Supporting Information for:

Assessing sources of human methylmercury exposure using stable mercury isotopes

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10 pages (including cover page and references)

3 Tables (S1, S2, S3)

2. Methods

2.3. Hg concentrations and stable isotope analyses

Previous studies have found that washing human hair with deionized water, soap, acetone, or HCl does not remove Hg that is externally adsorbed to the hair.[1, 2] Therefore, because we had limited quantities of hair, we did not wash it prior to preparation and isotopic analysis. Hg concentrations were measured in the final solutions after thermal combustion and transfer of the recovered Hg to a secondary trap. Human hair standards (BCR CRM 397, n = 8), tuna fish standards (ERM CE-464, n = 9), and procedural blanks (n = 5) were processed according to the same methods. Mercury recoveries for the procedural standards were consistently >80% (mean hair standard Hg recovery = 83.6%, SD = 3.3%, n = 8; mean tuna fish Hg recovery = 93.8%, SD = 5.6%, n = 9). The procedural blanks contained only small quantities of Hg that was entirely attributable to the 1% $KMnO_4$ solutions (mean = 0.005±0.001 ng Hg per g solution, SD, n = 5). We were also able to analyze four of the hair samples from the Faroese whalers and two of the hair samples from the Gulf of Mexico anglers in duplicate. Hg concentrations measured in these replicate samples were very similar (mean percent difference = 7.4%, SD = 5.3%, n = 6). Hg isotope ratios measured in these replicates were also very similar within the analytical uncertainty determined using the procedural standards with the exception of one sample (Mixed 2). It is likely the replicates of this sample displayed more variable Hg isotope ratios because the hair was bisected in half instead of being evenly divided along the entire length of hair. As a result, the two samples may not have been duplicates and may have instead recorded intake of MeHg from two different time periods.

3. Results

3.3. MeHg exposure sources calculated from hair Hg isotopes

We estimated the fraction of MeHg in each Gulf of Mexico angler's hair sample that resulted from exposure to different seafood sources in two ways using a simple two-end-member mixing model (Equations 1-2). We calculated estimates of the fraction of MeHg in each individual's hair that resulted from exposure to oceanic fish using the $\Delta^{199}Hg_h$ value of their hair (f_{MIF}), the $\delta^{202}Hg_h$ value of their hair (f_{MDF}) and dietary recall.

$$\Delta^{199}Hg_{oc} \times f_{MIF} + \Delta^{199}Hg_c \times (1-f_{MIF}) = \Delta^{199}Hg_h \quad (1)$$

$$[\delta^{202}Hg_{oc} \times f_{MDF} + \delta^{202}Hg_c \times (1-f_{MDF})] + MDF = \delta^{202}Hg_h \quad (2)$$

$$f_r = \sum_{oc} m_{oc} \times C_{oc} / (\sum_{oc} m_{oc} \times C_{oc} + \sum_c m_c \times C_c) \quad (3)$$

In this model, the reported average Hg isotope ratios in oceanic ($\delta^{202}Hg_{oc}$, $\Delta^{199}Hg_{oc}$) and coastal ($\delta^{202}Hg_c$, $\Delta^{199}Hg_c$) fish from the northern Gulf of Mexico are as follows[3]: $\delta^{202}Hg_{oc}$ = 0.41‰; $\Delta^{199}Hg_{oc}$ = 1.74‰; $\Delta^{199}Hg_c$ = 0.53‰; $\delta^{202}Hg_c$ = -0.54‰. f_{MIF} and f_{MDF} are the estimated fractions of Hg in the hair that originated from consumption of oceanic fish based on $\Delta^{199}Hg_h$ and $\delta^{202}Hg_h$, respectively. We assume that no MIF occurs during demethylation within the human body[4-7] and that the Δ^{199}Hg value of ingested MeHg is retained in the hair samples. We also assume a consistent offset in δ^{202}Hg values between human dietary MeHg sources and human hair. Here we applied the offset in δ^{202}Hg that we observed in the Faroese whaler's hair samples (MDF=1.75‰) to estimate f_{MDF}. The results from this model are presented in Table S2. Equation 3 shows how we calcuated the fractions of Hg derived from oceanic fish (f_r) based on dietary recall. The summed product of individual consumed masses (*m*) of oceanic (*oc*) and coastal (*c*) fish species reported over a three month period and their respective MeHg concentrations (*C*) were used to estimate the fraction of MeHg from ocean fish consumed by each angler. The three month recall period represents the same exposure time period as that represented by the hair samples collected at the base of the scalp.[8]

