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Letter

Chemical Forms of Mercury in Blue Marlin Billfish: Implications for Human Exposure

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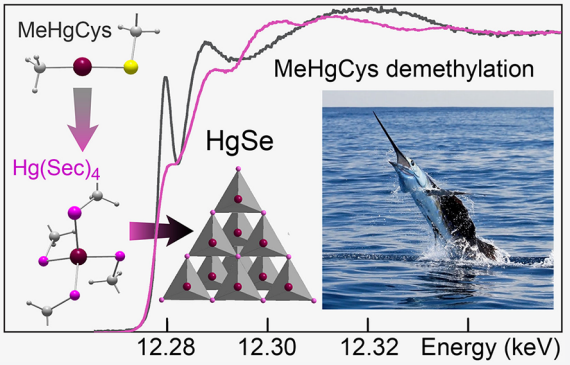
ABSTRACT: Although fish is an important source of nutrients, including some of the healthiest proteins, long-chain fatty acids, and essential selenium, species at the top of the food chain frequently contain large amounts of toxic mercury (Hg). The provisional tolerable weekly intake (PTWI) of Hg from fish consumption is calculated from the total concentration of Hg and assuming that all Hg is speciated as organic methylmercury (MeHg). Using high energy-resolution X-ray absorption near-edge structure (HR-XANES) spectroscopy, we show that blue marlin (*Makaira* sp.), a common top predator consumed by humans, contains high concentrations of inorganic Hg(II) complexed as 57 ± 10% Hg-tetraselenolate [$Hg(Sec)_4$] and 43 ± 10% tiemannite (HgSe). The stable Hg−Se chemical bond likely attenuates the bioavailability of Hg and counteracts some of its health hazards to consumers. Thus, monitoring the concentration of MeHg, rather than total Hg, in top predators such as marlin would provide a more robust measure of potential Hg exposure and may be sufficient for food safety controls. The bonding of Hg atoms to four selenocysteine (Sec) residues in the $Hg(Sec)_4$ complex severely depletes the stock of bioavailable Se, and quantification shows that blue marlin is not a chief source of dietary Se essential to selenoenzyme synthesis and activity.



INTRODUCTION

Eating piscivore marine fish warrants caution.[1] Whereas seafood is the main source of essential selenium (Se) for proper brain function and for the development of spermatozoa,[2−4] top predators can contain toxic concentrations of mercury (Hg).[5−7] Marlin, a billfish consumed by large segments of the Indo-Pacific tropical and subtropical populations and those from the west Atlantic coast, commonly contains several milligrams of Hg/kg of wet weight (ww),[8−14] which exceeds the maximum level of 0.5−1.0 mg/kg ww established by the U.S. Environmental Protection Agency, the U.S. Food and Drug Administration (2017), and Health Canada (2014). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) set a provisional tolerable weekly intake (PTWI) of 1.3 μg of Hg/kg of body weight (bw) for the organic methylmercury form (MeHg)[15] and 4.0 μg of Hg/kg bw for the inorganic mercuric form [Hg(II)].[16,17] MeHg is much more extensively and rapidly absorbed than Hg(II) in the gastrointestinal track,[18] which is why organic and inorganic Hg have different PTWI values. It is the first guideline value of 1.3 μg of Hg/kg bw that is usually considered, as nearly all of the Hg (≳90%) generally occurs as MeHg in muscles of predatory fish.[19−22]

Blue marlin (*Makaira* sp.) is known to accumulate Hg predominantly in inorganic Hg(II) form. This observation was made in the 1970s in marlin captured off Hawaii.[8] The total Hg concentration ($[Hg]_{tot}$) was 4.3 ± 3.9 mg/kg fish ww (n = 35), only 15.3 ± 10.2% of which was organic (MeHg). The average PTWI is 1.3 × 15.3% + 4.0 × 84.7% = 3.6 μg of Hg/kg bw. The PTWI of a 75 kg human is reached upon consumption of $(3.6 \times 10^{-3} \times 75)/4.3$ = 63 g ww blue marlin. Consumers of a large amount of predatory fish may have a considerably higher intake of Hg and largely exceed the PTWI. However, this estimate does not consider the large variability in Hg bioavailability, which diminishes upon cooking,[23,24] among other factors.

