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Demethylation of methylmercury in bird, fish, and earthworm

Alain Manceau^{1*}, Jean-Paul Bourdineaud², Ricardo B. Oliveira³, Sandra L.F. Sarrazin³, David P. Krabbenhoft⁴, Collin A. Eagles-Smith⁵, Joshua T. Ackerman⁶, A. Robin Stewart⁷, Christian Ward-Deitrich⁸, M. Estela del Castillo Busto⁸, Heidi Goenaga-Infante⁸, Aude Wack¹, Marius Retegan⁹, Blanka Detlefs⁹, Pieter Glatzel⁹, Paco Bustamante¹⁰, Kathryn L. Nagy¹¹ & Brett A. Poulin^{12,13*}

1. Université Grenoble Alpes, ISTerre, CNRS, Grenoble, France
2. Université de Bordeaux, Institut Européen de Chimie et Biologie, CNRS, Pessac, France
3. Universidade Federal do Oeste Pará, LabBBEx, Santarém, Pará, Brazil
4. U.S. Geological Survey, Upper Midwest Water Science Center, Middleton, Wisconsin, USA
5. U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Corvallis, Oregon, USA
6. U.S. Geological Survey, Western Ecological Research Center, Dixon Field Station, Dixon, California, USA
7. U.S. Geological Survey, Water Resources Mission Area, Menlo Park, California, USA
8. National Measurement Institute, LGC Limited, Teddington, Middlesex, United Kingdom
9. European Synchrotron Radiation Facility (ESRF), Grenoble, France
10. Université La Rochelle, CNRS, Littoral Environnement et Sociétés, La Rochelle, France
11. University of Illinois at Chicago, Department of Earth and Environmental Sciences, Chicago, Illinois, USA
12. U.S. Geological Survey, Water Resources Mission Area, Boulder, Colorado, USA
13. Department of Environmental Toxicology, University of California Davis, Davis, CA 95616, USA

Correspondence to: alain.manceau@univ-grenoble-alpes.fr; bapoulin@ucdavis.edu

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Abstract. Toxicity of methylmercury (MeHg) to wildlife and humans results from its binding to cysteine residues of proteins, forming MeHg-cysteinate (MeHgCys) complexes that hinder biological functions. MeHgCys complexes can be detoxified *in vivo*, yet how this occurs is unknown. We report that MeHgCys complexes are transformed into selenocysteinate (Hg(Sec)₄) complexes in multiple animals from two phyla (a waterbird, freshwater fish, and earthworms) sampled in different geographical areas and contaminated by different Hg sources. In addition, high energy-resolution X-ray absorption spectroscopy (HR-XAS) and chromatography-ICP mass spectrometry of the waterbird liver support the binding of Hg(Sec)₄ to selenoprotein P and biomineralization of Hg(Sec)₄ to chemically inert nanoparticulate mercury selenide (HgSe). The results provide a foundation for understanding mercury detoxification in higher organisms, and suggest that the identified MeHgCys to Hg(Sec)₄ demethylation pathway is common in nature.

INTRODUCTION

Mercury (Hg) enters ecosystems as inorganic divalent (Hg(II)) and elemental (Hg(0)) forms through atmospheric deposition at the global scale, and through anthropogenic point sources at the local scale. Inorganic Hg(II) is subject to methylation (to methylmercury (MeHg)) by microorganisms¹ and is transformed to MeHg-cysteinate (MeHgCys) when taken up in multicellular organisms. MeHgCys has been reported in skeletal muscle of fish,^{2,3} and whales⁴ as well as in human brain⁵ and hair.⁶ In marine mammals and seabirds, in which the Hg burden is exceptionally high and selenium (Se) abundant, MeHgCys is understood to be demethylated in the form of HgSe and Hg(S,Se) granules.⁷⁻¹² No other detoxified form of MeHgCys is yet known. Attempts to characterize the HgSe granules by X-ray absorption spectroscopy have been deceptive. In crystalline HgSe, the Hg atoms are tetrahedrally coordinated to 4 Se atoms at 2.635 Å and surrounded by 12 Hg neighbors at 4.30 Å. While Hg-Se bond lengths between 2.59 Å and 2.63 Å have been reported in liver and pituitary tissues from beluga whale

and in human brain by extended X-ray absorption fine structure (EXAFS) spectroscopy,^{5, 13} no Hg-Hg pairs have ever been detected in animals and human. The possibility exists that MeHgCys is detoxified to organic selenide forms prior to nanoparticulate HgSe nucleation.

To address this question, we investigated *in vivo* chemical forms of Hg in liver and extrahepatic tissues of the waterbird Clark's grebe (*Aechmophorus clarkii*) and several species of freshwater fish, and in whole bodies of earthworms. The Clark's grebe was collected from Lake Berryessa, which is impacted by mining activity in the California Coast Range (Table 1). Five piscivore fish, including three peacock bass (two *Cichla temensis* and one *C. monoculus*) and two pescada branca (*Plagioscion squamosissimus*), were collected in the Tapajós River in Brazil contaminated by artisanal gold mining activity (Tables S1 and S2). Earthworms ($n=13$) were collected from two French soils impacted by Hg deposition from a chlor-alkali plant¹⁴ and analyzed with and without digestive tracts (Table S3).

Mercury speciation analysis was performed using high energy-resolution (HR) X-ray absorption spectroscopy (HR-XAS).¹⁵ In X-ray absorption near-edge structure (HR-XANES) mode, Hg ligands are identifiable with enhanced chemical sensitivity compared to standard XANES, and Hg-Hg pairs are detectable in biological tissues at sub-ppm Hg concentrations (<1 mg total Hg kg^{-1}),^{6, 16, 17} even when the pairs occur in nanometric HgSe precipitates. HR-XAS also provides high quality HR-EXAFS data for complementary insight into the bonding environment and local structure of Hg. The parasitic Se K-absorption edge, which limits the energy range of the Hg L₃-edge EXAFS signal to 374 eV ($k \sim 9.6 \text{ \AA}^{-1}$)¹⁸⁻²¹ unless Ga K-edge absorption filters are used,^{22, 23} is not observed at high energy-resolution.²⁴⁻²⁶ The speciation of Hg and Se in Clark's grebe liver and muscle was also investigated by double affinity chromatography high performance liquid chromatography coupled to inductively coupled mass spectrometry (AF-HPLC-ICPMS).^{27, 28}

MATERIALS AND METHODS

Sampling and sample preparation. *Clark's grebe.* An adult male Clark's grebe (*Aechmophorus clarkii*; band # 2097-56064) was collected from Lake Berryessa (California, United States; 38°36'31"N 122°14'33"W NAD 83) in September 2012²⁹ and necropsied at the U.S. Geological Survey (Dixon, CA) to obtain the following tissues: head feather, breast feather, brain, pectoral muscle, kidneys, and liver. Tissues were lyophilized and homogenized. Clark's grebes are piscivorous, nest inland on lakes and migrate to coastal climates over the winter, and exhibit some of the highest Hg concentrations among bird species in western North America.³⁰

Fish. Peacock bass (*Cichla sp.*), called "tucunaré" in the Amazon region, is a piscivore fish in the adult state, and lakes are its main habitat. The fish species are given in Table S1. Tucunaré weighs up to 10 kg and there is no protection from harvesting during the period of reproduction, therefore it is sold on local markets all year long. Tucunaré is not a migratory species and the fish live generally in groups; therefore, the Hg content reflects local dietary intake. *Plagioscion squamosissimus*, called "pescada branca" in the Amazon region, is also a piscivore fish which can exceed 10 kg. It is a migratory fish that can swim many kilometers up and down river. The specimens used in this study were fished by artisanal fishermen in September 2018 in lakes near the community of São Luis do Tapajós (4°25'28.8"S 56°14'36.5"W NAD 83) located on the Tapajós River, which borders artisanal gold mining areas where liquid Hg⁰ is used for gold mining.

Earthworm. Earthworms were collected in 2017-2018 from two soils (S1: 45°05'13.1"N, 5°44'27.5"E NAD 83; S2: 45°05'26.1"N, 5°44'25.5"E NAD 83) near the chlor-alkali plant in Champ-sur-Drac located ~10 km south of Grenoble, France. Site S1 is situated approximately 250 meters to the southeast and site S2 approximately 300 meters to the northeast of the electrolytic cells. Soil samples of the topsoils (A-horizon) were taken for total Hg and Se analyses and pH determination. They were freeze-dried, the undecomposed organic material removed under a microscope, then sieved to < 2 mm and < 63 µm. Soil pH was measured on the < 2 mm fraction after 45 min agitation of 6 g

soil in 15 mL Milli-Q water. Soil S1 had a pH of 7.7 and soil S2 had a pH of 8.0. Hg and Se concentrations were measured on the < 63 μm fraction. Total Hg concentrations of soils were S1 = $0.20 \pm 0.01 \text{ mg kg}^{-1}$ and S2 = $27.6 \pm 0.3 \text{ mg kg}^{-1}$. A concentration of $0.23 \pm 0.02 \text{ mg kg}^{-1}$ was reported previously in a tilled soil layer situated 900 m north of the plant.¹⁴ The origin of Hg at this site is probably atmospheric. The high concentration at the second site probably results from a Hg spill. The Hg concentration was high enough for determination of Hg speciation by HR-XANES spectroscopy. Figure S1 shows that Hg is mainly in the form of nanoparticulate $\beta\text{-HgS}$. Total Se concentration of soil was lower at site S1 ($0.36 \pm 0.04 \text{ mg kg}^{-1}$) compared to S2 ($0.78 \pm 0.08 \text{ mg kg}^{-1}$).

Five endogeic and one anecic earthworms were sampled at site S1 and five endogeic and two anecic at site S2 (Table S3). Eleven out of the thirteen earthworms were rapidly frozen in liquid nitrogen then freeze-dried and crushed in a mortar for chemical and HR-XANES analysis. The digestive tract were removed from one endogeic earthworm from site S1 (EarthW-endo-1, $1.5 \pm 0.2 \text{ mg Hg kg}^{-1} \text{ dw}$), and one endogeic (EarthW-endo-9, $5.4 \pm 0.5 \text{ mg Hg kg}^{-1} \text{ dw}$) and one anecic (EarthW-anecic-1, $1.2 \pm 0.1 \text{ mg Hg kg}^{-1} \text{ dw}$) earthworm from site S2, and the dissected earthworms were freeze-dried. All dry powders were preserved in a desiccator until analysis. The total Hg concentrations of the 13 earthworms ranged from 0.53 and 10.1 mg kg^{-1} . The mean Hg concentration of endogeic earthworms is $1.9 \pm 1.0 \text{ mg kg}^{-1}$ ($n=5$) at site S1 and $5.8 \pm 2.5 \text{ mg kg}^{-1}$ ($n=5$) at site S2. Earthworms from the S2 site contained higher proportion of MeHg than those from the S1 site.

Methods. A detailed description of the experimental methods, including chemical, PCR, and protein analyses, SeLP amino acid sequences, and HR-XANES and HR-EXAFS measurements and data reduction, is given in the Supporting Information (SI). Briefly, all samples were measured for total Hg and Se concentration by chemical analysis, and for Hg speciation by HR-XANES spectroscopy. Only the Clark's grebe liver tissue contained high enough Hg ($43.1 \pm 4.3 \text{ mg kg}^{-1}$) for HR-EXAFS measurement. The speciation of Hg and Se in Clark's grebe liver and muscle tissues was also measured

by AF-HPLC-ICPMS. PCR analysis was performed on soil and earthworm microflora extracted from the digestive tract for the presence of Hg methylation genes. The predicted tertiary structures of SeIP amino acid sequences across fish, birds, and mammals were modeled. Data generated during this study are available at <https://doi.org/10.5066/P96NP376>.³¹

RESULTS

Mercury and selenium concentrations. The Clark's grebe tissues exhibited total Hg and Se concentrations consistent with grebes from high Hg locations in the Western United States,³² and a hierarchy in both element concentrations similar to those of other vertebrates (brain < muscle < kidneys < liver; Table 1).³³ The Clark's grebe feathers had elevated total Hg ($40.7 \pm 4.1 \text{ mg kg}^{-1}$) and low Se levels ($1.04 \pm 0.10 \text{ mg kg}^{-1}$). In the three fish species, total Hg and Se concentrations were comparable (mean [Hg] = $2.6 \pm 1.4 \text{ mg kg}^{-1}$, mean [Se] = $3.0 \pm 3.0 \text{ mg kg}^{-1}$), Se concentrations were higher in liver compared to muscle (mean [Se]_{liver} = $4.9 \pm 3.3 \text{ mg kg}^{-1}$, mean [Se]_{muscle} = $1.0 \pm 0.1 \text{ mg kg}^{-1}$), and the relative concentration of total Hg in liver versus muscle samples varied across the five individuals (from 0.4 to 1.8). Total Hg and Se concentrations in earthworms ranged from 1.2-10.1 mg kg^{-1} and 1.0-28.3 mg kg^{-1} , respectively (mean [Hg] = $3.7 \pm 2.5 \text{ mg kg}^{-1}$, mean [Se] = $10.6 \pm 8.0 \text{ mg kg}^{-1}$) (Tables S2 and S3).

Identification of the Hg-selenocysteinate complex in Clark's grebe. Two dominant Hg species occur in the Clark's grebe tissues as indicated by isosbestic points in the HR-XANES spectra, points where all spectra have the same normalized absorption (Figure 1a). The feathers and brain spectra have an intense near-edge peak at 12279.8 eV characteristic of MeHgCys^{6, 34} (Figure S2a). Conversely, the liver spectrum has a weak near-edge peak and the top-edge occurs at lower energy (12312-12338 eV). These differences are diagnostic of a shift in Hg coordination from linear in feathers and brain to tetrahedral in liver (Figure S2b).³⁵ Muscle and kidney tissues contain variable proportions of these two

species. The liver spectrum does not match exactly any known Hg spectrum, and of the tetrahedral compounds in our extended spectral database (ref.³⁵ and refs. therein) corresponds most closely to nanoparticulate tiemannite (HgSe_{NP}) (Figure 1b). However, the spectrum lacks oscillations in the top-edge region (12300-12340 eV) that arise from multiple scattering events of the photoelectron on the 12 Hg atoms surrounding a central Hg atom in particulates. Such oscillations would be intense in well-crystallized HgSe and less intense in nanoparticulate materials, which have wide ranges of angles and bond lengths as commonly observed for metal clusters in proteins.³⁵ Instead, the top-edge region is bell-shaped, which indicates that little of the Hg occurs as HgSe granules of any size. The seleniol nature of the new tetrahedral Hg species was confirmed by HR-EXAFS spectroscopy (Figure 2). The liver HR-EXAFS data were fit with Hg bound on average to 2.6 ± 0.6 Se atoms at 2.61 ± 0.01 Å and 0.8 ± 0.2 S atoms at 2.35 ± 0.03 Å in the first coordination shell, consistent with a four-fold coordination of Hg to Se³⁶ for the dominant seleniol species (Figure S3a) and a two-fold coordination of Hg to S for minor MeHgCys (Table S4). The minor contribution of MeHgCys was confirmed at 18% by chemical analysis (Figure S4). We conclude that the inorganic Hg species is a tetrahedral selenolate complex with selenocysteine residues ($\text{Hg}(\text{Sec})_4$).

The HR-XANES spectrum of $\text{Hg}(\text{Sec})_4$ was obtained by iterative transformation factor analysis (ITFA) of all data from bird, fish, and earthworm tissues, in which varying proportions of the $\text{Hg}(\text{Sec})_4$ complex were observed together with MeHgCys and a Hg dithiolate complex $\text{Hg}(\text{SR})_2$ (Figure S5). Cysteine is the most likely biological thiol associated with either a peptide (e.g., glutathione, GSH) or protein. Using the $\text{Hg}(\text{Sec})_4$ spectrum, the best two-component fit of the grebe liver spectrum was obtained with $86 \pm 5\%$ $\text{Hg}(\text{Sec})_4$ + $14 \pm 5\%$ MeHgCys, the latter represented by the Clark's grebe feather (Figure S6a and Table S5). Although the $\text{Hg}(\text{Sec})_4$ spectrum, like the grebe liver spectrum, lacks the oscillations in the top-edge region observed in HgSe_{NP} (Figure 1b), several lines of evidence point to the co-presence of individual $\text{Hg}(\text{Sec})_4$ complexes and disordered $\text{Hg}_x(\text{Se},\text{Sec})_y$ clusters in the grebe

liver, as in hepatic and extrahepatic tissues of giant petrels *Macronectes* spp.¹² Careful study of the top-edge region of the Hg(Sec)₄ spectrum shows that it slightly deviates from the Clark's grebe liver spectrum at energies where HgSe exhibits intense oscillations (arrows in Figure S6a). For this reason, a modest improvement in fit quality (10%) was achieved by including 6% HgSe in the fit of the grebe liver (Figure S6b), which decreased the proportion of Hg(Sec)₄ from 86% to 80%. It is likely that more than 6% of the total Hg is present as Hg_x(Se,Sec)_y clusters in the grebe liver, as (1) the HgSe reference is a proxy for well-crystallized HgSe granules and does not account for disordered Hg_x(Se,Sec)_y clusters (Figure S7), and (2) the molar ratio of Se and Hg (Se:Hg_{tot}) in the grebe liver is 1.1 ± 0.2 (Figure S8a). The existence of Hg_x(Se,Sec)_y clusters in the grebe liver is further supported by second coordination shell fitting of the HR-EXAFS spectrum, which detects 2.1 Hg-Hg pairs at 4.46 ± 0.03 Å, compared to 12 pairs at 4.30 Å in crystalline HgSe (Figure 2b, Figure S9, and Table S4).

Mercury speciation in the two animal phyla. The Hg(Sec)₄ complex was observed in the two animal phyla, and proportions of Hg species were obtained by least squares fitting of all HR-XANES spectra with linear combinations of MeHgCys, Hg(Sec)₄, and Hg(SR)₂. In the Clark's grebe, the amounts of mercury in the major Hg(Sec)₄ complex plus minor Hg_x(Se,Sec)_y/HgSe_{NP} are 37.1 ± 4.3 mg kg⁻¹ in liver, 12.7 ± 1.7 mg kg⁻¹ in kidneys, and 0.7 ± 0.3 mg kg⁻¹ in muscle (Table 1 and Table S5). The Hg(Sec)₄ species is also present in fish liver and earthworm samples in varying mixtures with MeHgCys and Hg(SR)₂ (Tables S2 and S3), but fish muscle tissues contain exclusively MeHgCys, in line with previous XANES studies.^{2,3} In the fish, the proportion of total Hg in the liver that is Hg(Sec)₄ correlates positively with the [Hg]_{liver}: [Hg]_{muscle} ratio (Figure S10) and was as high as $62 \pm 5\%$ in the liver of the fish with highest Hg concentration (*Cichla temensis*, 5.5 mg Hg kg⁻¹), suggesting that Hg(Sec)₄ is a product of demethylation. The MeHgCys and Hg(Sec)₄ species occur in all earthworms in proportions of $17-60 \pm 10\%$ and $10-72 \pm 10\%$, respectively, whereas Hg(SR)₂ is either absent or present in proportions up to $73 \pm 10\%$ (Table S3). Total inorganic Hg (Hg(Sec)₄ and Hg(SR)₂) in

earthworms ranges from 39-83%, which is lower than the 90-94% reported in earthworms from 34 natural forest soils throughout Switzerland,³⁷ suggesting that Hg methylation occurs in the two contaminated soils. The *hgcA* gene, which is required for Hg methylation, was observed in the soil and within the digestive tracts of the earthworms (Figures S11, S12, and Table S6). The majority of MeHg in earthworm samples is interpreted to be in the tissue because earthworms with and without digestive tracts had comparable amounts of MeHgCys (Table S3).

Spectra with similar proportions of Hg(Sec)₄ from the two phyla show a high degree of similarity, as demonstrated for the fish liver (62 ± 5%, PeacockBass-3-L), an earthworm (72 ± 10%, EarthW-anecic-2), and Clark's grebe kidneys (59 ± 5%) (Figure 3a,b). The proportion of Hg(Sec)₄ derived from a linear least squares fit of the fish and earthworm spectra to the grebe kidneys spectrum is 64% (Figure 3c). Agreement between the separate estimates of 64% and 59% Hg(Sec)₄ in the Clark's grebe kidneys serves as an internal consistency check on the quantification of the Hg species (Table 1 and Table S5). Further, the accuracy of the spectral fit results was evaluated by comparing the fractional amount and concentration of MeHg obtained by HR-XANES versus chemical extraction and atomic fluorescence detection (Figure S4 and Table S7). Good agreement was observed between the two independent methods for relative abundance and concentration of MeHg (Figure S4).

DISCUSSION

The data provide strong evidence for demethylation of MeHg as a tetraselenolate complex in the two animal phyla. This species can be formed with low molecular weight (LMW) seleniol molecules and with selenoproteins. Selenoneine is a LMW Se-containing molecule³⁸ small enough to form a Hg(Sec)₄ complex (Figure S3b), as demonstrated with a synthetic analogue.³⁶ Of selenoproteins, only selenoprotein P (SelP) contains at least four selenocysteine residues.³⁹ Selenoneine has been reported

in fish,³⁸ and Hg-bound SelP was identified in the plasma of Inuit adults⁴⁰ and miners exposed to high concentration of Hg.⁴¹

SelP is mainly produced in the liver and secreted into the blood plasma where it transports Se to peripheral tissues.^{33, 42} SelP expression was highly upregulated in the miners, supporting the idea that SelP may protect against Hg toxicity. SelP is also produced in lesser amounts in extrahepatic tissues. In vertebrates (goat - *Capra hircus*, mouse - *Mus musculus*), mRNA expression of SelP decreased in the order liver > kidneys > muscle > brain.^{43, 44} The variation of the biosynthesis activity of SelP mRNA among tissues coincides with the percentage fraction of Hg(Sec)₄ in the Clark's grebe tissues derived from HR-XANES: 86 ± 5 % in liver, 59 ± 5 % in kidneys, 11 ± 5 % in muscle, and ≤ 5% in brain (Table S5). The speciation analysis of protein extracts²⁷ of Clark's grebe liver and muscle tissues by AF-HPLC-ICPMS supports association of Hg and Se with SelP (Figure 4). Good agreement between biochemistry and spectroscopy is strong evidence for the binding of Hg to selenoprotein P in the cells of the Clark's grebe and fish. Similar binding configurations have been identified between Sec residues and metal coenzymes.⁴⁵ Less conclusive is the selenolate ligand in earthworm given the absence of SelP in annelids;⁴⁶ perhaps it is selenoneine.

Multiple selenocysteine (Sec) alignments of SelP from fish, birds, and mammals (including humans) show that the C-terminal domain contains the consensus XUXUX⁶UXUX motif (single-letter amino acid code, where U is Sec and X is any other amino acid) (Figure S13). This U- or Sec-rich region is the most plausible binding site for Hg, given the proximity of the protein chain to the cytosol. This hypothesis was evaluated by modeling the tertiary structure of the XUXUX⁶UXUX amino acid region of SelP for the great crested grebe (*Podiceps cristatus*) and zebrafish (*Danio rerio*) by iterative threading assembly refinement (I-TASSER⁴⁷, Figures S14 and S15). The three bird and fish models with the highest prediction score all consist of a loop with the X⁶ sequence forming the turn and the UXU motifs facing each other on each strand (Figure 5 and Figure S15). The predicted conformations

are highly favorable to bind the Hg^{2+} ion in a tetrahedral configuration. The geometric similarity of bird and fish selenolate sites, despite having relatively low amino acid sequence identity (35% in the C-terminal domain), suggests that this binding site is preserved in vertebrates and may have a common origin.