We conducted a sensitivity analysis using Crystal Ball (Fusion Edition, Oracle Corporation) to determine how variability in the fish Hg isotope ratios used in the isotope mixing model (i.e., $\delta^{202}Hg_{c}$, $\delta^{202}Hg_{oc}$, $\Delta^{199}Hg_{c}$, $\Delta^{199}Hg_{oc}$) influences the estimates of f_{MIF} and f_{MDF}. In each simulation trial, the analysis took random draws of each Hg isotope ratio within the range of ± 1 SD of the mean isotope ratios previously measured[3] (SD: $\delta^{202}Hg_{oc}$ = 0.18‰, $\delta^{202}Hg_{c}$ = 0.32‰, $\Delta^{199}Hg_{oc}$ = 0.48‰ and $\Delta^{199}Hg_{c}$ = 0.11‰). These values were used as input parameters to the isotope mixing model (Equation 1 and 2) and the corresponding estimates of f_{MIF} and f_{MDF} were then calculated. After 1,000 trials for each individual, we believe that the simulation captured the full range (minimum to maximum) of estimated f_{MIF} and f_{MDF} due to observed variability in fish Hg isotope ratios. Estimates of f_{MIF} and f_{MDF} made using average Hg isotope ratios of Gulf of Mexico fish and the full range of modeled fish isotope ratios are shown in Table S2.

Table S1. Mercury concentrations and isotope ratios in standards and samples (a: Faroese whalers and pilot whales; b: GOM anglers). The number of analyses for the UM-Almadén standard is the total number of analytical sessions and presented isotope ratios are averages of the mean value for each session. The number of analyses for procedural standards is the total number of processed standards and presented isotope ratios are averages of the mean value for each session. Analytical uncertainties for the UM-Almadén standard and procedural standards are 2 s.d. of analytical session averages. Analytical uncertainties for samples are 2 s.d. of multiple analyses within one analytical session.

(a)

