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Khouloud El Hanafi, Zoyne Pedrero, Laurent Ouerdane, Claudia Marchán Moreno, Silvia Queipo-Abad, et al.. First Time Identification of Selenoneine in Seabirds and Its Potential Role in Mercury Detoxification. Environmental Science and Technology, 2022, 56 (5), pp.3288-3298. 10.1021/acs.est.1c04966 . hal-03591549

HAL Id: hal-03591549

<https://univ-pau.hal.science/hal-03591549>

Submitted on 16 Sep 2022

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First time identification of selenoneine in seabirds and its potential role in mercury detoxification

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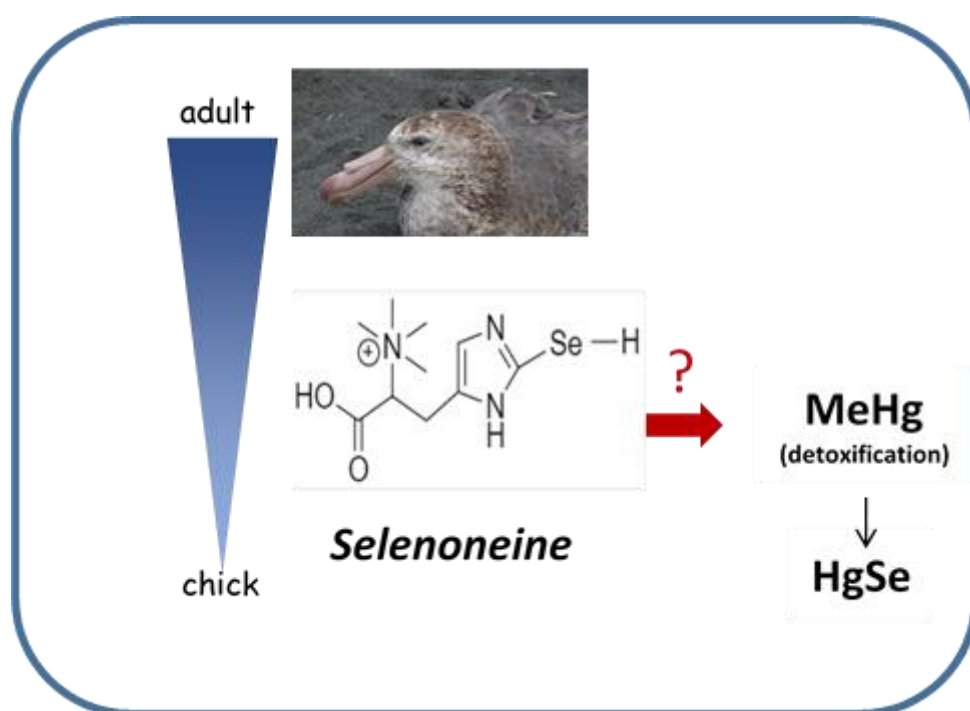
Abstract:

Birds are principally exposed to selenium (Se) through their diet. In long-lived and top predator seabirds, such as the giant petrel, extremely high concentrations of Se are found. Selenium speciation in biota has aroused great interest in recent years, however there is a lack of information about the chemical form of Se in (sea)birds. The majority of publications focus on the growth performance and antioxidant status in broilers in relation to Se dietary supplementation. The present work combines elemental and molecular mass spectrometry for the characterization of Se species in wild (sea)birds. A set of 8 giant petrel (*Macronectes* sp.) individuals of a wide age range from the Southern Ocean was studied. Selenoneine (2-selenyl-N,N,N-trimethyl-L-histidine), a Se-analog of ergothioneine was identified for the first time in wild avian species. This novel Se-compound, previously reported in fish, constitutes the major Se species in the water-soluble fraction of all the internal tissues and blood samples analyzed. The levels of selenoneine found in giant petrels are the highest reported in animal tissues until now, supporting the trophic transfer in the marine food web. The characterization of selenoneine in brain, where it represents between 78 and 88% of the total Se, suggests a crucial role in the nervous system. The dramatic diminishment of selenoneine (from 70 to 5 %) with the increase of Hg concentrations in the liver strongly supports the hypothesis of its key role in Hg detoxification.

Keywords: selenoneine, procellariiform, petrels, mercury, selenium, brain, liver

Synopsis: First time identification of selenoneine in internal organs and blood of seabirds: food web transfer and its potential role in mercury detoxification

Abstract art



Introduction

Selenium (Se) is an essential element for living organisms, including humans,^{1,2} due to its central function into selenoenzymes and selenoproteins.³ Upwards of 25 selenoproteins have been identified from archaea to mammals.⁴ This element plays key metabolic roles,^{3,5} it has been recognized as antioxidant^{6,7} and its presence is related with the reduction of certain types of cancer and other diseases^{4,8–10} Notwithstanding its beneficial effect on living organisms, Se exhibits an ambivalent behaviour due to a narrow concentration range that separates the deficiency dietary intake and toxicity.¹¹ Not only its concentration, but also the chemical form (speciation) determines its potential toxic/beneficial effects.^{12,13}

Birds are principally exposed to Se through the diet¹⁴ and its bioaccumulation is influenced by both intrinsic (e.g., gender, age, long-life span)^{15,16} and extrinsic (e.g., feeding habitat, diet, migration) factors.^{17,18} Seabirds usually exhibit higher Se levels than terrestrial birds^{19,20} and

because of this they have been used as bioindicators of metallic^{21–23} and organic pollutants.²⁴ Seabirds belonging to the Procellariiformes order include albatrosses and petrels which are at the top of the list of living organisms presenting maximum Se concentrations.²⁵ These high concentrations in seabird tissues (for example, values over 1100 µg.g⁻¹ dry weight (dw) in the liver of giant petrels *Macronectes* spp.)²⁶ are likely due to their high position in the marine food web and to their long life-span.^{16, 18}

