Journal of Hazardous Materials 431 (2022) 128582

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Letter to the editor

Reply to the comment on "New insights into the biomineralization of mercury selenide nanoparticles through stable isotope analysis in giant petrel tissues" by A. Manceau, J. Hazard. Mater. 425 (2021) 127922. doi: 10.1016/j.jhazmat.2021.127922

Silvia Queipo-Abad [a], Zoyne Pedrero [a,*], Claudia Marchán-Moreno [a], Khouloud El Hanafi [a], Sylvain Bérail [a], Warren T. Corns [b], Yves Cherel [c], Paco Bustamante [d,e], David Amouroux [a]

[a] Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les matériaux, Pau, France
[b] PS Analytical, Arthur House, Crayfields Industrial Estate, Main Road, Orpington, Kent BR5 3HP, UK
[c] Centre d'Etudes Biologiques de Chizé, UMR 7372 CNRS - La Rochelle Université, 79360 Villiers-en-Bois, France
[d] Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS - La Rochelle Université, 2 rue Olympe de Gouges, 17000 La Rochelle, France
[e] Institut Universitaire de France (IUF), 1 Rue Descartes, 75005 Paris, France

ARTICLE INFO

Keywords
Mercury
Seabirds
Isotopic fractionation
HgSe nanoparticles
MeHg demethylation

ABSTRACT

In the comments reported by A. Manceau [1], relating to our recent paper on mercury (Hg) species-specific isotopic characterization in giant petrel tissues [2] two critical questions were raised. Firstly, according to A. Manceau, our method of extraction and isolation of nanoparticles was not able to efficiently isolate mercury selenide nanoparticles (HgSe NPs) and therefore the δ^{202}Hg values measured are not species-specific, but rather δ^{202}Hg of mixtures of complexes such as MeHgCys, $Hg(Sec)_4$, and HgSe. Secondly, he suggests that our main findings showing that no isotopic fractionation is induced during the HgSe NPs biomineralization step from the precursor-demethylated species is erroneous because it contradicts the conclusion of two recent articles by A. Manceau and co-workers [3,4]. In this reply we defend our scientific findings and respectively respond to the questions and comments raised by A. Manceau.

1. Discussion

Firstly, we address the comment questioning the purity of the isolated HgSe NPs fraction. Manceau is suggesting that this fraction is a mixture of MeHgCys, $Hg(Sec)_4$ and HgSe, therefore leading to a non-species-specific HgSe NPs isotopic characterization. We would like to point out that the NPs isolation approach, as detailed in the manuscript, is based on the published research by Bolea-Fernández and co-workers for Hg isotopic characterization of tiemannite in pilot whale tissues (Bolea-Fernandez et al., 2019; Gajdosechova et al., 2016). We have done slight modifications to their method including a heating step at 85 °C for 2 h with formic acid to remove any organic molecules. Even if a small amount of Hg could be found in the solid residual resulting fraction, it is also a product of Hg (bio)demethylation. In addition, as detailed in our article, the cut-off filter with isolated HgSe NPs fraction was abundantly washed with MQ-water until complete elimination of total soluble Hg was achieved and verified by CV-AFS measurements. Therefore, by using such sample treatment, the absence of the concomitant Hg species mentioned by Manceau is guaranteed and it is unlikely it will modify the interpretation of the obtained data. Manceau supports his criticism regarding HgSe NPs purity, probably based on those values where the $\delta^{202}Hg_{bulk}$ is equivalent to species-specific δ^{202}Hg of isolated HgSe NPs. However, in more than 50% of the samples the absolute shift δ^{202}Hg (Bulk - HgSe NPs) is larger than 0.20‰, all having been treated with the same procedure and demonstrating that labile-Hg was removed from that fraction.