Standard Name				n	δ^{204}Hg (‰)	2σ (‰)	δ^{202}Hg (‰)	2σ (‰)	δ^{201}Hg (‰)	2σ (‰)	δ^{200}Hg (‰)	2σ (‰)	δ^{199}Hg (‰)	2σ (‰)	Δ^{204}Hg (‰)	2σ (‰)	Δ^{201}Hg (‰)	2σ (‰)	Δ^{200}Hg (‰)	2σ (‰)	Δ^{199}Hg (‰)	2σ (‰)
UM-Almadén standard				58	-0.86	0.08	-0.58	0.06	-0.47	0.06	-0.28	0.04	-0.17	0.04	0	0.06	-0.04	0.03	0.01	0.03	-0.02	0.03
BCR CRM 397 (human hair standard)				8	0.22	0.12	0.14	0.03	0.09	0.06	0.07	0.04	0	0.06	0	0.1	-0.02	0.05	-0.01	0.04	-0.03	0.06
ERM CE-464 (fish procedural standard)				9	0.94	0.06	0.7	0.04	2.49	0.06	0.43	0.05	2.55	0.08	-0.1	0.05	1.96	0.06	0.07	0.04	2.38	0.07
Individual ID	**Location**	**Primary diet**	**Hair [Hg] (µg/g)**	**n**	**δ^{204}Hg (‰)**	**2σ (‰)**	**δ^{202}Hg (‰)**	**2σ (‰)**	**δ^{201}Hg (‰)**	**2σ (‰)**	**δ^{200}Hg (‰)**	**2σ (‰)**	**δ^{199}Hg (‰)**	**2σ (‰)**	**Δ^{204}Hg (‰)**	**2σ (‰)**	**Δ^{201}Hg (‰)**	**2σ (‰)**	**Δ^{200}Hg (‰)**	**2σ (‰)**	**Δ^{199}Hg (‰)**	**2σ (‰)**
HSPH-1	Faroe Islander	Pilot whale	37.00	2	4.59	0.08	3.12	0.08	3.36	0.09	1.62	0.04	2.03	0.03	-0.07	0.04	1.01	0.03	0.05	0	1.24	0.06
HSPH-2	Faroe Islander	Pilot whale	25.23	2	5.05	0.14	3.42	0.13	3.6	0.05	1.76	0.06	2.09	0.02	-0.04	0.05	1.03	0.05	0.04	0.01	1.23	0.02
HSPH-3	Faroe Islander	Pilot whale	12.52	1	4.72		3.22		3.49		1.66		2.11		-0.08		1.06		0.05		1.29	
HSPH-3 REP	Faroe Islander	Pilot whale	13.52	1	4.77		3.2		3.48		1.68		2.14		0		1.08		0.07		1.33	
HSPH-4	Faroe Islander	Pilot whale	10.71	2	5.15	0.03	3.47	0.08	3.63	0.08	1.8	0.06	2.16	0.13	-0.03	0.15	1.02	0.02	0.06	0.02	1.28	0.11
HSPH-4 REP	Faroe Islander	Pilot whale	10.56	2	5.04	0.15	3.43	0.02	3.62	0.04	1.76	0.01	2.15	0.02	-0.08	0.12	1.04	0.03	0.04	0.02	1.29	0.02
HSPH-7	Faroe Islander	Pilot whale	22.13	2	5.26	0.07	3.54	0.03	3.71	0.01	1.82	0.06	2.14	0	-0.03	0.03	1.04	0.01	0.04	0.05	1.25	0.01
HSPH-7 REP	Faroe Islander	Pilot whale	19.22	2	5.37	0.03	3.68	0.03	3.82	0.05	1.92	0.04	2.22	0.01	-0.12	0.07	1.06	0.03	0.07	0.05	1.29	0.01
HSPH-10	Faroe Islander	Pilot whale	18.12	2	4.55	0.01	3.1	0.06	3.36	0.07	1.63	0.01	2.06	0.02	-0.07	0.09	1.04	0.03	0.07	0.02	1.28	0
HSPH-10 REP	Faroe Islander	Pilot whale	16.30	2	4.41	0.02	2.94	0.12	3.26	0.19	1.55	0.01	2.02	0.1	0.03	0.2	1.05	0.1	0.07	0.05	1.28	0.07
Pilot whales			**Whale wet wt conc (µg/g)**	**n**	**δ^{204}Hg (‰)**	**2σ (‰)**	**δ^{202}Hg (‰)**	**2σ (‰)**	**δ^{201}Hg (‰)**	**2σ (‰)**	**δ^{200}Hg (‰)**	**2σ (‰)**	**δ^{199}Hg (‰)**	**2σ (‰)**	**Δ^{204}Hg (‰)**	**2σ (‰)**	**Δ^{201}Hg (‰)**	**2σ (‰)**	**Δ^{200}Hg (‰)**	**2σ (‰)**	**Δ^{199}Hg (‰)**	**2σ (‰)**
28/80-2006, 753	G. melas, Huaunasund		5.66	2	2.07	0.01	1.46	0.03	2.05	0.03	0.8	0.03	1.53	0	-0.12	0.06	0.95	0.06	0.07	0.01	1.16	0.01
2818-2006, 759	G. melas, Huaunasund		4.6	2	1.95	0	1.32	0.03	1.88	0.13	0.69	0.07	1.44	0.07	-0.02	0.04	0.88	0.11	0.02	0.05	1.11	0.06
2818-2006, 759 REP	G. melas, Huaunasund		4.77	2	1.97	0.08	1.29	0.09	1.88	0.17	0.68	0.05	1.5	0.02	0.04	0.06	0.91	0.1	0.03	0	1.18	0
2818-2006, 760	G. melas, Huaunasund		4.33	2	2.13	0.03	1.42	0.01	2.01	0.03	0.78	0.01	1.53	0.03	0.01	0.02	0.94	0.03	0.06	0.02	1.17	0.02
2818-2006, 761	G. melas, Huaunasund		3.93	2	1.95	0.06	1.32	0.05	1.96	0.06	0.69	0.06	1.51	0	-0.02	0.13	0.97	0.02	0.02	0.04	1.17	0.01

