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Comment on “New insights into the biomineralization of mercury selenide nanoparticles through stable isotope analysis in giant petrel tissues”

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Abstract

Some birds and cetaceans can demethylate the toxic methylmercury cysteinate (MeHgCys) complex into inert mercury sulfide (HgSe) through the formation of an intermediate tetrahedral selenolate complex with selenocysteine (Sec) residues (Hg(Sec)₄). The nucleation of the HgSe biominerals involves the substitution of the Se ligand for the Sec residues, which is considered to occur in the form of multinuclear Hg_x(Se,Sec)_y clusters mediated by proteins. Queipo-Abad et al. [1] isolated HgSe nanoparticles from the biological tissues of giant petrels and measured the mass-dependent fractionation of the ²⁰²Hg isotope (δ²⁰²Hg). They concluded that the δ²⁰²Hg values of the HgSe nanoparticles from each tissue of individual petrels are specific to the HgSe species alone and that the Hg(Sec)₄ → HgSe reaction occurs without fractionation of the ²⁰²Hg isotope. We show (1) that the HgSe nanoparticles are mixtures of MeHgCys, Hg(Sec)₄, and HgSe, and therefore that the δ²⁰²Hg values are not species-specific, and (2) that the ²⁰²Hg isotope is actually fractionated during the Hg(Sec)₄ → HgSe reaction, and therefore that this isotope can be used to trace the Hg metabolic pathways between tissues in a single individual and in different animals.

It was shown recently that the Clark’s grebe, giant petrels, long-finned pilot whales, and blue marlins detoxify the organic methylmercury cysteinate (MeHgCys) complex by the stepwise MeHgCys → Hg(Sec)₄ → Hg_x(Se,Sec)_y → HgSe demethylation reaction [2–5]. Hg(Sec)₄ is a four-coordinate selenocysteine complex and HgSe is tiemannite. The inorganic Hg(Sec)₄ complex is likely bonded to selenoprotein P (SeIP), for this protein was shown to be associated with Hg in

Clark's grebe using double affinity chromatography high-performance liquid chromatography coupled to inductively coupled mass spectrometry (AF-HPLC-ICPMS) [2]. SelP-bound Hg has been identified also in the plasma of Inuits [6], and in the plasma of rats exposed simultaneously to sodium selenide (Na_2SeO_3) and mercury chloride (HgCl_2) [7–9]. HgSe nanoparticles (NPs) were imaged in liver, kidneys and muscle of giant petrels [3], and their formation involves the replacement of selenocysteine ligands with selenide ligands, likely through the nucleation of multinuclear $\text{Hg}_x(\text{Se},\text{Sec})_y$ clusters ($y < 4x$). The production of biominerals mediated by metalloproteins is common in nature [10], and we show below that the results of Queipo-Abad et al. [1] support the composite nature of the HgSe NPs, as observed for other biominerals [11–13].

Queipo-Abad et al. [1] isolated 37 HgSe NPs from the biological tissues of 11 giant petrels and measured the mass-dependent fractionation (MDF) of the ^{202}Hg isotope, expressed as $\delta^{202}\text{Hg}$ [14], with the objective to characterize how MeHg is detoxified into insoluble HgSe. They observed that NPs from specific tissues in a single petrel had different $\delta^{202}\text{Hg}$ signatures. They hypothesized that $\text{Hg}(\text{Sec})_4$ (denoted as Hg:Se (1:4)) and HgSe NPs have the same MDF in a given tissue, and therefore that the $\text{Hg}(\text{Sec})_4 \rightarrow \text{HgSe}$ reaction occurs without fractionation of the $\delta^{202}\text{Hg}$ isotope in each tissue. Thus, they considered that a $\delta^{202}\text{Hg}$ value measured on the extracted NPs is specific to the HgSe species alone, and that this species-specific $\delta^{202}\text{Hg}$ value varies from one tissue to another.