Selenium speciation in biota has aroused great interest in recent years,²⁷ however there is a lack of information about the chemical form of Se in (sea)birds. The literature concerning Se speciation in birds^{26,28–33} has been almost exclusively focused on experimental birds and broilers.^{28–30,33} Most of these studies compared the growth performance, meat yield and antioxidant status in broilers depending on the Se dietary sources.^{28,29,34–36} In general terms, a supplementation with organic forms, usually selenomethionine, is more efficiently incorporated than selenite, leading also to improved growth and antioxidant status.^{29,33,35} Selenium speciation studies in experimental birds (by High-Performance Liquid Chromatography coupled to Inductively Coupled Plasma–Mass Spectrometry (HPLC-ICP-MS)) reveal a dissimilar metabolization of inorganic and organic Se sources, as well as an association of Se with high and medium-molecular weight species.^{28,30,33} Only a few investigations considered Se speciation in wild birds, these being limited to the identification of its association with Hg.^{26,31,32} In this respect, Hg bound to tetraselenolate (Hg(SeR)₄) likely acting as a precursor of tiemannite (HgSe).^{26,31} This latter compound is considered the final product of methylmercury (MeHg) detoxification.³⁷ However, the metabolic pathways involved in the formation of tiemannite remain unknown.

In the present work, the combination of elemental and molecular mass spectrometry was exploited on the characterization of soluble Se species for the first time in wild seabirds. Specifically, giant petrels (*Macronectes* sp.) of a wide age range were investigated. Different internal tissues, including the liver, kidneys, muscle, brain, testicles and also blood of this seabird species were studied. The potential role of Se species in Hg metabolism of giant petrels was addressed.

Materials and methods

Sample collection

Eight dead individuals of giant petrels were opportunistically collected from Kerguelen archipelago (Southern Indian Ocean) and Adélie Land (Antarctic continent). All details

regarding sampling data set are presented in Table S1. The age of these individuals could not be precisely determined, but one of the seabirds collected in Adélie Land was a fully-fledged chick the P-P1 (viz. less than 1 year old). In addition to this, samples of three other *M. giganteus* (see details in Renedo et al., 2021)⁵¹ were also considered in the present study (PGA01, PGA02 and PGA03).

The samples from Adélie Land were in good shape for adults (P-10 & P-11) who died after a collision against an electric pole, but the chick (P-P1) was very emaciated suggesting it died from starvation. For all the others, the cause of death is unknown, in general they were in good shape, except P1 and P3 (emaciated). All of them were collected freshly after their death.

After dissection, all the samples were stored at -80°C . The internal tissues analyzed in this study (liver $n=7$; kidneys $n=5$; brain $n=3$; pectoral muscles $n=6$ and testicles $n=1$) were sampled, weighed and stored individually in plastic bags. Blood samples were obtained by collecting clotted blood from heart atria and stored in Eppendorf microtubes.

Reagents and standards

All solutions were prepared using Milli-Q (ultrapure) water ($18.2\text{ M}\Omega\text{ cm}$, Millipore Bedford, MA) and all analytical reagent grade chemicals were purchased from Sigma Aldrich (Saint Quentin, Fallavier, France). Trace metal grade acids (HNO_3 and HCl) were purchased from Fisher Scientific (Illkirch, France) and Se standard from SCP SCIENCE (France). Working standard solutions were daily prepared by appropriate dilution of stock standard solutions in 2% HNO_3 and stored at 4°C until use.

Instrumentation

Liquid chromatographic separations were carried out with an Agilent 1100 liquid chromatography (Agilent, Wilmington, DE) equipped with an autosampler and a binary HPLC pump was used. An Agilent inductively coupled plasma mass spectrometer (ICP-MS) 7500ce (Yokogawa Analytical Systems, Tokyo, Japan) was used for Se and Hg detection after liquid chromatography separation and for Se quantification after acid digestion of the biological samples. Two size exclusion chromatography (SEC) columns were employed: Superdex 200 and Superdex peptide HR 10/30 ($10\times 300\text{ mm}$, $13\text{ }\mu\text{m}$, GE Healthcare, Uppsala, Sweden). The hydrophilic interaction chromatography (HILIC) separation system with TSK gel amide 80

column (250×1 mm, 5 µm, Tosoh Biosciences, Stuttgart, Germany) was coupled to ICP-MS or to an electrospray hybrid linear trap quadrupole Orbitrap Velos mass spectrometer (ESI LTQ Orbitrap MS) from Thermo Fisher Scientific (Bremen, Germany) by means of a heated electrospray ionization source (HESI II) (Thermo Fisher Scientific)^{39,40}. An ultrasonic probe—Vibracell (USP)75115 (Biorblock Scientific, Illkirch) instrument with a 3 mm diameter and a nominal power of 500 W—was used for water-soluble fractions extraction. A centrifugation system using a centrifuge mini spin plus model (Eppendorf, Hamburg, Germany) at 14100 x g was used. Total Hg was determined using cold vapour- atomic fluorescence spectrometry (PSA Millennium Merlin 10.025).

Total Se and Hg determination

Freeze-dried and homogenized tissue samples (0.05-0.2 g) corresponding to the bulk and to the water-soluble fraction (details in the section below) were digested (in triplicate) with 1.5 mL of HNO₃ (ca. 63%, analytical grade) after overnight pre-digestion at room temperature, in an Ultrawave, Milestone Srl system (Table S2). For Se analyses, the resulting solutions were appropriately diluted with Milli-Q water and analysed by ICP-MS according to the conditions given in Table S3. The measurement of total Hg (THg) in the digests was performed by atomic fluorescence with previous Cold Vapor Generation of Hg(0) by reduction with SnCl₂ 3% (Scharlab, Barcelona, Spain) in 10% HCl (J.T.Baker, Fisher scientific, Illkirch, France) in Milli-Q water. The samples were then diluted in HCl 5% for analyses and the quantification was performed by standard calibration. Measurements were validated using certified reference materials (DOLT-5, CE-101, BCR-464 and TORT-2). The information relative to the quality assurance of the analyses of Hg and Se is found in Table S4. Concentrations of both elements are further reported in µg g⁻¹ dw.

Se speciation

The water-soluble fraction was extracted from fresh samples (approximately 0.1 g) by ultrasonication (30 s at 21% at 100 W) in 3 mL of 100 mM ammonium acetate (pH 7.4) followed by centrifugation, as described elsewhere.³⁸ The resulting water-soluble fraction was analysed by size exclusion chromatography (SEC) –coupled to ICP-MS under conditions compiled in Table S3.

