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

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14

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16 **Abstract:**

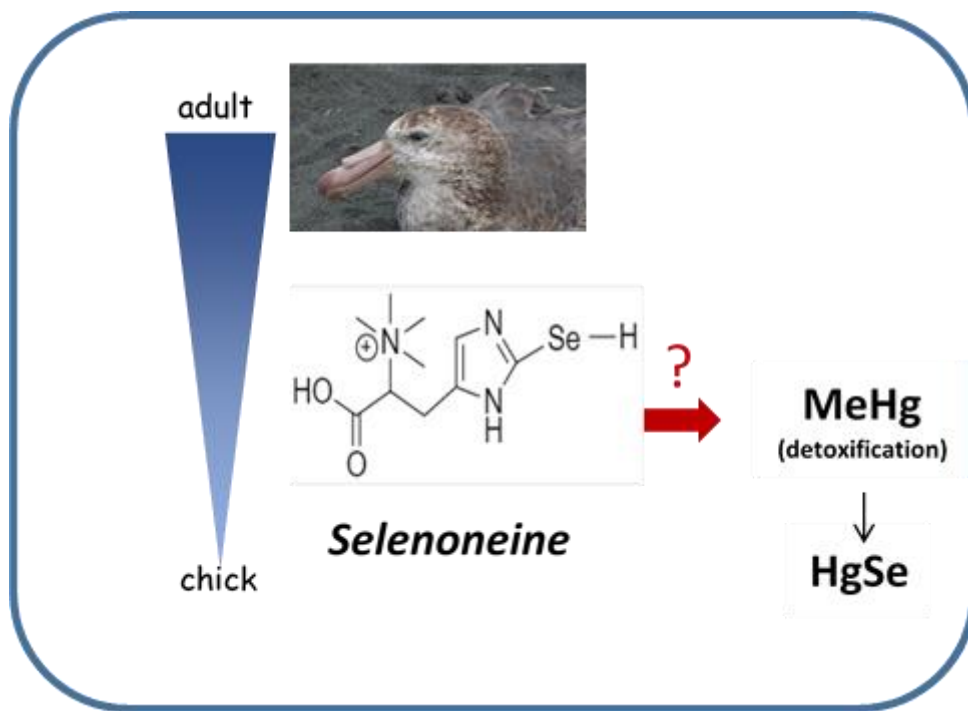
17 Birds are principally exposed to selenium (Se) through their diet. In long-lived and top predator
18 seabirds, such as the giant petrel, extremely high concentrations of Se are found. Selenium
19 speciation in biota has aroused great interest in recent years, however there is a lack of
20 information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

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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



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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

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

61 Selenium speciation in biota has aroused great interest in recent years,²⁷ however there is a lack
62 of information about the chemical form of Se in (sea)birds. The literature concerning Se
63 speciation in birds^{26,28–33} has been almost exclusively focused on experimental birds and
64 broilers.^{28–30,33} Most of these studies compared the growth performance, meat yield and
65 antioxidant status in broilers depending on the Se dietary sources.^{28,29,34–36} In general terms, a
66 supplementation with organic forms, usually selenomethionine, is more efficiently incorporated
67 than selenite, leading also to improved growth and antioxidant status.^{29,33,35} Selenium speciation
68 studies in experimental birds (by High-Performance Liquid Chromatography coupled to
69 Inductively Coupled Plasma–Mass Spectrometry (HPLC-ICP-MS)) reveal a dissimilar
70 metabolization of inorganic and organic Se sources, as well as an association of Se with high
71 and medium-molecular weight species.^{28,30,33} Only a few investigations considered Se
72 speciation in wild birds, these being limited to the identification of its association with Hg.^{26,31,32}
73 In this respect, Hg bound to tetraselenolate ($\text{Hg}(\text{SeR})_4$) likely acting as a precursor of tiemannite
74 (HgSe).^{26,31} This latter compound is considered the final product of methylmercury (MeHg)
75 detoxification.³⁷ However, the metabolic pathways involved in the formation of tiemannite
76 remain unknown.