The second comment addressed by Manceau questions our main hypothesis that HgSe NPs biomineralization from the precursor-demethylated species does not induce Hg isotopic fractionation due to its conflict with two research investigations conducted by himself and

* Corresponding author.
E-mail address: zoyne.pedrerozayas@univ-pau.fr (Z. Pedrero).

https://doi.org/10.1016/j.jhazmat.2022.128582
Received 27 January 2022; Accepted 23 February 2022
Available online 28 February 2022

Table 1
Estimation of the $\delta^{202}Hg_{bulk}$ based on the species-specific $\delta^{202}Hg$ of the three main species according to HR-XANES measurements. The measured $\delta^{202}Hg_{bulk}$ value for comparison has been calculated as an average of the two published values for these tissues. The $\delta^{202}Hg_{HgSe\ (1:4)}$ has been approximated to the $\delta^{202}Hg_{HgSe}$ experimentally measured in the corresponding tissues.

		$\delta^{202}Hg_{bulk}$ (‰)(Manceau et al., 2021a)	$\delta^{202}Hg_{bulk}$ (‰)	Mean $\delta^{202}Hg_{bulk}$ (‰)	$\delta^{202}Hg_{MeHg}$ (‰)(Manceau et al., 2021a)	$\delta^{202}Hg_{HgSe} = \delta^{202}Hg_{HgSe\ (1:4)}$ (‰)(Queipo-Abad et al., 2021)	f MeHg (%)(Manceau et al., 2021c)	f HgSe (1:4) (%)(Manceau et al., 2021c)	f HgSe (%) (Manceau et al., 2021c)	$\delta^{202}Hg_{bulk\ estimated}$ (‰)
Individual 1	Kidneys	0.15 ± 0.02	0.11(Queipo-Abad et al., 2021)	0.15 ± 0.03	2.70 ± 0.03	-0.08	0.07 ± 0.03	0.32 ± 0.09	0.61 ± 0.08	0.11
	Muscle	-0.76 ± 0.03	-0.48(Queipo-Abad et al., 2021)	-0.76 ± 0.20	2.78 ± 0.03	-0.70	–	0.67 ± 0.08	0.33 ± 0.08	-0.70
Individual 2	Liver	0.04 ± 0.04	-0.08(Queipo-Abad et al., 2021)	0.04 ± 0.08	1.87 ± 0.03	-0.05	–	0.09 ± 0.06	0.91 ± 0.06	-0.05
	Muscle	-0.57 ± 0.02	-0.51(Queipo-Abad et al., 2021)	-0.54 ± 0.04	2.07 ± 0.03	-0.55	0.07 ± 0.03	0.60 ± 0.09	0.33 ± 0.09	-0.37
Individual 3	Muscle	-0.73 ± 0.06	–	-0.73 ± 0.06	0.90 ± 0.04	–	–	0.40 ± 0.08	0.60 ± 0.08	–
Individual 4	Brain	0.50 ± 0.04	0.10(Poulin et al., 2021)	0.30 ± 0.28	2.58 ± 0.04	-0.32	0.13 ± 0.05	0.16 ± 0.10	0.71 ± 0.08	0.06

$\delta^{202}Hg_{\ bulk\ estimated} = f_{MeHg}$ x $\delta^{202}Hg_{MeHg} + f_{HgSe}\ \delta^{202}Hg_{HgSe} + f_{HgSe\ (1:4)}\ \delta^{202}Hg_{HgSe\ 1:4}$ (‰).
Individual 1: P2 (Manceau et al., 2021c)=P3 (Queipo-Abad et al., 2021) ; Individual 2: P3 (Manceau et al., 2021c)=P4 (Queipo-Abad et al., 2021); Individual 3: P5 (Manceau et al., 2021c)=P8 (Queipo-Abad et al., 2021); Individual 4: P8 (Manceau et al., 2021c)=PGA03 (Queipo-Abad et al., 2021; Renedo et al., 2021)

his co-workers (Manceau et al., 2021a, 2021b). In this regard, we would like to state that our premise is solidly based on high precision measurements of the Hg isotopic composition in the tissues of 11 giant petrels (40 tissues in total, with HgSe NPs extracted from 37 tissues, all except three blood samples). In contrast, the two recent publications by Manceau and co-workers (Manceau et al., 2021a, 2021b), based their hypothesis on a mathematical calculation approach which has not yet been experimentally validated by measurements of species-specific Hg isotopic composition.