In parallel, to identify low molecular weight Se species, an aliquot of the water-soluble fraction was diluted with acetonitrile (1:2 v/v). The mixture was centrifuged at 14000×g for 10 min and

the obtained supernatant was directly analyzed on Hydrophilic Interaction Liquid Chromatography (HILIC) coupled to ICP-MS and to Electrospray ionization (ESI)-MS.^{39,40} Derivatized samples were also prepared, with iodoacetamide and tris(2-carboxyethyl) phosphine (TCEP) as detailed by Klein et al.⁴⁰

ESI-MS conditions : μ HILIC ESI-MS

The gradient used for the chromatographic separations is described in Table S3. The ESI-MS was operated in the positive ion mode under the optimum following settings: ion spray voltage, 2.80 kV; capillary temperature 300 °C, capillary temperature 285 °C, source heater temperature 120 °C, nitrogen sheath gas flow 15, auxiliary gas flow 5 and sweeps gas 1 (arbitrary units), S-lens RF level 95 %. Mass spectrometer calibration was performed with a mixture of caffeine (m/z 195.08765), Met-Arg-Phe-Ala (MRFA) (m/z 524.26499) and Ultramark polymer (m/z 1221.99063) dissolved in 50 % acetonitrile and 0.1 % formic acid solution. Mass spectra data were processed with Xcalibur 2.1 software (Thermo Fisher). In full scan mode, an m/z 120 to 1800 range was scanned for the detection of Se bio-compounds at a resolution of 100000 (m/ Δ m, FWHM at m/z 400). The Se isotopic patterns were searched using Thermo MetWorks 1.2.1 software.

Results and discussion

Se concentrations in blood and internal tissues

The total Se concentrations determined in the internal tissues and blood of eight giant petrels are presented in Table 1. Despite the high variability in the Se concentrations found in the analysed samples, there is a clear trend of highest bioaccumulation of Se in the liver. The hepatic Se concentrations range between 19 to 426 $\mu\text{g g}^{-1}$ dw. Se concentrations in kidneys are in general slightly lower than in the liver, followed by brain, blood and muscle. A similar trend was reported in other procellariiform seabirds.^{26,41,42}

Se concentrations in all the tissues of the single chick are much lower than in the adult ones, with, for example, only 19 $\mu\text{g g}^{-1}$ in the liver (i.e., around 5 to 20 times lower than in adults). The increase of Se content in internal tissues with age has been previously reported in other (marine) animal species,⁴³ being attributed to the bioaccumulation of this element during their life span.⁴⁴⁻⁴⁷ The principal source of Se in birds is dietary exposure.^{14,48} Specifically in the case

183 of chicks, the Se source is probably a mixture of dietary exposure during the chick-rearing
184 period and the initial maternal Se transfer in the egg.^{24,49,50}

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Table 1. Concentrations of Se, Hg and selenoneine (SEN) in the different tissues (dry weight) and blood (wet weight) of giant petrels and the percentages of Se and SEN in their water-soluble fractions.

Tissue	Individual	Se $\mu\text{g g}^{-1}$	Hg $\mu\text{g g}^{-1}$	molar ratio Se:Hg	Water-soluble Se (% of total Se)	SEN (% of water-soluble Se)	SEN (% of total tissue Se)	SEN $\mu\text{g Se g}^{-1}$
Liver	P-P1**	19 \pm 3	1.11 \pm 0.04	44	70	96	68	13 \pm 2
	P-10	115 \pm 17	109 \pm 19	2.7	45	99	45	52 \pm 7
	P-11	99 \pm 21	99 \pm 22	2.5	41	99	40	40 \pm 9
	PGA-01	113 \pm 15	214 \pm 34*	1.3	15	98	15	17 \pm 2
	P-1	142 \pm 3	324 \pm 68	1.1	22	86	19	27 \pm 1
	PGA03	222 \pm 27	405 \pm 7*	1.4	18	98	17	38 \pm 5
	P-3	426 \pm 3	928 \pm 73	1.2	3	97	3	12.8 \pm 0.1
Kidneys	P-P1**	41 \pm 5	0.55 \pm 0.15	185	76	95	72	30 \pm 4
	P-10	109 \pm 10	25 \pm 3	11	81	96	78	85 \pm 8
	P-11	116 \pm 21	32 \pm 1	18	78	97	76	88 \pm 13
	PGA02	80 \pm 7	39.9 \pm 0.4*	5.1	43	76	32	26 \pm 2
	PGA03	131 \pm 10	50.8 \pm 0.7*	6.5	53	91	49	64 \pm 5
Muscle	P-P1**	4 \pm 3	0.10 \pm 0.03	92	57	85	48	2 \pm 1
	P-10	12 \pm 2	1.0 \pm 0.2	30	74	93	68	8 \pm 2
	P-11	13 \pm 3	1.4 \pm 0.2	24	70	93	65	9 \pm 2
	PGA01	25 \pm 4	4.1 \pm 0.1*	15	70	99	69	25 \pm 3
	P-1	30 \pm 1	37 \pm 6	2.1	25	81	20	6.0 \pm 0.1
	PGA03	31 \pm 3	29.2 \pm 0.3*	2.8	58	98	56	18 \pm 2
Brain	P-P1**	6 \pm 5	0.19 \pm 0.11	77	84	93	78	5 \pm 4
	P-10	40 \pm 5	1.40 \pm 0.66	74	86	98	85	35 \pm 4

	P-11	40±2	3.1±0.1	33	89	99	88	35±2
	P-P1**	3±1	0.07±0.02	90	80	76	61	1.2±0.4
Blood	P-10	27±2	0.5±0.1	129	94	96	90	24±1
	P-11	25±3	0.5±0.1	126	93	98	91	23±3

192 Results expressed as mean value ± S.D. (n = 3).

193 *values previously reported by Renedo et al.⁵¹

194 ** chick

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198 **Se species screening in the water-soluble fraction**

199 Se species in the water-soluble fraction were initially screened in a size exclusion
200 chromatographic column (Superdex 200), with a linear separation range between 3 and 600
201 kDa. The obtained chromatograms (Figure 1) reveal that Se elutes in a main fraction at
202 approximately 30 min, therefore in the low molecular weight range. Interestingly, a similar
203 profile is observed in all the internal tissues (liver, kidneys, muscle, brain and testicles) and also
204 in the blood.