This interpretation conflicts with two recent Hg isotope studies performed on 22 petrel tissues from 8 individuals and 89 whale tissues from 28 individuals [4,15]. The $\delta^{202}\text{Hg}$ value of a tissue ($\delta^{202}\text{Hg}_t$) is the weighted sum of the MDF of each Hg species (Sp_i) expressed as follows:

$$\delta^{202}\text{Hg}_t = \sum_i f(\text{Sp}_i)_t \times \delta^{202}\text{Sp}_i \quad (1)$$

in which $f(\text{Sp}_i)_t$ is the fractional amount of species i in tissue t , and $\delta^{202}\text{Sp}_i$ is the species-specific MDF. Through a mathematical inversion analysis of the petrel and whale isotopic data, Manceau et al. [4,15] showed that the 22 $\delta^{202}\text{Hg}_t$ petrel values and the 89 $\delta^{202}\text{Hg}_t$ whale values, could be calculated with just three $\delta^{202}\text{Sp}_i$ values, each associated with the MeHgCys, $\text{Hg}(\text{Sec})_4$, and HgSe species. Therefore, Equation 1 was reduced to

$$\begin{aligned} \delta^{202}\text{Hg}_t = & \sum [f(\text{MeHgCys})_t \times \delta^{202}\text{MeHgCys}] \\ & + [f(\text{Hg}(\text{Sec})_4)_t \times \delta^{202}\text{Hg}(\text{Sec})_4] + [f(\text{HgSe})_t \times \delta^{202}\text{HgSe}] \end{aligned} \quad (2)$$

with $\delta^{202}\text{MeHgCys} = 2.69 \pm 0.04 \text{ ‰}$, $\delta^{202}\text{Hg}(\text{Sec})_4 = -1.37 \pm 0.06 \text{ ‰}$, and $\delta^{202}\text{HgSe} = 0.18 \pm 0.02 \text{ ‰}$ for the petrel data, and $\delta^{202}\text{MeHgCys} = 1.21 \text{ ‰}$, $\delta^{202}\text{Hg}(\text{Sec})_4 = -1.37 \text{ ‰}$, and $\delta^{202}\text{HgSe} = 0.00 \text{ ‰}$

for the whale data. The two sets of $\delta^{202}\text{Hg}(\text{Sec})_4$ and $\delta^{202}\text{HgSe}$ values were remarkably similar, and the difference of $\delta^{202}\text{MeHgCys}$ value was attributed primarily to metabolic differences between organisms in the rate of demethylation relative to new dietary MeHg intake and MeHg depuration, as giant petrels demethylate MeHg with extreme efficiency producing uncommonly large amounts of HgSe in liver, kidneys, brain, and even muscle.

We show below that the Queipo-Abad et al. [1] data can be reinterpreted in light of the findings of the recent petrel and whale studies [4,15], which concluded that the three Hg species have a specific isotopic composition which is homogenized throughout the body. The demonstration is performed in two steps. We first provide evidence for a mixture of Hg species in the HgSe NPs, then we show that the $\delta^{202}\text{Hg}(\text{Sec})_4 = \delta^{202}\text{HgSe}$ hypothesis is unsupported.

Three (PGA01, PGA02, PGA03) of the six brain tissues studied by Queipo-Abad et al. [1] had been analyzed previously for Hg speciation using X-ray absorption spectroscopy [3]. PGA01 contains $40 \pm 6\%$ MeHgCys + $24 \pm 15\%$ Hg(Sec)₄ + $36 \pm 13\%$ HgSe, PGA02 contains $83 \pm 5\%$ MeHgCys + $9 \pm 9\%$ Hg(Sec)₄ + $8 \pm 8\%$ HgSe, and PGA03 contains $13 \pm 5\%$ MeHgCys + $16 \pm 10\%$ Hg(Sec)₄ + $71 \pm 8\%$ HgSe. The high contrast in Hg speciation between the three samples correlates with the Hg concentration. As expected from a detoxification standpoint, the brain with the highest amount of Hg (PGA03: $13.23 \pm 0.22 \mu\text{g/g}$) has the highest proportion of HgSe ($71 \pm 8\%$) and the lowest proportion of MeHgCys ($13 \pm 5\%$) and, vice versa, the brain with the lowest amount of Hg (PGA02: $1.58 \pm 0.02 \mu\text{g/g}$) has the lowest proportion of HgSe ($8 \pm 8\%$) and the highest proportion of MeHgCys ($83 \pm 5\%$). When plotting $\delta^{202}\text{HgSe}$ against total $\delta^{202}\text{Hg}$ for the three brain tissues, one observes that the data are linearly correlated (Fig. 1A, $R^2 = 0.998$), which indicates that the NPs are not pure Hg species, but mixtures. This interpretation can be extended to all tissues by plotting $\delta^{202}\text{HgSe}$ and total $\delta^{202}\text{Hg}$ for all samples studied by Queipo-Abad et al. [1] (Fig. 1B). The correlation is lower ($R^2 = 0.796$), but is higher within each tissue. Further, Bolea-Fernandez et al. [16] extracted 7 HgSe NPs from the liver and muscle of long-finned pilot whales, and their data match well those of the giant petrels, which adds support to our interpretation.