The most direct interpretation of the findings is that the XUXUX⁶UXUX SelP site in birds and fish, and selenoneine or an unknown LMW species in earthworms, bind Hg(II) as the product of MeHgCys demethylation. The coexistence of $\text{Hg}(\text{SR})_2$ with $\text{Hg}(\text{Sec})_4$ in numerous earthworms (Table S3) and the Clark's grebe muscle and kidney tissues (Table 1) could arise from an additional, though less prominent, demethylation pathway producing $\text{Hg}(\text{SR})_2$ or a deficiency in available selenolate. Although the $\text{Hg}(\text{SR})_2 \rightarrow \text{Hg}(\text{Sec})_4$ reaction cannot be dismissed in theory, the formation of $\text{Hg}(\text{Sec})_4$ through this pathway has not been documented. More work is ultimately required to document thiolate versus selenolate competition for MeHg and Hg(II) at the protein level.²³ In terms of reactivity, selenolate (as selenocysteine) outcompetes thiolate (as cysteine) for association with MeHg,⁴² and selenolate addition to MeHgCys is exergonic and cleaves both the Hg-C and the Hg-SR bonds.^{36, 48, 49} In addition, the protolytic cleavage of the Hg-C bond is promoted when the coordination of Hg increases from two in the reactant (MeHgCys) to four in the product ($\text{Hg}(\text{SR})_4$,⁵⁰ and here, $\text{Hg}(\text{Sec})_4$). The strictly conserved XUXUX⁶UXUX motif of SelP may serve as a catalytic site for the demethylation of MeHg, analogous to cysteine residues in the organomercurial lyase MerB.⁵¹ MeHg demethylation by selenoneine has been demonstrated *in vitro* in fish blood cells and human embryonic kidney cells.⁵²

Placed in a broader context, the results presented here fuel the idea that the C-terminal domain of SelP acts as a nucleation center for the formation of HgSe in vertebrates, driving *in vivo* MeHgCys detoxification. This finding is based on the following observations: (1) Hg is four-coordinate in $\text{Hg}(\text{Sec})_4$ and HgSe; (2) the Se:Hg_{tot} molar ratio of the HgSe grains in the liver of pilot whales is above

1 as the grain size diminishes and in tissue surrounding the grain aggregates,¹¹ which suggests that the grains are embedded in a SelP-rich matrix having a Se:Hg_{tot} ratio approaching and above 4; (3) here, the Se:Hg(Sec)₄ ratio is always greater than 4, as expected if there is a Hg(Sec)₄ complex, except in the Clark's grebe liver (Se:Hg(Sec)₄ = 1.3 ± 0.2) and kidneys (Se:Hg(Sec)₄ = 2.1 ± 0.3) (Figure S8b), the former confirmed to contain Hg_x(Se,Sec)_y/HgSe_{NP} (Figure S9); (4) the C-terminal domain of SelP contains six UXU motifs, which could seed the formation of Hg_x(Se,Sec)_y clusters.¹² These considerations show how Hg speciation results, elemental Se:Hg ratios, and protein analyses can be reconciled to provide a foundation for understanding the process of HgSe formation. A similar nucleation mechanism has been proposed for the formation of Hg₄(Cys)₁₂ clusters from a Hg(Cys)₂ complex and five proximal CXC motifs in metallothionein.³⁵ In SelP, we propose that Hg binds to the XUXUX⁶UXUX site at low concentration, forms highly disordered Hg_x(Se,Sec)_y clusters through metallophilic interactions^{16, 35, 53, 54} in the C-terminal domain at higher concentration, and finally precipitates as HgSe by self-assembly of the Hg_x(Se,Sec)_y scaffolds in highly exposed or older vertebrates. Hg_x(Se,Sec)_y growth may occur through catalysis of MeHgCys demethylation at nanoparticle surfaces. Structural sketches of the Hg_x(Se,Sec)_y biotemplates, inspired from the topology of the metal clusters in vertebrate, invertebrate, and bacterial metallothioneins, are shown in Figures S7a and S16. Biomineralization by protein assemblies with high symmetry is common in nature⁵⁵. The proposed reaction pathway is probably how Hg is detoxified as HgSe in human brain⁵ and seabirds.¹² The binding of Hg to SelP may, however, deplete neuronal and glial cells in organo-Se essential to selenoenzyme synthesis and activity.⁴² It may also alter the antioxidant function of SelP.^{33, 42}

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Materials and methods, supplementary tables and figures, Cartesian coordinates of the Hg(selenoneine)₄ complex and the Hg₁₀(SeMe)₂₀ cluster (PDF).

HR-XANES spectra (Excel).

Data availability

All data supporting the findings of this study are available within the paper and have been deposited in the U.S. Geological Survey repository ScienceBase.³¹ The deposit includes all HR-XANES spectra, the Hg L₃-edge HR-EXAFS spectrum of the Clark's grebe liver, and the Cartesian coordinates of the Hg(selenoneine)₄ complex and the Hg₁₀(SeMe)₂₀ cluster.

AUTHOR INFORMATION

Corresponding Authors

E-mail: alain.manceau@univ-grenoble-alpes.fr

E-mail: bapoulin@ucdavis.edu

ORCID

Alain Manceau: 0000-0003-0845-611X

Kathryn Nagy: 0000-0002-4997-7547
Pieter Glatzel: 0000-0001-6532-8144
Jean-Paul Bourdineaud: 0000-0002-1619-7050
Christian Ward-Deitrich: 0000-0002-5204-2443
M.Estela del Castillo Busto: 0000-0001-6595-5236
Heidi Goenaga-Infante: 0000-0002-2416-9666
Brett Poulin: 0000-0002-5555-7733
David Krabbenhoft: 0000-0003-1964-5020
Collin Eagles-Smith: 0000-0003-1329-5285
Joshua Ackerman: 0000-0002-3074-8322
A. Robin Stewart: 0000-0003-2918-546X

Notes

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Legends of Figures

Figure 1. Mercury coordination in the Clark's grebe derived from Hg L₃-edge HR-XANES. a) Spectra from the grebe tissues. b) Spectrum of the Hg tetraselenocysteine complex (Hg(Sec)₄) with spectra

from well-crystallized and nanoparticulate HgSe (HgSe_{NP}). The linear coordination of Hg in MeHgCys and a portion of the HgSe structure are represented in inset. Dark red, purple, yellow, gray, and light gray spheres represent Hg, Se, S, C, and H, respectively.

Figure 2. Mercury L₃-edge HR-EXAFS data of the Clark's grebe liver. a) HR-EXAFS spectrum fit with Hg-Se and Hg-S pairs in the first coordination shell and Hg-Hg-pairs in the second coordination shell, and fit residual. b) Fourier transform of the HR-EXAFS spectrum and fit. Interatomic distances are not corrected for phase-shift (ΔR).

Figure 3. Mercury coordination in peacock bass and earthworm derived from Hg L₃-edge HR-XANES. a) Comparison of the fish liver spectrum from the individual having the highest proportion of Hg(Sec)₄ (PeacockBass-3-L; $62 \pm 5\%$, Table S2) with the spectrum from the Clark's grebe kidneys ($59 \pm 5\%$ Hg(Sec)₄). b) Comparison of the earthworm spectrum from the individual having the highest proportion of Hg(Sec)₄ ($72 \pm 10\%$, Table S3) with the spectrum from the Clark's grebe kidneys. c, Fit of the Clark's grebe kidneys spectrum with 60% fish spectrum and 40% earthworm spectrum. The calculated proportion of Hg(Sec)₄ is $(0.60 \times 0.61) + (0.40 \times 0.69) = 64\%$, close to the nominal value of 59%. *NSS* is the normalized sum-squared residual expressed as $\Sigma[(y_2 - y_1)^2] / \Sigma(y_2^2)$.

Figure 4. Double affinity chromatograms of Se (red lines) and Hg (blue lines) of Clark's grebe liver extract (a) and Clark's grebe muscle extract (b) by AF-HPLC-ICPMS. The Se species of each peak are as follows: peak 1, P1 (glutathione peroxidase 3; GPX3 and other non-retained species, e.g., inorganic Se and LMW Se species), peak 2, P2 (heparin-binding selenoproteins; likely selenoprotein P, SelP) and peak 3, P3 (Se-albumin; SeAlb). Chromatograms show that, of the Se species, the majority of Hg is associated with P2 ($87.8 \pm 0.4\%$ and $84.3 \pm 0.7\%$ in liver and muscle, respectively).

Figure 5. Modeled tertiary structure of SelP. a) Ribbon representation of the SelP protein for zebrafish (*Danio rerio*), as a proxy for peacock bass. b,c) Amino-acid sequence and predicted structure of the UXUX⁶UXU region of the C-terminal domain of SelP for the great crested grebe (*Podiceps cristatus*), as a proxy for the Clark's grebe (b), and zebrafish, as a proxy for peacock bass (*Cichla sp.*) and pescada branca (*Plagioscion squamosissimus*) (c). U is the single-letter selenocysteine (Sec) code. Purple, red, and blue represent selenol, carboxyl/hydroxyl, and amino groups from amino-acid side chains, respectively. Other models are shown in Figure S16.

Table 1. Total concentrations (mg kg^{-1}) of Hg and Se determined by chemical analysis, and concentrations of MeHg, Hg tetraselenolate, and Hg dithiolate determined by HR-XANES in Clark's grebe (mg Hg kg^{-1} dry weight).

Tissue	[Hg] _{Tot}	[Se] _{Tot}	[MeHgCys]	[Hg(Sec) ₄] ^a	Hg(SR) ₂
Breast feather	40.7 ± 4.1	1.04 ± 0.10	40.7 ± 4.6	0.0 ± 2.1	0.0 ± 2.1
Brain	3.1 ± 0.3	1.55 ± 0.16	3.1 ± 0.3	0.0 ± 0.2	0.0 ± 0.2
Muscle	6.5 ± 0.7	2.31 ± 0.23	4.3 ± 0.5	0.7 ± 0.3	1.5 ± 0.4
Kidneys	21.6 ± 2.2	10.6 ± 1.0	6.0 ± 1.2	12.7 ± 1.7	2.6 ± 1.1
Liver	43.1 ± 4.3	19.3 ± 1.9	6.0 ± 2.2	37.1 ± 4.3	0.0 ± 2.1

^aMinor $\text{Hg}_x(\text{Se},\text{Sec})_y/\text{HgSe}_{\text{NP}}$ species are not modeled independent of $\text{Hg}(\text{Sec})_4$, because the amount cannot be quantified.

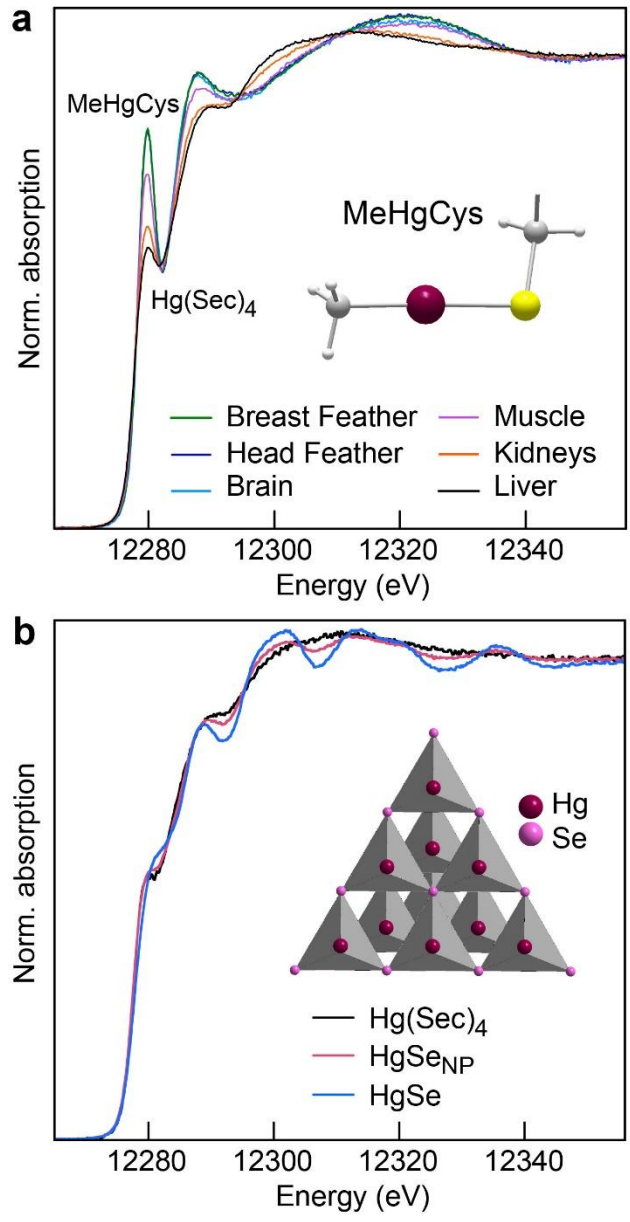


Figure 1

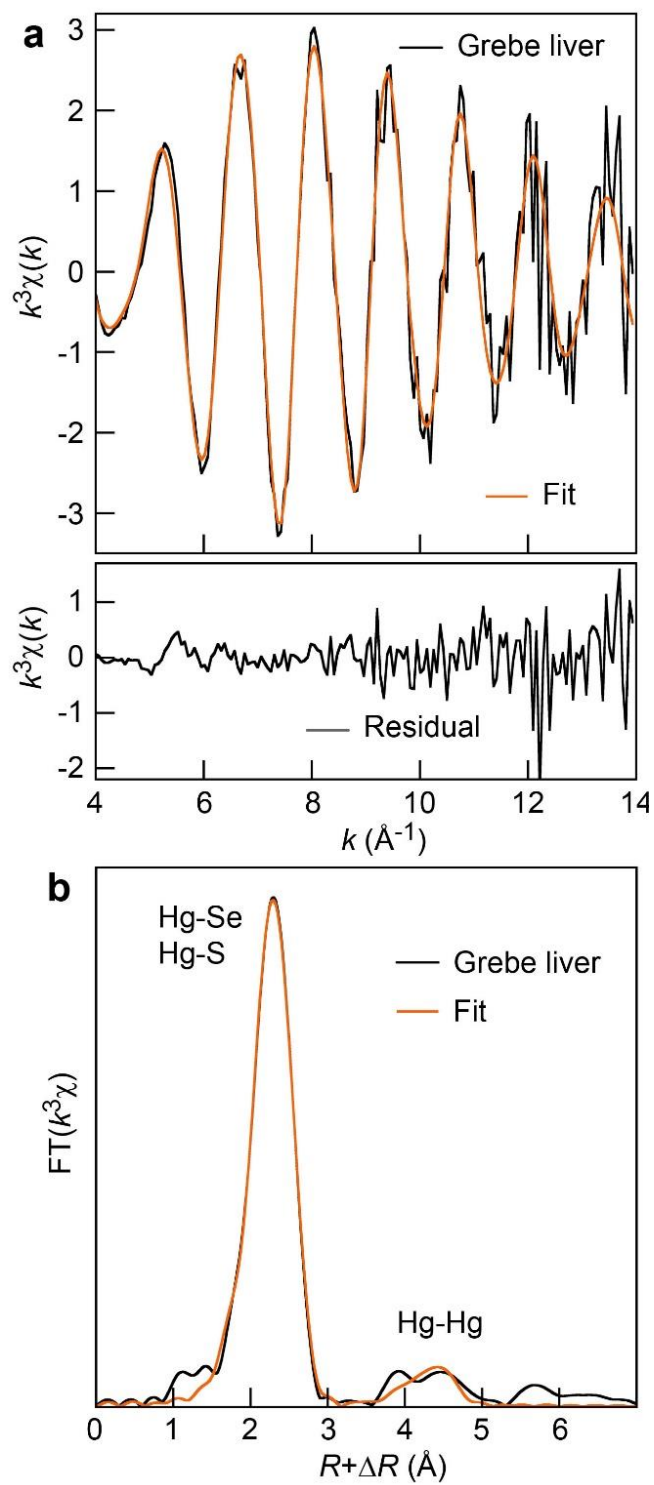


Figure 2

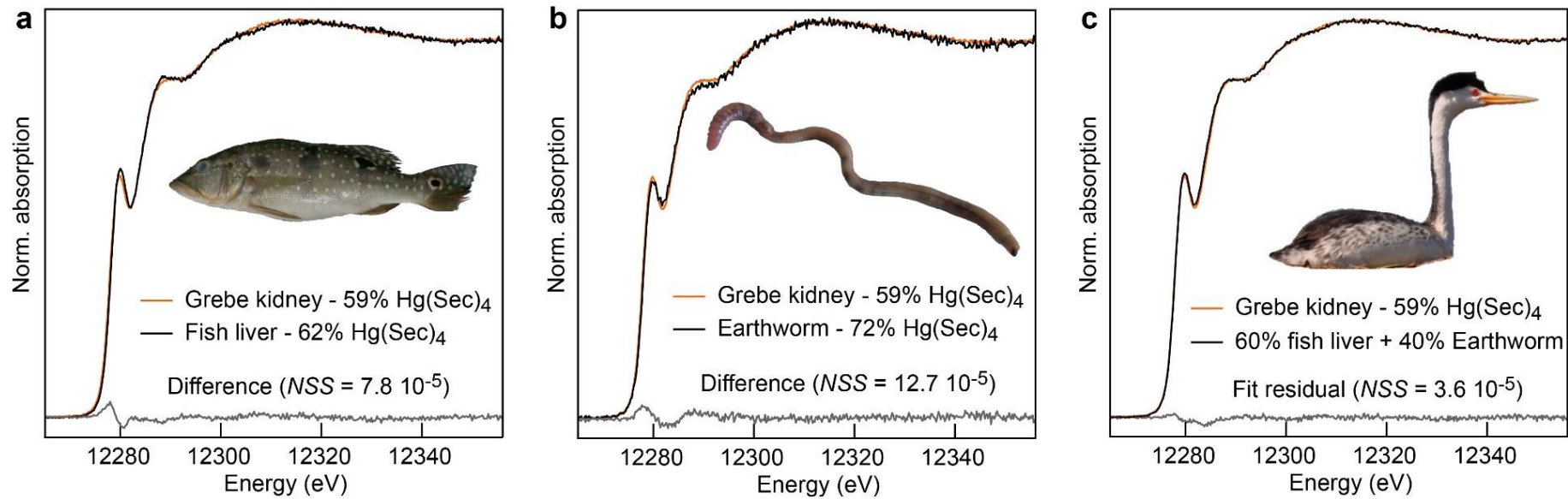


Figure 3

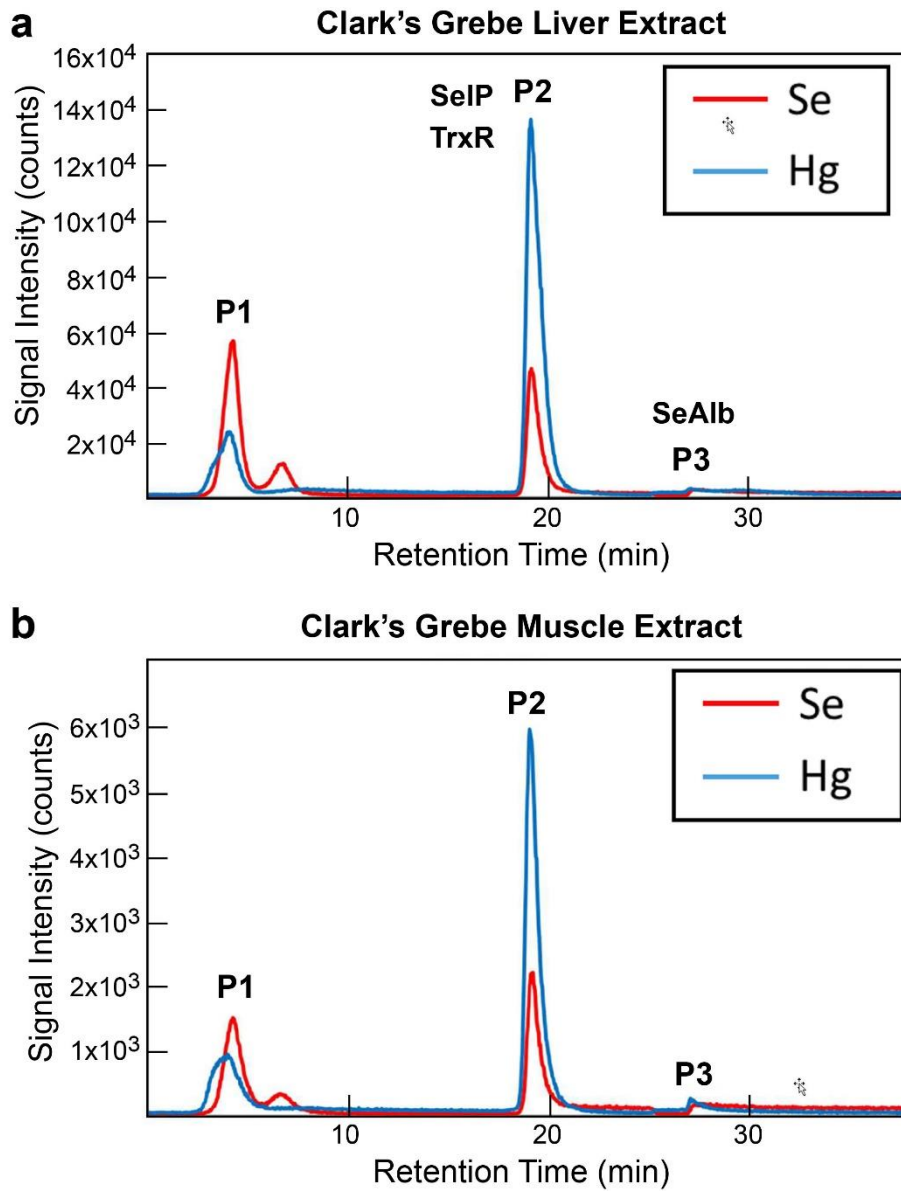


Figure 4

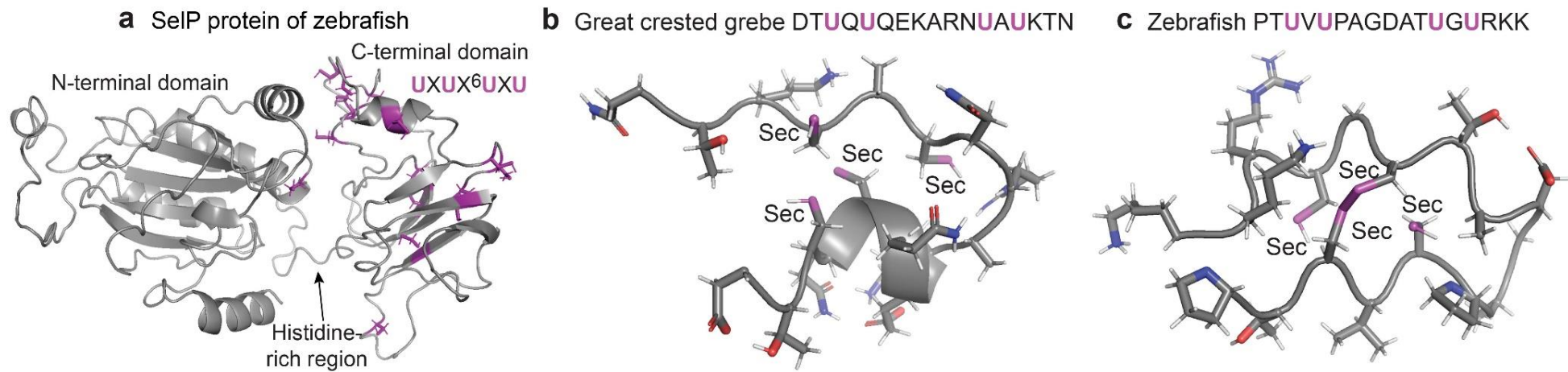
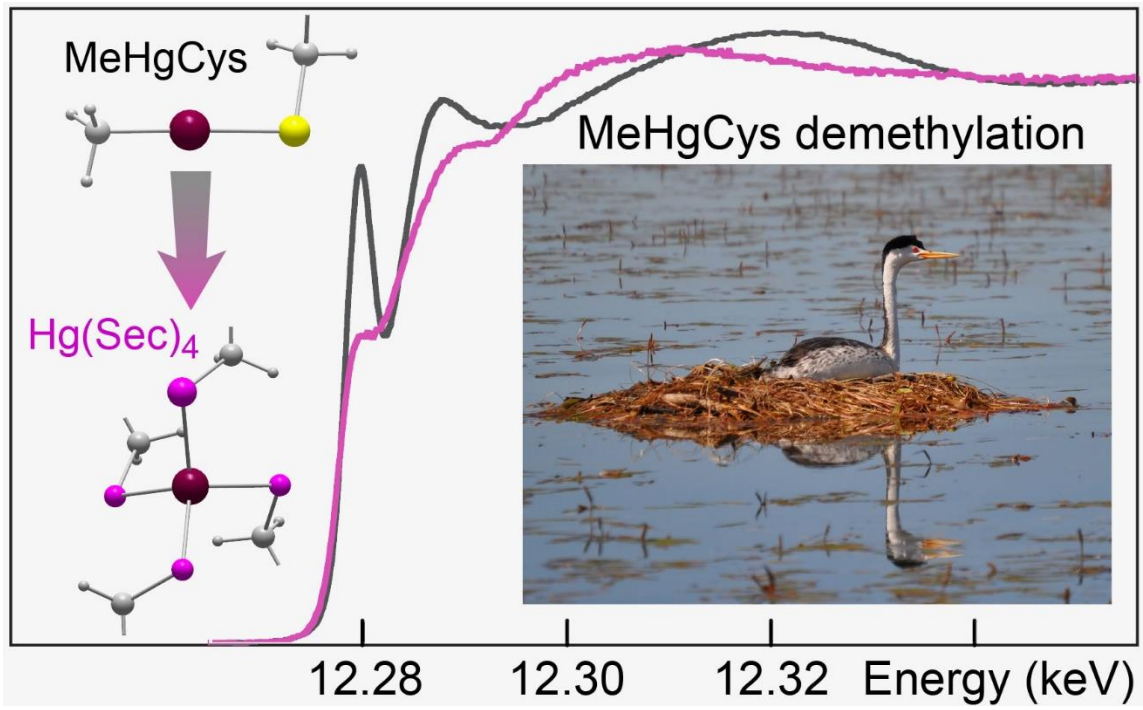