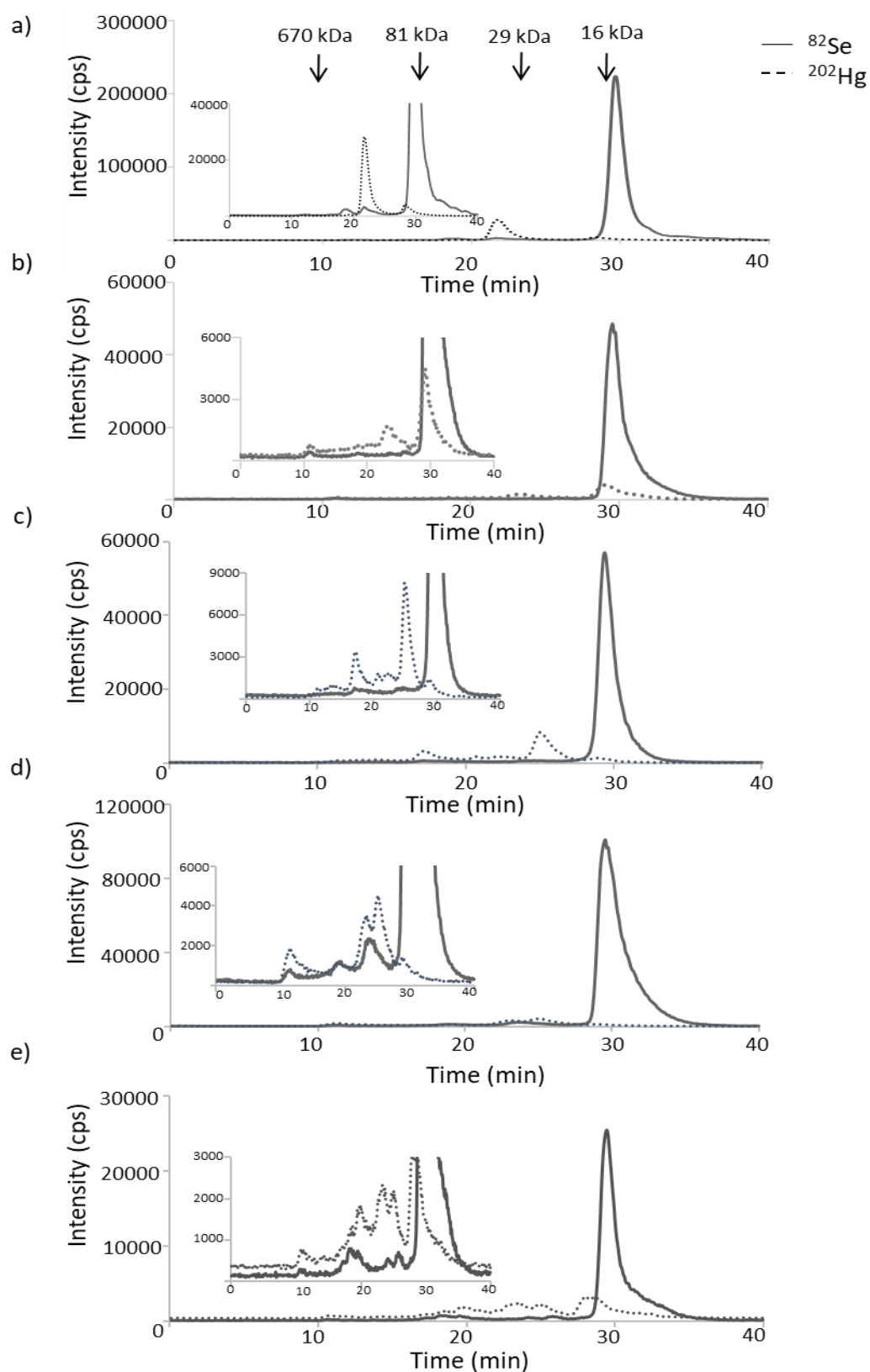
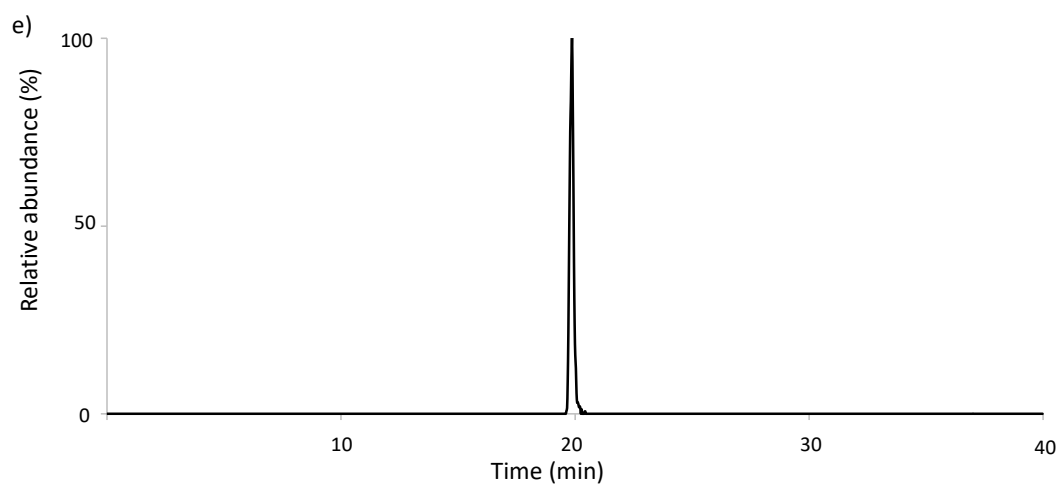
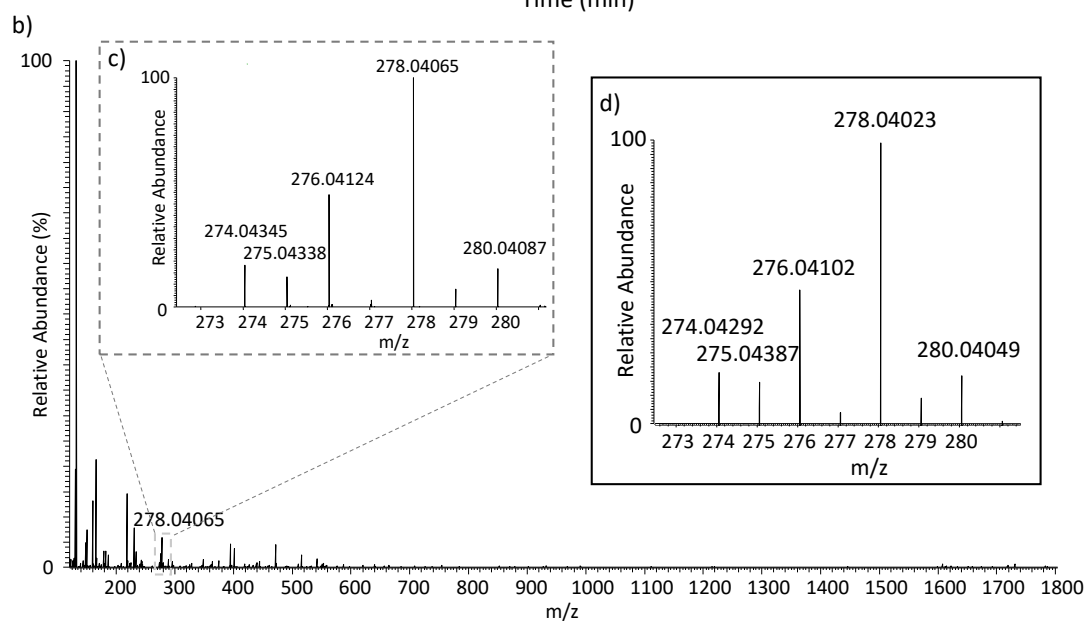
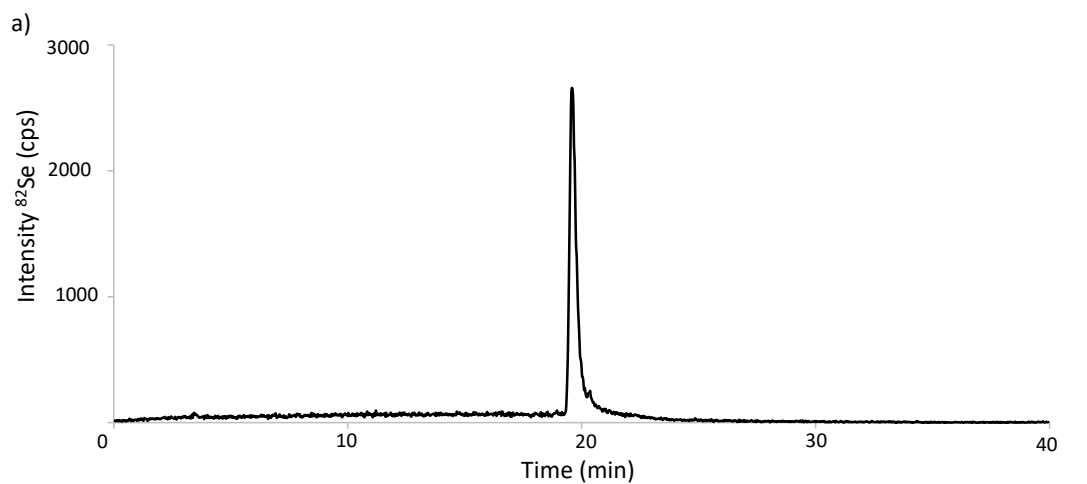