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

83 **Materials and methods**

84 **Sample collection**

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

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

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

96
97 After dissection, all the samples were stored at -80°C . The internal tissues analyzed in this
98 study (liver n=7; kidneys n=5; brain n=3; pectoral muscles n=6 and testicles n=1) were sampled,
99 weighed and stored individually in plastic bags. Blood samples were obtained by collecting
100 clotted blood from heart atria and stored in Eppendorf microtubes.

101

102 **Reagents and standards**

103 All solutions were prepared using Milli-Q (ultrapure) water (18.2 M Ω cm, Millipore Bedford,
104 MA) and all analytical reagent grade chemicals were purchased from Sigma Aldrich (Saint
105 Quentin, Fallavier, France). Trace metal grade acids (HNO₃ and HCl) were purchased from
106 Fisher Scientific (Illkirch, France) and Se standard from SCP SCIENCE (France). Working
107 standard solutions were daily prepared by appropriate dilution of stock standard solutions in
108 2% HNO₃ and stored at 4 $^{\circ}$ C until use.

109

110 **Instrumentation**

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

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

128

129 **Total Se and Hg determination**

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

143

144 **Se speciation**

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

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

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

156

157 **ESI-MS conditions : μ HILIC ESI-MS**

158 The gradient used for the chromatographic separations is described in Table S3. The ESI-MS
159 was operated in the positive ion mode under the optimum following settings: ion spray voltage,
160 2.80 kV; capillary temperature 300 °C, capillary temperature 285 °C, source heater temperature
161 120 °C, nitrogen sheath gas flow 15, auxiliary gas flow 5 and sweeps gas 1 (arbitrary units), S-
162 lens RF level 95 %. Mass spectrometer calibration was performed with a mixture of caffeine
163 (m/z 195.08765), Met-Arg-Phe-Ala (MRFA) (m/z 524.26499) and Ultramark polymer (m/z
164 1221.99063) dissolved in 50 % acetonitrile and 0.1 % formic acid solution. Mass spectra data
165 were processed with Xcalibur 2.1 software (Thermo Fisher). In full scan mode, an m/z 120 to
166 1800 range was scanned for the detection of Se bio-compounds at a resolution of 100000
167 (m/ Δ m, FWHM at m/z 400). The Se isotopic patterns were searched using Thermo MetWorks
168 1.2.1 software.

169

170 **Results and discussion**

171 **Se concentrations in blood and internal tissues**

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

178 Se concentrations in all the tissues of the single chick are much lower than in the adult ones,
179 with, for example, only 19 $\mu\text{g g}^{-1}$ in the liver (i.e., around 5 to 20 times lower than in adults).
180 The increase of Se content in internal tissues with age has been previously reported in other
181 (marine) animal species,⁴³ being attributed to the bioaccumulation of this element during their
182 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}