Figure 5



TOC

Supplementary Information

Demethylation of methylmercury in bird, fish, and earthworm

Alain Manceau^{1*}, Jean-Paul Bourdineaud², Ricardo B. Oliveira³, Sandra L.F. Sarrazin³, David P. Krabbenhoft⁴, Collin A. Eagles-Smith⁵, Joshua T. Ackerman⁶, A. Robin Stewart⁷, Christian Ward-Deitrich⁸, M. Estela del Castillo Busto⁸, Heidi Goenaga-Infante⁸, Aude Wack¹, Marius Retegan⁹, Blanka Detlefs⁹, Pieter Glatzel⁹, Paco Bustamante¹⁰, Kathryn L. Nagy¹¹ & Brett A. Poulin^{12,13*}

1. Université Grenoble Alpes, ISTerre, CNRS, Grenoble, France
2. Université de Bordeaux, Institut Européen de Chimie et Biologie, CNRS, Pessac, France
3. Universidade Federal do Oeste Pará, LabBBEx, Santarém, Pará, Brazil
4. U.S. Geological Survey, Upper Midwest Water Science Center, Middleton, Wisconsin, USA
5. U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Corvallis, Oregon, USA
6. U.S. Geological Survey, Western Ecological Research Center, Dixon Field Station, Dixon, California, USA
7. U.S. Geological Survey, Water Resources Mission Area, Menlo Park, California, USA
8. National Measurement Institute, LGC Limited, Teddington, Middlesex, United Kingdom
9. European Synchrotron Radiation Facility (ESRF), Grenoble, France
10. Université La Rochelle, CNRS, Littoral Environnement et Sociétés, La Rochelle, France
11. University of Illinois at Chicago, Department of Earth and Environmental Sciences, Chicago, Illinois, USA
12. U.S. Geological Survey, Water Resources Mission Area, Boulder, Colorado, USA
13. Department of Environmental Toxicology, University of California Davis, Davis, CA 95616, USA

Supporting information includes 17 Figures, 7 Tables and 43 pages.

Data generated during this study are available at <https://doi.org/10.5066/P96NP376>.

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S1. Methods

S.1.1. Chemical analysis

The total Hg concentration of the blood sample of the Clark's grebe was quantified on a wet weight basis (1.70 ± 0.17 mg Hg kg⁻¹ wet weight), and converted to a dry weight basis (7.04 ± 0.70 mg Hg kg⁻¹ dry weight) using the average moisture content of grebe blood (75.9%).¹ Total Hg and MeHg were quantified at the U.S. Geological Survey Mercury Research Laboratory (Middleton, Wisconsin) on tissues on a dry weight basis. Tissues were processed using a nitric acid (4.5 M) extraction and heated at 55 °C for 8 hours. First, MeHg was quantified on an aliquot of extracts by aqueous phase ethylation, trapping on Tenax® (Buchem B.V.), isothermal gas chromatography separation, and cold vapor atomic fluorescence spectroscopy (CVAFS) detection using a Brooks Rand MERX-M following U.S. Environmental Protection Agency Method 1630. Second, tissue extracts were further oxidized with sodium persulfate (2% of final volume) overnight and brominated to a final volume of 10% bromine monochloride (BrCl). Oxidized extracts were measured for total Hg via stannous chloride reduction and gold amalgamation coupled to CVAFS detection using a Brooks Rand MERX-T following U.S. Environmental Protection Agency Method 1631. The standard deviation between digestion triplicates for MeHg was $\leq 5.2\%$ ($n=3$), the recovery of MeHg from IAEA-436 reference ($n=9$; certified at 0.200 ± 0.010 mg MeHg kg⁻¹ dry weight) averaged 92%, and the recovery of MeHg from quality control spikes averaged 99% ($n=17$). The standard deviation between digestion triplicates for total Hg was $\leq 8.6\%$ ($n=4$), the recovery of Hg from IAEA-436 reference ($n=9$; certified at 0.220 ± 0.010 mg Hg kg⁻¹ dry weight) averaged 98%, and the recovery of Hg from quality control spikes averaged 99% ($n=14$). The total error in percent MeHg (%MeHg) by chemical analysis was estimated by propagation of uncertainty associated with MeHg and total Hg concentrations (assigned at 10% for each measurement).

Total Hg was quantified on the < 2 mm soil fraction (Site S1 and S2) using a DMA-80 Hg analyzer (Milestone Dual-cell). The detection limit was 0.003 ng Hg and accuracy was confirmed by analysis of the IAEA-436 reference from the International Atomic Energy Agency. The established value is 4.19 ± 0.36 mg kg⁻¹ dry weight and the determined value was 3.96 ± 0.13 mg Hg kg⁻¹ dry weight ($n = 30$).

Selenium concentrations were determined on all samples by isotope dilution-hydride generation-inductively coupled plasma-mass spectrometry (ID-HG-ICP-MS)^{2, 3} at the U.S. Geological Survey (Menlo Park, California), and on three tissues from the Clark's grebe also at University of La Rochelle (muscle, kidneys, liver). At the U.S. Geological Survey, quality assurance and quality control were

assessed by procedural blanks (run in triplicate), duplicate digestions ($n = 10$), and certified reference materials that spanned a range of sample matrices (run in triplicate with each run). Se recoveries for certified reference materials averaged 106% ($n = 6$) for National Institute of Science and Technology (NIST2976) mussel tissue, 102% ($n = 6$) for National Research Council Canada (NRCC) dogfish muscle (DORM2), 96% ($n = 6$) for NRCC dogfish liver (DOLT3), 93% ($n=3$) for NRCC lobster hepatopancreas (TORT3), and 115% ($n = 3$) for NRCC marine sediment (MESS-3). At the University of La Rochelle, tissues were digested in duplicate (Clark's grebe muscle and kidneys) and triplicate (Clark's grebe liver) in ultrapure HNO_3 in polytetrafluoroethylene reactors with a MARS-6 microwave. Sample digests were analyzed with an Agilent 7500cx ICP-MS with Octopole Reaction System. Certified reference materials included European Reference Material ERM-CE278k mussel tissue (recovery of 108%, $n=1$) and NRCC dogfish liver (DOLT5) (recovery of 101%; $n=1$). Analytical calibration blanks quantifying instrument noise and procedural blanks were subtracted from sample concentrations. Procedural blanks were always less than 10% of sample concentrations and averaged 0.010 mg kg^{-1} dry weight (based on an average sample mass of 10 mg) and precision for samples run in duplicate averaged 3.0% (calculated as the absolute deviation divided by the mean, as a percent). Average Se concentrations are reported on Clark's grebe tissues when data from both laboratories is available. The uncertainty in Se concentrations was assigned at 10% for all measurements, the maximum deviation in recovery of certified reference materials.

Molar ratios of Se to Hg were calculated using total Se concentration and (1) the total Hg concentration ($\text{Se:Hg}_{\text{tot}}$) and (2) the Hg concentration as $\text{Hg}(\text{Sec})_4$ ($\text{Se:Hg}(\text{Sec})_4$). The latter was calculated by multiplying the percentage of Hg as $\text{Hg}(\text{Sec})_4$ as determined by HR-XANES by the total Hg concentration, and therefore is only reported for tissues where $\text{Hg}(\text{Sec})_4$ was quantified. The total error in $\text{Se:Hg}_{\text{tot}}$ and $\text{Se:Hg}(\text{Sec})_4$ values was estimated by propagation of uncertainty associated with Se and total Hg concentrations and HR-XANES spectral fits. Figure S8 presents the $\text{Se:Hg}_{\text{tot}}$ and $\text{Se:Hg}(\text{Sec})_4$ of the bird, fish, and earthworm tissues as a function of the total Hg concentration. Horizontal dashed lines present stoichiometric ratios of 4 (red) and 1 (gray) for reference of ratios of $\text{Hg}(\text{Sec})_4$ and HgSe species, respectively. Stoichiometric ratios of $\text{Se:Hg}_{\text{tot}}$ less than 1 are observed in the feather and muscle of the Clark's grebe and several muscle tissues of fish (*Plagioscion squamosissimus* and *Cichla sp.*, $n=4$) (Figure S8a). These samples either had no detectable $\text{Hg}(\text{Sec})_4$ (Clark's grebe feather, fish muscle tissues) or $\text{Hg}(\text{Sec})_4$ was a minor species (Clark's grebe muscle, $11 \pm 5\% \text{ Hg}(\text{Sec})_4$). All other tissues ($n=22$) exhibited $\text{Se:Hg}_{\text{tot}}$ greater than 1. Of the tissues where $\text{Hg}(\text{Sec})_4$ was quantified, the $\text{Se:Hg}(\text{Sec})_4$ was greater than 4 in all samples aside from the Clark's

grebe kidneys ($\text{Se:Hg}(\text{Sec})_4 = 2.1 \pm 0.3$) and liver tissues ($\text{Se:Hg}(\text{Sec})_4 = 1.3 \pm 0.2$) (Figure S8b). We attribute the $\text{Se:Hg}(\text{Sec})_4$ less than 4 in these two tissues to the presence of $\text{Hg}_x(\text{Se,Sec})_y$ clusters/nanoparticulate Hg selenide, as detected in the HR-EXAFS spectra of the Clark's grebe liver (Figure S9). The inverse relationship between $\text{Se:Hg}(\text{Sec})_4$ and total Hg concentration (Figure S8b) may reflect a continuum in Hg speciation from $\text{Hg}(\text{Sec})_4$ at low total Hg concentration to $\text{Hg}_x(\text{Se,Sec})_y$ clusters at high total Hg concentration across the various tissues, but further work is ultimately needed to resolve the biochemical reasons for these observations.

S.1.2. PCR analysis

DNA extraction from soil and earthworm microflora. For the soil sample (site S2), the DNeasy PowerSoil kit (Qiagen, Product 12888) was used to extract bacterial genomic DNA from 0.25 g of soil following the manufacturer's recommendations. For earthworms, individual earthworms were isolated in a plastic box devoid of soil for 24 h and then dissected and the digestive tract opened. With a sterile spatula, the interior of the digestive tract was transferred to a 12 mL tube containing sterile LB medium. Cultures were incubated static at 16 °C for 3 d. After centrifugation, genomic DNA was extracted from the bacterial pellet with a solution of EDTA, Tris, and lysozyme, followed by proteinase K and N-laurylsarkosinate.⁴ The genomic DNA was purified by precipitating in 60% ethanol and rinsing several times in 70% ethanol. The DNA was rinsed a final time with 70% ethanol overnight at -20 °C, air dried, and dissolved in a solution of 10 mM Tris (pH 8) and 1 mM EDTA. The concentration of DNA was measured at 260 nm.

Amplification of *hgcA* gene fragments from environmental samples. The *hgcA* gene fragments were amplified from environmental DNA samples using a combination of a forward primer (*hgcA*-F) targeting the highly conserved putative cap helix motif and a reverse primer (*hgcA*-912R) targeting a motif between the third and fourth predicted transmembrane helices near the C-terminus⁵ (Table S6). These two primers allow the amplification of a product from the *hgcA* gene around 650 base pairs (bp).⁶ The control amplification of an *hgcA* gene fragment (650 bp) from a plasmid containing *hgcA* was confirmed using *hgcA*-F and *hgcA*-912R primers and DreamTaq polymerase reagent (Figure S11a); the *hgcA* gene fragment was provided by Hee-Sung Bae (University of Florida). Upon receipt, the plasmid was extracted and purified after alkaline lysis on columns containing a silica membrane (kit NucleoSpin plasmid, Macherey-Nagel, Product 740588.250).

The PCR amplification of environmental samples was performed using identical cycling conditions to that previously described⁷ in 20 μL volumes with 10 μL DreamTaq polymerase (Thermo Scientific;

Products K1071), 0.2 mM of dNTP, 0.75 μ M of each primer, and the indicated amount of plasmid or genomic DNA. The final concentration of $MgCl_2$ was 1.5 mM. Amplification products were verified by gel electrophoresis on a 1% agarose gel. PCR products between approximately 620-660 bp were excised from the gel and purified on a column containing a silica membrane (kit NucleoSpin Gel and PCR Clean-up, Macherey-Nagel, Product 740609.250). The isolated PCR products were ligated into pGEM T-Easy vector (Promega) overnight at 4 °C according to manufacturer's recommendations. Thereafter, *E. coli* TOP10 cells (Invitrogen Thermo Fisher Scientific, Product C404010), rendered chemically competent for transformation through $CaCl_2$ treatment, were transformed with 2 μ L of the ligation mixture. Transformants were selected on ampicillin-containing jellified LB medium (50 μ g/mL). Positive recombinants were selected using PCR: 10 colonies were picked out from the Petri dish and each was suspended in a mixture made of 10 μ L of sterile milliQ water, 0.5 μ L (50 ng) of M13 forward primer, 0.5 μ L (50 ng) of M13 reverse primer, and 12.5 μ L of 2 x concentrated DreamTaq blend. The thermocycling program was as follows: initial denaturation step at 94 °C for 10 min, 36 cycles at 94 °C for 60 s, 55 °C for 60 s, and 72 °C for 140 s, followed by a final extension step at 72 °C for 10 min. Positive transformants were cultured on LB medium containing ampicillin (50 μ g/mL) and the harbored plasmids were purified using the kit NucleoSpin plasmid (Macherey-Nagel, Product 740588.250). The fragment contained in two recombinant plasmids was sequenced using M13 forward and reverse primers (GATC Biotech, Eurofins Genomics Company).

PCR results. Soil from site S2 contained bacterial DNA with the *hgcA* gene with an amplified gene fragment length of approximately 630 bp (Figure S11b, lane 3). After purification of total genomic DNA from earthworm microflora, amplified *hgcA* gene fragments of approximately 630 to 650 bp were identified for earthworms from sites S1 and S2 (Figure S11c). At site S1, the *hgcA* fragments were from anecic earthworm microflora (Figure S11c, panel i, lane 3), and at site S2 they were from both anecic and endogeic earthworm microflora (Figure S11c, panel ii, lanes 3 and 4, respectively).

Only a single *hgcA* sequence was retrieved from the excised and purified bands from both the soil and earthworm microflora samples. The retrieved *hgcA* sequence (633 nucleotides, 211 amino acid residues; Figure S12a) matches *hgcA* sequences belonging to the *Geobacter* genus. Since earthworms ingest soil, the *hgcA* gene-harboring bacteria detected in the gut microflora of earthworms are interpreted to have originated in the soil. At the nucleotide level, the retrieved *hgcA* sequence best matched the *hgcA* sequence of *Geobacteraceae* clone B2_FE06 (Figure S12b), which was amplified from the total bacterial genomic DNA of a temperate forested wetland in southern Sweden.⁶ The retrieved *hgcA* sequence contained 437 of 633 identical nucleotides to that of *Geobacteraceae* clone

B2_FE06 (identity percentage of 69%). At the protein level, the amino acid sequence of the retrieved *hgcA* gene best matched a sequence of *Geobacteraceae* isolated from paddy soil in the Guizhou province of southwest China⁸; 43 of 211 amino acid residues are identical (identity percentage of 68%; Figure S12c).

S.1.3. HR-XANES and HR-EXAFS measurement

Freeze-drying a frozen tissue does not change the speciation of chemically-bound metals,⁹ which we verified previously on MeHgCys.¹⁰ All freeze-dried samples were pressed into 5.0 mm diameter and 2.5 mm thick pellets, mounted in a polyether ether ketone (PEEK) sample holder sealed with Kapton tape, and maintained in a desiccator until their transfer into the liquid helium cryostat of the beamline. Mercury L₃-edge HR-XANES and HR-EXAFS spectra were measured with high-reflectivity analyzer crystals¹¹ on beamline ID26 at the European Synchrotron Radiation Facility (ESRF). The storage ring was operated in the 7/8 + 1 filling mode, with 200 mA current. Rejection of higher harmonics and reduction of heat load were achieved with a white beam Pd-coated, flat mirror working under total reflection at 2.5 mrad deflecting angle. The energy of the incoming beam was selected by the 111 reflection of a Si double crystal monochromator, and the beam was focused horizontally by a second Pd-coated mirror and vertically by a third Pd-coated mirror. The flux on the sample was approximately 10¹³ photon/s in a beam footprint of ~700 (H) x 80 (V) μm² full width at half maximum (FWHM). The Hg L_{α1} (3d_{5/2} → 2p_{3/2}) fluorescence line was selected using the 555 reflection of five spherically bent (radius = 0.5 m)¹¹ Si analyzer crystals (diameter = 100 mm) aligned at 81.8° Bragg angle in a vertical Rowland geometry. The diffracted intensity was measured with a Si drift diode detector (SDD) in single photon counting mode. The effective energy resolution, obtained by convoluting the total instrument energy bandwidth (spreads of the incident and emitted rays) and the 3d_{5/2} core-hole width from the L_{α1} line was about 3.0 eV, compared to an intrinsic line broadening of about 6.1 eV in conventional fluorescence yield measurement with a solid-state detector (conventional XANES and EXAFS).

The incident energy was scanned from 12260 eV to 12360 eV in 0.2 eV steps in HR-XANES mode, and the spectra were normalized to unity at E = 12360 eV. The incident energy was scanned from 12210 eV to 13050 eV in 2.0 eV steps in HR-EXAFS mode. The stability in energy of the incident beam was monitored by measuring frequently a fresh MeHgCys reference. The photon energy is referenced to the maximum of the near-edge peak of MeHgCys at 12279.8 eV. The precision of the calibrated spectra is 0.1 eV. Spectra were collected at a temperature of 10-15 K and a scan time of 15

s to reduce exposure, and repeated at different pristine positions on the sample to increase the signal-to-noise ratio. Radiation damage, which occurs when the beam is positioned on the same spot for too long, depends on the chemical form of Hg and, for a given form, varies with the kinetic energy of the photoelectron (i.e., energy of the incident beam). Bound states, probed at low kinetic energy, are more sensitive to radiation exposure than unbound states in the continuum. This dependence is illustrated in Figure S17 with MeHgCys, which is the most radiation sensitive Hg species. Hg(SR)₂ is more stable, and nanoparticulate β-HgS and HgSe resist damage during long exposure time.

S.1.4. HR-XANES and HR-EXAFS data analysis and accuracy of the fit results

The number of Hg species was determined by principal component analysis,^{12, 13} and their nature subsequently by iterative transformation factor analysis (ITFA¹⁴) for the Hg(Sec)₄ species, and by target transformation analysis¹⁵ for the MeHgCys and Hg(SR)₂ species. Target transformation analysis was conducted with reference spectra from a large dataset of Hg minerals (α-HgS, β-HgS, β-HgS_{NP}, HgSe, HgSe_{NP}), and Hg(II) and MeHg model complexes described previously (Ref.¹⁰ and references therein). All reference spectra were considered as a basis for identification, but only diagnostic spectra are discussed in the article. The proportions of the Hg species were evaluated using least-squares fitting (LSF) of the data with linear combinations of the Clark's grebe breast feather spectrum, the Hg(Sec)₄ spectrum, and the spectrum of the Hg(L-glutathione)₂ complex (Hg(GSH)₂) at pH 7.4.^{16, 17} In Hg(GSH)₂, Hg(II) is bonded approximately linearly to the cysteinyl sulfur atoms at 2.33 Å from the two γ-Glu-Cys-Gly peptides and surrounded in trans-equatorial position by a carboxyl oxygen from a Gly residue at 2.62 Å, the backbone carbonyl oxygen at 2.88 Å from the Gly-Cys peptide bond (Gly-NH-CO-Cys) of the same GSH molecule, and an amide group (-NH) at 3.01 Å from the second GSH molecule.¹⁸ This reference is a good proxy for the Hg(SR)₂ coordination in biota.¹⁸ The precision of estimation of a fit component was estimated to be equal to the variation of its value when the fit residual (*NSS*) was increased by 20%. *NSS* is the normalized sum-squared difference between two spectra expressed as $\Sigma[(y_2 - y_1)^2] / \Sigma(y_2^2)$. The principal component analysis (PCA) and linear fit programs from beamline 10.3.2 at the Advanced Light Source were used.^{12, 19}

The accuracy of spectral fit results was evaluated by comparing the percentage and concentration of MeHg obtained by chemical analysis and HR-XANES. The relative abundance of MeHg as determined by HR-XANES (%MeHgCys_{XANES}) and chemical analysis (%MeHg_{Chem}) show good agreement between the two independent methods (Figure S4a), demonstrating a high level of accuracy in spectral fit results. In Figure S4a, a generic Y-axis error bar represent the precision of HR-XANES

spectral fits and the X-axis error bar represent the total propagation of error in %MeHg_{Chem} measurements. Across the tissues a moderately lower percent MeHg was observed by chemical analysis compared to HR-XANES. We attribute this discrepancy primarily to the lower recovery of MeHg in tissue digests during the ethylation step of the analysis (average of 92% MeHg recovery for IAEA-436, $n=9$). In contrast, HR-XANES analysis does not require sample modification prior to measurement, is sensitive to all Hg atoms in a tissue sample, MeHgCys has a distinctive spectral signature with an intense near-edge peak, and the percentage MeHgCys is obtained directly from a spectral fit. To assess the uncertainty of estimation further, the concentrations of MeHg determined by HR-XANES ($[\text{MeHgCys}]_{\text{XANES}} = \% \text{MeHgCys} \times [\text{Hg}]_{\text{Tot}}$) and chemical analysis ($[\text{MeHg}]_{\text{Chem}}$) were compared (Figure S4b and Table S7). Good agreement was observed between $[\text{MeHgCys}]_{\text{XANES}}$ and $[\text{MeHg}]_{\text{Chem}}$, demonstrating also high certainty in the quantification of MeHgCys by the two methods.