To be bioavailable during the digestion process, Hg needs first to be released in a bioaccessible form from the food matrix and to cross afterward the intestinal epithelium to reach the bloodstream. In a comprehensive review of 20 studies of human dietary exposure to seafood, Bradley and co-workers[24] estimated a bioaccessibility range of ∼2−100% for MeHg and 0.2−94% for Hg(II). The large variability in effective Hg

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bioavailability can be explained in the first instance by the diversity of the complexation forms of Hg. Mercury has a high chemical affinity for (1) reduced sulfur [S(-II) present as thiol groups in organic matter] and (2) cysteine (Cys) residues in peptides [e.g., glutathione (GSH)] and proteins, with formation of MeHgCys and $Hg(Cys)_2$ complexes.[20,25] Nutrients rich in either sulfur functionality modify the bioaccessibility of Hg. Under experimental conditions, MeHgCys proved to be less toxic to zebrafish than methylmercury chloride (MeHgCl),[20] and complexation of Hg(II) to thiol groups reduced its absorption across model intestinal epithelium compared to $HgCl_2$.[26] Furthermore, Hg has a higher affinity for reduced Se [Se(-II)] than for S(-II),[27,28] and Hg bonded to Se has a negligible in vivo absorption rate.[29] Thus, the degree of exposure to Hg depends on not only $[Hg]_{tot}$ and the MeHg/Hg(II) ratio but also the molecular identity of Hg in the gut, which in turn depends on its form in raw and cooked fish meat.

Here, the main study goal was to assess the toxicological risk involved in the intake of Hg from the consumption of blue marlin by determining the identity and quantity of the different Hg species in raw muscle using high energy-resolution X-ray absorption near-edge structure (HR-XANES) spectroscopy.[25,30] XANES spectra measured at high energy-resolution (HR) provide increased chemical and structural sensitivity on Hg speciation in biomolecules at natural concentration (Figure S1).[31−35]

MATERIALS AND METHODS

Samples. Eleven fresh fish muscles, otherwise intended for human consumption, were obtained in 2016 and 2017 from a fishmonger on the island of Moorea (French Polynesia). Each fillet was rapidly freeze-dried, ground to a fine powder, and stored in polyethylene vials for further analyses. The average moisture content of fresh fish was 72.53 ± 1.72%.

Molecular Analyses for Identification of Fish Fillets at the Species Level through DNA Barcoding. Genomic DNA was extracted from the 11 fillet powders using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). The gene of the 5′-end fragment for mitochondrial cytochrome *c* oxidase subunit I (COI) was amplified using primers FishF1 and FishR1.[36] PCRs were performed using Type-It Microsatellite (Qiagen) in a 10 μL final volume containing 1× Master Mix, 0.1× Q-solution, each primer at 0.25 μM, and 50−150 ng of DNA template. PCRs were achieved in an Eppendorf tube with 3 min at 94 °C, 37 cycles at 94 °C for 1 min, 48 °C for 1 min, and 72 °C for 90 s, and then a final step at 72 °C for 10 min. Polymerase chain reaction products were sent to Genoscreen (Lille, France) for sequencing on an ABI 3730XL genetic analyzer (Applied Biosystems, Carlsbad, CA) using the forward primer. Sequences of 638 base pairs (bp) of a portion of the mtDNA COI gene were retrieved from sequence chromatograms using BioEDIT.[37] The 11 samples shared the exact same COI sequence, which was submitted to the Barcode of Life Data System (BOLD)[38] for genus and species identification. The COI sequence is >99% identical with all *Makaira nigricans* sequences deposited in public databases, and we therefore confirmed that the 11 marlin samples are all *Makaira mazara* (Jordan and Snyder, 1901), the Indo-Pacific blue marlin, not genetically differentiated from the Atlantic blue marlin *M. nigricans* at the COI gene.[39] The nucleotide sequence was deposited in GenBank (http://www.ncbi.nlm.nih.gov) as entry MW323444.

Hg and MeHg Analyses. Total Hg concentrations were determined on 5−15 mg of homogenized freeze-dried muscle using an Advanced Mercury Analyzer (AMA-254, Altec, Czech Republic) calibrated using gravimetric dilution of a Certified Reference Material (CRM) standard solution (1000 μg/g, Trace Select, Merck). Accuracy was assessed on certified reference material IAEA-436A (tuna fish flesh, IAEA). The absolute mass detection limit was 0.1 ng of Hg, which corresponds to 10 ng/g of total Hg for a 10 mg sample. Muscle tissues were analyzed in triplicate with a recovery of 102 ± 2% (n = 9). The standard uncertainty (RSD) was 4−6%, and the expanded uncertainty (k = 2) was 12%.

MeHg was analyzed on ∼300 mg of muscle digested in an alkaline solution using an automated methylmercury analyzer (MERX Brooks Rand, Seattle, WA) following the established procedure.[40] The instrument was calibrated by external calibration using gravimetric dilution of a CRM standard solution (1000 μg/g, Alfa Aesar). Accuracy was assessed with the IAEA-436A reference. The detection limit was 0.5 pg of Hg, which corresponds to 3 ng/g for a 300 mg sample. Muscle tissues were analyzed in triplicate with a recovery of 104 ± 8% (n = 8). The standard uncertainty (RSD) was 5−10%, and the expanded uncertainty (k = 2) was 15%.