In each tissue, the bulk Hg isotopic composition results from the contribution of different Hg species as outlined in Eq. (1):

$$\delta^{202}Hg_{bulk} = \sum_{i=Hg\ species} f_i \times \delta^{202}Hg_i(‰) \quad (1)$$

where $\delta^{202}Hg_{bulk}$ represents the isotopic composition of total mercury in the tissue, f_i is the fraction of i-species in the corresponding tissue, and $\delta^{202}Hg_i$ represents the species-specific isotopic composition of Hg in i-species. The resolution of this equation requires the knowledge of a series of data that have been over simplified in the aforementioned mathematical works (Manceau et al., 2021a, 2021b) with the following assumptions: i) the isotopic composition of each Hg species is only species-dependent, and it does not vary between individuals or between tissues; ii) Hg species in tissues are exclusively limited to three different forms, viz.: MeHgCys, $Hg(Sec)_4$ and HgSe. Regarding the invariance of Hg isotopic composition, to the best of our knowledge there is no research to date that supports the theory that there is a specific $\delta^{202}Hg$ value that is solely dependent on the nature of the species. So far, in cases where different Hg species have been extracted, no unique and constant $\delta^{202}Hg$ value has been obtained for all tissues or in all individuals studied. This has not been experimentally proven neither for MeHg (Manceau et al., 2021a; Masbou et al., 2013; Poulin et al., 2021; Perrot et al., 2016) or HgSe NPs (Queipo-Abad et al., 2021; Bolea-Fernandez et al., 2019) in different living organisms. A study developed by our group (IPREM CNRS Pau) and co-workers is the only one to jointly report Hg isotopic compositions for MeHg and inorganic mercury (iHg) (Perrot et al., 2016) in aquatic mammal tissues. These values reflect a large variability between individuals and tissues for the species-specific $\delta^{202}Hg$ values in beluga whales ($\delta^{202}Hg_{MeHg}$ variation of ~3.5‰) and seals ($\delta^{202}Hg_{MeHg}$ variation of ~1.7‰). This aspect of the mathematical approach has also been recently questioned by Wiederhold and Jiskra (Wiederhold and Jiskra, 2022), which makes it clear that this assumption remains questionable for the seabird tissues. So far the number of studies dealing with Hg species-specific isotopic composition is limited mainly due to the great challenge associated with the extraction/isolation of the different Hg species while preserving the original isotopic pattern (Queipo-Abad et al., 2021; Bolea-Fernandez et al., 2019; Masbou et al., 2013; Poulin et al., 2021; Perrot et al., 2016; Rodríguez-González et al., 2009). The Hg species-specific approach in animal tissues has been mainly applied on the isotopic characterization of MeHg (Manceau et al., 2021a; Masbou et al., 2013; Poulin et al., 2021; Perrot et al., 2016). Meanwhile Bolea-Fernández and co-workers together with our article (Queipo-Abad et al., 2021; Bolea-Fernandez et al., 2019) reported the unique Hg species-specific isotopic composition relative to HgSe NPs. The obtained Hg species-specific isotopic data has been key to obtain information on metabolic processes (Queipo-Abad et al., 2021; Bolea-Fernandez et al., 2019; Masbou et al., 2013; Poulin et al., 2021; Perrot et al., 2016).

The comments of Manceau about the $\delta^{202}Hg$ in HgSe NPs values experimentally determined in our article comes from the differences with the values estimated by a mathematical approach considering exclusively three species of Hg (MeHgCys, $Hg(Sec)_4$ and HgSe). However, the analysis of the water-soluble fraction from the different seabird tissues by size exclusion chromatography (SEC)-ICP-MS presented in Figure 4 of our recent article (Queipo-Abad et al., 2021), evidenced that Hg binds several (unknown) biomolecules that probably play key roles on MeHg demethylation. The unambiguous characterization of Hg binding biomolecules/proteins represents an important analytical challenge, which explains the limited number of publications reporting Hg-metabolites (Krupp et al., 2008; Krupp et al., 2009; Trümpler et al., 2014; Pedrero Zayas et al., 2014; Pedrero et al., 2012; Garcia-Calleja et al., 2021; Mangal et al., 2019; Strohmidel et al., 2018). The crucial role of speciation in understanding metabolic processes is undoubtedly an additional dimension to Hg isotopic characterisation. Thus, we consider that a mathematical approach which simplifies the number of Hg species and the possibly large variability of $\delta^{202}Hg$ in different tissues and individuals, cannot be used in a general way to estimate Hg isotopic values to improve our understanding of the pathways and fate of Hg in biota.