The HR-EXAFS spectrum from the Clark's grebe liver was analyzed with WinXAS²⁰ using amplitude and phase shift functions generated by FEFF 7.0.2²¹ and HgSe as structure model²² (Table S4). The amplitude reduction factor S_0^2 was fixed to 0.9. EXAFS lacks sensitivity to the C atom at about 2.07 Å from the methyl group of MeHgCys and misses Hg atoms at about 4.46 Å as a result of the high disorder of the Hg_x(Se,Sec)_y clusters. Thus, the coordination number (CN) of Hg at 4.46 Å is an apparent number.

S.1.5. Protein extraction and analysis

Protein extraction from Clark's grebe tissues. To assess if Hg and Se were associated with SeIP, a protein extraction was performed on the Clark's grebe liver and muscle tissues.²³ In triplicate, 0.1 g of lyophilized, homogenized tissue was mixed with 0.4 g of protein extraction buffer (50 mM TRIS-HCl, CAS 1185-53-1; 0.01 g/g SigmaFast Protease Inhibitor Cocktail, Sigma Aldrich) in 1.5 mL Eppendorf tubes. Procedural extraction blanks were performed in triplicate. At 4 °C, samples were homogenized (Omni Tissue Homogenizer with plastic disposable tips) using 5 repeated cycles of a 10 second homogenization and 50 second pause. Protein extracts were separated from residual tissues by centrifugation at 10,000 g at 4 °C for 30 minutes. The supernatant was immediately frozen at -80 °C and shipped at -80 °C to LGC Group (Teddington, United Kingdom) for selenoprotein analysis.

Speciation of Hg and Se in Clark's grebe tissues. Protein extracts from Clark's grebe liver tissue ($n = 3$) and muscle tissue ($n = 3$) and extraction blanks ($n = 3$) were screened for the co-elution of Hg and Se with selenoproteins by double affinity (AF) high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICPMS^{3, 24}). All samples were measured in parallel in the

same analytical run. Each sample was measured in duplicate. The double AF-HPLC chromatographic system achieves separation of three major Se protein species by the combination of two AF columns (HiTrap Heparin High Performance, 1mL; Hi Trap Blue High Performance, 1 mL) that retain SeIP and SeAlb, respectively. Peak 1 elutes forms of Se not retained on the columns, including glutathione peroxidase 3 (GPX3), inorganic Se species, and LMW Se species (retention time of approximately 3.3 minutes). Peak 2 elutes at the retention time of SeIP (approximately 19.3 minutes), known to have high affinity to heparin. Peak 3 elutes at the retention time of Se-albumin (Se-Alb; approximately 28.0 minutes). The plasma reference material NIST SRM 1950 was used as the quality control material for chromatographic quality assessment (Se species elution distribution and order), because of the lack of any certified material for Se-species in tissue. It should be noted that thioredoxin reductase (TrxR) elutes at the same retention time as SeIP, based on the analysis of a protein standard of TrxR from rat liver. Se species were eluted using ammonium acetate buffers (0.05 and 1.5 M, pH 7, 0.5 mL min⁻¹). Eluent from the AF-HPLC was introduced to a collision cell ICP-MS/MS for measurement of Se and Hg (8800 ICP-QQQMS, Agilent Technologies, UK). The ICP-MS/MS was operated in oxygen mode, and therefore Se was determined by measurement of ⁸⁰Se as ⁹⁶SeO. Mercury was measured by monitoring ²⁰²Hg. Relative elemental percentages were calculated using the peak area distribution of Peaks 1-3. Hg mass balance of tissue extracts associated with Se species was not performed.

Good repeatability in Hg and Se chromatograms of triplicate tissue extracts was observed. An example of Hg and Se chromatograms of the Grebe liver and muscle extracts are presented in Figure 4. In the liver and muscle tissues, the majority of detected Hg co-eluted with Peak 2 (SeIP + TrxR; 87.8 ± 0.4% and 84.3 ± 0.7%, respectively), and the remainder of Hg co-eluted with Peak 1 (GPX3, inorganic Se species, LMW Se species; 11.5 ± 0.4% and 13.7 ± 0.6% respectively). Similarly, Se chromatograms show that Se co-eluted with Peak 1 (71.4 ± 0.7% and 62.5 ± 2.4% in liver and muscle tissue extracts, respectively) and Peak 2 (27.3 ± 0.8% and 35.5 ± 2.3%, respectively). No considerable Hg or Se in either tissue extract was observed to co-elute with Peak 3 (Se-Alb; ≤ 2%). The Se and Hg chromatograms of extraction blanks contained only minor peaks for Hg also observed in the eluent blank (signal ≤ 3% of tissue extracts), confirming that Hg and Se chromatograms of tissue extracts were free of contamination. In summary, this speciation analysis shows that Hg co-elutes primarily with SeIP and TrxR and minorly with GPX3 in Grebe liver and muscle tissues, where HR-XANES quantified 86% and 11% of Hg as Hg(Sec)₄ (Table S5). Based on biostructural considerations, formation of Hg(Sec)₄ is incompatible with TrxR because this homodimeric protein containing a single Sec residue per subunit²⁵, therefore binds Hg linearly.²⁶ Similarly, the minor elution of Hg with Peak

1 is considered unlikely to be a result of Hg association with GPX3, given that GPX3 is a tetramer containing one Sec residue in each subunit²⁷ too far apart to form a Hg(Sec)₄ complex. In addition, the HR-XANES spectra of MeHgSec and Hg(Sec)₂ species are clearly distinguished from MeHgCys and Hg(GSH)₂ (Figure S2c, d), further supporting that co-elution of Hg and Se with Peak 2 is likely as Hg(Sec)₄.

S.1.6. SelP amino acid sequence

Crested grebe (*Podiceps cristatus*). The partial protein sequence has been established from GenBank: KL268852.1 by joining two translated nucleotides fragments from locations 15116-15235 and 16132-16350. This incomplete sequence is also found under the protein accession number GenBank: KFZ63143.1. The trace of the first nucleotidic fragment is underlined below.

Incomplete SelP protein:

RCGRLVYHLGLPYSFLSFQYVEESIKIAYCENKCGNCSYTEPAIDDICENITKTADEKLAEPKPTGQHS
HHNLHRHRHHHHHHHREGSRHRSKNENHQNSSETQRHHPHSGHNRHDHTGSHEQVDTVPPGESVEISQDKKLUKK
GKTSCKNQLTUNWQTASDSASSUCCHURHLLFEELGNSVTUQCRGALPNSCRUHGQLSAEDITESUQUURLLT
AAUQSPAAGASETSDTUQUQEKARNUAUKTN

C-terminal region used to model the Hg-binding site

DTUQUQEKARNUAUKTN

Zebrafish (*Danio rerio*). The SelP sequence has been established from the protein accession number GenBank: NP_840082.3.

SelP protein:

MWKALSLTLALCLLVGCSAESETEGARCKLPPEWKVGDVEPMKNALGQVTVVAYLQASULFCLEQASKLNDLL
LKLEKQGYPNIAYMVVNNREERSQRLHLLQERLLNITLYAQDLSQPDWQAVNAEKDDILVYDRCGRLTYHL
SLPYTILIHVPHVEEAIKHTYCDRICGECSSLESSAQLEECKKATEEVNKPVEEEPRQDHGHHEQGHHEHQGEAE
RHRHGHHHPHHHHHHHRGQQQVDVDQVLSQVDFGQVAVETPMMKRPUAKHSRUKVQYSUQQGADSPVASUCU
HURQLFGGEGNGRVAGLUHCDEPLPASUPUQGLKEQDNHIKETUURPAPPAEUELSQPTUVUPAGDATUGUR
KK

C-terminal region used to model the Hg-binding site

PTUVUPAGDATUGURKK

Fish, birds, and mammals. We performed BLAST analyses with known SelP sequences in fish, birds, and mammals as queries to search protein databases for distant SelP homologs. The sequences

identified served as new search entries for SelP homologs in nucleotide sequence databases. Multiple alignments of SelP sequences (Figure S13) were carried out using the DNAMAN software.

S2. Supplementary figures

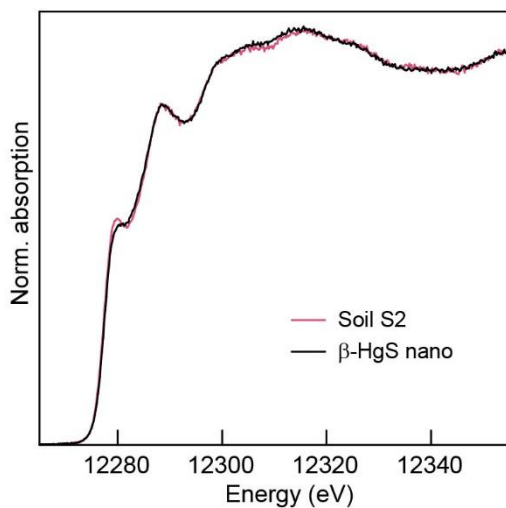


Figure S1. Mercury L₃-edge HR-XANES spectra of the <63 μm fraction of soil S2 (27.6 mg Hg kg⁻¹ dw) and the clay-size fraction (0.1 – 2.0 μm) from the A horizon soil (191 mg Hg kg⁻¹ dw) studied in Ref.²⁸. Hg is speciated as nanoparticulate β-HgS in the A horizon sample.

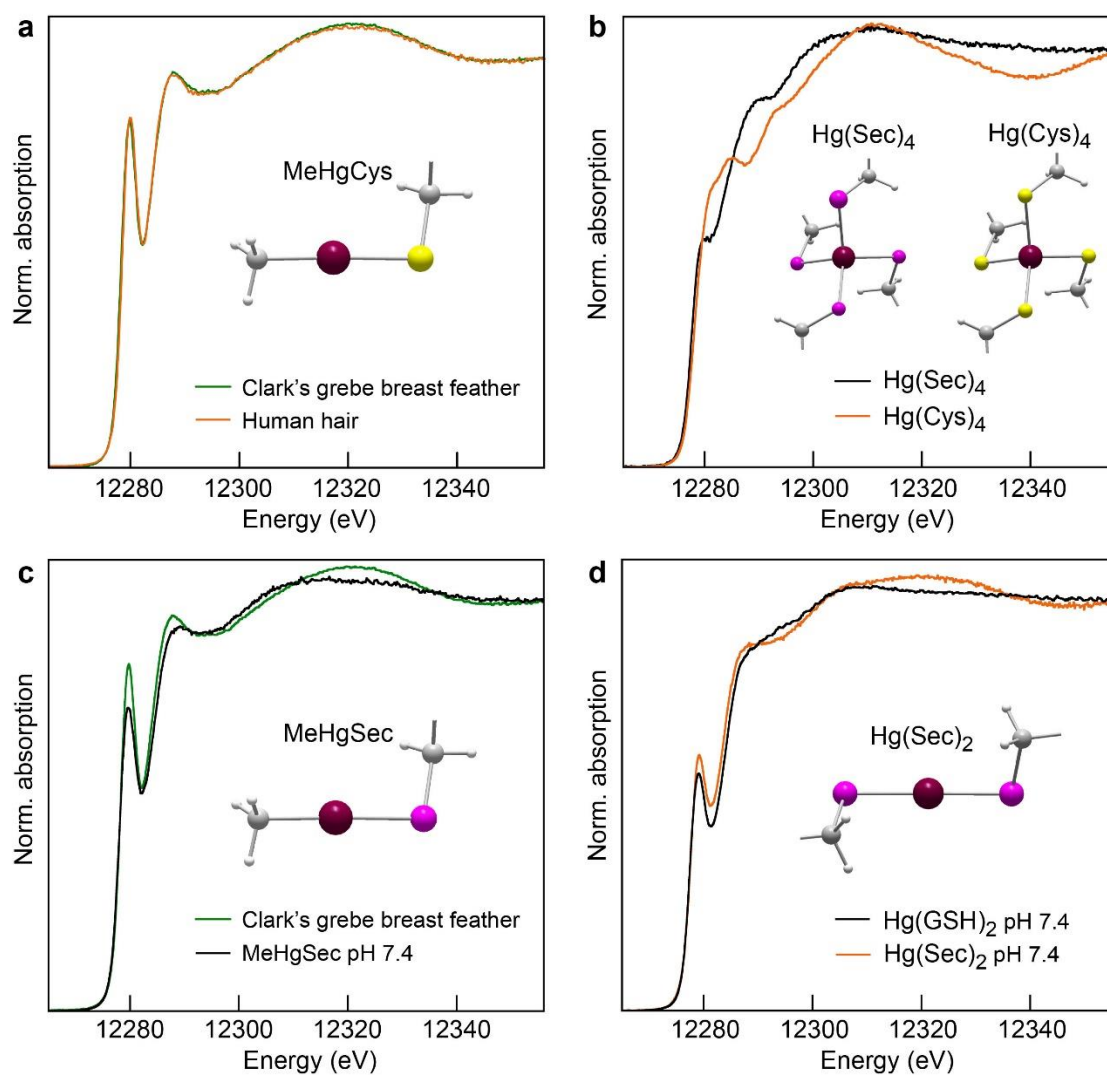


Figure S2. a) HR-XANES spectra of MeHgCys in human hair and Clark's grebe breast feather. The human hair spectrum corresponds to sample 1MeHg13.8 ([Hg] = 13.8 ppm) from Figure 3 of Ref.²⁹. b-d) HR-XANES spectra of cysteine and selenocysteine Hg complexes illustrating the high sensitivity of HR-XANES to the coordination and ligation of Hg.

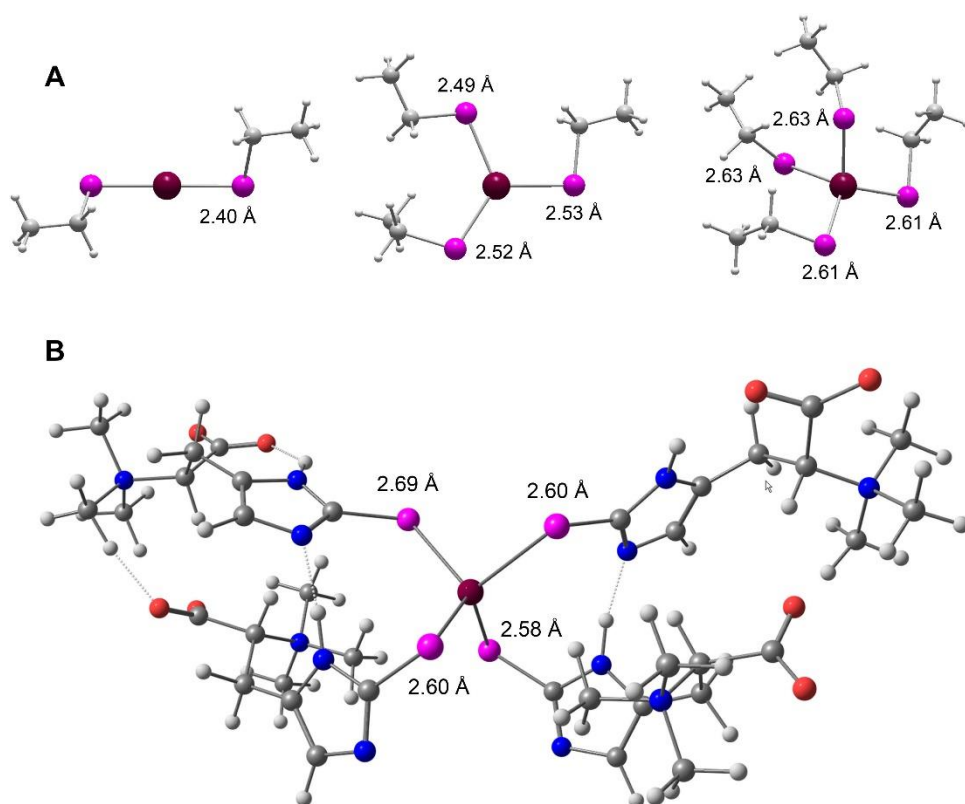


Figure S3. Geometry optimization of the Hg di-, tri-, and tetraselenolate complexes (modeled with ethylselenol) (a) and Hg tetraselenoneine complex ($\text{Hg}(\text{selenoneine})_4$) (b) performed at the second-order Møller-Plesset perturbation theory (MP2)³⁰ level using ORCA 4.2.1³¹ and a computational scheme tested previously on the modeling of the structure and stability of monomeric Hg-thiolate complexes^{29, 32}. The Ahlrichs polarized def2-TZVP (Hg, Se) and def2-SVP (C, H) basis sets were used in combination with the auxiliary def2-TZVP/C^{33, 34} Coulomb and exchange fitting basis to accelerate the MP2 calculations with the resolution of identity (RI) approximation^{35, 36}. For Hg atoms, scalar relativistic effects were accounted with an effective core potential (ECP)³⁷, as obtained from the Stuttgart pseudopotential library (60 core electrons, $l_{\text{max}}=3$). The valence basis set used in connection with the ECP is based on a (8s8p6d1f)/[6s5p3d1f] contraction scheme. The water solvent was represented with the SMD model³⁸. The convergence for the self-consistent field (SCF) calculations was set to 10^{-8} a.u. Dark red, purple, gray, blue, red, and light gray spheres represent Hg, Se, C, N, O, and H, respectively.

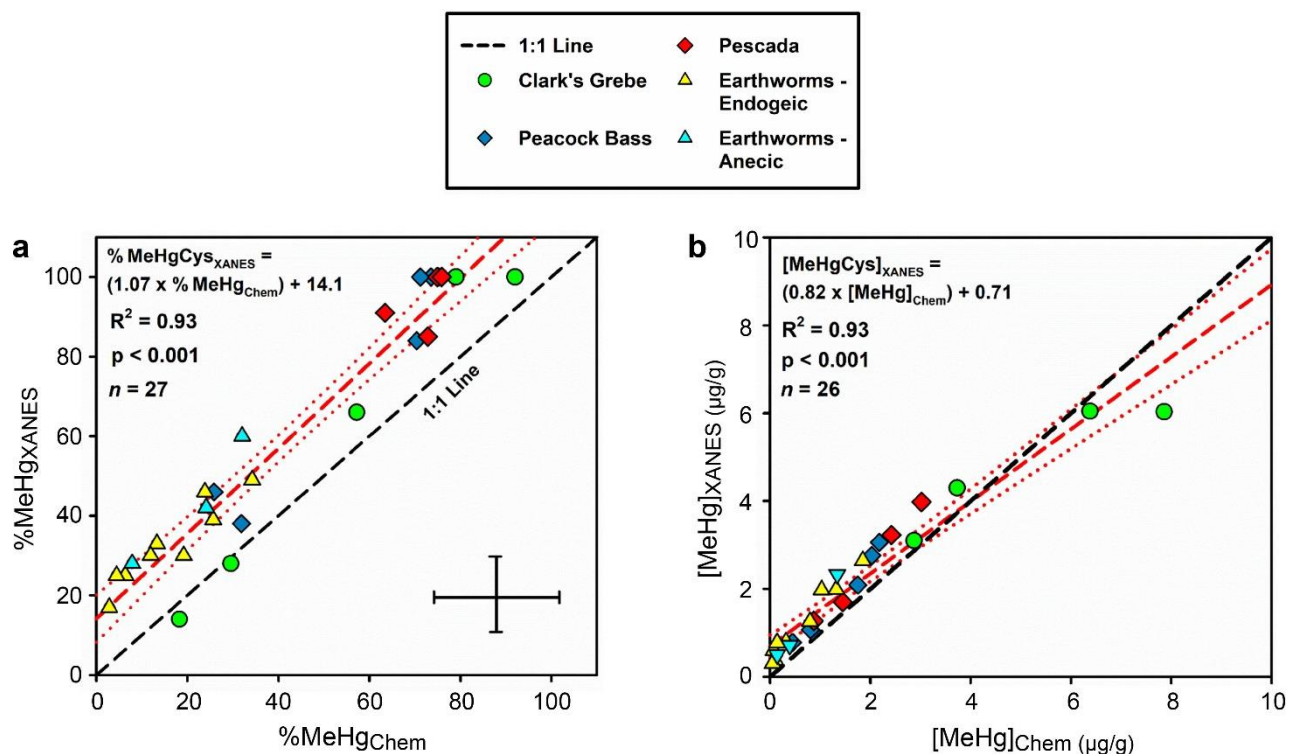


Figure S4. a) The accuracy of spectral fit results was evaluated by comparing the percentage of Hg as MeHg-thiolate complex determined by HR-XANES ($\%MeHgCys_{XANES}$) and percentage of MeHg determined by chemical analyses ($\%MeHg_{Chem}$). Maximum uncertainties in measurements are expressed as a generic error bar. b) The uncertainty in spectral fit results was evaluated by comparing the concentrations of MeHg determined by HR-XANES ($[MeHgCys]_{XANES} = \%MeHgCys_{XANES} \times [Hg]_{Tot}$) and chemical analysis ($[MeHg]_{Chem}$). The dashed black line presents the 1:1 line, the dashed red line presents the linear fit of data, and the dotted red lines present the 95% confidence intervals of the fit.