Turning now our attention to the lack of fractionation of the $\delta^{202}\text{Hg}$ isotope during the Hg(Sec)₄ → HgSe reaction, Queipo-Abad et al. [1] wrote: “In the giant petrel group exhibiting the highest Hg hepatic concentrations, there is a perfect match between the isotopic signature of THg ($\delta^{202}\text{Hg}_{\text{bulk}}$) and those in HgSe NPs ($\delta^{202}\text{Hg}_{\text{HgSe}}$) for the liver, kidneys and muscles.” Afterward the authors state that the bulk samples contain dominantly inorganic Hg in the forms of Hg(Sec)₄ and HgSe, and they conclude from this and from bulk $\delta^{202}\text{Hg} \approx \delta^{202}\text{HgSe}$ that $\delta^{202}\text{Hg}(\text{Sec})_4 = \delta^{202}\text{HgSe}$.

We selected the five liver, four kidney, and two muscle tissues for which bulk $\delta^{202}\text{Hg} \approx \delta^{202}\text{HgSe}$ (Fig. 2A, $R^2 = 0.993$), and plotted their proportions of MeHg measured chemically by the authors (%MeHg) as a function of the total Hg concentration (Fig. 2B). The subset of samples does not include those with the highest Hg hepatic concentrations, but covers a large range of Hg concentration, from $0.56 \pm 0.15 \mu\text{g/g}$ to $928.49 \pm 73.31 \mu\text{g/g}$, and includes the most diluted liver ($1.11 \pm 0.04 \mu\text{g/g}$) and kidney ($0.56 \pm 0.15 \mu\text{g/g}$) tissues. These two tissues have as much as $28.55 \pm 2.43 \%$ MeHg and $27.34 \pm 2.71 \%$ MeHg, respectively. Another tissue from the subset of samples with bulk $\delta^{202}\text{Hg} \approx \delta^{202}\text{HgSe}$ has about 25.9 %MeHg for $[\text{Hg}] = 35.8 \mu\text{g/g}$, two other tissues have about 20 %MeHg for $[\text{Hg}] \approx 8 - 60 \mu\text{g/g}$, and one has 7.5 %MeHg for $[\text{Hg}] = 36.6 \mu\text{g/g}$. The last four tissues are also poor in Hg in comparison to those containing several hundreds $\mu\text{g/g}$ Hg and for which bulk $\delta^{202}\text{Hg} \neq \delta^{202}\text{HgSe}$. Clearly, the identity between bulk $\delta^{202}\text{Hg}$ and $\delta^{202}\text{HgSe}$ in tissues containing a high proportion of MeHg and low total Hg cannot be explained by a mixing of just two dominant Hg species, HgSe and $\text{Hg}(\text{Sec})_4$, and by the assumption that the HgSe NPs are pure HgSe. Therefore, not only are the HgSe NPs mixtures of MeHgCys, $\text{Hg}(\text{Sec})_4$, and HgSe, but we also conclude from this that the $\delta^{202}\text{Hg}(\text{Sec})_4 = \delta^{202}\text{HgSe}$ hypothesis is incorrect.