Moreover, we would also like to highlight this statement by Manceau: "*that the ^{202}Hg isotope is actually fractionated during the $Hg(Sec)_4 \rightarrow HgSe$ 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*". This argument is independent of whether there is isotopic fractionation at that stage, and even transcends this study, as $\delta^{202}Hg$ (MDF) has already been used in several works to trace metabolic pathways in living organisms (Bolea-Fernandez et al., 2019; Perrot et al., 2016; Feng et al., 2015; Li et al., 2020; Meng et al., 2020; Masbou et al., 2018; Le Croizier et al., 2020; Renedo et al., 2021). Even the fact that there is no isotopic fractionation of Hg at this stage characterizes the (metabolic) process, therefore, this statement for us means a trivialization of the scientific results of this research.

Additionally, our hypothesis related to the absence of isotopic fractionation during the biomineralization step from the precursor (demethylated) species, can be supported by the combination of $\delta^{202}Hg$ values measured in giant petrels and estimated with Eq. (1). The $\delta^{202}Hg$ species-specific experimental data (Table 1) reported (Queipo-Abad et al., 2021; Manceau et al., 2021a; Renedo et al., 2021; Manceau et al., 2021c) for MeHg ($\delta^{202}HgMeHg$) and for HgSe NPs ($\delta^{202}Hg_{HgSe}$) were combined by applying Eq. (2) (adapted from Eq. (1)) in the estimation of $\delta^{202}Hg_{bulk}$ as follows:

$$\delta^{202}Hg_{bulkestimated} = f(MeHg)x\delta^{202}Hg_{MeHg} + f(HgSe)x\delta^{202}Hg_{HgSe} + f(HgSe(1:4))x\delta^{202}Hg_{HgSe1:4}(‰) \quad (2)$$

where $\delta^{202}Hg_{bulk}$ represents the estimation of isotopic composition of total mercury in the tissue and f is the fraction of the three main species (MeHg, precursor HgSe (1:4), and HgSe NPs) determined by HR-XANES (Manceau et al., 2021c). The $\delta^{202}Hg_{HgSe\ (1:4)}$ will be considered equivalent to $\delta^{202}Hg_{HgSe}$, in line with our observation in the preceding article. The obtained $\delta^{202}Hg_{\ bulk\ estimated}$ reported in Table 1 and the measured values (average between $\delta^{202}Hg_{\ bulk}$ measurements in different sections of the tissues) (Queipo-Abad et al., 2021; Manceau et al., 2021a) show a good agreement when assuming the lack of Hg isotopic fractionation in the biomineralization and approximating the $\delta^{202}Hg_{HgSe\ (1:4)}$ to the $\delta^{202}Hg_{HgSe}$.

In summary, the complexity of Hg pathways in biota calls for a combination of complementary analytical techniques to contribute to their elucidation. The analytical approaches addressed on this discussion (species-specific isolation, liquid chromatography separation, HR-XANES identification and high precision isotopic analyses) demonstrates the potential of such synergy to go further on the understanding of Hg processes in biota.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Bolea-Fernandez, E., Rua-Ibarz, A., Krupp, E.M., Feldmann, J., Vanhaecke, F., 2019. High-precision isotopic analysis sheds new light on mercury metabolism in long-finned pilot whales (Globicephala melas). Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-019-43825-z.

Feng, C., Pedrero, Z., Gentès, S., Barre, J., Renedo, M., Tessier, E., Berail, S., Maury-Brachet, R., Mesmer-Dudons, N., Baudrimont, M., Legeay, A., Maurice, L., Gonzalez, P., Amouroux, D., 2015. Specific pathways of dietary methylmercury and inorganic mercury determined by mercury speciation and isotopic composition in zebrafish (Danio rerio). Environ. Sci. Technol. 49, 12984–12993. https://doi.org/10.1021/acs.est.5b03587.