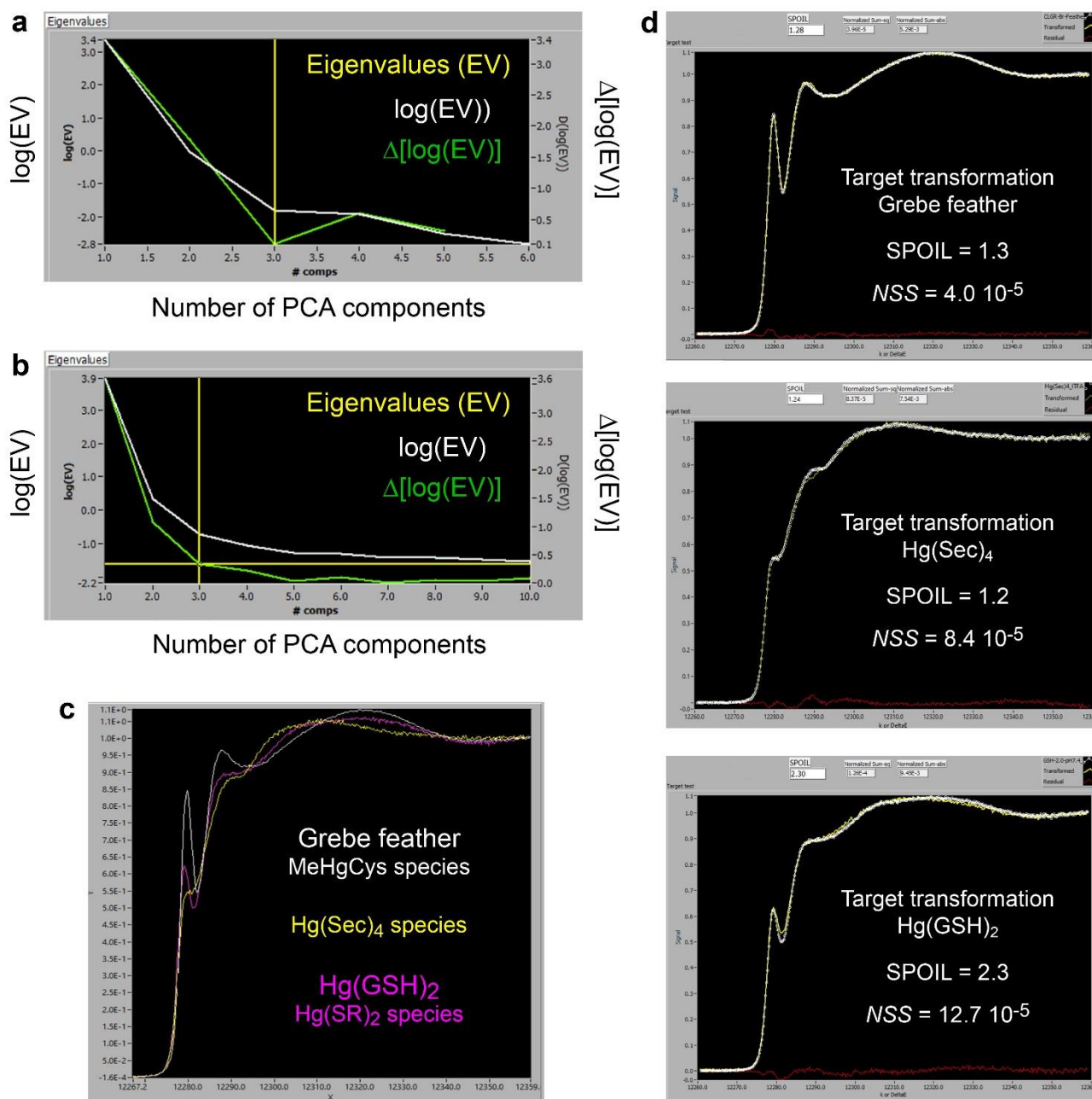


Figure S5. a,b) Estimation by principal component analysis (PCA) of the number of significant abstract components contained in the six HR-XANES spectra from the grebe (a), and the 10 fish + 13 earthworm spectra (b). The curves are a scree plot of the components and the associated eigenvalues (EV)³⁹. The variance of each dataset can be accounted for with three components (i.e., Hg species). See ref.¹³ for details. c, Spectrum of Hg(Sec)₄ identified by iterative transformation factor analysis (ITFA)¹⁴, and spectra of MeHgCys and Hg(SR)₂ identified by target transformation analysis¹⁵ of a large library of Hg organic and inorganic model compounds^{10, 17, 18, 28, 29, 32}. The Hg(SR)₂ species was identified previously in fish contaminated by inorganic Hg(II)¹⁸. d) Target transformed spectra of the three Hg species. The quality of the reconstruction is evaluated with the *SPOIL* criteria¹⁵ and *NSS* value. *SPOIL* measures the degree to which replacing an abstract component with the candidate spectrum would increase the fit error. This is a non-negative dimensionless number for which values

< 1.5 are considered excellent, 1.5-3 good, 3-4.5 fair, 4.5-6 poor, and > 6 unacceptable. See ref.¹² for details.

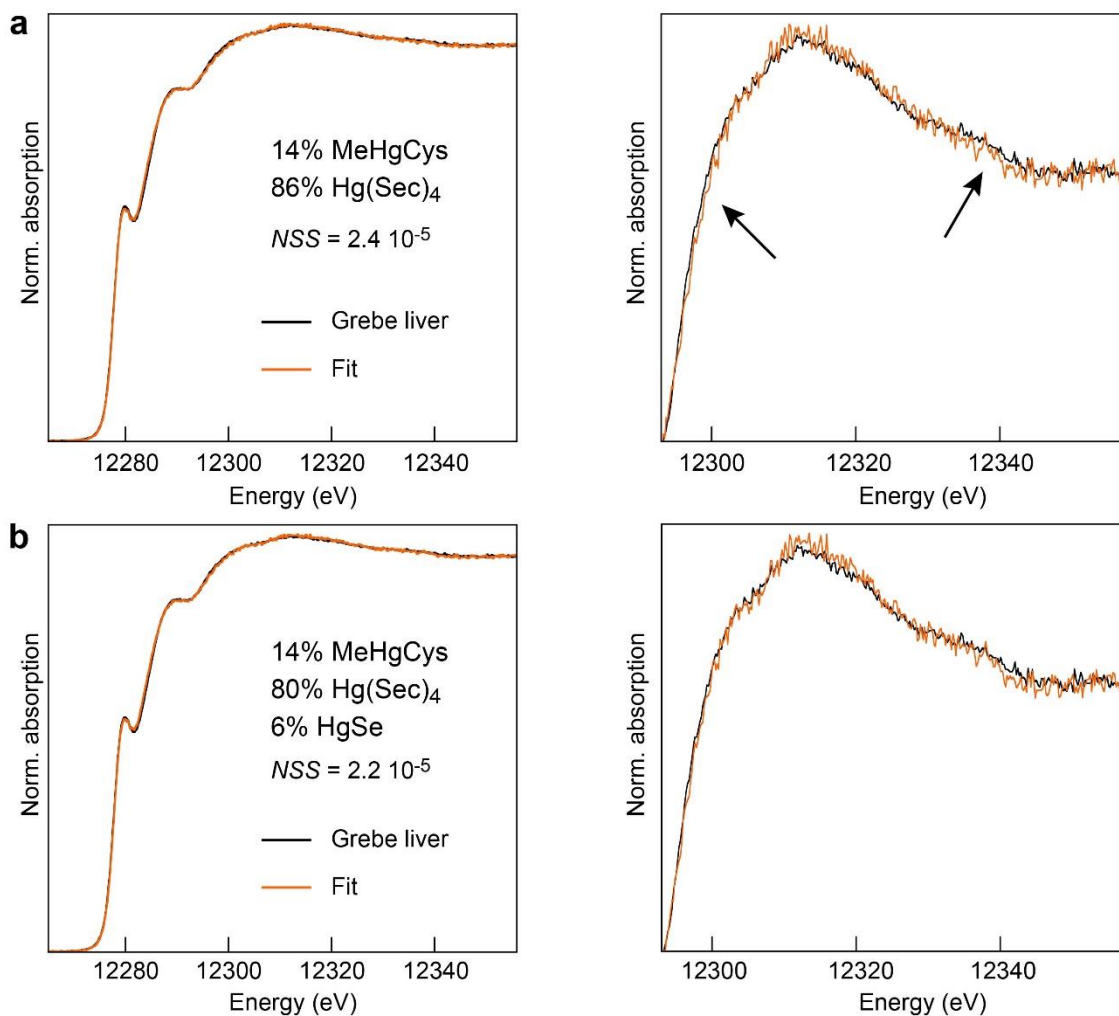


Figure S6. Least-squares fit of the liver HR-XANES spectrum of the Clark’s grebe without HgSe (a) and with HgSe (b). The spectra on the right are an enlargement of the top-edge region where the HgSe reference gives a distinctive signal at the energies indicated with arrows. The fit lacks intensity at the diagnostic energies in the absence of the HgSe reference. Including HgSe provides a better fit of the entire grebe liver spectrum including in the diagnostic region where the data and reconstructed spectrum are now statistically superimposed.

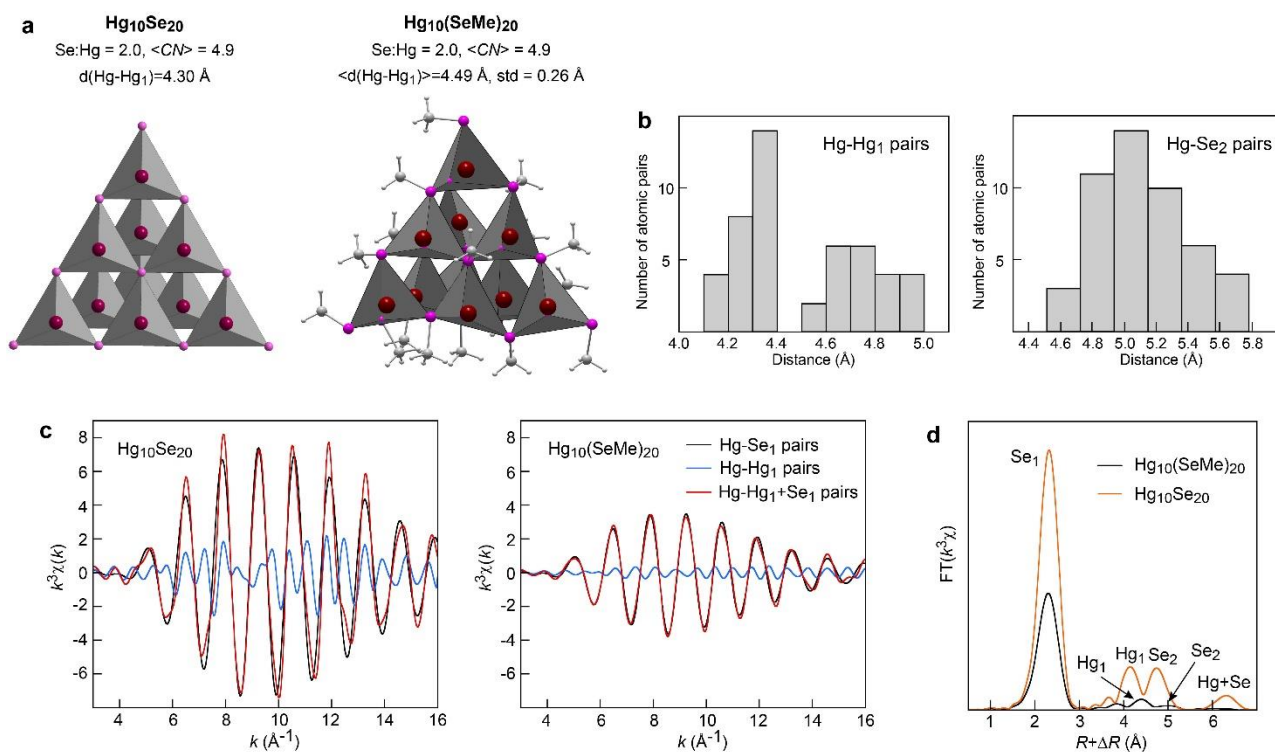


Figure S7. a) Polyhedral representation of a Hg₁₀Se₂₀ portion of the HgSe structure²², and the Hg₁₀(Sec)₂₀ cluster optimized geometrically. The Hg₁₀(Sec)₂₀ cluster has a predicted first shell Hg-Hg₁ distance of 4.49 Å on average, close to the HR-EXAFS distance of 4.46 Å in the grebe liver (Table S4), and 0.19 Å longer than the crystallographic distance in HgSe (4.30 Å). The optimization was performed at the PBE0-D3 DFT quantum level with def2-TZVP (Hg, Se) and def2-SVP (C, H) basis sets using ORCA 4.2.1³¹. The water solvent was represented with the SMD model³⁸. Sec residues were modeled with methaneselenolate (CH₃Se⁻). Dark red, Hg; purple, Se; gray, C, light gray, H. b) Population histograms of the predicted first shell Hg-Hg₁ and second shell Hg-Se₂ distances in Hg₁₀(Sec)₂₀. Interatomic distances are counted in intervals of 0.1 Å (Hg-Hg₁) and 0.2 Å (Hg-Se₂). The distributions of the interatomic distances are non-Gaussian with considerable asymmetry toward long distances. The Hg-Hg₁ and Hg-Se₂ distances partly overlap, whereas they are separated by 5.04 – 4.30 = 0.74 Å in HgSe. c,d) Calculated EXAFS photoelectron waves using FEFF7⁴⁰ of the Hg-Hg₁ plus Hg-Se₁ pairs for Hg₁₀Se₂₀ and Hg₁₀(Sec)₂₀ and Fourier transforms (radial structure function). The Hg-Hg₁ pair is extremely weak on the radial structure function, as a result of the large dispersion and asymmetry of the Hg-Hg₁ distances and the Hg-Hg₁/Se₂ overlap. Few Hg-Hg₁ pairs are detected, despite the presence, on average, of 4.9 Hg₁ atoms around each Hg atom in the Hg-selenolate cluster. The Hg-Se₁ bond lengths are also unequal and asymmetrical in Hg₁₀(Sec)₂₀.

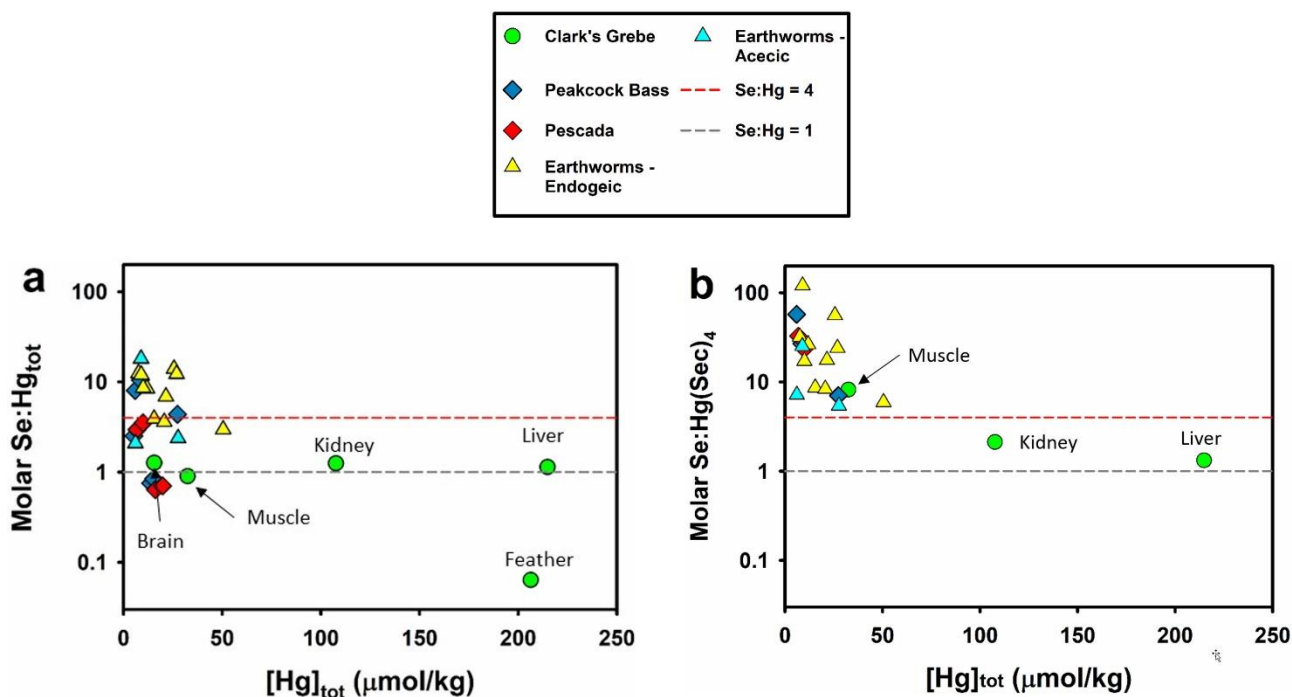


Figure S8. Molar ratios of Se to total Hg ($\text{Se:Hg}_{\text{tot}}$) versus Hg_{tot} concentration (a) and Se to Hg as a tetraselenolate complex ($\text{Se:Hg}(\text{Sec})_4$) versus Hg_{tot} concentration (b) of the Clark's grebe, fish, and earthworm samples. Horizontal dashed lines present stoichiometric ratios of 4 (red) and 1 (gray) for reference of ratios of $\text{Hg}(\text{Sec})_4$ and HgSe species, respectively. Identities of the various Clark's grebe tissues are provided. All tissues are presented in plot (a), whereas only tissues with $\text{Hg}(\text{Sec})_4$ quantified by HR-XANES are presented in plot (b). Plot (b) shows that tissues containing $\text{Hg}(\text{Sec})_4$ have a $\text{Se:Hg}(\text{Sec})_4 \geq 4$ except for the Clark's grebe kidneys ($\text{Se:Hg}(\text{Sec})_4 = 2.1 \pm 0.3$) and liver tissues ($\text{Se:Hg}(\text{Sec})_4 = 1.3 \pm 0.2$), the latter confirmed to contain $\text{Hg}_x(\text{Se},\text{Sec})_y$ / nanoparticulate Hg selenide by HR-EXAFS analysis (Figure S9).

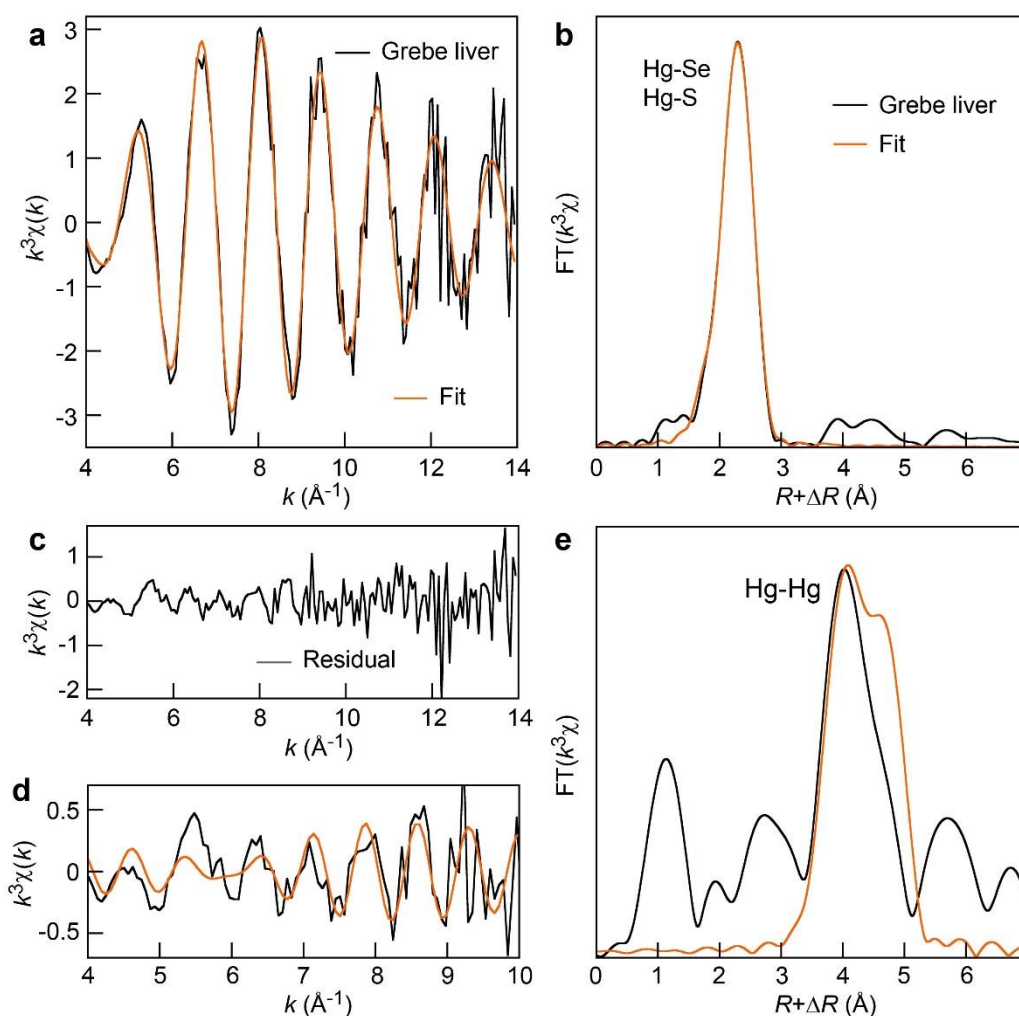


Figure S9. a,b) Fit of the HR-EXAFS spectrum and Fourier transform (radial structure function) for the Clark’s grebe liver with Hg-Se and Hg-S pairs in the first coordination shell and no Hg-Hg-pairs in the second coordination shell. c, Residual of the HR-EXAFS fit, showing clear evidence for significant spectral structures above noise level that are not accounted for by the Hg-(Se,S) model-fit. c,d) Fit of the HR-EXAFS residual and Fourier transform with Hg-Hg pairs, demonstrating their detection by HR-EXAFS. HR-EXAFS detects 2.1 ± 0.5 Hg atoms at 4.46 ± 0.03 Å with a static disorder of $\sigma = 0.1$ Å (Table S4). The distribution of the Hg-Hg distances was considered to be Gaussian in the analysis, which is incorrect when the local structure is highly disordered (e.g., $\sigma \geq \sim 0.1$ Å^{41,42}) (Figure S7b). An analysis of disordered systems using symmetrical distributions results in “lost” atoms, which means that Hg is in all likelihood surrounded by more than 2.1 Hg atoms. If all the Hg-Hg pairs were from the 6% HgSe detected by HR-XANES (Figure S6b), the number of Hg atoms would be $12 \times 0.06 = 0.7$. We conclude from this analysis that the majority of the Hg atoms detected by HR-EXAFS are from Hg_x(Se,Sec)_y clusters. Their existence is also supported by the Hg-Hg distance of 4.46 ± 0.03 Å, which is 0.16 Å longer than the Hg-Hg distance in HgSe, and in agreement with the predicted average Hg-Hg distances for disordered Hg_x(Se,Sec)_y cluster models (Figures. S7 and S16).

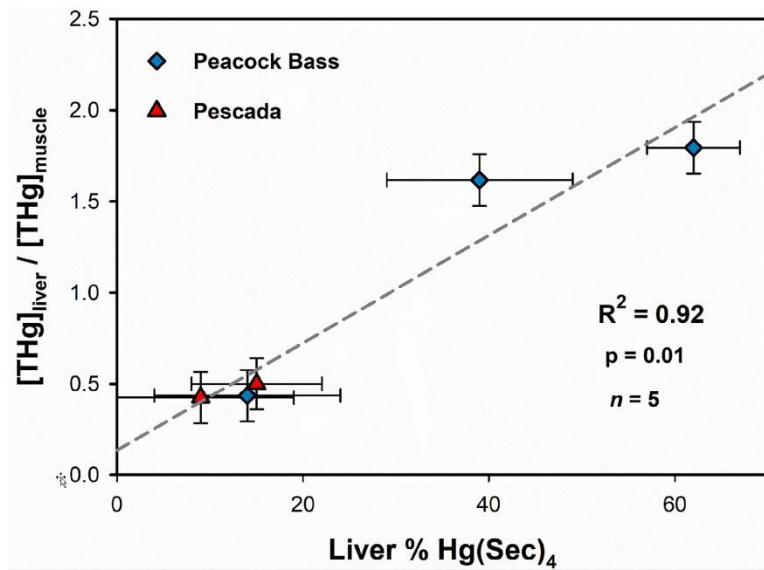
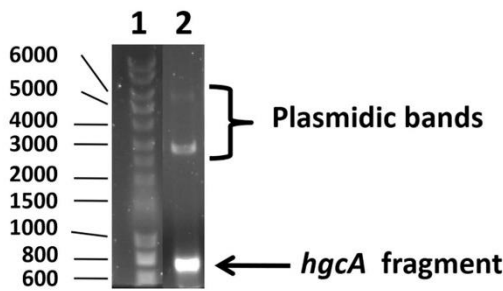
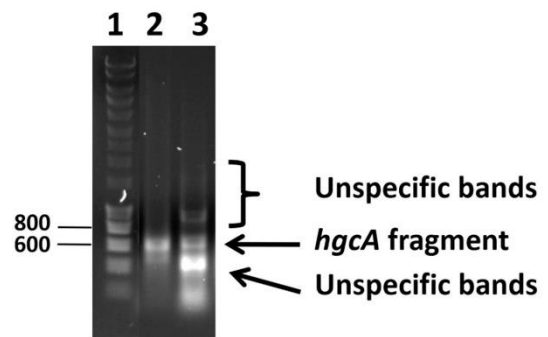


Figure S10. Positive correlation between the total Hg concentration ratio between liver and muscle ($[Hg]_{liver}/[Hg]_{muscle}$) and the percentage of the demethylated fraction of Hg ($\%Hg(Sec)_4$) in the liver tissue of peacock bass. Horizontal error bars present uncertainty in spectral fit data, and vertical error bars present the total propagated error for the ratio of total Hg measurements. The dashed gray line presents the linear fit of data.