References

- [1] S. Queipo-Abad, Z. Pedrero, C. Marchán-Moreno, K. El Hanafi, S. Bérail, W.T. Corns, Y. Cherel, P. Bustamante, D. Amouroux, New insights into the biomineralization of mercury selenide nanoparticles through stable isotope analysis in giant petrel tissues, *J. Hazard. Mater.* 425 (2022) n° 127922.
- [2] A. Manceau, J.P. Bourdineaud, R.B. Oliveira, S.L.F. Sarrazin, D.P. Krabbenhoft, C.A. Eagles-Smith, J.T. Ackerman, A.R. Stewart, C. Ward-Deitrich, M.E.D. Busto, H. Goenaga-Infante, A. Wack, M. Retegan, B. Detlefs, P. Glatzel, P. Bustamante, K.L. Nagy, B.A. Poulin, Demethylation of methylmercury in bird, fish, and earthworm, *Environ. Sci. Technol.* 55 (2021) 1527–1534.
- [3] A. Manceau, A.C. Gaillot, P. Glatzel, Y. Cherel, P. Bustamante, In vivo formation of HgSe nanoparticles and Hg-tetraselenolate complex from methylmercury in seabird – Implications for the Hg-Se antagonism, *Environ. Sci. Technol.* 55 (2021) 1515–1526.
- [4] A. Manceau, R. Brossier, B.A. Poulin, Chemical forms of mercury in pilot whales determined from species-averaged mercury isotope signatures, *ACS Earth Space Chem.* 5 (2021) 1591–1599.

- [5] A. Manceau, S. Azemard, L. Hédouin, E. Vassileva, D. Lecchini, C. Fauvelot, P.W. Swarzenski, P. Glatzel, P. Bustamante, M. Metian, The chemical forms of mercury in blue marlin billfish: Implications for human exposure, *Environ. Sci. Technol. Lett.* 8 (2021) 405–411.
- [6] A. Achouba, P. Dumas, N. Ouellet, M. Lemire, P. Ayotte, Plasma levels of selenium-containing proteins in Inuit adults from Nunavik, *Environ. Intern.* 96 (2016) 8–15.
- [7] C. Sasakura, K. Suzuki, Biological interaction between transition metals (Ag, Cd and Hg), selenide/sulfide and selenoprotein P, *J. Inorg. Biochem.* 71 (1998) 159–162.
- [8] K. Suzuki, C. Sasakura, S. Yoneda, Binding sites for the (Hg-Se) complex on selenoprotein P, *Biochim. Biophys. Acta.* 1429 (1998) 102–112.
- [9] S. Yoneda, K. Suzuki, Equimolar Hg-Se complex binds to selenoprotein P, *Biochem. Biophys. Res. Commun.* 231 (1997) 7–11.
- [10] P.M. Dove, J.J. De Yoreo, S. Weiner, *Biom mineralization*, Mineralogical Society of America, Washington DC, 2003.
- [11] S.L. Zhang, J.C. Zang, W.M. Wang, H. Chen, X.R. Zhang, F.D. Wang, H.F. Wang, G.H. Zhao, Conversion of the native 24-mer ferritin nanocage into its non-native 16-mer analogue by insertion of extra amino acid residues, *Angew. Chem. Int. Edit.* 55 (2016) 16064–16070.
- [12] B. Maity, S. Abe, T. Ueno, Observation of gold sub-nanocluster nucleation within a crystalline protein cage, *Nat. Commun.* 8 (2017) n° 14820.
- [13] C. Pozzi, S. Ciambellotti, C. Bernacchioni, F. Di Pisa, S. Mangani, P. Turano, Chemistry at the protein-mineral interface in L-ferritin assists the assembly of a functional (μ^3 -oxo)Tris (μ^2 -peroxo) triiron(III) cluster, *Proc. Natl. Acad. Sci. U.S.A.* 114 (2017) 2580–2585.
- [14] B.A. Bergquist, J.D. Blum, Mass-dependent and -independent fractionation of Hg isotopes by photoreduction in aquatic systems, *Science.* 318 (2007) 417–420.
- [15] A. Manceau, R. Brossier, S.E. Janssen, T.J. Rosera, D.P. Krabbenhoft, Y. Cherel, P. Bustamante, B.A. Poulin, Mercury Isotope Fractionation by Internal Demethylation and Biom mineralization Reactions in Seabirds: Implications for Environmental Mercury Science, *Environ. Sci. Technol.* 55 (2021) 13942–13952.
- [16] E. Bolea-Fernandez, A. Rúa-Ibarz, E.M. Krupp, J. Feldmann, F. Kvanhaecke, High-precision isotopic analysis sheds new light on mercury metabolism in long-finned pilot whales (*Globicephala melas*), *Sci. Rep.* 9 (2019) n° 7262.