1319-2006, 783	G. melas, Torshaun	2.81	2	2.61	0.1	1.78	0.18	2.35	0.12	0.97	0.12	1.67	0.11	-0.04	0.17	1.01	0.01	0.07	0.03	1.22	0.06
1319-2006, 790	G. melas, Torshaun	4.76	2	2.07	0.11	1.51	0.06	2.14	0	0.81	0.09	1.56	0.06	-0.18	0.02	1	0.04	0.05	0.06	1.18	0.05
1319-2006, 911	G. melas, Torshaun	3.83	2	2.39	0.06	1.65	0.05	2.3	0.04	0.88	0.07	1.62	0.03	-0.07	0.14	1.06	0	0.05	0.04	1.2	0.02
1319-2006, 914	G. melas, Torshaun	3.02	2	2.77	0.15	1.93	0.03	2.36	0.08	0.98	0	1.71	0.09	-0.11	0.1	0.91	0.06	0.02	0.01	1.22	0.08
1319-2006, 9171	G. melas, Torshaun	2.94	2	2.26	0.05	1.58	0.13	2.17	0.11	0.82	0.08	1.61	0.04	-0.09	0.13	0.98	0.02	0.03	0.02	1.21	0.01

(b)

Standard Name				**n**	**δ^{204}Hg (‰)**	**2σ (‰)**	**δ^{202}Hg (‰)**	**2σ (‰)**	**δ^{201}Hg (‰)**	**2σ (‰)**	**δ^{200}Hg (‰)**	**2σ (‰)**	**δ^{199}Hg (‰)**	**2σ (‰)**	**Δ^{204}Hg (‰)**	**2σ (‰)**	**Δ^{201}Hg (‰)**	**2σ (‰)**	**Δ^{200}Hg (‰)**	**2σ (‰)**	**Δ^{199}Hg (‰)**	**2σ (‰)**
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BCR CRM 397 (human hair standard)				8	0.22	0.12	0.14	0.03	0.09	0.06	0.07	0.04	0	0.06	0	0.1	-0.02	0.05	-0.01	0.04	-0.03	0.06
ERM CE-464 (fish procedural standard)				9	0.94	0.06	0.7	0.04	2.49	0.06	0.43	0.05	2.55	0.08	-0.1	0.05	1.96	0.06	0.07	0.04	2.38	0.07
Individual ID	**Location**	**Primary diet**	**Hair [Hg] (µg/g)**	**n**	**δ^{204}Hg (‰)**	**2σ (‰)**	**δ^{202}Hg (‰)**	**2σ (‰)**	**δ^{201}Hg (‰)**	**2σ (‰)**	**δ^{200}Hg (‰)**	**2σ (‰)**	**δ^{199}Hg (‰)**	**2σ (‰)**	**Δ^{204}Hg (‰)**	**2σ (‰)**	**Δ^{201}Hg (‰)**	**2σ (‰)**	**Δ^{200}Hg (‰)**	**2σ (‰)**	**Δ^{199}Hg (‰)**	**2σ (‰)**
Oceanic 1	Louisianna fisherman	oceanic fish	1.78	2	3.92	0.04	2.71	0.16	3.72	0.06	1.42	0.14	2.79	0.04	-0.12	0.2	1.69	0.06	0.06	0.06	2.11	0.08
Oceanic 2	Louisianna fisherman	oceanic fish	1.46	2	3.5	0.12	2.39	0.02	3.24	0.03	1.3	0.03	2.41	0.02	-0.07	0.16	1.43	0.01	0.1	0.01	1.81	0.01
Coastal 1	Louisianna fisherman	coastal fish	1.67	1	2.88		2.05		3.07		1.06		2.46		-0.18		1.52		0.03		1.94	
Coastal 2	Louisianna fisherman	coastal fish	1.8	1	2.14		1.46		1.91		0.82		1.36		-0.04		0.81		0.09		1	