Gajdosechova, Z., Lawan, M.M., Urgast, D.S., Raab, A., Scheckel, K.G., Lombi, E., Kopittke, P.M., Loeschner, K., Larsen, E.H., Woods, G., Brownlow, A., Read, F.L., Feldmann, J., Krupp, E.M., 2016. In vivo formation of natural HgSe nanoparticles in the liver and brain of pilot whales. Sci. Rep. 6, 1–11. https://doi.org/10.1038/srep34361.

Garcia-Calleja, J., Cossart, T., Pedrero, Z., Santos, J.P., Ouerdane, L., Tessier, E., Slaveykova, V.I., Amouroux, D., 2021. Determination of the intracellular complexation of inorganic and methylmercury in cyanobacterium synechocystis sp. PCC 6803. Environ. Sci. Technol. 55, 13971–13979. https://doi.org/10.1021/acs.est.1c01732.

Krupp, E.M., Milne, B.F., Mestrot, A., Meharg, A.A., Feldmann, J., 2008. Investigation into mercury bound to biothiols: Structural identification using ESI-ion-trap MS and introduction of a method for their HPLC separation with simultaneous detection by ICP-MS and ESI-MS. Anal. Bioanal. Chem. 390, 1753–1764. https://doi.org/10.1007/s00216-008-1927-x.

Krupp, E.M., Mestrot, A., Wielgus, J., Meharg, A.A., Feldmann, J., 2009. The molecular form of mercury in biota: Identification of novel mercury peptide complexes in plants. Chem. Commun. 28, 4257–4259. https://doi.org/10.1039/b823121d.

Le Croizier, G., Lorrain, A., Sonke, J.E., Jaquemet, S., Schaal, G., Renedo, M., Besnard, L., Cherel, Y., Point, D., 2020. Mercury isotopes as tracers of ecology and metabolism in two sympatric shark species. Environ. Pollut. 265, 114931 https://doi.org/10.1016/j.envpol.2020.114931.

Li, M., Juang, C.A., Ewald, J.D., Yin, R., Mikkelsen, B., Krabbenhoft, D.P., Balcom, P.H., Dassuncao, C., Sunderland, E.M., 2020. Selenium and stable mercury isotopes provide new insights into mercury toxicokinetics in pilot whales. Sci. Total Environ. 710, 136325 https://doi.org/10.1016/j.scitotenv.2019.136325.

Manceau, A., Brossier, R., Janssen, S.E., Rosera, T.J., Krabbenhoft, D.P., Cherel, Y., Bustamante, P., Poulin, B.A., 2021. Mercury isotope fractionation by internal demethylation and biomineralization reactions in seabirds: implications for environmental mercury science. Environ. Sci. Technol. 55, 13942–13952. https://doi.org/10.1021/acs.est.1c04388.

Manceau, A., Brossier, R., Poulin, B.A., 2021. Chemical forms of mercury in pilot whales determined from species-averaged mercury isotope signatures. ACS Earth Sp. Chem. 5, 1591–1599. https://doi.org/10.1021/acsearthspacechem.1c00082.

Manceau, A., Gaillot, A.C., Glatzel, P., Cherel, Y., Bustamante, P., 2021. In Vivo Formation of HgSe Nanoparticles and Hg-Tetraselenolate Complex from Methylmercury in Seabirds - Implications for the Hg-Se Antagonism. Environ. Sci. Technol. 55, 1515–1526. https://doi.org/10.1021/acs.est.0c06269.

Mangal, V., Phung, T., Nguyen, T.Q., Guéguen, C., 2019. Molecular characterization of mercury binding ligands released by freshwater algae grown at three photoperiods. Front. Environ. Sci. 6, 1–11. https://doi.org/10.3389/fenvs.2018.00155.

Masbou, J., Point, D., Sonke, J.E., 2013. Application of a selective extraction method for methylmercury compound speci fi c stable isotope analysis (MeHg-CSIA) in biological materials †. J. Anal. . Spectrom. 28, 1620–1628. https://doi.org/10.1039/c3ja50185j.

Masbou, J., Sonke, J.E., Amouroux, D., Guillou, G., Becker, P.R., Point, D., 2018. Hg-stable isotope variations in marine top predators of the western arctic ocean. ACS Earth Sp. Chem. 2, 479–490. https://doi.org/10.1021/acsearthspacechem.8b00017.