Figure caption

Fig. 1. $\delta^{202}\text{Hg}$ values measured on whole tissues and the corresponding isolated HgSe nanoparticles. A) Three brain tissues of giant petrels studied by Queipo-Abad et al. [1] with linear fit ($y = -0.396 + 0.63x$, $R^2 = 0.998$). B) All tissues of giant petrels studied by Queipo-Abad et al. [1], and liver and muscle tissues of long-finned pilot whales studied by Bolea-Fernandez et al. [16] with linear fit of the two independent sets of data ($y = -0.134 + 0.57x$, $R^2 = 0.656$). The outlier liver of petrel P-11 ($\delta^{202}\text{Hg} = 0.32$, $\delta^{202}\text{HgSe} = 1.43$) was omitted.

Fig. 2. A) $\delta^{202}\text{Hg}$ values measured on whole tissues of giant petrels and the corresponding isolated HgSe nanoparticles, for which bulk $\delta^{202}\text{Hg} \approx \delta^{202}\text{HgSe}$, with linear fit ($y = 0.007 + 1.04x$, $R^2 = 0.993$). B) Molar fraction of MeHg to total Hg against the total weight concentration of Hg in the same tissues as in A).

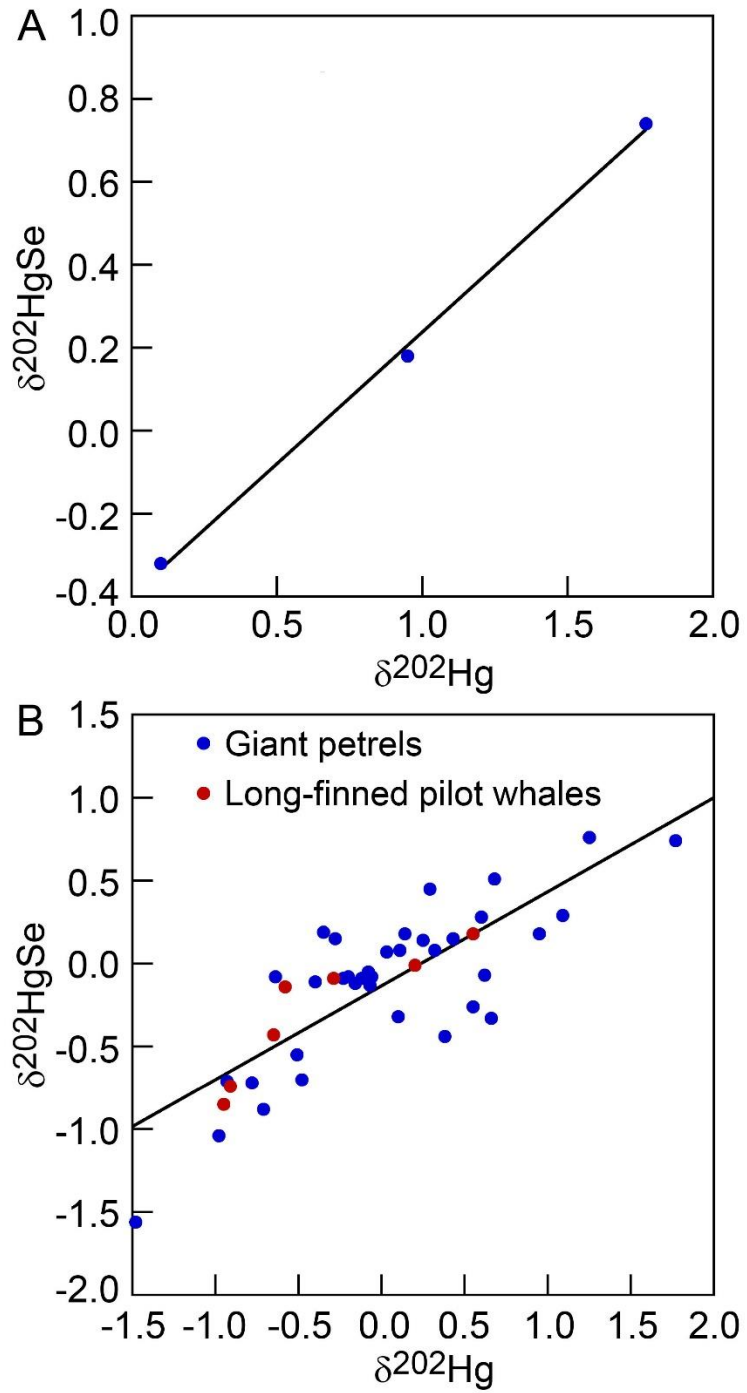


Figure 1

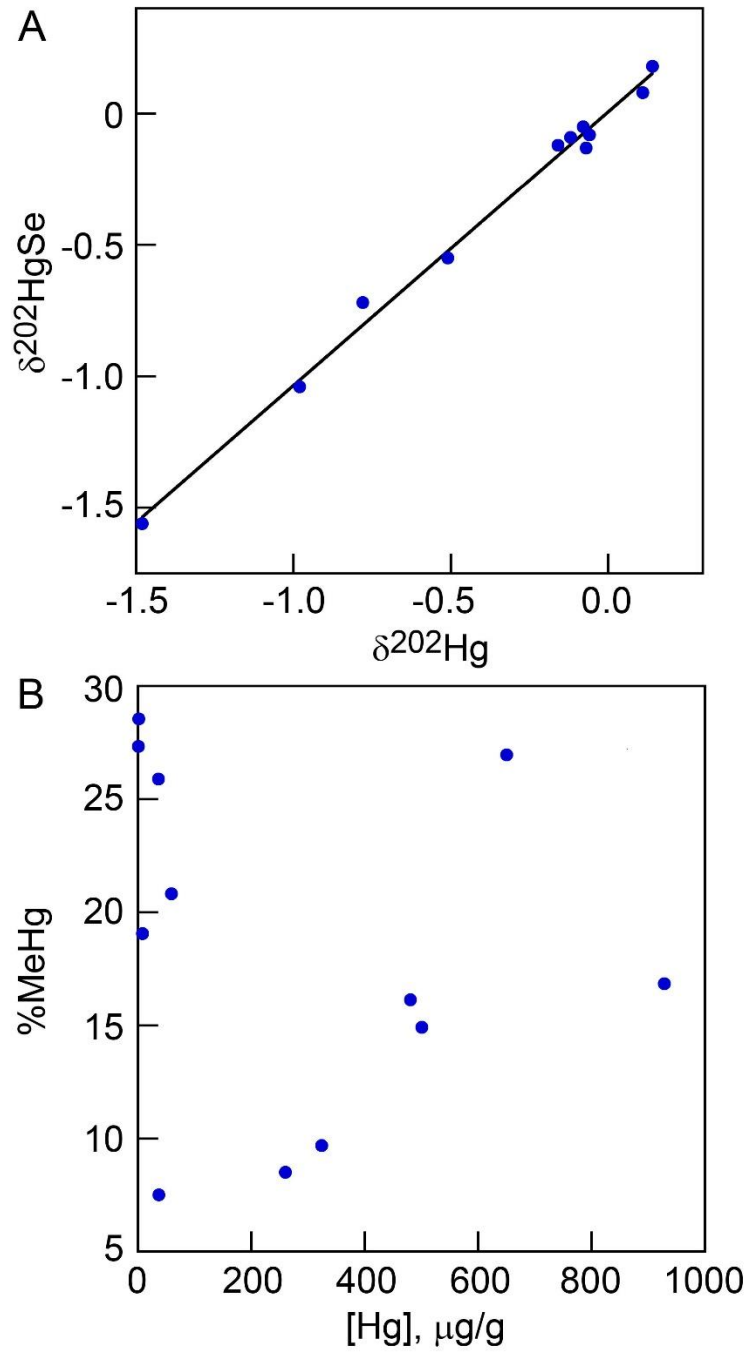


Figure 2