Coastal 3	Louisianna fisherman	coastal fish	0.65	2	3.94	0.11	2.66	0	3.05	0.02	1.46	0.02	1.98	0	-0.02	0.11	1.05	0.02	0.12	0.02	1.31	0
Coastal 4	Louisianna fisherman	coastal fish	0.5	2	2.38	0.08	1.67	0.03	2.72	0.13	0.91	0.07	2.27	0.04	-0.11	0.04	1.46	0.11	0.07	0.06	1.85	0.05
Mixed 1	Louisianna fisherman	mixed oceanic/coastal fish	0.45	1	3.46		2.41		3.03		1.32		2.26		-0.14		1.22		0.11		1.65	
Mixed 2	Louisianna fisherman	mixed oceanic/coastal fish	0.48	1	2.6		1.77		2.19		0.96		1.56		-0.04		0.86		0.07		1.12	
Mixed 2 REP	Louisianna fisherman	mixed oceanic/coastal fish	0.53	1	2.19		1.45		1.87		0.76		1.32		0.04		0.79		0.04		0.95	
Mixed 3	Louisianna fisherman	mixed oceanic/coastal fish	1.42	1	2.45		1.67		2.08		0.93		1.53		-0.03		0.82		0.09		1.11	
Mixed 4	Louisianna fisherman	mixed oceanic/coastal fish	0.5	1	2.33		1.58		1.81		0.82		1.23		-0.03		0.62		0.03		0.83	
Mixed 5	Louisianna fisherman	mixed oceanic/coastal fish	1.48	2	2.63	0.03	1.83	0.08	2.7	0.03	0.99	0.07	2.1	0.03	-0.1	0.08	1.32	0.03	0.08	0.03	1.64	0.01
Mixed 5 REP	Louisianna fisherman	mixed oceanic/coastal fish	1.49	2	2.6	0.25	1.77	0.12	2.64	0.13	0.93	0.07	2.04	0.02	-0.05	0.07	1.31	0.04	0.04	0.01	1.59	0.05
Fw 1	Louisianna fisherman	freshwater fish	1.05	1	2.18		1.46		1.32		0.86		0.86		0		0.22		0.13		0.49	

Fw 2	Louisianna fisherman	freshwater fish	3.65	2	2.6	0.02	1.77	0.09	1.92	0.01	0.95	0.02	1.26	0.04	-0.04	0.15	0.59	0.06	0.07	0.02	0.81	0.01
Shell 1	Louisianna fisherman	shellfish	0.63	1	3.84		2.52		2.69		1.32		1.6		0.07		0.79		0.05		0.96	
Shell 2	Louisianna fisherman	shellfish	0.38	1	4.74		3.22		3.32		1.56		1.82		-0.06		0.9		-0.05		1.01	

Table S2.
Comparison of estimated fractions of MeHg from oceanic fish based on dietary recall (f_{recall}) and the isotope mixing model based on $\Delta^{199}Hg_{hair}$ and $\delta^{202}Hg_{hair}$ (f_{MIF} and f_{MDF}). The ranges of f_{MIF} and f_{MDF} based on the sensitivity analysis with variable fish Hg isotope ratios (±SD) are shown in parenthses.