Figure 1. Typical SEC₂₀₀-ICP-MS chromatograms of ⁸²Se (solid line) and ²⁰²Hg (dotted line) corresponding to the water-soluble fraction of a) blood, b) brain, c) liver, d) kidneys and e) muscles of adult giant petrels. Insert corresponds to a zoom.

210 Considering that Se in the water-soluble fraction from the different tissues is principally found
211 at low molecular weight, an additional column (Superdex peptide) with a better resolution
212 (operational separation range 0.1-7 kDa) was used. The obtained chromatograms (Figure S1)
213 revealed once again, the presence of a major Se fraction, eluting at 27 min in all the samples.

214 In order to determine the identity of the observed Se containing fraction, an additional
215 hyphenated approach was performed through the coupling of HILIC to ICP-MS and ESI-MS
216 (Figure 2). A single Se containing peak, eluting at 21 min was observed. The spectra obtained
217 by HILIC ESI-MS at the mentioned retention time revealed the presence of a unique Se isotopic
218 pattern at m/z 278.04065, corresponding to selenoneine (2-selenyl-N α , N α , N α -trimethyl-L-
219 histidine). In addition, the same samples were reduced with TCEP and derivatized with
220 iodoacetamide, as detailed by Klein et al. ⁴⁰, before analysis to check for the potential presence
221 of additional selenoneine bound to high molecular weight fractions (e.g. bound to protein
222 thiols). However, no significant differences were observed compared to underivatized samples
223 (data not shown). Derivatization was also tested before and after extraction and no significant
224 differences were detected. In the investigated set of samples, the dimer of selenoneine and the
225 methylated form of this Se species, methylselenoneine, were not found.

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Figure 2. Liver extract after acetonitrile treatment analysed by a: HILIC-ICP-MS (m/z ^{82}Se) and HILIC ESI-MS (b: mass spectrum obtained at 19.8 min and e: extracted ion chromatogram of m/z 278.0407 ± 0.02). Inserts correspond respectively to c: a zoom of the mass spectra showing the isotopic pattern of Se obtained by ESI-MS for the selenoneine ion and d: the theoretical isotopic pattern of selenoneine ion ($\text{C}_9\text{H}_{16}\text{N}_3\text{O}_2\text{Se}^+$).