Se Analysis. Selenium concentrations were determined using inductively coupled plasma quadrupole mass spectrometry (Q-ICP-MS, XSERIES, Thermo Fisher Scientific). First, ∼200 mg of freeze-dried tissues was digested with a microwave system (Mars X-press, CEM) in PTFE reactors containing 5 mL of HNO_3 (Ultrex, T. T. Baker, Phillipsburg, NJ) and 2 mL of H_2O_2 (p.a., Merck). Internal standards (rhenium and rhodium) were added to calibration standards and to sample solutions to correct for matrix interference and for potential instrument drifts as a result of temperature variations during ICP-MS measurements. Quality assurance and quality control were assessed by procedural test blanks (n = 6) and analysis of CRM IAEA-407 (fish tissue, IAEA) prepared in the same way as the samples. Se recovery for the CRM averaged 95 ± 6% (n = 3). Procedural blanks were always below the detection limit (0.013 μg/g), and the precision of triplicate measurements was 3−6%.

HR-XANES Spectroscopy. Freeze-dried powder of the marlin skeletal muscles was pressed into pellets, and the Hg L_3-edge XANES spectra were measured at high energy-resolution (HR-XANES) with high-luminosity analyzer crystals[41] on beamline ID26 at the European Synchrotron Radiation Facility (ESRF). Spectra were collected at 10−15 K and a scan time of 15 s to reduce exposure and repeated at new positions on the sample to increase the signal-to-noise ratio. Scans were monitored carefully for any evidence of radiation damage. The incident energy was scanned from 12260 to 12360 eV in 0.2 eV steps, and the spectra were normalized to unity at E = 12360 eV. The identities and relative proportions of the mercury species were determined using a spectral database.[25,32−35] All reference spectra were considered as a basis for identification, but only diagnostic spectra are discussed herein. The precision of the fit components was estimated to be equal to the variation of their best-fit values when the fit residual (NSS) was increased by 20%. NSS is the normalized sum-squared difference between the data and fit expressed as $\sum[(y_{data} - y_{fit})^2/\sum(y_{data}^2)$. Further details about data acquisition and analysis can be found elsewhere.[25,32,33]

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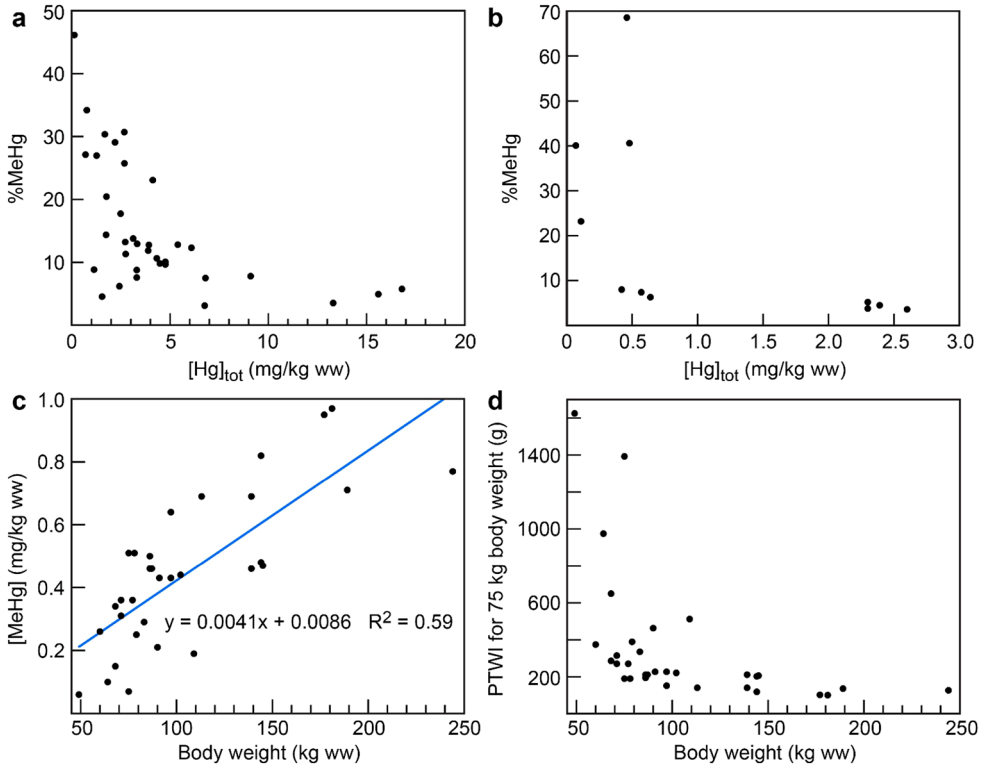

Figure 1. Chemical analyses. Proportion of methylmercury (MeHg) vs total Hg concentration in blue marlin muscle from (a) Hawaii and (b) French Polynesia. (c) Regression analysis of the MeHg concentration in muscle tissue of Hawaii marlin vs fish weight, showing that the MeHg content increases over time. One outlier fish specimen weighing 415 kg has been omitted. (d) Provisional tolerable weekly intake (PTWI) of Hawaii marlin for a 75 kg human as a function of fish weight. Data from the Hawaii fish are from ref 8.