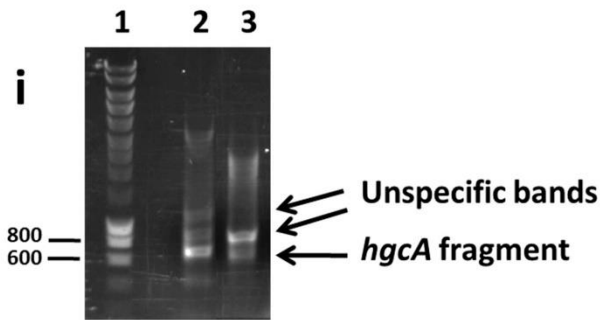
a Control (plasmid *hgcA*)



b Soil (Site S2)



C Earthworms: Site S1



Earthworms: Site S2

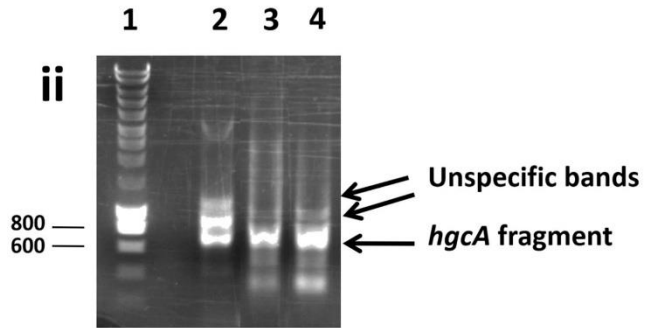


Figure S11. Agarose gel electrophoresis results. a) Shows the control amplification of an *hgcA* gene fragment of 650 bp from a plasmid harboring a cloned fragment of the *hgcA* gene using primers *hgcA*-F and *hgcA*-912R and DreamTaq polymerase. Lane 1 – molecular ladder; lane 2 – PCR made up with 200 ng of plasmid and primers *hgcA*-F + *hgcA*-912R; band at approximately 650-700 bp. b) Shows the amplification of an *hgcA* gene fragment of approximately 630 bp from total genomic DNA extracted from soil at site S2. Lane 1 – molecular ladder; lane 2 – control PCR with *hgcA*-harboring plasmid (40 ng per reaction); lane 3 – PCR made up with total genomic DNA extracted from the site S2 soil (120 ng of genomic DNA per reaction were used). c) Shows the amplification of *hgcA* gene fragments of approximately 630 to 650 bp from earthworm digestive microflora from sites S1 (panel i) and S2 (panel ii). Panel i, Lane 1 – molecular ladder; lane 2 – control PCR with *hgcA*-harboring plasmid (40 ng per reaction); lane 3 - PCR of the total genomic DNA extracted from the microflora of an anecic earthworm from site S1. Panel ii, Lane 1 – molecular ladder; lane 2 – control PCR with *hgcA*-harboring plasmid (40 ng per reaction); lanes 3 and 4 – PCRs of the total genomic DNA extracted from the microflora of earthworms from site S2, either anecic (lane 3) or endogeic (lane 4).

a. Nucleotide (633 bp) and translated protein sequences (211 amino acids) of the *hgcA* gene fragment amplified from soil and earthworm DNA

```

gggggtcaatgtatgggtgcgcgccggcgaagggaccttcgggcacgacggaactgctggcc
G V N V W C A A G K G T F G T T E L L A
cagatcagcgcgaccaatctcgccgccatcgtcagccaccgccagctgatcctgccgatc
Q I S A T N L A A I V S H R Q L I L P I
ctcggcgcgccgggagtgggcggcgcacgaggtaaagcaacagagcggccttcaccgctccgc
L G A P G V A A H E V K Q Q S G F T V R
tacgggacgatccgcgctgccgatctccccgactatctcgacaacgggtctgggtcactacc
Y G T I R A A D L P D Y L D N G L V T T
ccggcaatgcaggagctgaccttcaactctctacgaacggctgggtcctcctcccggctcgag
P A M Q E L T F T L Y E R L V L L P V E
atcatccacgcccctgaaatcggcggcagtcatcaccctgagcctcttccctcctcggcctc
I I H A L K S A A V I T L S L F L L G L
cttgccggcggggaacgccagcgcgccagccgggatgatcctcgcctatctcgggtgccctcttc
L A G G N A S A S R M I L A Y L G A L F
agcggcatcgctcgctcgggccccctcctcctccctggtgcccggggcggagtttcgccgctc
S G I V V G P L L L P W L P G R S F A V
aaggggggagtcgctcgggtctactctggctcgtttctctgctatcgattgatcgggtgctgac
K G G V V G L L W S F L C Y R L I G A D
tggaacctggcccagacgctggcggccgggtctggcgtgcccggcgtgagcgttttttac
W N L A Q T L A A G L A L P A L S A F Y
accctcaactacaccggctgcacccccctacacc
T L N Y T G C T P Y T

```


C. Comparison of amino acid sequences from the *hgcA* gene fragment amplified from soil and earthworm (211 residues) and the best match from the NCBI database (AHL38020, *Geobacteraceae*).

```

This study          GVNWCAAGKGTFTTELLAQISATNLAAIVSHRQLILPILGAPGVAAHEVKQSGFTVR
AHL38020           GVNWCAAGKGTFTGELVRQIAASGLAQVVSHRLLLLPILGAPGVAAHEVARRSGFKVS
***** ** : ** : * : . ** : ***** : * : ***** : ***** : : **** . *

This study          YGTIRAADLPDYLDNGLVTTTPAMQELFTFLYERLVLLPVEI IHALKSAAVITLSLFLGL
AHL38020           YAIRAADLPRYLDNGMVTVPGMQQLSFTLYERLVLPVELVLALKSISLACFALFVLF
* . : ***** ***** : ** . * . ** : * : ***** : ***** : : **** : : : : ** : *

This study          LAGGNASASRMILAYLGALFSGIVVGPLLLPWLPGRSFAVKGGVVGLLWSFLCYRLIG-A
AHL38020           VAGGPAAGITAALAWLGACLTGIVIGPLLLPWLPGRSFAVKGAVAGLAWSVLFYLLTGGD
: *** * : .      ** : *** : : *** : ***** : ***** : . * * * * *

This study          DWNLAQTLAAGLALPALSIFYTLNYTGCTPYT-----
AHL38020           AWSAWLTVAALLALPAVSIFYTLNFTGCTPFTSISGVKKE
* .      * : ** ***** : ***** : ***** : *

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Figure S12. a) The nucleotide sequence (633 bp) and translated single-letter amino acid sequence (211 residues) of the amplified *hgcA* gene fragment from soil and earthworms. b) A comparison between the nucleotide sequence of the amplified *hgcA* gene fragment from soil and earthworms and the best matching sequence (NCBI web site, KJ021090, *Geobacteraceae* clone B2_FE06). c) A comparison between the amino acid sequence of the amplified *hgcA* gene fragment from soil and earthworms and the best matching sequence (NCBI web site, AHL38020, *Geobacteraceae*). The asterisks, colon, and period denote residues that are identical, have strongly similar properties, and have weakly similar properties, respectively.

b

Multiple sequence alignment (various bird species)

```

grebe      MWAGLGLVLALCLLPGGGTEIQNCKEPPPEWHIGEENPMLSSRGSVTVVALLQASUYMCLL
crane      MWAGLGLVLALCLLPGGGTEIQNCKEPPPEWHIGEENPMLNTRGSVTVVALLQASUYLCLL
pelican    MWAGLGLVLALCLLPGGGTEIQNCKEPPPEWHIGEENPMLNSRGSVTVVALLQASUYLCLL
hoatzin    MWAGLGLVLALCLLPGGGTEIQNCKEAPPEWRIGEEDPMLNSRGSVTVVALLQASUYLCVL
penguin    MWAGLGLVLALCLLGGGTEIQNCKEPPPEWRIGEENPMLNSRGSVTVVALLQASUYLCLL
emperor    MWAGLGLVLALCLLGGGTEIQNCKEPPPEWRIGEENPMLNSRGSVTVVALLQASUYLCLL
cormorant  MWAGLGLVLALCLLPGGGTEIQNCKEPPPEWRIGEENPMLNSRGSVTVVALLQASUYLCLL
ibis       MWAGLGLVLALCLLPGGGTEIQNCKEPPPEWRIGEENPMLNSRGSVTVVAPLQASUYLCLL
tropicbird MWAGLGLVLAVCLLPGGGTEIQNCKEPPPEWRIGEENPMLNSRGSVTVVALLQASUYLCLL
loon       MWAGLGLVLALCLLPGGGTEIQNCKEPPPEWRIGEENPMLNSRGSVTVVALLQASUYLCLL
fulmar     -----

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*****:***:***.*.*****:*.***:***:***.:***** *****:***:

```

grebe      QASRLEDLRVKLENEGLVNI SYVVVNHQGSYSQRKFHLLKERVSNYITVYQQDEQQADVW
crane      QASRLEDLRVKLENKGLFNISYVVVNHQGTYSQKRFHLLKERVSEYITVYQQDEQQDDVW
pelican    QASRLEDLRVKLQNEGLVNI SYVVVNHQGTYSQRKFHLLKESVSDYITVYQQDEQQADVW
hoatzin    QASRLEDLRVKLVNEGLVNI SYVVVNHQGPSSRRKFHLLKESVSDSITVYQQDEQQDDVW
penguin    QASRLEDLRVKLENEGLVNI SYVVVNHQGTYSQRKFHLLKESVSDYITVYQQDEQQADVW
emperor    QASRLEDLRVKLENEGLVNI SYVVVNHQGTYSQRKFHLLKESVSDYITVYQQDEQQADVW
cormorant  QASRLEDLRVKLENEGLVNI SYVVVNHQGTSSQRKIHLKASVSDYVTVYQQDEQQADVW
ibis       QASRLEELRVKLENEGLVNI SYVVVNHQGTYSQRKFHLLKESVSDYITVYQQDEQQADVW
tropicbird QASRLEDLRVKLQNEGLVNI SYVVVNHQGTSSQRKFHLLKESVSDYITVYQQDEQQADVW
loon       QASRLEDLRVKLENEGLVNI SYVVVNHQGTSSQRKFHLLKESVSDSITVYQQDEQQADVW
fulmar     -----M

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*****:***** *:**.*****. *:**:***** **: :***** **

```

grebe      TTLNGNKDDFLIYR-CGRLVYHLGLPYSFSLFQYVEESIKIAYCENKCGNCSYTEPAIDD
crane      TTLNGNKDDFLIYDRCGRLVYHLGLPYSFSLFQYVEESIKIAYCENKCGNCSYTEPDIDV
pelican    TTLNGNKDDFLIYDRCGRLVYHLGLPYSFSLFQYVEESIKIAYCENKCGNCSYTEPDIDG
hoatzin    TTLNGNKDDFLIYDRCGRLVYHLGLPYSFSLFQYVEDSIKIAYCENKCGNCSYTEPGIDG
penguin    TTLNGNKDDFLIYDRCGRLVYHLGLPYSFSLFQYVEESIKIAYCENKCGNCSYMEPEIDG
emperor    TTLNGNKDDFLIYDRCGRLVYHLGLPYSFSLFQYVEESIKIAYCENKCGNCSYMEPEIDG
cormorant  TTLNGSKDDFLIYDRCGRLVYHLGLPYSYLSFQYVEESIKIAYCENKCGNCSYMEPDIDG
ibis       TTLNGNKDDFLIYDRCGRLVYHLGLPYSFSLFQYVEESIKIAYCENKCGNCSYTEPDIDG
tropicbird TTLNGNKDDFLIYDRCGRLVYHLGLPYSFSLFQYVAESIKIAYCENKCGNCSYMEPDIDG
loon       ATLNGNKDDFLIYDRCGRLVYHLGLPYSFSLFQYVEESIKIAYCENKCGNCSYMEHDIDG
fulmar     GQIGTIKIPSRTLDRCGRLVYHLGLPYSFSLFQYVEESIKIAYCENKCGNCFYMEPDIDG

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```

grebe      ICENITKTADEKLAIEPKPTGQSHHHNLHRHRHHHHHHHREGSRHSHKNENHQNSSETQ
crane      ICENITKKTDEKLEIEPKPTGQSHHHSLHRHRHHHHHHR-EGGRHSHKNENHQTPSESQ
pelican    VCKNITQKADEKLEIEPKPTGQSHHHSLHRHRQHRRHREG---SRHSHKNENHQASSETQ
hoatzin    ACENITKKADEKLAIEPIPTGQSHHHNPHRHRHHHHHHH-EGSHHSHKNENHRAPSETQ
penguin    ICENMTKKADEKLAIEVEPKPTGQLSHHHNLHRHRQHRRHRE--GSHHAKNENRQAPSETQ
emperor    LCENITKKADEKLAIEPKPTGQSHHHNLHRHRQHRRHRE--GSHHSHKNENHQAPSETQ
cormorant  ICENITKQADEKRAEIEPSPTGQRSHDHNTHRHIKHHQHRE--GSHHSHKNENHQAPSETR
ibis       ICENITKKADEKLAERELQPADQSHHHDPHRHGHHHHHRE--GSHHSHKNENHQALSETQ
tropicbird ICENITKKADEKLAIEVEPKPTGQSHHHSLHRHRHHHHHHE--SSRSHKNENHQSPSETQ
loon       VCENITKKADEKLAIEPTPTGQSHHHNLHRHRQHRRHRE--SSRSHKNENHQAPSETQ
fulmar     ICENITKKAEEKLAIEPKPTGQSHHHNLHRHRQHRRHRE--GSRHSHKNENHQAPSETQ

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*:***: **:** * * *:. * ***. ** *:***. :.***:***: **:

```

grebe      RHHPHS-----GHNHRDHTGS-HEQVDTVPPGESVEISQDKKLUKKGKTSCKNQLTUN
crane     RHHPHSGQRRRVFGHNHRDHTGS-HEQVDTAPPGESVEITQDKKLUKKGKNSCKNQLTUN
pelican   RHHPHSGQRRRVFGNRNRHDQTGN-HEQVDTVPPGESVEITQDKKLUKKGKTSCKNQLTUN
hoatzin   RHHPRSGQRHRVSGHNRRHQGTGR-DAQVDTVPPGESVEITQDKKLUKKGKTSCKNQLSUN
penguin   RHHPHSGRRHRVFGHNRRDQTDDSHQVDTVPPGESVEITQDKKLUKKGKTSCKNQLTUN
emperor   RHHPHSGRRHRVFGHSRRDQADS-QEQVDSVPPGESVEITQDKKLUKKGKTSCKNQLTUN
cormorant RHHSDSSQRHRAFGRNRHVQTGS-HEQVDTVPPGESVEITQDKKLUKKGKISCKNQLTUN
ibis      RRHAHSSRRHRVFGNRNRHDQTGS-HEQVDTVPPGESVDITQDKKLUKKGKTSCKNQLTUN
tropicbird RHHPHSSRRHRVFGHNTHDQTGS-HEQVDIGPPGESVEMTQDKKLUKKGKNSCKNQLTUH
loon      RHHPHSGRRHRVLGRTRHDQTGS-HEHVDTVPPGESVEITQDKKLUKKGKTSCKKQLTUN
fulmar    RHHPHSGRRHRVFGNRNRHDQTGS-HEQVDTVPPGESVEITQDKKLUKKGKTSCKNQLTUS
*:. *      *:. : :. . : ** *.*****::***** ***:**

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```

grebe      WQTASDSASSUCCHURHLLFEELGNSVTUQCRGALPNSCRUHGQLSAEDITESUQURLL
crane     WQTASDSTSSUCCHCRHLLFEELGNSVTUQCRGALPNSCRUHGQLSAEDITESUQURLL
pelican   WQTASDSTSSUCCHURHLLFEELGNSVTUQCRGALPNSCRUHGQLPAEDITESUQURLL
hoatzin   WQTASDSNSSUCCHCRHLLFEELGNSVTUQCRGALPNSCRUHGQLAAEDITESUQCRLL
penguin   WQTASDPTSSUCCHCRHLLFEELGNSVTUQCHGALPNSCSUHGQLSAEDITESUQURLL
emperor   WQTASDSTSSUCCHCRHLLFEELGNSVTUWCHGALPNSCSUHGQLSAEDITESUQURLL
cormorant WQTASDSTSSUCCHCRHLLFEELGNSVTUQCRGALPNSCRURUHGQLSAEDITESUQURLL
ibis      WQTASDSTSSUCCHCRHLLFEELGNSVTUQCRGALPNSCRUHGQLSAEDITESUQURLL
tropicbird WQTASDSASSNUCCHCRHLLFEELGNSVTUQCRGALPSSCRUHGQLSAEDITESUQURLL
loon      WQTASDLTSSUCCHCRHLLFEELGNSVTUQCRGALPNSCRUHGQLSAEDITESUQURLL
fulmar    WQTASDSASSUCCHCRHLLFEELGNSVTUQCRGALPNSCRUHGQLSAEDITESUQURLL
*****   *.*****:***** *:*:*:*:*: *****.*****:***

```

```

grebe      TAAUQSPAAGASETSDTUQUQEKARNUAUKTN
crane     TAAUQSPAAGTSEASDTUQUQEKARNUAUKTN
pelican   TAAUQSPAA--SETSDTUEUQEKARNUAUKTN
hoatzin   TAAUQSPAAGGSETSDTUQUQENARNUAUKTN
penguin   TAAUQSPAA--SETRDTUQUQEKARNUAUKTN
emperor   TAAURSPAA--SETSDTUQUQEKARNUAUKTN
cormorant TAAUQSPA--TSETSDTUHUQEKARNUAUKTN
ibis      TAAUQSPAAGVSDSSDTUQUQEKARNUAUKTN
tropicbird TAAUQSPAAGATETSDTUQUQEKARNUAUKTN
loon      TAAUQSPAAGASETSDTUQUQEKARNUAUKTN
fulmar    TD-UQSPTAGASETSDTUQUQEKARNUAUKTN
*   *:*:*:*: :   *** ***:*****