Selenoneine is a selenoamino acid reported for the first time in 2010,⁵² identified so far in a reduced number of samples. Selenoneine has been reported in different biological matrices of animal origin, precisely in sea turtles⁵³ and dolphin liver,³⁹ in the blood, liver, spleen, heart, and muscles of several fish (i.e., Pacific mackerel, tilapia, sardine and tuna), chicken liver, squid digestive gland,^{52,54} beluga skin,⁵⁵ and human blood^{40,55–57} and urine⁴⁰. To the best of our knowledge, this is the first study that identifies selenoneine in seabirds and, more generally, in organs like testicles and brain.

Considering that a single Se fraction was found in both size exclusion chromatographic columns (Figure 1 and Figure S1) and also in the specific analyses by HILIC-ICP/ESI-MS/MS (Figure 2), the proportion of Se present as selenoneine (Table 1) was estimated by calculating the percentage of the peak area in the size exclusion chromatogram, after verification of quantitative Se recovery from the (Superdex 200) chromatographic column (details in Table S5). It should be highlighted that the reported values correspond to an estimation of the content of selenoneine, exploited for comparison between the individuals from this set of samples. Quantification approaches by post-column isotopic dilution⁵⁷ and/or external calibration⁵⁵ would provide more accurate values. It should be mentioned that so far there is no commercial standard of selenoneine. The mean percentage of Se as selenoneine in the water-soluble fraction of the set of samples analysed was $93 \pm 7\%$ (Table 1). Among the different tissues, the highest concentration of selenoneine was found in kidneys ($88 \mu\text{g Se g}^{-1}$).

Even though the age of all the individuals could not be precisely estimated, P-P1 is a chick meanwhile P10 and P11 are adults. The analyses of the tissues from these individuals, in which blood and internal organs were sampled, clearly shows a noticeable increase of selenoneine from chick to adults (Table 1). Only one chick was investigated in this work, therefore the conclusions of this study should not be extrapolated. However, considering that so far there are no studies in individuals of different ages and the rarity of this kind of samples from remote areas, it is worthwhile to present the results of this preliminary comparison. Unequivocally, definitive conclusions on the effect of age require a study with a larger population.

The highest shift with age is observed in blood, adults presenting values around 20 times higher than that of the chick. The Se chromatograms corresponding to the water-soluble fraction clearly shows the described age raising effect (Figure 3 and Table 1). In the case of chick's blood (Figure 3), some Se containing fractions are observed at high molecular weight (HMW), eluting around 10 and 22 minutes whereas selenoneine exhibits the lowest percentage of all the analysed samples, i.e., 76% (Table 1). In adults, the intensity of the Se fraction at HMW remains similar, meanwhile a significant increase of the peak corresponding to selenoneine was observed (Figure 3). The Se compounds eluting between 10 and 22 min, are probably constituted by selenoproteins that are known to play key physiological roles, like selenoprotein P, glutathione peroxidase and/or selenium-containing proteins as selenoalbumin,⁵⁷⁻⁵⁹ among others. Interestingly, the intensity of the mentioned Se-proteins was maintained at similar levels in chick and adults, suggesting their regulation and essential implication in metabolism. Considering that blood reflects the recent dietary uptake,^{60,61} the differences observed between selenoneine levels in chick and adults could be attributed to the divergent dietary sources according to the animals' age. It should be highlighted that the investigated set of samples regarding the comparison of adults and chicks is quite limited. The results discussed should therefore be considered as preliminary. In addition, the Se profile specifically observed in the chick could be also consequence of emaciation of this individual. Further studies are required to precisely know if the presence of selenoneine in blood is also a result of internal organ redistribution.

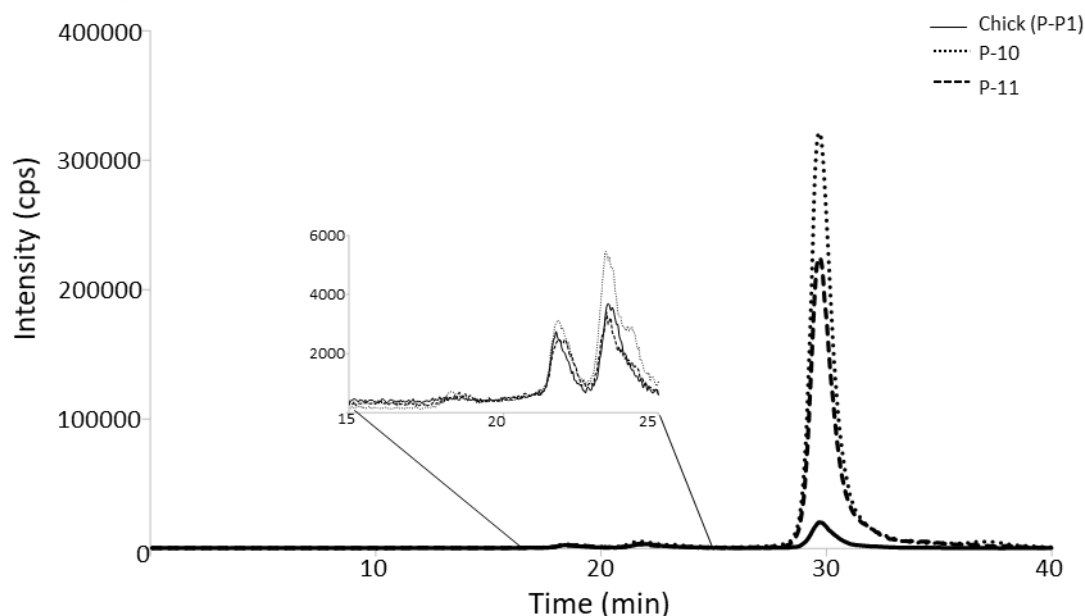


Figure 3. SEC₂₀₀-ICP-MS chromatograms (⁸²Se) corresponding to the water-soluble fraction of blood of chick (solid line) and adults (dotted line) of giant petrels.

