4: Summary of $\delta^{13}C_{CH3Hg}$ values obtained for the CH_3Hg primary standard solution, comparing short term and long term experiments using derivatization and halogenation methods. The consensus mean value represents the mean of all long-term propylated and halogenated $\delta^{13}C_{CH3Hg}$ measurements. The long-term reproducibility was evaluated by repeated injection of a 36 ng of CH_3Hg (8 ng as C) propylated CH_3Hg standard over a period of 3 days and of a 37-283 ng of CH_3HgI (2 to 16 n g as C) over a period 6 days.

	Propylation experiments* $CH_3Hg(C_2H_5)$	Ethylation experiments* $CH_3Hg(C_3H_7)$	Long term reproducibility propylation 8 ng.C /3days	Halogenation experiments (CH_3HgI)	Long term reproducibility halogenation 7 ng.C /3days	Long term reproducibility halogenation 2-16 ng.C /6days	Consensus Mean
$\delta^{13}C_{CH3Hg}$ ±2SD (‰) (n)	-54.8±1.8 (11)	-54.9±2.2 (10)	-53.9±1.5 (8)	-54.0±0.7 (12)	-53.6±0.7 (7)	-53.7±0.9 (24)	-53.8±1.1 (32)

* Data obtained from figure 3 and ESI_figure 1

Figure S1: Influence of the amount and type of derivatizing agent ($NaBEt_4$ and $NaBPr_4$) in solution on the derivatization efficiency of CH_3Hg and iHg, and associated determinations of $\delta^{13}C_{CH3Hg}$ values. In both experiments, the derivatization of 21 µg of CH_3Hg (1.6 µg as C) and 20 µg of iHg was performed into 1.75ml MQ water. The derivatized compounds were backextracted into 0.5 mL of hexane. The results showed the absence of a significant effect of the amount and type of derivatization agent used on the determination of $\delta^{13}C_{CH3Hg}$ values. Quantitative derivatization yields are reached while using 2 mg of $NaB(C_2H_5)_4$ and 0.7 mg of $NaB(C_3H_7)_4$. Consensus mean values (plain line) and their uncertainties (dashed lines (±2SD)) are based on all measurements.

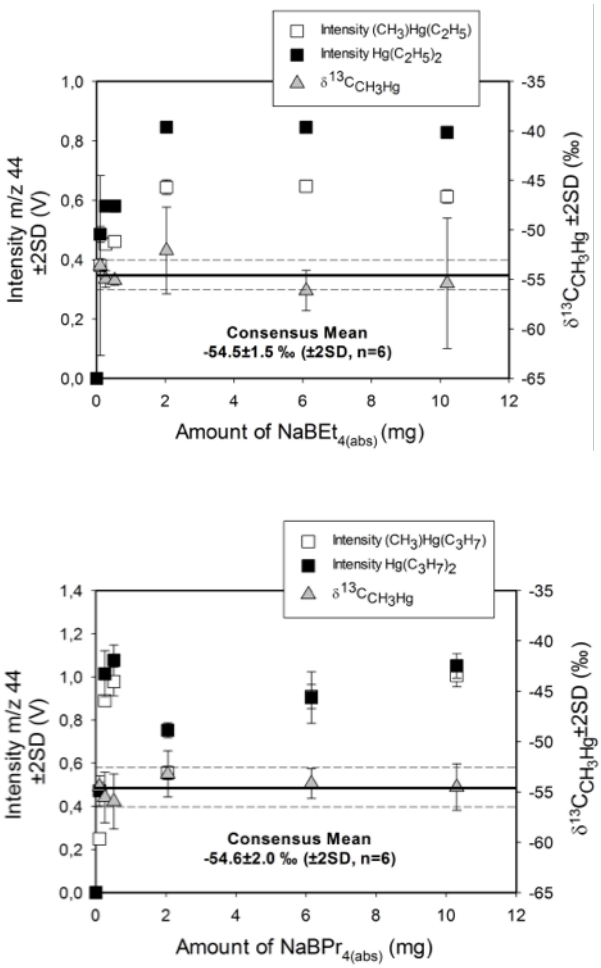
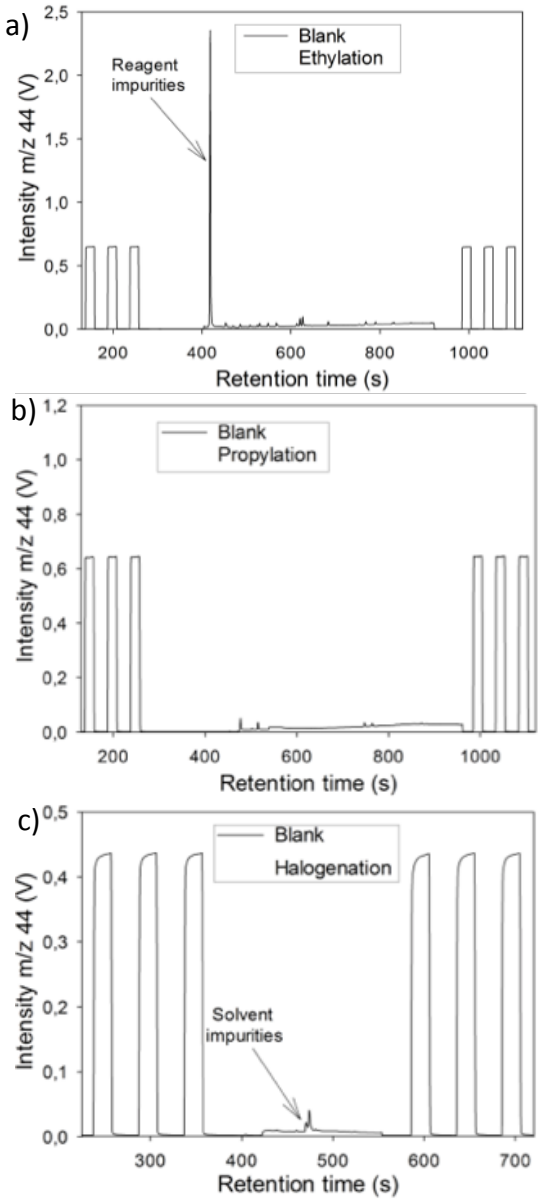


Figure S2. GC-C-IRMS blank chromatograms of ethylation (a), propylation (b) and halogenation (c) conditions



References

(1) Masbou, J.; Point, D.; Sonke, J. E. *Journal of Analytical Atomic Spectrometry* **2013**.