Meng, M., Sun, R., Liu, H., Yu, B., Yin, Y., Hu, L., Chen, J., Shi, J., Jiang, G., 2020. Mercury isotope variations within the marine food web of Chinese Bohai Sea: Implications for mercury sources and biogeochemical cycling. J. Hazard. Mater. 384, 121379 https://doi.org/10.1016/j.jhazmat.2019.121379.

Pedrero, Z., Ouerdane, L., Mounicou, S., Lobinski, R., Monperrus, M., Amouroux, D., 2012. Identification of mercury and other metals complexes with metallothioneins in dolphin liver by hydrophilic interaction liquid chromatography with the parallel detection by ICP MS and electrospray hybrid linear/orbital trap MS/MS. Metallomics 4, 473–479. https://doi.org/10.1039/c2mt00006g.

Pedrero Zayas, Z., Ouerdane, L., Mounicou, S., Lobinski, R., Monperrus, M., Amouroux, D., 2014. Hemoglobin as a major binding protein for methylmercury in white-sided dolphin liver. Anal. Bioanal. Chem. 406, 1121–1129. https://doi.org/10.1007/s00216-013-7274-6.

Perrot, V., Masbou, J., Pastukhov, M.V., Epov, V.N., Point, D., Bérail, S., Becker, P.R., Sonke, J.E., Amouroux, D., 2016. Natural Hg isotopic composition of different Hg compounds in mammal tissues as a proxy for in vivo breakdown of toxic methylmercury. Metallomics 8, 170–178. https://doi.org/10.1039/C5MT00286A.

Poulin, B.A., Janssen, S.E., Rosera, T.J., Krabbenhoft, D.P., Eagles-smith, C.A., Ackerman, J.T., Stewart, A.R., Kim, E., Kim, J., Manceau, A., 2021. Isotope Fractionation from In Vivo Methylmercury Detoxification in Waterbirds. ACS Earth Sp. Chem. 5, 990–997. https://doi.org/10.1021/acsearthspacechem.1c00051.

Queipo-Abad, S., Pedrero, Z., Marchán-Moreno, C., El Hanafi, K., Bérail, S., Corns, W.T., Cherel, Y., Bustamante, P., Amouroux, D., 2021. New insights into the biomineralization of mercury selenide nanoparticles through stable isotope analysis in giant petrel tissues. J. Hazard. Mater. 425, 127922 https://doi.org/10.1016/j.jhazmat.2021.127922.

Renedo, M., Pedrero, Z., Amouroux, D., Cherel, Y., Bustamante, P., 2021. Mercury isotopes of key tissues document mercury metabolic processes in seabirds. Chemosphere 263, 127777. https://doi.org/10.1016/j.chemosphere.2020.127777.

Rodríguez-González, P., Epov, V.N., Bridou, R., Tessier, E., Guyoneaud, R., Monperrus, M., Amouroux, D., 2009. Species-specific stable isotope fractionation of

mercury during Hg(II) methylation by an anaerobic bacteria (Desulfobulbus propionicus) under dark conditions. Environ. Sci. Technol. 43, 9183–9188. https://doi.org/10.1021/es902206j.

Strohmidel, P., Sperling, M., Karst, U., 2018. Investigations on the binding of ethylmercury from thiomersal to proteins in influenza vaccines. J. Trace Elem. Med. Biol. 50, 100–104. https://doi.org/10.1016/j.jtemb.2018.06.011.

Trümpler, S., Meermann, B., Nowak, S., Buscher, W., Karst, U., Sperling, M., 2014. In vitro study of thimerosal reactions in human whole blood and plasma surrogate samples. J. Trace Elem. Med. Biol. 28, 125–130. https://doi.org/10.1016/j.jtemb.2014.01.006.

Wiederhold, J.G., Jiskra, M., 2022. Comment on "Mercury Isotope Fractionation by Internal Demethylation and Biomineralization Reactions in Seabirds: Implications for Environmental Mercury Science": Principles and limitations of Source Tracing and Process Tracing with Stable Isotope Signatur. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.1c07656.