RESULTS AND DISCUSSION

Mercury Concentration. The 11 fish contained $[Hg]_{tot}$ = 1.12 ± 1.03 mg/kg ww and $[MeHg]/[Hg]_{tot}$ = 19.2 ± 21.6% (Table S1). Although the French Polynesia fish have less $[Hg]_{tot}$ on average than the Hawaiian fish, the two sample lots have similar %MeHg (15.3 ± 10.2% and 19.2 ± 21.6%, respectively), and they both exhibit a negative power-law variation of %MeHg with $[Hg]_{tot}$ (Figure 1a,b). Older (i.e., higher weight) fish specimens contain more $[Hg]_{tot}$ and $[MeHg]_{tot}$[8] (Figure 1c), as a result of their lifespan bioaccumulation of Hg, but much less %MeHg. The power-law relationship of %MeHg against $[Hg]_{tot}$ has been observed previously in the brain of southern giant petrel seabirds.[33] Using HR-XANES, the decrease in %MeHg with an increase in $[Hg]_{tot}$ in petrel brains was shown to result from the detoxification of MeHgCys as tiemannite (HgSe),[33] which is an insoluble form of Hg ($K_{sp} = 10^{-56.6\pm0.2}$).[42]

Mercury Speciation. The chemical forms of Hg were determined in the two fish (specimens 1 and 3) having the lowest proportion of MeHg [3.6% and 3.8%, respectively (Table S1)]. Fish specimen 1 has $[Hg]_{tot}$ = 2.60 mg/kg ww, and specimen 3 has $[Hg]_{tot}$ = 2.30 mg/kg ww. Their HR-XANES spectra are indistinguishable, indicating similar or identical Hg speciation (Figure 2a). They closely resemble the spectrum of a petrel muscle containing 26.6 mg of Hg/kg of dry weight (dw) speciated as 67 ± 8% $Hg(Sec)_4$ + 33 ± 8% HgSe (Figure 2b). $Hg(Sec)_4$ is a four-coordinate selenocysteinate $[Hg(Sec)_4]$ complex, and an intermediate species in the detoxification of MeHgCys into HgSe according to the stepwise MeHgCys → $Hg(Sec)_4$ → $Hg_x(Se,Sec)_y$ → HgSe demethylation reaction.[33] Note that Hg is tetrahedrally coordinated to four Se atoms in the three selenious compounds. The two marlin spectra were fit with 57 ± 10% $Hg(Sec)_4$ + 43 ± 10% HgSe, with a detection limit of 6% for MeHgCys and 12% for $Hg(Cys)_2$ (Figure 2c). The frequency of the top edge of metacinnabar (β-HgS) is shifted relative to that of HgSe (Figure S1d), which excludes the existence of a HgS_xSe_{1-x} solid solution. Adding the β-HgS reference to the $Hg(Sec)_4$ + HgSe two-component fit yielded a negative fraction for β-HgS. The MeHgCys spectrum has a sharp absorption line at 12279.8 eV in the rising part of the absorption edge, well observed in albacore tuna (*Thunnus alalunga*) containing >95% MeHg (Figure 2c and Figure S2). The fit residual of the marlin spectra has a small amplitude at this position, which confirms that a small proportion of Hg is methylated in the two fish specimens (arrow in Figure 2c). The higher proportion of HgSe in marlin (43 ± 10%) relative to petrel (33 ± 8%) is seen on the marlin spectra as significant absorption dips at three energies denoted with arrows in Figure 2b. These positions correspond to minima in the HR-XANES of HgSe that are not observed in $Hg(Sec)_4$ (Figure 2d). Although the marlin spectra are well reconstructed with the $Hg(Sec)_4$ and HgSe references, the fit results are compatible with the occurrence of multinuclear $Hg_x(Se,Sec)_y$ clusters ($y < 4x$), as HR-XANES cannot differentiate $Hg_x(Se,Sec)_y$ from a mixture of $Hg(Sec)_4$ and HgSe, and even $Hg_x(Se,Sec)_y$ from $Hg(Sec)_4$ if the structure of the nanoclusters is highly disordered (Figure 2e).[33] The biomineralization of MeHgCys as HgSe implies the demethylation capability of the muscle tissue of marlin via the formation of the $Hg(Sec)_4$ complex that was known to exist only in the liver of freshwater fish,[32] in liver,