```

C

Multiple sequence alignment (fish species)

```
amberjack      -MWACLSLLLTLCLLHGGGAESDGDGPRCQPPPAWKIGEVDPMKEALGQVTVVALLQASU
eel            -MWACLSLLLPLCLLHGGRAESEGVGPRCQLPPAWNIGEVPEPMKGTMGQVTVVALLQASC
perch         -MWRGLSLLLTLCLLHGGGAESDGGGPRCQLPPPAWKIGEVPEPMTQAMGRVTVLALFQASU
tilapia      -MWAGLSLLLTLCLLHGGGAESEGGGPRCQLPSDWRIGDVEPMKGSVGRVTVVALLQASU
pike         -MWAGLSLLLALCLLPGGGTESEGEGRCKPPIWSIGEVPEPMKEAMGQVTVVALLQASU
trout        MMWVGLSLLLALCLLPGGGTESEGEGRCKQPPGWSIGEVPEPMKEVMGQVTVVALLQASU
salmon       -MKAGLSLLLALCLLPGGGAESEGEGRCKPPAGWSIGEVPEPMKGVMGQVTVVALLQASU
zebrafish    -MWKALS LTLALCLLVGCSAESETEGARCKLPPEWKVGDVEPMKNALGQVTVVAYLQASU
              *   ***  :.**** *   :*:  *.** :. *  :*:**  :*:***: * :*:
              :

amberjack     LFCLVQASRVDGLRQRLESQGLKDVTYMVINHQGEAQRLHAVLAQRLSENITLYKQEEQ
eel           LFCLVQASRMDSLHQMMESQGLKNVAYMVINHQGEQAQLLHPMLAQRMSSENITLYKQDQQ
perch        LFCLVQASRMDPLRNKLESQGLKNVYVMVNVHQQEHAQRLHPMLADRLSENITLYKQDEL
tilapia     LFCLVQASRLDGLQKLERQGLKNVYVMVNVHQQEQRHLHPLLEAKLSKNIILYKQDGH
pike        LFCLVQASLLDGLRLKLEGQGLKNVHYMVNVHQQEKAQRLHKLKQLSEKITLYKQEPF
trout       LFCLVQASLLDGLRLKLEGQGLDNVTYMVNVHQQEQAQRLHTLLRQKLSSENITLYKQPK
salmon      LFCLVQASLLDELRLKLEGQGLDNVTYMVNVHQQDQAQHLHTLLSQKLSENIILYKQEPK
zebrafish   LFCLEQASKLNDLLLKLEKQGYPNIAVMVNNREERSQRLHLLQERLLN-ITLYAQDLS
              **** *.  : : *   : * **  : : ****: : : . : : ** : *   : : : * ** * :

amberjack     QPDVWQTLSGEKDDFLIYDRCGRLTHHISLPYSIIGQGHVEGAIKDITYCKRICGNCTHES
eel           QVDVWKTLLGGQKDDFLIYDRCGRLTHHISLPYSIIGQGHVEGAIRDITYCNRICGDCTHES
perch        QPDVWKSLSNAQKDDFLIYDRCGRLTHHISLPYSIIGQGHVESAIKEAYCKRMCGDCTHES
tilapia     QPDVWQTLAGEKDDFFIYDRCGRLTYRISLPYSIIGEGHIEKAIKDTYCKRLCGDCTHES
pike        QVDVWQSLAGQKDDFLIYDRCGRLTYHISLPYSIMSIPYVENAIKETYCARICGNCKHES
trout       QEDVWQTLAGEKDDFLIYDRCGRLTYHISLPYSILGTPYVENAIKETYCTRVCGDCTYES
salmon      RADVWQALAGKKDDFLIYDRCGRLTHHIFLPFSILGTPYVENAIKETYQOSICGDCTYES
zebrafish   QPDAWQAVNAEKDDILVYDRCGRLTYHLSLPYTTILHHPHVEEAIKHTYCDRICGECLES
              : *.** : : . : **** : : ***** : : ** : : : : : : ** : : ** : **

amberjack     TDTPEECKGKADAQPTTDGTPAVEEDTGHGHGHGHGHGHGHLG---HGHHG-----
eel           TETPEECKGKADVQPDADGTPAIEHNTGHGHHHGHGHGHGHGHDNSGFHHG-----
perch        ADIPEECRETADAQPGADSPPAVEEGTQHGHSQDHAHGHHRRHHHHHGHGHGQDNNDFQS
tilapia     AEIPEECKDNAGVQPDVPAEQDDTR-HDHHHGHGHGHGHGHGHGHGHGHGHDNDVHP
pike        VEIPAECNGTAEATSEGEDKPTTVEPTHGQHRHHHHHHH-----HHDDKHGDHGD
trout       KEIPAECNRTVEAKPEGEEKPVTGRETTGGHGHGHGHGHGHGNGNRHGHGNGNRHGHGHHGE
salmon      TEIPAECNRMVEVKPEGEEKPVTGGDTPHGGRGHGHGHGHGNGH-----HHSKSHGHGHGHE
zebrafish   SAQLEECKKATEEVNKPVEEPEPRQDHGHEQGHHEHQGEAERHR-----
              **.  .  :  .  .  .
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dolphin      APDNPEHPPPSGLHRHHRHRGHQRQGHSENUDALQLSFPQKKLURSGCINQLLUKFPKDS
tursiops     APDNPEHPPPSGLHRHHRHRGHQRQGHSENUDALQLSFPQKKLURSGCINQLLUKFPKDS
orca         APDNPEHPPPSGLHHHHRHRGHQRQGHSENUDALQLSFPQKKLURSGCINQLLUKFPKDS
beluga       APDNPEHPPPSGLHRHHRHRGHQRQGHSENUDALQLSLPQKKLURSGCINQLLUKFPKDS
porpoise     APDNPEHAPPSSLHRHHRHRGHQRQGHSENUDALQLSLPQKKLURSGCINQLLUKFPKDS
cachalot     APDNPEHTPPSGLHRDHRHRGHQRQGHSENUDTLQLSLPQKKLURVRCVNLUKFPRDS
lipotes      APDNPEHPPPSGLHHHHRHRGHQRQGHSENUDTLQISLPQKKLURSGCINQLLUKFPKDS
*****.*****:*****:***:***:***** **:******:***

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dolphin      ESALSSUUUHRHLIFEKTGSAVTUQCKDNLP SLCSUQGLLAEHVTESUQURLPPAACH
tursiops     ESALSSUUUHRHLIFEKTGSAVTUQCKDNLP SLCSUQGLLAEHVTESUQURLPPAACH
orca         ESALSSUUUHRHLIFEKTGSAVTUQCKDNLP SLCSUQGLLAEHVTESUQURLPPAACH
beluga       ESALSSUCUHRHLIFEKTGSAVTUQCKQNLPSLCSUQGLLAEHVTESUQURLPPAACH
porpoise     ESALSSUCUHRHLIFEKTGSAVTUQCKENLP SLCSUQGLLAEHVTESUQURLPPAACH
cachalot     ESALSSUUUHRHLIFEKTGSAVTUQCKENLP SLCSUQGLLAEHVTESUQURLPRAACP
lipotes      ESALSSUCUHRHLIFEKTGSAVTUQCKENLP SLCSUQGLLAEHVTESUQURLPPAACQ
***** *****:*****:***** ***** **

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dolphin      SSQQLKPTAETKUSUEKKAEMUKUPSD
tursiops     ATQQLKPTAETKUSUEKKAEMUKUPSD
orca         ASQQLKPTAETKUSUEKKAEMUKUPSD
beluga       ASQQLKPTAETKUSUEKKAEMUKUPSN
porpoise     ASQQLKPTAETKUSUEKKAEMUKUPSN
cachalot     ASQQLNPTEASTKUSUKKKAEMUKUPSN
lipotes      ASQQPNPTEASTKUSUKKKAEMUKUPSN
:;* :****.*****:*****:

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e Multiple sequence alignment (various pinniped species)

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fur seal      MWRSLGLALALCLLPWGAESQGQSSLCCKPPPAWSIGDQNPVMKSSGSVTVVALLQASUY
sea lion     MWRSLGLALALCLLPWGAESQGQSSFCCKPPPAWSIGDQNPVNSSGSVTVVALLQASUY
walrus       MWRSLGLALALCLLPWGAESQGQSSFCCKPPPAWSIGDQNPVNSSGSVTVVALLQASUY
Weddell seal MWRSLGLALALCLLPWGAESQEQSSFCCKPPPAWSIGDQNPMLNSSGSVTVVALLQASUY
monk seal    MWRSLGLALALCLLPWGAESQEQSSFCCKPPPAWSIGDQNPMLNSSGSVTVVALLQASUY
***** *****:***:*** *****:*****:*****

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fur seal      LCILQASRLEDLRVKLEKEGYLNI SYVVVNHQGLSSQLKYMHLKKNVSEYIAVYQQEENQ
sea lion     LCILQASRLEDLRVKLEKEGYLNI SYVVVNHQGLSSQLKYMHLKKNVSEYIAVYQQEENQ
walrus       LCILQASRLEDLRVKLEKEGYLNI SYVVVNHQGLSSQLKYMHLKKNVSEYIAVYQQEENQ
Weddell seal LCILQASRLEDLRVKLEKEGYLNI SYVVVNHQGLSSQLKYMHLKKNVSEYIPVYQQEENQ
monk seal    LCILQASRLEDLRVKLEKEGYLNI SYVVVNHQGLSSQLKYMHLKKNVSEYIPVYQQEENQ
***** *****:*****:***** *****

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fur seal      TDVWMLLNGKKEDFLIYDRGRLVYHLGLPYSFLTFPYIEEAIKITYCKEKGCSLMTL
sea lion     TDVWMLLNGKKEDFLIYDRGRLVYHLGLPYSFLTFPYIEEAIKITYCKEKGCSLMTL
walrus       TDVWMLLNGKKEDFLIYDRGRLVYHLGLPYSFLTFPYVEEAIKITYCKEKGCSLMTL
Weddell seal TDVWMLLNGKKEDFLIYDRGRLVYHLGLPYSFLTFPYVEEAIKITYCKEKGCSLMTL
monk seal    TDVWMLLNGKKEDFLIYDRGRLVYHLGLPYSFLTFPYVEEAIKITYCKEKGCSLMTL
**** *****:*****:*****:*****:***:*** ** **

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fur seal      ESEDFCKMVSSATVGNTTEAPQP---HHHHHHHHRHHKHHGHQHGHGIDGVSENQQPEAP
sea lion     EAEDFCKMVSLTVGNTTEAPQPH--HHHHHHHCHHHKHHGHQHGHGIDGVSENQQPEAP
walrus       EAEDFCKMVCLATVGDTTEAPQPHHHHHHHHHRHHKHHGHQHGHGIDGVSENQQPEAP
Weddell seal EAADHCKMVSSATVGNTTEAPQP----HHHHHHHGHHLKHEHGHGHGIDGVSENQQPEAP
monk seal    EAEDHCKMVSSATVGNTTEAPQP----HHHHHHHGHHLKHEHGHGHGIDGVSENQQPEAP
*: *.****. :***:***** ***** ** * *****

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fur seal      DAAEHPTP-----
sea lion     DAAERPTPXGLHCHHKHKNHQTEGHPETUDLPAGSESLQPSVPQKRLUQKGCINQLLCKL
walrus       DTAEHPTPXGLHRHHKHKHKNHQREGHPDTUDLPAGSESSQPSVPQKRLURKGCINQLLCKL
Weddell seal DAAEHPTPXGLHHHKKHKNHQREGHPETUDLPAGSESLQPSVPQRRLUUKGCINQLLCKL
monk seal    DAAEHPTPXGLHRHHKHKHKNHQREGHPETUDLPAGSESLQPSVPQRRLUUKGCINQLLCKL
*:*:*:***** *:*:*:***** *:*:*:***** *:*:*:*****

fur seal     -----
sea lion     PKDSGLAPNSCCUHURHLIFEKTGSAVTUQCKESLPSLCSUQGLWAEENVIESUQURLPP
walrus       PKDSGLAPNSCCUHURHLIFEKTGSAITUQCKESLPSLCSUQGLWAEENVIESUQURLPP
Weddell seal PKDSGLAPNSCCUHURHLIFEKTGSAITUQCKETLPSLCSUQGLWAEENVIESUQURLPP
monk seal    PKDSGLAPNSCCUHURHLIFEKTGSAITUQCKETLPSLCSUQGLWAEENVIESUQURLPP
*****:*****:*****:*****

fur seal     -----
sea lion     AAUQASQQLQPTEASTNUSUKHKAKM
walrus       AAUQASQQLQPTEASTNUSUKHKAKM
Weddell seal AAUQASQQLQPTEASASUSUKHKAKM
monk seal    AAUQASQQLQPTEASASUSUKHKAKM
*****:*****

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Figure S13. Multiple sequence alignments of SelP proteins. Selenocysteine residues (U) are highlighted in yellow. The conserved XUXUX⁶UXUX motif is present in cetaceans, birds, and mammals. This motif is also present in some fish species. The UXUX⁶UX⁷UXU motif is found in many species and pinnipeds have the following motif: UXUX⁶UX¹⁴UXU. All these motifs can form a tetraselenolate complex with Hg. The asterisks, colon, and period denote residues that are identical, have strongly similar properties, and have weakly similar properties, respectively. GenBank access numbers are in parenthesis.

a) *Homo sapiens* (AAH15875.1); house mouse: *Mus musculus* (NP_001036079.1); great crested grebe: *Podiceps cristatus* (KL268852.1); zebrafish: *Danio rerio* (NP_840082.3).

b) great crested grebe: *Podiceps cristatus* (KL268852.1); grey crowned crane: *Balearica regulorum gibbericeps* (XP_010311294.1); Dalmatian pelican: *Pelecanus crispus* (XP_009484102.1); Adélie penguin: *Pygoscelis adeliae* (XP_009322697.1); emperor penguin: *Aptenodytes forsteri* (XP_009272808.2); great cormorant: *Phalacrocorax carbo* (XP_009511852.1); crested ibis: *Nipponia nippon* (XP_009472359.1); white-tailed tropicbird: *Phaethon lepturus* (XP_010284851.1); red-throated loon: *Gavia stellata* (XP_009818154.1); Arctic fulmar: *Fulmarus glacialis* (XP_009579693.1).

c) greater amberjack: *Seriola dumerili* (XP_022613554.1); swamp eel: *Monopterus albus* (XP_020449352.1); climbing perch: *Anabas testudineus* (XP_026199022.1); Nile tilapia: *Oreochromis niloticus* (XP_013127321.2); northern pike: *Esox lucius* (XP_012992107.1); rainbow trout: *Oncorhynchus mykiss* (CCX35038.1); Atlantic salmon: *Salmo salar* (AXS67852.2); zebrafish: *Danio rerio* (NP_840082.3).

d) Pacific white-sided dolphin: *Lagenorhynchus obliquidens* (XP_026956984.1); common bottlenose dolphin: *Tursiops truncatus* (XP_019785640.1); killer whale: *Orcinus orca* (XP_004266005.2); beluga whale: *Delphinapterus leucas* (XP_022453898.1); narrow-ridged finless porpoise: *Neophocaena asiaeorientalis* (XP_024596120.1); sperm whale: *Physeter catodon* (XP_007119423.2); Yangtze River dolphin: *Lipotes vexillifer* (XP_007447694.1).

e) northern fur seal: *Callorhinus ursinus* (XP_025723121.1); California sea lion: *Zalophus californianus* (XP_027460417.1); Pacific walrus: *Odobenus rosmarus divergens* (XP_004409206.2);

Weddell seal: *Leptonychotes weddellii* (XP_006747601.1); monk seal: *Neomonachus schauinslandi* (XP_021556282.1).

MWKALSLTLALCLLVGCSAESETEGARCKLPPEWKVGDVEPMKNALGQVTVVAY
 LQASULFCLQASKLN~~DL~~LLKLEKQGYPNIA~~Y~~MVVNNREERSQRLHLLQERLLNI
 TLYAQDLSQPDAWQAVNAEKDDILVYDRCGR~~L~~TYHLSLPY~~T~~ILIH~~P~~HVEEA~~I~~KHTYC
 DRICGEC~~S~~LESSAQL~~E~~ECKKATEEVNKPVE~~E~~EP~~R~~QD~~H~~GHHEQGHHEHQGEAER
 HRHGHHHPHHHHHHHRGQQQVDVDQ~~V~~LSQVDFGQVAVETPMMKRPUAKHS
 RUKVQYSUQQGADSPVASUCUHURQLFGGEGNGRVAGLUHCDELPASUPUQ
 GLKEQDNHIKETUQURPAPPAEUELSQTUVUPAGDATUGURKK

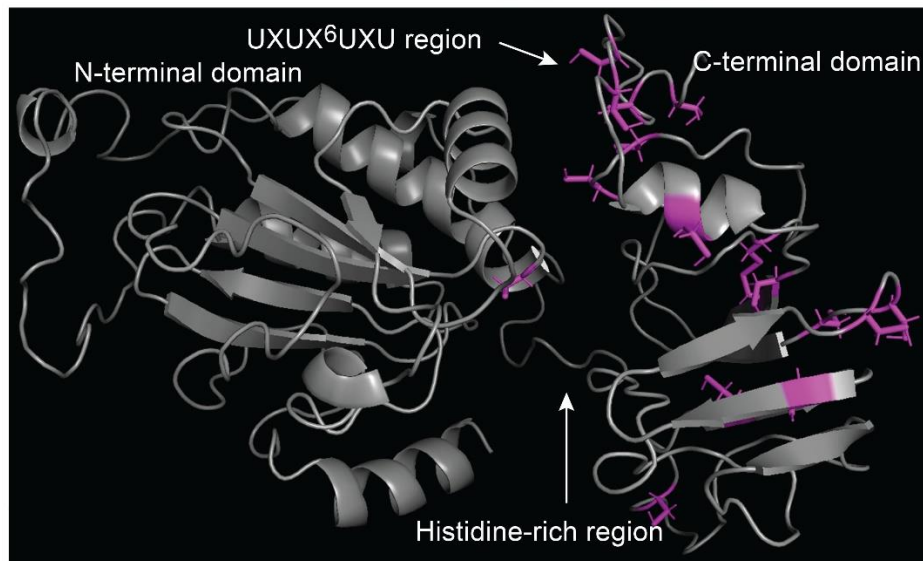


Figure S14. Predicted tertiary structure from I-Tasser^{43, 44} of SelP for zebrafish. SelP is made up of a N-terminal domain containing 1 Sec residue (U) and a C-terminal domain containing 16 U residues in zebrafish (and 13 U residues in the grebe). The two domains are connected through a histidine-rich region. The N-terminal domain has redox enzymatic properties and the C-terminal domain supplies Se to extrahepatic tissues⁴⁵. The 17 side chains from the Sec residues in the two domains are represented by purple squares on the left side of the figure. The figure on the right side presents the C-terminal domain. Only the 6 Sec side chains located on the flexible loop after the α -helix are represented. The position of these residues in the amino acid sequence, represented in single-letter code, is denoted with a U symbol. The structures were visualized with PyMol⁴⁶.

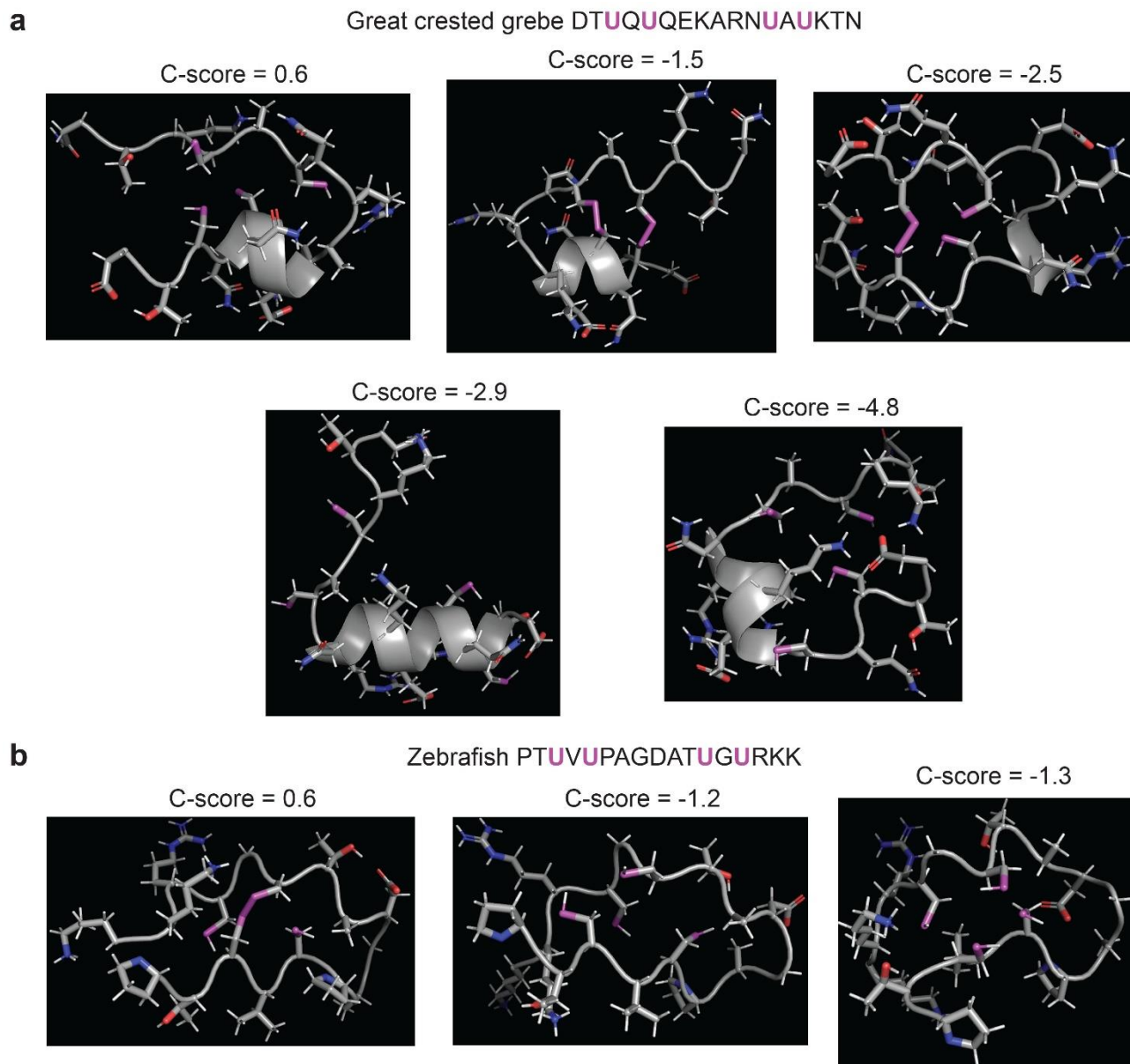


Figure S15. Tertiary structure of the UXUX⁶UXU region of the C-terminal domain of SelP for the great crested grebe and zebrafish generated by I-Tasser^{43, 44}. I-Tasser ranks predicted models according to the C-score confidence parameter and the cluster size. a) Top five predicted models for the great crested grebe. The Sec residues of the three highest C-score models form a cage conducive to binding Hg. Some of the Sec residues are linked through Sec-Sec bridges. b) I-Tasser listed only three models of zebrafish because the top homologous structure templates were similar to each other. This means that the simulation is well converged, thus the structure prediction is of high quality. All conformations were modeled on cysteine-substituted sequences. The amino-acids in each sequence are represented with a single-letter code, with U corresponding to selenocysteine. Purple, red, and blue represent selenol, carboxyl/hydroxyl, and amino groups from amino-acid side chains, respectively.

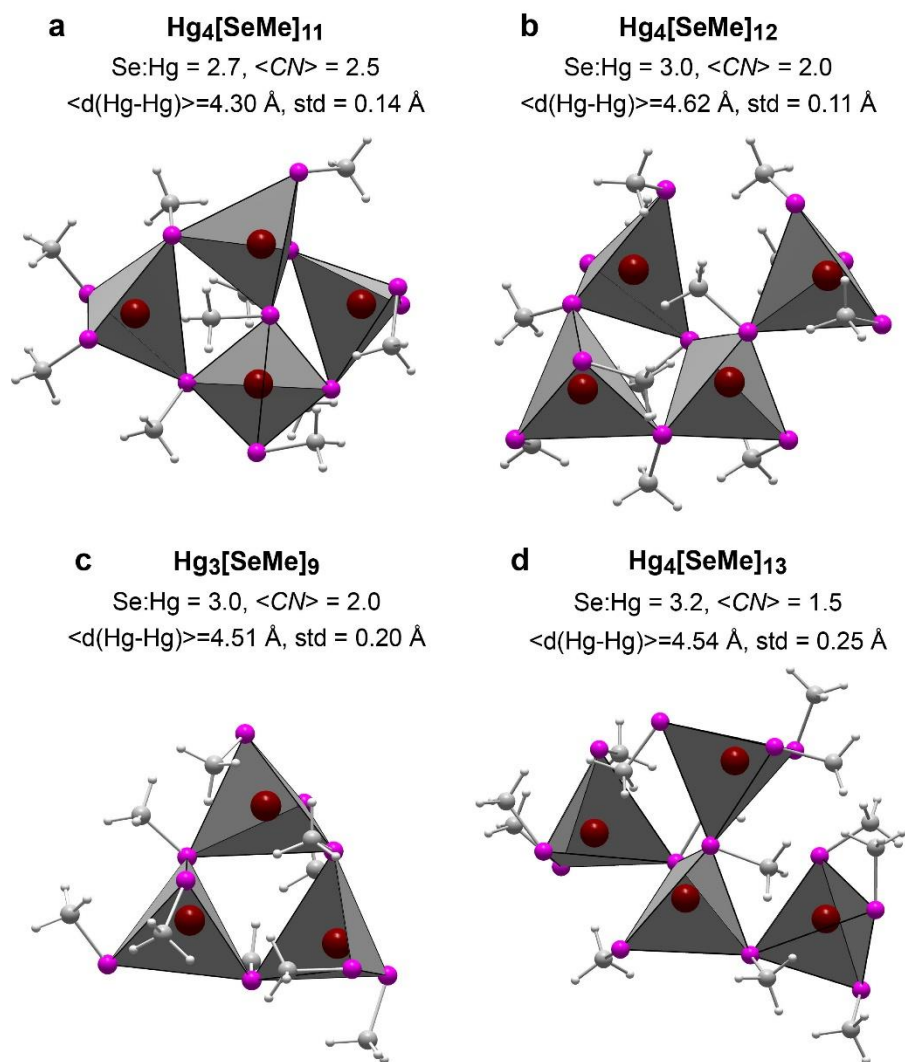


Figure S16. Theoretical topologies of $\text{Hg}_x(\text{Se},\text{Sec})_y$ clusters inspired from metallothionein metal clusters with average Hg-Hg coordination numbers (<CN>) and distances (<d(Hg-Hg)>). a) $\text{Hg}_4(\text{Sec})_{11}$ cluster inspired from the $\alpha\text{-(Zn,Cd)}_4(\text{Cys})_{11}$ cluster of metallothionein in vertebrate⁴⁷. b) $\text{Hg}_4(\text{Sec})_{12}$ cluster inspired from the $\text{Cd}_4(\text{Cys})_{12}\text{-}\alpha$ cluster of the metallothionein for *Mytilus galloprovincialis*⁴⁸. c,d) $\text{Hg}_3(\text{Sec})_9$ and $\text{Hg}_4(\text{Sec})_{13}$ clusters inspired from the $(\text{Zn,Cd})_x(\text{Cys})_y$ clusters in bacterial metallothionein⁴⁹. The Hg atom adopts tetrahedral tetraselenolate coordination geometry in all clusters, but the connectivity varies. The Se:Hg ratio can be diminished by substituting a Cys for a Sec ligand. Note that the C-terminal domain of SelP has one Cys residue (Figure S14). Because Hg has an affinity for Se that is approximately two order of magnitude higher than for sulfur⁵⁰, the thiol ligand of MeHgCys complexed at the surface of a $\text{Hg}_x(\text{Se},\text{Sec})_y$ cluster can be replaced with a selenolate ligand, catalyzing the demethylation of MeHg and promoting the growth of the clusters. All clusters were optimized geometrically at the PBE0-D3 DFT quantum level with def2-TZVP (Hg, Se) and def2-SVP (C, H) basis sets using ORCA 4.2.1³¹. The water solvent was represented with the SMD model³⁸. Sec residues were modeled with methaneselenolate (CH_3Se^-). Dark red, Hg; purple, Se; gray, C, light gray, H.

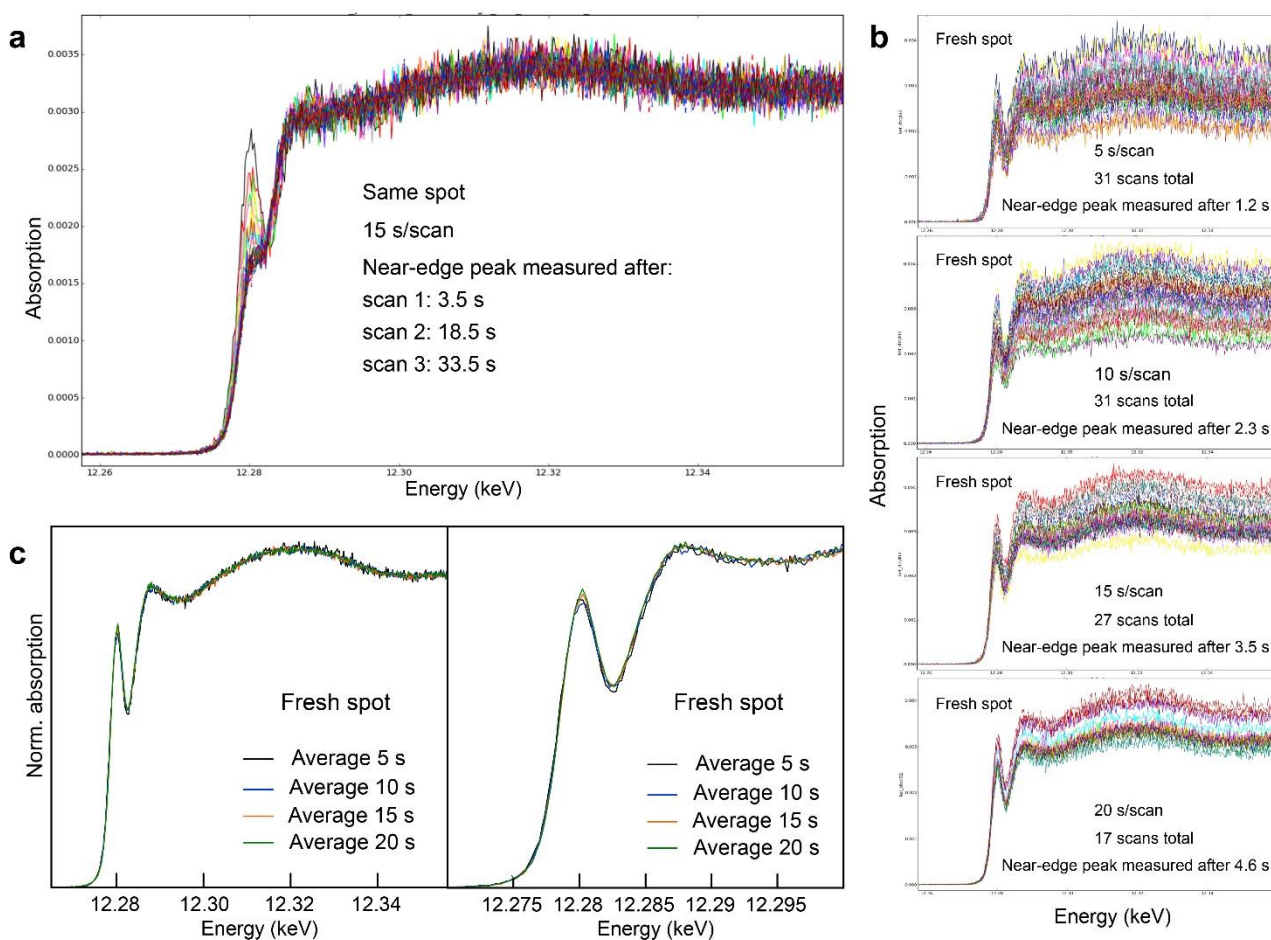