The studies that report the presence of selenoneine in biota are reduced to a few examples and there is a lack of information about the sources of this selenocompound. Dietary exposure seems to be the principal source of selenoneine for animals.^{40,56,62} The bioaccumulation pathway of selenoneine is expected to be similar to ergothioneine (its sulfur analog) based on their chemical similarity.⁶⁸ Ergothioneine is primarily synthesized by fungal and bacterial species^{63–65} and largely accumulated in animals through the food chain.⁶⁶

The identification of selenoneine in the blood of Inuits from Nunavik is one of the key studies that support the trophic transfer of this selenocompound.^{55,57} Selenoneine is bioaccumulated in this human population through beluga *mattaaq* consumption, a delicacy highly praised by Inuits consisting of the skin with the underlying layer of fat, where selenoneine represent up to 74% of the total Se content.⁵⁵ Se speciation in the liver of turtles revealed that selenoneine is exclusively found in marine species, but not detected in terrestrial species,⁵³ which has been attributed to the trophic transfer through the marine food webs.^{54,56,67}

The comparison of selenoneine content in the tissues and blood of giant petrels (Table 1) supports its trophic transfer in the marine ecosystems. Giant petrels, being top predators, could bioaccumulate selenoneine from their large variety of prey that comprises fish, other seabirds and stranded marine mammals.^{23,68–71} The levels of selenoneine found in giant petrels are the highest reported in animal tissues until now, with values reaching up to 88 $\mu\text{g Se g}^{-1}$ (Table 1).

The current work reports for the first time a potential increase of selenoneine concentrations from juvenile (chicks) to adults. The highest values found in adults could be justified by their dietary intake. Several studies report an increase of ergothioneine with age until adulthood, followed by a decrease with aging (senescence).⁷²

Brain exhibits the highest fraction of Se as selenoneine of all internal organs. The percentage of selenoneine, comprising the chick and two adults, is quite homogeneous in this organ (78-88%), suggesting that selenoneine plays a crucial role in the (protection/functioning) nervous system. The functions of Se in brain are considered vital⁷³⁻⁷⁵ and its homeostasis in this organ evidenced in several studies^{73,75}. The deficiency of this element leads among others, to irreversible neuronal tissue injury causing several diseases.⁷⁴⁻⁷⁶ The transfer of selenoneine in a well-established *in vitro* model of the blood–brain barrier has been recently demonstrated⁷⁷ and our study reveals its presence in this organ for the first time.

In humans, the enhancement effects on cognitive function associate with an increase mushroom consumption evidenced the crucial role of ergothioneine in brain.⁷⁸ Several studies demonstrated that this compound could promote neurogenesis and acts against oxidative damage and neuroinflammation.^{72,79-81} The precise role of selenoneine in this organ is not known so far. However, considering its similarity with ergothioneine, already characterized in brain, the role of selenoneine could be related to protective and antioxidant effect in the nervous system.

Potential role of selenoneine in (methyl)mercury detoxification

As previously mentioned, the identification of selenoneine in biota is relatively recent and the metabolic mechanisms in which this compound is involved remain unknown. Some studies evoked a potential role of this selenoamino acid in the detoxification of MeHg,^{39,55,57,62,82} however, the mechanism has not yet been characterized. *In vitro* studies in zebrafish embryo evidenced a decrease of both MeHg accumulation and toxicity in the presence of selenoneine and a selenoneine-specific transporter, organic cations/carnitine transporter-1 (OCTN1).⁶² The hypothesis is that selenoneine acts in a protection mechanism through a direct conjugation with MeHg, leading to HgSe formation.⁶² Human studies also speculate about the involvement of selenoneine in MeHg detoxification.^{55,57} Selenoneine is the major Se species in red blood cells of Nunavimmiut and has been related to MeHg demethylation in blood, potentially reducing its distribution to other organs.⁵⁵

HgSe nanoparticles have been recently identified in several organs of giant petrels, including liver, kidneys, muscles and brain, representing up to 95% of the total Hg in the latter tissue.²⁶ In addition to tiemannite, another Hg-Se complex, a tetraselenolate complex with a molar ratio 1:4 (Hg:Se) was reported in these samples.²⁶ According to Palmer et al. ⁸²MeHg demethylation could be mediated by a structural analogue of selenoneine (1-methyl-1,3-dihydro-2H-benzimidazole-2-selone) through a Hg-Se complex exhibiting a similar Hg:Se ratio (1:4). The selenoamino acid complexes readily promote MeHg induced protolytic cleavage of Hg-C bonds.⁸² Therefore, considering that: *i.* selenoneine is the major water soluble Se species in giant petrel samples and *ii.* the coordination number of Hg with selenoneine is four,⁸² we could speculate that the identified selenoamino acid is directly involved on the MeHg demethylation step prior to HgSe precipitation.

Laboratory experiments with a variety of selenoamino acids such as selenocysteine, selenogluthathione, selenopenicillamine and selenomethionine, already demonstrated their chemical MeHg demethylation capabilities through the formation of unstable complexes before HgSe precipitation.^{83,84} A remarkable diminishment of the proportion of Se as selenoneine with increasing liver Hg content was found in giant petrels (Figure 4a).