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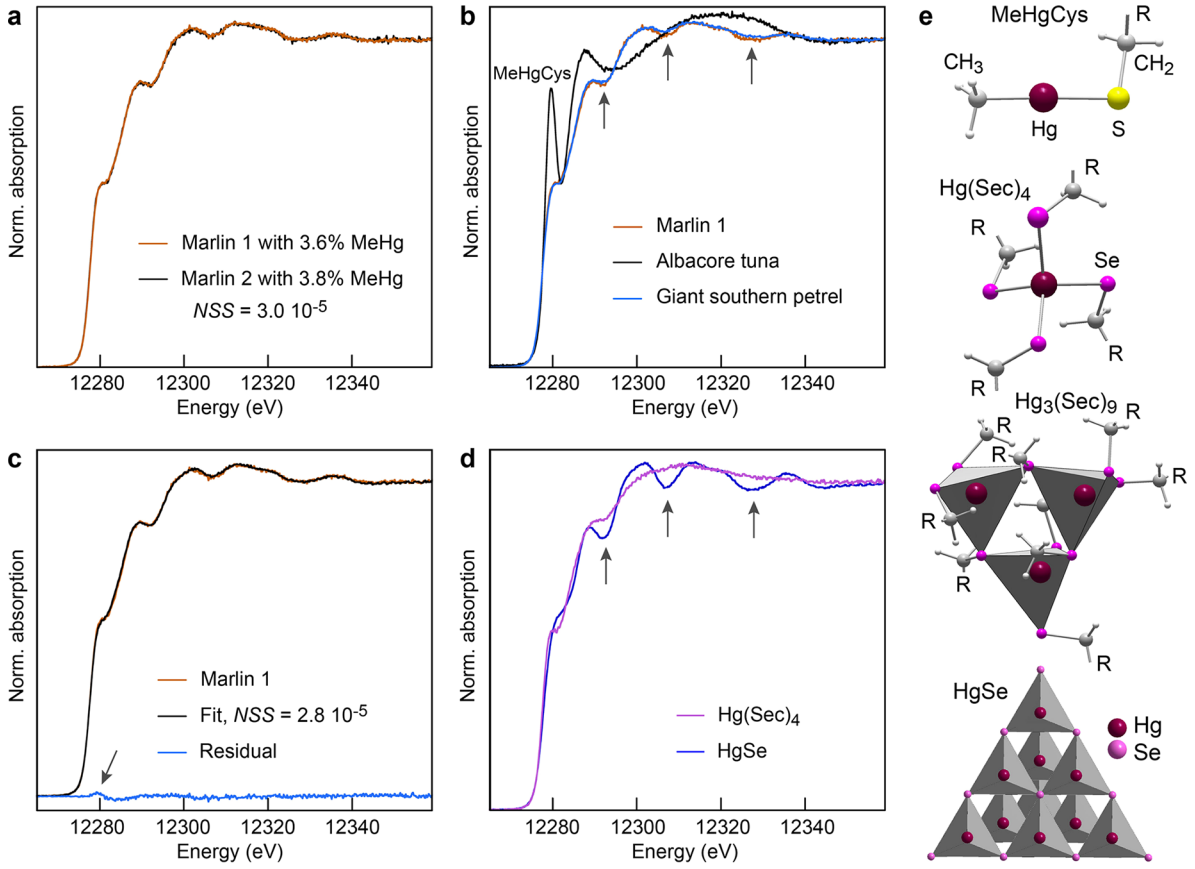

Figure 2. Chemical forms of Hg in marlin muscle derived from Hg L_3-edge HR-XANES spectroscopy. (a) Spectra from the two fish specimens having the lowest proportion of MeHg (Table S1). (b) Spectrum of marlin with muscle spectra from albacore tuna ($[Hg]_{tot}$ = 0.31 mg/kg ww = 1.17 mg/kg dw) and giant southern petrel ($[Hg]_{tot}$ = 42.0 mg/kg dw).[33] (c) Fit of the marlin spectrum with 57 ± 10% $Hg(Sec)_4$ + 43 ± 10% HgSe. (d) Spectra of the $Hg(Sec)_4$ complex and HgSe. NSS is the normalized sum-squared residual expressed as $\sum[(y_2 - y_1)^2/\sum(y_2^2)$. (e) Ball-and-stick representations of the linear coordination of Hg in MeHgCys and its 4-fold coordination to selenocysteine in $Hg(Sec)_4$, polyhedral representation of the $Hg_3(Sec)_9$ cluster as an example of a $Hg_x(Se,Sec)_y$ cluster, and portion of the HgSe structure. Dark red, purple, yellow, gray, and light gray spheres represent Hg, Se, S, C, and H, respectively.

kidneys, muscle, and brain of giant petrel seabirds,[33] and in liver, kidneys, and muscle of the Clark's grebe waterbird.[32]

Toxicological Risk. The speciation results provide a more precise evaluation of the toxicological risk resulting from the consumption of marlin. Because Hg−Se species are either not bioavailable or exhibit little or no toxicity if they are bioavailable,[28,43−46] the amount of toxic Hg reaching the bloodstream can be estimated from the concentration of only MeHg. The PTWI of a 75 kg human is reached for the consumption of $(1.3 \times 10^{-3} \times 75)/0.093$ = 1048 g ww blue marlin 1, where 1.3 is the MeHg PTWI per bw and $[MeHg]_{tot}$ = 0.093 mg/kg (Table S1). Alternatively, if the actual form of Hg is ignored and all inorganic Hg is considered to be speciated as $Hg(Cys)_2$, the average PTWI is 1.3 × 3.6% + 4 × 96.4% = 3.9 μg of Hg/kg bw, where %MeHg = 3.6% and % $Hg(Cys)_2$ = 96.4%. The PTWI of a human of 75 kg is reached for the consumption of $(3.9 \times 10^{-3} \times 75)/2.60$ = 112 g ww blue marlin 1, where $[Hg]_{tot}$ = 2.60 mg/kg. Thus, omitting the actual form of Hg overestimates by 10-fold the dietary exposure to Hg (1.1/0.11). The tolerable weekly meal weight co-varies with $[MeHg]_{tot}$. For the 11 marlins studied here, it ranges between 505 and 3750 g for a 75 kg human (Table S1). For the Hawaiian fish, the range is 103−1625 g. The heavier the fish, the lower the tolerable weekly meal, as $[MeHg]_{tot}$ increases with body weight (Figure 1d).