Figure S17. Tests of beam damage. a) The near-edge peak of MeHgCys, which corresponds to transitions of the Hg $2p_{3/2}$ photoelectron to hybridized Hg $6s5d - S 3sp$ bound-states from the Hg-S bond and to hybridized Hg $6s5d - C 2sp$ bound-states from the Hg-C bond³², decreases in intensity after 18.5 s of exposure, and even more so at longer exposure time. The other regions of the HR-XANES spectrum appear stable. In this test, the near-edge peak was first measured (1st scan) after 3.5 s of sample illumination, a duration perhaps already too long to prevent any radiation damage. This possibility was tested next by measuring multiple scans of 5 s, 10 s, 15 s, and 20, each on a fresh spot (b). In this test, the near-edge peak was always measured after 1.2 s (5s scan), 2.3 s (10 s scan), 3.5 s (15 s scan), and 4.6 s (20 s scan) of exposure time. Then each scan series was summed up and normalized to unit step, and the four spectra compared to one another (c). Careful evaluation of spectra in c shows that the near-edge peak is statistically invariant. We conclude that the samples did not suffer any detectable radiation damage over the 15 s dwell time of all measurements. Furthermore, radiation damage decreases the amplitude of the HR-XANES peaks without changing their energy position, as shown previously in Figure S4 from Ref.²⁹. Plots (a) and (b) are screenshots from the PyMCA software⁵¹.

S3. Supplementary tables

Table S1. Body weight, size, and length of fish

Individual	Genus and species	Weight (g)	Body size (cm) ^a
PeacockBass-1	<i>Cichla monoculus</i>	356	24.3
PeacockBass-2	<i>Cichla temensis</i>	284	24.0
PeacockBass-3	<i>Cichla temensis</i>	304	25.5
Pescada-1	<i>Plagioscion squamosissimus</i>	225	28.5
Pescada-2	<i>Plagioscion squamosissimus</i>	190	27.8

^aExcluding the caudal fin.

Table S2. Total concentrations of Hg and Se determined by chemical analysis (mg kg⁻¹) and proportions of organic (MeHgCys) and inorganic (Hg(Sec)₄ and Hg(SR)₂) Hg determined by HR-XANES in fish

Individual and tissue ^a	Genus and species	[Hg] _{Tot} mg kg ⁻¹	[Se] _{Tot} mg kg ⁻¹	%MeHgCys	%Hg(Sec) ₄	%Hg(SR) ₂	%Precision	NSS x 10 ⁻⁵
PeacockBass-1-M	<i>Cichla monoculus</i>	2.8 ± 0.3	0.82 ± 0.08	100	0	0	5	-
PeacockBass-1-L	<i>Cichla monoculus</i>	1.2 ± 0.1	3.79 ± 0.38	86	14	0	10	14.8
PeacockBass-2-M	<i>Cichla temensis</i>	1.1 ± 0.1	1.06 ± 0.11	100	0	0	5	-
PeacockBass-2-L	<i>Cichla temensis</i>	1.7 ± 0.2	7.13 ± 0.71	46	39	16	10	6.1
PeacockBass-3-M	<i>Cichla temensis</i>	3.1 ± 0.3	1.01 ± 0.10	100	0	0	5	-
PeacockBass-3-L	<i>Cichla temensis</i>	5.5 ± 0.5	9.44 ± 0.94	38	62	0	5	3.2
Pescada-1-M	<i>Plagioscion squamosissimus</i>	3.2 ± 0.3	0.81 ± 0.08	100	0	0	5	-
Pescada-1-L	<i>Plagioscion squamosissimus</i>	1.4 ± 0.1	1.59 ± 0.16	91	9	0	10	19.5
Pescada-2-M	<i>Plagioscion squamosissimus</i>	4.0 ± 0.4	1.10 ± 0.11	100	0	0	5	-
Pescada-2-L	<i>Plagioscion squamosissimus</i>	2.0 ± 0.2	2.75 ± 0.27	85	15	0	7	9.5

^aM denotes muscle tissue and L denotes liver tissue.

Table S3. Total concentrations of Hg and Se determined by chemical analysis (mg kg⁻¹) and proportions of organic (MeHgCys) and inorganic (Hg(Sec)₄ and Hg(SR)₂) Hg determined by HR-XANES in earthworms

Individual	Sampling site	[Hg] _{Tot} mg kg ⁻¹	[Se] _{Tot} mg kg ⁻¹	%MeHgSR	%Hg(Sec) ₄	%Hg(SR) ₂	%Precision	NSS x 10 ⁻⁵
EarthW-endo-1 ^a	S1	1.5 ± 0.2	7.51 ± 0.75	25	39	36	10	17.6
EarthW-endo-2	S1	2.4 ± 0.2	7.93 ± 0.79	33	32	34	10	20.3
EarthW-endo-3	S1	1.8 ± 0.2	8.58 ± 0.86	17	10	73	10	10.2
EarthW-endo-4	S1	2.0 ± 0.2	6.76 ± 0.68	30	50	19	10	18.7
EarthW-endo-5	S1	3.1 ± 0.3	4.77 ± 0.48	25	45	28	10	12.2
EarthW-endo-6	S2	4.3 ± 0.4	11.7 ± 1.2	46	39	15	10	10.7
EarthW-endo-7	S2	4.2 ± 0.4	5.88 ± 0.59	30	43	27	10	7.3
EarthW-endo-8	S2	5.1 ± 0.5	28.3 ± 2.8	39	25	36	10	14.7
EarthW-endo-9 ^a	S2	5.4 ± 0.5	26.0 ± 2.6	49	51	0	10	6.0
EarthW-endo-10	S2	10.1 ± 1.0	11.8 ± 1.2	50	50	0	10	9.1
EarthW-aneccic-1 ^a	S2	1.2 ± 0.1	1.00 ± 0.10	60	29	10	10	12.4

EarthW-aneccic-2	S1	1.8 ± 0.2	12.7 ± 1.3	28	72	0	10	5.6
EarthW-aneccic-3	S2	5.6 ± 0.6	5.19 ± 0.52	42	44	13	10	7.3

^aDigestive tract removed.

Table S4. Structural parameters of the Clark's grebe liver derived from Fourier-filtered Hg L₃-edge HR-EXAFS analysis

	Hg-Se			Hg-S			Hg-Hg			ΔE_0 (eV)	<i>Res</i>
	<i>R</i> (Å)	<i>CN</i>	σ^2 (Å ²)	<i>R</i> (Å)	<i>CN</i>	σ^2 (Å ²)	<i>R</i> (Å)	<i>CN</i>	σ^2 (Å ²)		
Grebe liver	2.61	2.6	0.008 ^a	2.36	0.8	0.008 ^a	4.46	2.1	0.01 ^b	4.6	5.6 ^c
Confidence ^d	0.008	0.6	0.0001	0.026	0.2	0.0001	0.029	0.5	-	1.6	-

^aParameters constrained to decrease correlations between $\sigma(\text{Se,S})$ and *CN*(Se,S). ^bValue fixed to suppress the correlation between $\sigma(\text{Hg})$ and *CN*(Hg). ^c*Res* = $[\sum(|\chi_{\text{exp}} - \chi_{\text{fit}}|)/\sum(|\chi_{\text{exp}}|)] \times 100$. ^dConfidence limits of fitting parameters determined from the variation of the fit residual (*Res*) within 95 %.

Table S5. Total concentrations of Hg and Se determined by chemical analysis (mg kg⁻¹) and proportions of organic (MeHgCys) and inorganic (Hg(Sec)₄ and Hg(SR)₂) Hg determined by HR-XANES in Clark's grebe

Tissue	[Hg] _{Tot} mg kg ⁻¹	[Se] _{Tot} mg kg ⁻¹	%MeHgCys	%Hg(Sec) ₄	%Hg(SR) ₂	%Precision	<i>NSS</i> × 10 ⁻⁵
Head feather	-	-	100	0	0	5	-
Breast feather	40.7 ± 4.1	1.04 ± 0.10	100	0	0	5	-
Brain	3.1 ± 0.3	1.55 ± 0.16	100	0	0	5	-
Muscle	6.5 ± 0.7	2.31 ± 0.23	66	11	23	5	1.1
Kidneys	21.6 ± 2.2	10.6 ± 1.0	28	59	12	5	1.9
Liver	43.1 ± 4.3	19.3 ± 1.93	14	86	0	5	2.4

Table S6. Sequences of the oligonucleotides used for PCR amplification

Primers	5'→3' sequences	References
hgcA-F	GGNRTYAA YRTNTGGTGYGC	Ref. ⁷
hgcA-912R	GGTGTAGGGGGTGCAGCCSGTRWARKT	Ref. ⁶

Table S7. Concentrations of MeHg as determined by HR-XANES and chemical analysis (mg Hg kg⁻¹ dry weight)

Individual	[MeHgCys] XANES ^a	Precision	[MeHg] chemical analysis	Precision
Clark's grebe				
Head feather	-	-	-	-
Breast feather	40.7	4.6	34.2	3.4
Brain	3.1	0.3	2.9	0.3
Muscle	4.3	0.5	3.7	0.4
Kidneys	6.0	1.2	6.4	0.6
Liver	6.0	2.2	7.9	0.8
Fish				
PeacockBass-1-M	2.8	0.3	2.0	0.2
PeacockBass-1-L	1.0	0.2	0.85	0.08
PeacockBass-2-M	1.1	0.1	0.81	0.08
PeacockBass-2-L	0.8	0.19	0.45	0.04
PeacockBass-3-M	3.1	0.3	2.2	0.2
PeacockBass-3-L	2.1	0.3	1.8	0.2
Pescada-1-M	3.2	0.3	2.4	0.2
Pescada-1-L	1.3	0.2	0.87	0.09
Pescada-2-M	4.0	0.4	3.0	0.3
Pescada-2-L	1.7	0.2	1.5	0.1
Earthworm				
EarthW-endo-1	0.38	0.16	0.10	0.01
EarthW-endo-2	0.79	0.25	0.32	0.03
EarthW-endo-3	0.31	0.18	0.05	0.01
EarthW-endo-4	0.60	0.21	0.07	0.01
EarthW-endo-5	0.78	0.32	0.14	0.01
EarthW-endo-6	2.0	0.5	1.0	0.1
EarthW-endo-7	1.3	0.4	0.80	0.08
EarthW-endo-8	2.0	0.6	1.3	0.1
EarthW-endo-9	2.6	0.5	1.9	0.2
EarthW-endo-10	5.1	1.1	-	-
EarthW-anecic-1	0.72	0.14	0.39	0.04
EarthW-anecic-2	0.50	0.19	0.14	0.01
EarthW-anecic-3	2.3	0.6	1.3	0.1

^a[MeHgCys]_{XANES} = %MeHgCys x [Hg]_{Tot}.

S4. Supplementary references

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S5. Cartesian coordinates of Hg(Selenoneine)₄ and Hg₁₀(SeMe)₂₀

Hg[Selenoneine] ₄			Hg ₁₀ (SeMe) ₂₀				
Hg	3.461701	4.146748	5.583562	Hg	6.014420	-0.058675	0.371545
Se	2.706204	2.013233	6.875411	Hg	-0.268486	6.515726	0.411108
Se	4.922708	4.001610	3.333475	Hg	-0.253160	0.430061	6.310613
Se	5.232110	5.278112	7.089261	Hg	5.938762	6.269286	6.175340
Se	1.193700	5.029105	4.657621	Hg	-0.155567	3.163920	3.116928
C	4.476669	1.607680	7.394439	Hg	5.984914	3.240218	3.043747
N	5.423897	1.224327	6.487590	Hg	2.548632	0.020246	2.977366
N	4.988642	1.697864	8.622831	Hg	3.151103	6.329468	2.859888
C	6.614491	1.079474	7.153129	Hg	2.920073	3.173796	-0.766157
C	6.314848	1.369774	8.480903	Hg	3.197494	3.087935	6.342012
C	7.870810	0.569132	6.523838	Se	1.444574	1.863416	1.431035
H	7.004447	1.354284	9.326655	Se	1.795140	4.497964	4.416517
H	5.237060	1.111153	5.464794	Se	4.350104	1.597700	4.401854
C	8.245636	1.102636	5.132285	Se	4.441839	4.573375	1.325447
H	7.771604	-0.513782	6.363167	Se	7.554977	1.392694	1.932403
H	8.701825	0.700246	7.234270	Se	7.328923	-1.522043	-1.319519
C	9.100078	0.031958	4.365074	Se	4.936997	1.842911	-1.452448
O	8.599791	-1.113986	4.414527	Se	3.892732	-1.393875	1.209287
O	10.145865	0.379327	3.760442	Se	1.814804	7.912470	1.251024
N	8.839878	2.507015	5.144193	Se	0.771487	4.391640	-1.227576
C	10.186464	2.544438	5.788929	Se	-1.579017	7.688114	-1.476559
C	7.936086	3.437658	5.889650	Se	-1.640263	5.149015	2.193377
C	8.935495	3.027086	3.741204	Se	1.106600	2.250149	7.660173
H	10.089642	2.212864	6.827122	Se	1.296942	-1.302379	4.801895
H	10.855192	1.891119	5.225267	Se	-1.777931	1.586978	4.434928
H	10.534462	3.582794	5.765039	Se	-1.487740	-1.257628	7.833693
H	6.928722	3.365774	5.463529	Se	7.688998	7.743364	7.381857

H	7.926431	3.167904	6.948110	Se	7.506932	4.885899	4.372863
H	8.327102	4.453916	5.761155	Se	4.016526	7.617318	4.974804
H	9.616915	2.388822	3.179986	Se	5.038407	4.267409	7.739551
H	7.930301	3.000866	3.307192	C	-2.577701	6.166898	-2.190697
H	9.299819	4.059148	3.791551	C	-2.763118	4.081739	1.008201
H	7.325735	1.209258	4.536808	C	2.866505	7.640337	-0.365368
C	5.314390	2.207598	2.909719	C	5.015315	9.102027	4.205908
N	6.062667	1.888638	1.816692	C	5.702831	5.550858	0.199722
N	4.938404	1.086620	3.544192	C	4.078636	0.522030	-2.601745
C	6.192786	0.524961	1.740697	C	0.316836	0.770403	0.277591
C	5.473034	0.041798	2.824503	C	8.774283	6.280457	8.094170
C	6.933547	-0.178036	0.650556	C	0.718600	5.677103	5.530074
H	5.334394	-0.996833	3.123961	C	7.533846	6.371537	3.113138
H	6.606628	2.507978	1.205970	C	6.502881	3.031870	7.392416
C	8.461269	0.049332	0.686000	C	-1.827617	0.079819	3.201773
H	6.534300	0.108159	-0.335675	C	5.670190	0.352466	5.112999
H	6.697426	-1.240994	0.778198	C	1.939028	1.144315	9.032010
C	8.853129	1.276894	-0.196569	C	-2.438132	-2.211425	6.415179
O	8.286205	2.339358	0.164199	C	2.724464	-1.425207	6.120654
O	9.647899	1.144029	-1.151784	C	4.724793	-2.714985	2.377020
N	9.229358	-1.236191	0.387777	C	8.407714	2.533342	0.598741
C	8.997964	-1.738080	-0.998742	C	9.076558	-0.652161	-1.210952
C	8.825028	-2.307698	1.355052	C	1.197052	5.351923	-2.868638
C	10.689050	-0.985822	0.618930	H	-1.905783	5.331305	-2.426378
H	7.922989	-1.895179	-1.137591	H	-3.068344	6.499632	-3.115035
H	9.376887	-0.996577	-1.702159	H	-3.343099	5.832244	-1.480246
H	9.527322	-2.692370	-1.099164	H	2.160965	5.865415	-2.782145
H	8.785675	-1.877118	2.364424	H	1.215043	4.648069	-3.708828
H	7.853047	-2.716692	1.068446	H	0.402830	6.097368	-3.013214
H	9.576852	-3.101977	1.295872	H	3.320857	-0.042712	-2.046112
H	11.018791	-0.203152	-0.064298	H	3.625598	1.023837	-3.464629
H	10.801274	-0.677730	1.664451	H	4.870700	-0.159148	-2.939445
H	11.224123	-1.922454	0.429380	H	9.412828	-0.587826	-0.168218
H	8.769237	0.281483	1.715532	H	9.036973	0.349996	-1.655232
C	4.072920	6.182070	8.280140	H	9.791109	-1.264360	-1.777040
N	2.999383	6.906641	7.848804	H	7.700523	2.804248	-0.194013
N	4.169523	6.215033	9.613408	H	9.254966	1.989107	0.165808
C	2.358402	7.421155	8.946524	H	8.778477	3.434425	1.103583
C	3.113676	6.986790	10.033404	H	6.509284	6.668405	2.860436
C	1.030548	8.092146	8.832323	H	8.079593	6.078526	2.208510
H	2.923583	7.180587	11.090468	H	8.054374	7.207914	3.596685
H	2.764584	7.122028	6.848721	H	9.658845	6.718935	8.574949
C	-0.093881	7.252069	8.213801	H	9.101352	5.615642	7.284195
H	1.106642	8.966946	8.163711	H	8.209649	5.708970	8.841960
H	0.715493	8.485069	9.807664	H	5.840712	8.745859	3.580634
C	-1.358582	8.136822	8.015376	H	4.322785	9.705796	3.606506
O	-1.982334	7.958819	6.942424	H	5.408324	9.705104	5.033794
O	-1.630013	8.944077	8.940645	H	2.951063	6.567454	-0.574571
N	-0.463185	5.979873	8.982234	H	3.857814	8.087540	-0.225905
C	-0.602789	6.239615	10.443706	H	2.349959	8.141534	-1.193229
C	0.552443	4.902638	8.743772	H	-3.543708	4.732072	0.595754
C	-1.765326	5.453439	8.462592	H	-2.169169	3.642667	0.197890
H	0.387067	6.445911	10.860475	H	-3.230954	3.287788	1.604503
H	-1.269955	7.096797	10.576328	H	-2.298219	0.400382	2.264552
H	-1.021166	5.338885	10.906317	H	-0.815419	-0.296865	3.012353
H	0.649966	4.774111	7.658422	H	-2.431511	-0.713754	3.658293
H	1.507475	5.188258	9.186681	H	-2.951959	-3.068435	6.870542
H	0.180134	3.985629	9.214665	H	-3.184353	-1.559457	5.943538

H	-2.571333	6.129948	8.759431	H	-1.731876	-2.579941	5.659929
H	-1.701657	5.388058	7.372097	H	1.137887	0.719148	9.649280
H	-1.921706	4.464143	8.905460	H	2.532136	0.338636	8.586185
H	0.206007	6.878728	7.225946	H	2.579041	1.785807	9.650208
C	1.384638	6.901459	4.696856	H	7.404312	3.432219	7.872218
N	0.441676	7.748462	4.205295	H	6.674932	2.939355	6.313917
N	2.395108	7.598284	5.230934	H	6.260575	2.054166	7.825434
C	0.839380	9.037902	4.444784	H	0.049655	6.262629	4.888000
C	2.065257	8.926150	5.085920	H	1.383194	6.348129	6.086926
C	-0.009938	10.208516	4.073822	H	0.130640	5.068963	6.227798
H	2.714215	9.727207	5.440882	H	5.219987	5.751736	-0.763293
H	-0.479433	7.549914	3.785547	H	6.587330	4.924801	0.038130
C	-1.440305	10.056503	4.642710	H	5.994666	6.492895	0.673539
H	-0.052853	10.331025	2.979355	H	5.284887	-0.112503	6.026284
H	0.490467	11.107074	4.456184	H	5.856528	-0.416584	4.354237
C	-2.370958	9.503238	3.527842	H	6.601344	0.886101	5.329208
O	-2.051544	8.338502	3.167480	H	3.926817	-3.285435	2.868528
O	-3.292554	10.205034	3.059991	H	5.330827	-3.388222	1.758390
N	-1.934200	11.308615	5.346992	H	5.360154	-2.234158	3.128720
C	-1.962584	12.499479	4.448331	H	2.321972	-1.949164	6.996786
C	-1.055172	11.596550	6.522633	H	3.558620	-2.001895	5.702565
C	-3.303404	11.040224	5.892413	H	3.059782	-0.422316	6.409620
H	-0.937738	12.739649	4.148426	H	-0.314238	1.437707	-0.321754
H	-2.578651	12.252913	3.581995	H	-0.304458	0.094089	0.871688
H	-2.388122	13.336519	5.012871	H	0.969588	0.185795	-0.382365
				H	-1.113486	10.747463	7.212104
				H	-0.027473	11.761020	6.189701
				H	-1.433117	12.504484	7.005355
				H	-3.990983	10.908874	5.056052
				H	-3.237230	10.131552	6.501737
				H	-3.592522	11.896897	6.510645
				H	-1.431745	9.303615	5.444663