ID	category by diet	f_{recall}	f_{MIF}	f_{MIF} range		f_{MDF}	f_{MDF} range	
Oceanic 1	oceanic fish consumer	99%	130%	(93%,	232%)	158%	(127%,	254%)
Oceanic 2	oceanic fish consumer	100%	105%	(74%,	187%)	125%	(101%,	180%)
Coastal 1	coastal fish consumer	3%	117%	(83%,	200%)	89%	(65%,	113%)
Coastal 2	coastal fish consumer	0%	39%	(23%,	68%)	26%	(-13%,	52%)
Coastal 3	coastal fish consumer	0%	64%	(43%,	107%)	153%	(123%,	243%)
Coastal 4	coastal fish consumer	0%	109%	(77%,	192%)	48%	(18%,	70%)
Mixed 1	mixed fish consumer	44%	93%	(64%,	157%)	127%	(106%,	187%)
Mixed 2	mixed fish consumer	55%	42%	(25%,	72%)	42%	(11%,	65%)
Mixed 3	mixed fish consumer	57%	48%	(30%,	82%)	48%	(19%,	70%)
Mixed 4	mixed fish consumer	66%	25%	(13%,	46%)	39%	(7%,	62%)
Mixed 5	mixed fish consumer	71%	90%	(63%,	157%)	62%	(34%,	83%)

Table S3. Percentages of total MeHg exposure from each fish species accounting for most of the MeHg exposure for each individual based on the dietary survey. Fish species with percentages <5% were not included.

ID	Category by diet	Primary species	Secondary species	Tertiary Species	Others			
Oceanic 1	oceanic fish consumer	Swordfish (61%)	Fresh tuna (24%)					
Oceanic 2	oceanic fish consumer	Wahoo (33%)	Fresh tuna (27%)	Blue marlin (17%)	Mahi Mahi (11%)			
Coastal 1	coastal fish consumer	Speckled trout (41%)	Redfish or Red drum (34%)	Crab (13%)				
Coastal 2	coastal fish consumer	Speckled trout (45%)	Redfish or Red drum (37%)	Crab (14%)				
Coastal 3	coastal fish consumer	Speckled trout (55%)	Redfish or Red drum 45%					
Coastal 4	coastal fish consumer	Speckled trout (49%)	Gaffotopsail-catfish (26%)	Shrimp (10%)	Redfish or Red drum (9%)			
Mixed 1	mixed fish consumer	Speckled trout (33%)	Sushi tuna (25%)	Sushi crab (10%)	Redfish or Red drum (9%)	Shrimp (7%)	Crab (7%)	Fresh tuna (6%)
Mixed 2	mixed fish consumer	Sushi tuna (35%)	Sushi yellowtail (14%)	Speckled trout (10%)	Yellowtail (9%)	Redfish or Red drum (8%)	Fresh tuna (8%)	
Mixed 3	mixed fish consumer	Fresh tuna (46%)	Speckled trout (20%)	Redfish or Red drum (16%)	Cobia (8%)			
Mixed 4	mixed fish consumer	Canned tuna (25%)	Speckled trout (24%)	Amberjack (18%)	Fresh tuna (13%)			
Mixed 5	mixed fish consumer	Sushi tuna (33%)	Sushi snapper (16%)	Canned tuna (10%)	Speckled Trout (10%)	Crab (9%)	Shrimp (9%)	Redfish or Red drum (8%)
Fw 1	freshwater fish consumer	Sacalait or Crappie (35%)	Largemouth bass (19%)	Goggle eye (10%)	Canned tuna (7%)	Brim or Bluegill (6%)	Crab (6%)	
Fw 2	freshwater fish consumer	Largemouth bass (53%)	Sacalait or Crappie (33%)					
Shell 1	shellfish consumer	Crab (35%)	Shrimp (24%)	Oyster (11%)	Freshwater catfish (11%)	Speckled Trout (9%)	Largemouth bass (8%)	
Shell 2	shellfish consumer	Crab (36%)	Oyster (15%)	Shrimp (10%)	Fresh tuna (9%)	Southern flounder (8%)	Crawfish (7%)	

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