Mercury−Selenium Antagonism. Mercury exerts its toxicity via complexation to cysteinyl (Cys) and selenocysteinyl (Sec) metal binding sites in proteins.[32,47] The binding of Hg to Sec residues reduces the Se supply for the biosynthesis of vital selenoproteins. It is usually considered that Hg is detoxified as HgSe and therefore is without toxicological consequences when the molar difference [Se] − [Hg] > 0, or the molar ratio $[Hg]_{mol}/[Se]_{mol}$ < 1, leaving sufficient bioavailable Se for seleoprotein synthesis and activity.[28,48] The $[Hg]_{mol}/[Se]_{mol}$ ratio represents the fraction of Se to total Se bound to Hg. This criterion breaks down when Hg is also bonded to four Sec residues $[Hg(Sec)_4]$, because Hg is no longer in equimolar stoichiometry with Se in the $Hg(Sec)_4$ complex (Se/Hg = 4), in contrast to HgSe. There is less bioavailable Se than what chemical analysis estimates. The chemical ratios of specimens 1 and 3 are $[Hg]^{chem}_{mol}/[Se]^{chem}_{mol}$ = 0.72 and 0.56, respectively, and the ratios of the other marlins are $0.05 \leq [Hg]^{chem}_{mol}/[Se]^{chem}_{mol} \leq 0.65$, suggesting that the amount of Se is large enough to counteract some of the health hazards of Hg upon selenoenzymes (Table S1). However, because 57% of the Se-bound Hg is speciated as $Hg(Sec)_4$ [and/or $Hg_x(Se,Sec)_y$], there is less bioavailable Se than what chemical analysis estimates. The biological $[Hg]^{biol}_{mol}/[Se]^{biol}_{mol}$ ratio derived from HR-XANES is higher than $[Hg]^{chem}_{mol}/[Se]^{chem}_{mol}$. If all inorganic Hg is speciated as $Hg(Sec)_4$ and HgSe,

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the biological ratio is[33] $[Hg]^{biol}_{mol}/[Se]^{biol}_{mol} = [Hg]^{chem}_{mol}/[Se]^{chem}_{mol} \times [\%HgSe + 4 \times \%Hg(Sec)_4]/100$. Specimen 1 has $[Hg]^{biol}_{mol}/[Se]^{biol}_{mol} = 0.72 \times (0.43 + 4 \times 0.57) = 1.9$, and specimen 2 has $[Hg]^{biol}_{mol}/[Se]^{biol}_{mol} = 0.56 \times (0.43 + 4 \times 0.57) = 1.5$. The two biological ratios exceed the threshold value of 1, suggesting that all Se is bonded to Hg. Another way to look at it is to calculate the concentration of bioavailable Se: $[Se]_{bio} = (1 - [Hg]^{biol}_{mol}/[Se]^{biol}_{mol}) \times [Se]_{tot}$.[33] $[Se]_{bio}$ is negative for the two fish specimens, meaning that the muscle tissues are depleted in bioavailable Se. Because $[Se]_{bio}$ obviously cannot be negative, the calculation supports the occurrence of $Hg_x(Se,Sec)_y$ because $y < 4x$. Thus, Se deficiency disorders may appear as little to no Hg-unbound Se appears to remain for biological processes.[3,28] We conclude that consumption of specimens 1 and 3 is not a chief source of dietary Se essential to selenoenzyme metabolism and detoxification of MeHgCys.

Fish Consumption Advisory. This study highlights the importance of considering more systematically the amount and chemical forms of inorganic Hg to evaluate human exposure and risk, as Se- and S-bound Hg(II) have quite different toxicological implications. Marlins fished in Hawaian and French Polynesian waters contain $Hg(Sec)_4$ and HgSe species, which are not thought to be very hazardous, and various amounts of MeHgCys, which is highly hazardous. Thus, monitoring the concentration of MeHg in commercial blue marlin appears to be sufficient for food safety control. A cheap methodology for measuring MeHg routinely does exist.[49] As a general recommendation, one should avoid regularly consuming the heaviest blue marlins ($\gtrsim$100 kg), as body weight (or length) is a good predicator of tissue MeHg with larger fish specimens having higher levels of MeHg than smaller specimens (Figure 1c), and one should cook the food, as boiling and grilling diminish the bioaccessibility of Hg in fish meat.[23,24]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.1c00217.