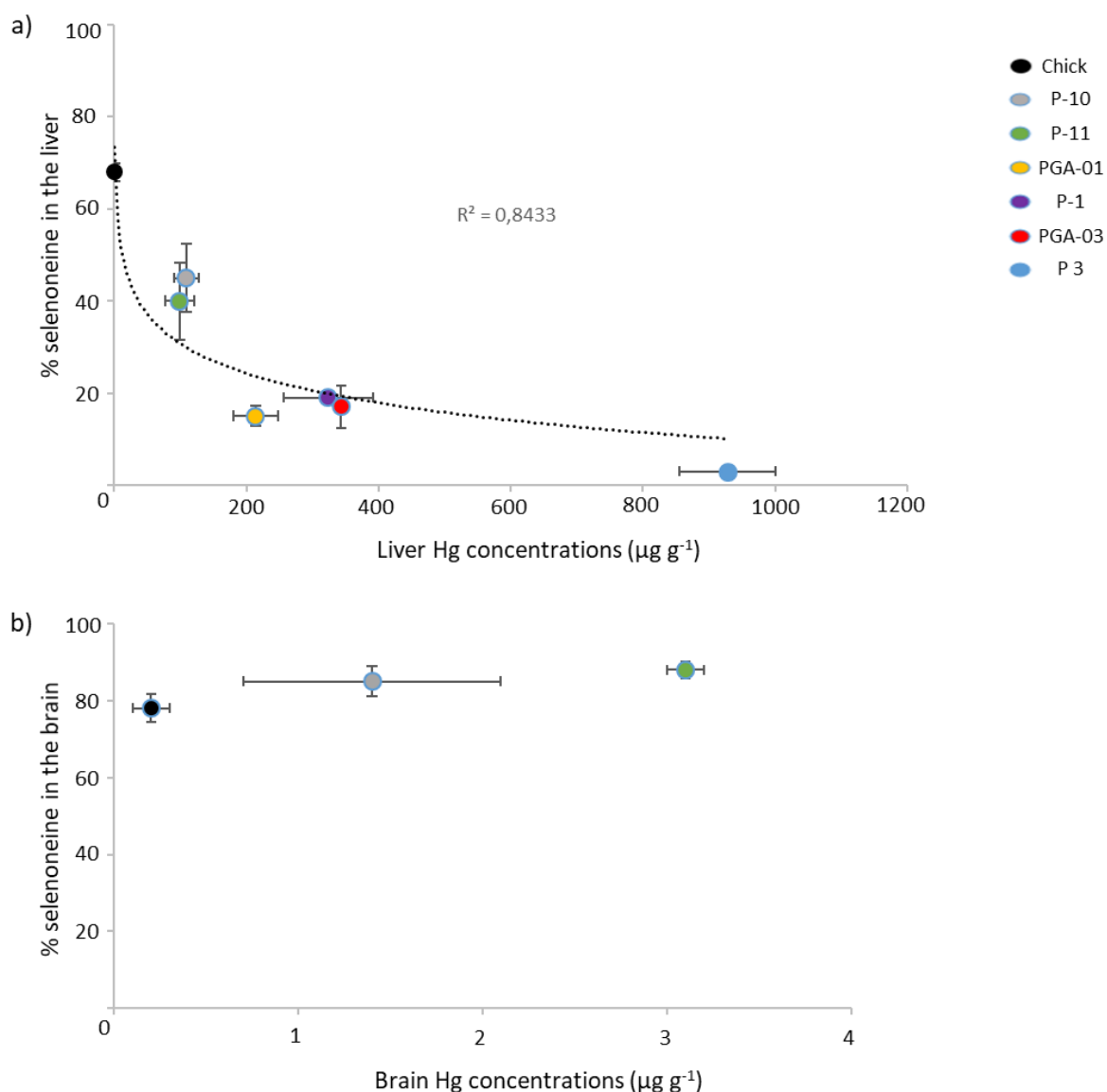


Figure 4. Relationship between the selenoneine fraction and Hg concentration in a) the liver (with a logarithmic curve) and b) brain of giant petrels.

The decrease of the selenoneine proportions (from 70 to 5%) with increasing Hg concentrations could be a consequence of the implication of this selenoamino acid in the demethylation of MeHg, leading to the co-degradation of MeHg and selenoneine resulting in HgSe formation, the main Hg compound (95%) in the liver.²⁶ In contrast, the percentage of selenoneine in brain remains stable irrespectively of the increment of Hg concentrations (Figure 4b). HgSe nanoparticles are also formed in the brain (approximately 35% of total Hg),²⁶ as the result of MeHg detoxification. Considering that this organ shows the highest Se:Hg molar ratio (33-77) and percentage of selenoneine (80%), we hypothesize that this novel selenocompound plays a crucial role in the nervous system, comprising probably a protection against Hg toxicity.

In summary, the main outcome of this study is the identification for the first time of selenoneine in seabirds. This relatively novel selenocompound represents the main Se species in the water-extractable fraction in all giant petrel samples studied (liver, kidney, blood, brain, and muscle). The relevance of this compound in biota is still unknown, our work claims for urgent studies of larger animal populations in order to investigate aspects such as the variation of selenoneine content with age and its potential role on MeHg demethylation. Selenoneine characterization of seabird prey represents another perspective of the present study, which should shed light on the trophic transfer process of this selenospecies.

Supporting Information

Additional experimental details, including tables and figures.

Acknowledgements:

The authors thank The Agence Nationale de la Recherche (MERSEL Project No. ANR-18-CE34-0004-01), CNRS (MITI-ISOISEAUX) and Communauté d'Agglomération Pau Béarn Pyrénées (CDAPBP, Project MERISOTOP) for funding. This project also received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101007962. S. Queipo and C. Marchan Moreno acknowledge the postdoctoral and PhD fellowships, respectively, funded by the Region Nouvelle-Aquitaine (BENESEL, N°2019-1R20127) and I-site E2S UPPA (contract 2020-17). Support was provided to Yves Cherel by the Institut Polaire Français Paul Emile Victor (IPEV programme no. 109, C. Barbraud) and the Terres Australes et Antarctiques Françaises and to Paco Bustamante by the Institut Universitaire de France (IUF). The authors would like to acknowledge PS Analytical for their instrumental and technical support.

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Table caption:

Table 1. Concentrations of Se, Hg and selenoneine (SEN) in the different tissues (dry weight) and blood (wet weight) of giant petrels and the percentages of Se and SEN in their water-soluble fractions.

Figure caption:

Figure 1. Typical SEC₂₀₀-ICP-MS chromatograms of ⁸²Se (solid line) and ²⁰²Hg (dotted line) corresponding to the water-soluble fraction of a) blood, b) brain, c) liver, d) kidneys and e) muscle of adult giant petrels. Insert corresponds to a zoom.

Figure 2. Liver extract after acetonitrile treatment analysed a: by HILIC-ICP-MS (m/z ⁸²Se) and b: HILIC ESI-MS (trace of m/z=278.0407). Insert corresponds to a zoom of the mass spectra showing the isotopic pattern of Se obtained by ESI-MS for the selenoneine ion.

Figure 3. SEC₂₀₀-ICP-MS chromatograms (⁸²Se) corresponding to to the water-soluble fraction of blood of chick (solid line) and adults (dotted line) of giant petrels.

Figure 4. Relationship between the selenoneine fraction and Hg concentration in a) the liver and b) brain of giant petrels.