Table S1 and Figures S1 and S2 (PDF)

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Notes

The authors declare no competing financial interest.

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Supplementary Information

The chemical forms of mercury in blue marlin billfish: Implications for human exposure

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Table S1. Total concentrations in mg/kg of Hg, MeHg, and Se, proportions of MeHg to total Hg, total Hg to total Se molar ratios, and provisional tolerable weekly intake for a consumer weighing 75 kg.

Specimen	$[Hg]_{tot}$ (dw)	$[Hg]_{tot}$ (ww)	[MeHg] (dw)	[MeHg] (ww)	%MeHg	$[Se]_{tot}$ (dw)	[Hg]/[Se]	PTWI (g)
1	9.45	2.60	0.340	0.093	3.6	5.15	0.72	1048
2	8.68	2.39	0.391	0.107	4.5	5.26	0.65	911
3	8.38	2.30	0.319	0.088	3.8	5.88	0.56	1108
4	8.37	2.30	0.434	0.119	5.2	-	-	819
5	2.34	0.64	0.148	0.041	6.3	3.10	0.30	2378
6	2.07	0.57	0.153	0.042	7.4	2.86	0.29	2321
7	1.73	0.48	0.704	0.193	40.6	2.64	0.26	505
8	1.68	0.46	1.150	0.316	68.6	-	-	309
9	1.53	0.42	0.123	0.044	8.0	-	-	2216
10	0.41	0.11	0.095	0.026	23.2	3.16	0.05	3750
11	0.27	0.07	0.108	0.030	40.1	-	-	3250
Mean	**4.08**	**1.12**	**0.360**	**0.099**	**19.2**	**4.01**	**0.40**	**1692**
SD	**3.74**	**1.03**	**0.321**	**0.088**	**21.6**	**1.36**	**0.24**	**1151**

Note: All concentrations were measured on freeze-dried powder (dw) and the Hg concentrations converted on a wet weight basis (dw). Marlin contains 72.53 ± 1.72 % of water (n = 13), which gives a conversion factor of 0.275 between dry and wet weights. All calculated results (ratio, percentages, mean, SD) are based on unrounded raw data, taking molar masses of 78.96 g/mol for Se and 200.59 g/mol for Hg. PTWI values of fish specimens 2, 5, 6, 7, and 10 were calculated by assuming that inorganic Hg is speciated as $Hg(Sec)_4$ and HgSe only (i.e., no $Hg(Cys)_2$).

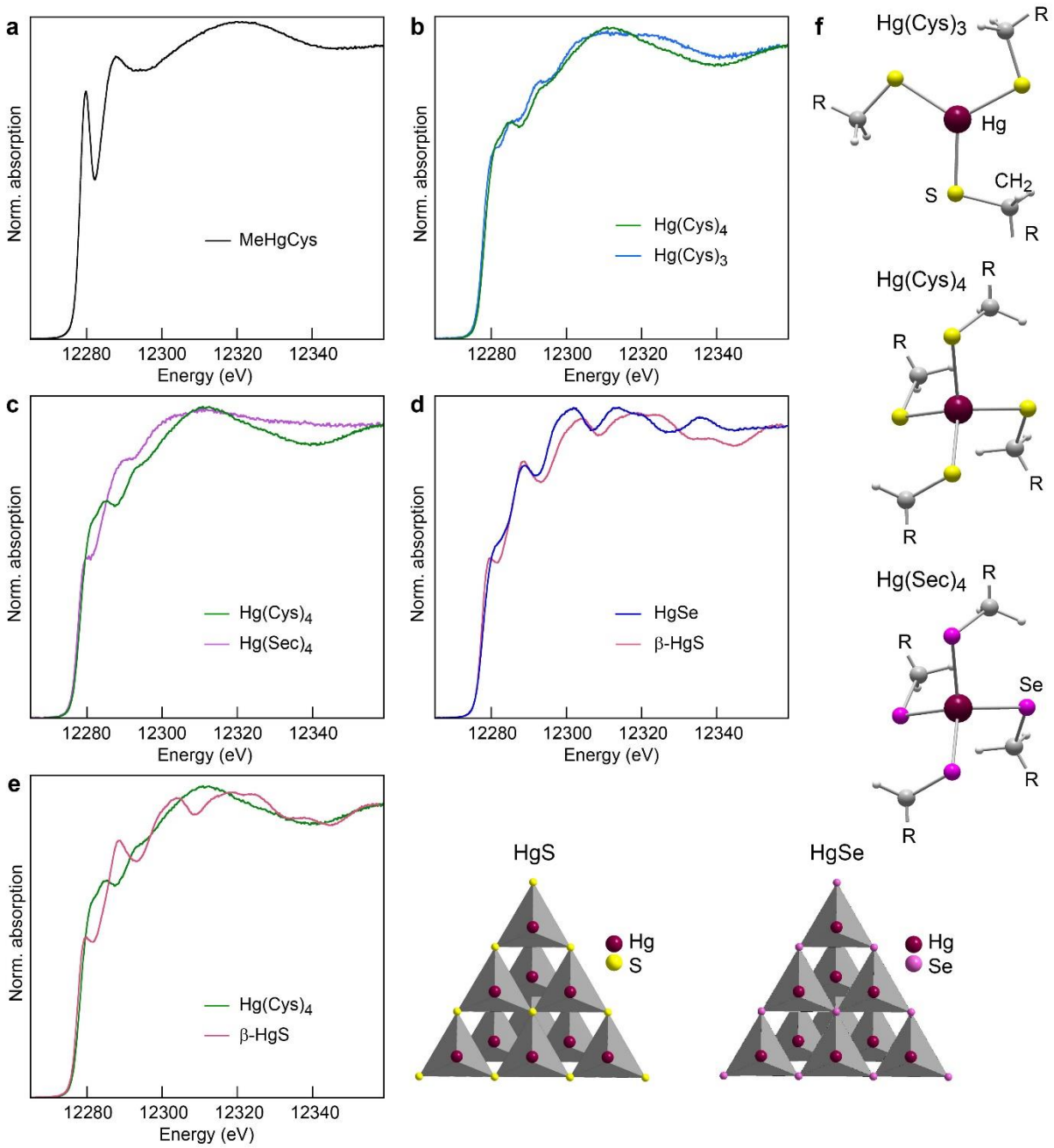

Figure S1. Sensitivity of Hg L_3-edge HR-XANES spectroscopy to Hg coordination. (a) Hg linearly coordinated to one methyl group and one cysteinate anion (MeHgCys complex). The digonal coordination of Hg gives a sharp absorption line in the near-edge region (i.e., rising part of the absorption spectrum). The spectrum is from a feather of giant southern petrel[1]. (b) Hg three-coordinated ($Hg(D\text{-}Pen)_3$[2, 3]) and four-coordinated ($Hg(Cys)_4$[3, 4]) to cysteinate anions. The energy of the trailing edge after the edge maximum depends on the Hg-S distance, it is shifted to lower energy in $Hg(Cys)_4$ which has a bond length of ~2.55Å, ~0.1 Å longer than in $Hg(Cys)_3$[5]. (c) Hg four-coordinated to cysteinate (Cys) and selenocysteinate (Sec[6]) anions. A higher absorption below the edge maximum and a flat absorption after the edge maximum distinguish the Hg-Se bond from the Hg-S bond. (d) Hg four-coordinated to sulfide anions in β-HgS and to selenide anions in HgSe.

The two spectra are similar, because the two mineral structures are isomorphic. The absorption oscillations in the top-edge region are displaced to lower energy in HgSe as the Hg-Se bonds are longer (2.63 Å[7]) than the Hg-S bonds (2.53 Å[8]). (e) Hg four-coordinated to S in $Hg(Cys)_4$ and β-HgS. The β-HgS trace oscillates on both sides of the $Hg(Cys)_4$ trace, a result of the crystalline structure of β-HgS with Hg-Hg pairs not existing in the $Hg(Cys)_4$ complex. This observation holds for the comparison of HgSe and $Hg(Sec)_4$ in Fig. 2d. (f) Bonding structure of Hg in $Hg(Cys)_3$, $Hg(Cys)_4$, and $Hg(Sec)_4$ complexes, and in portions of the β-HgS and HgSe structures. Dark red, purple, yellow, gray, and light gray spheres represent Hg, Se, S, C, and H, respectively.

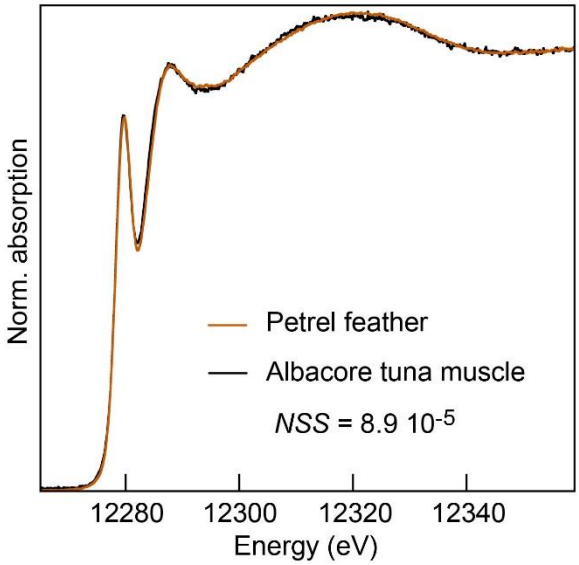

Figure S2. HR-XANES spectra of the muscle tissue from albacore tuna and feather from giant southern petrel. 100 ± 5% of Hg is methylated in the two tissues[1], in contrast to merlin muscle in which it is almost completely (>95%) demethylated (Fig. 2b).

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