185

186

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

190

191

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

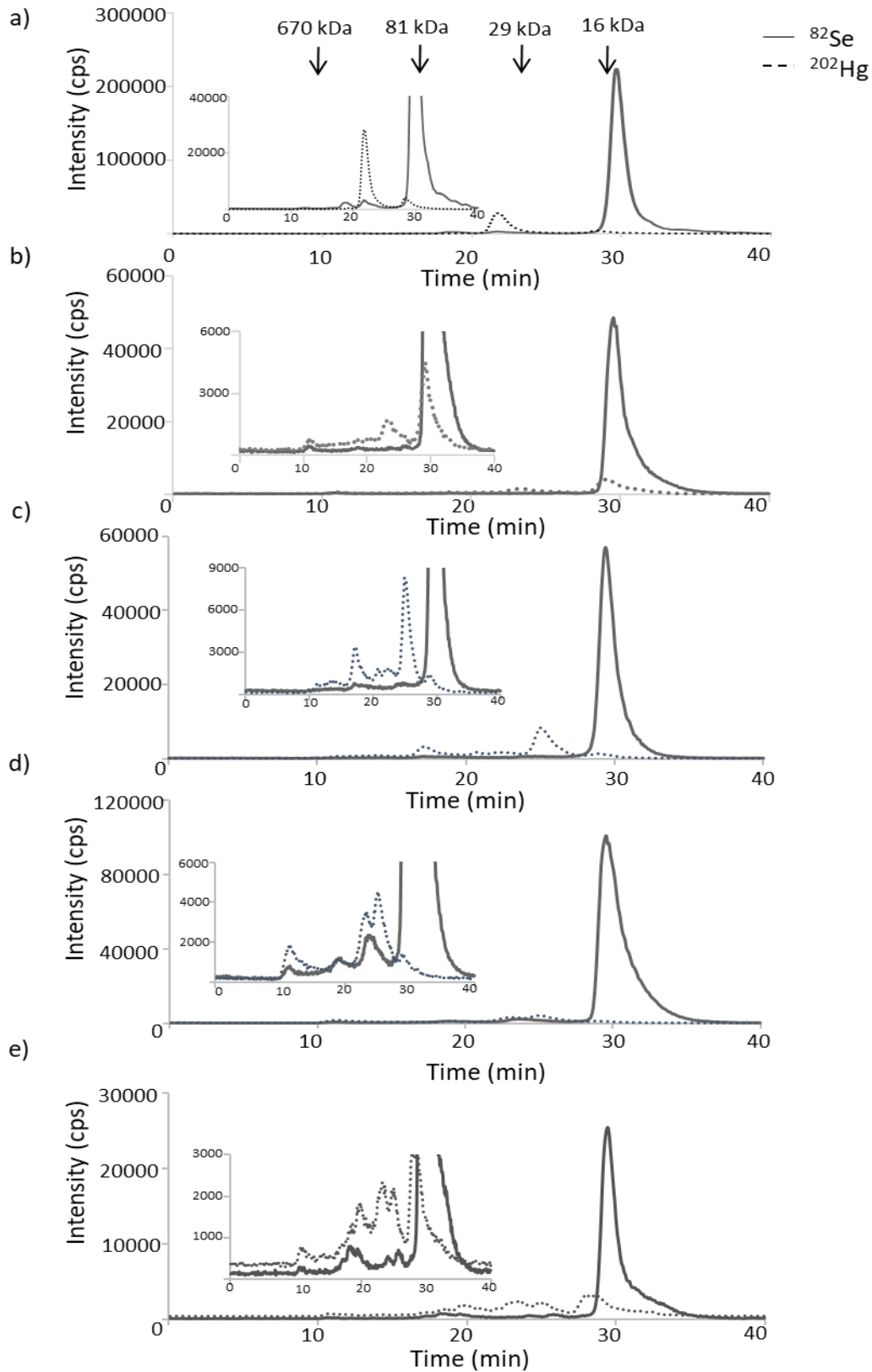
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198 **Se species screening in the water-soluble fraction**

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.



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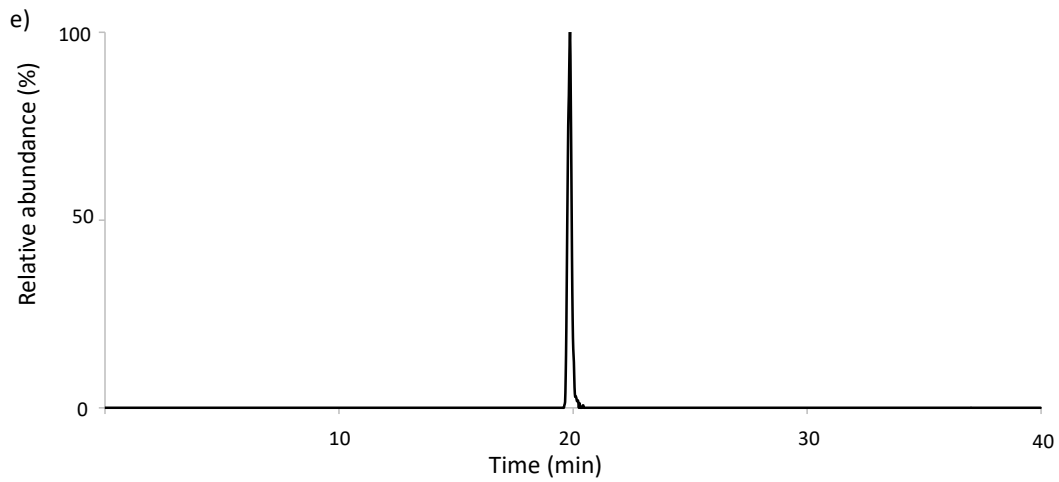
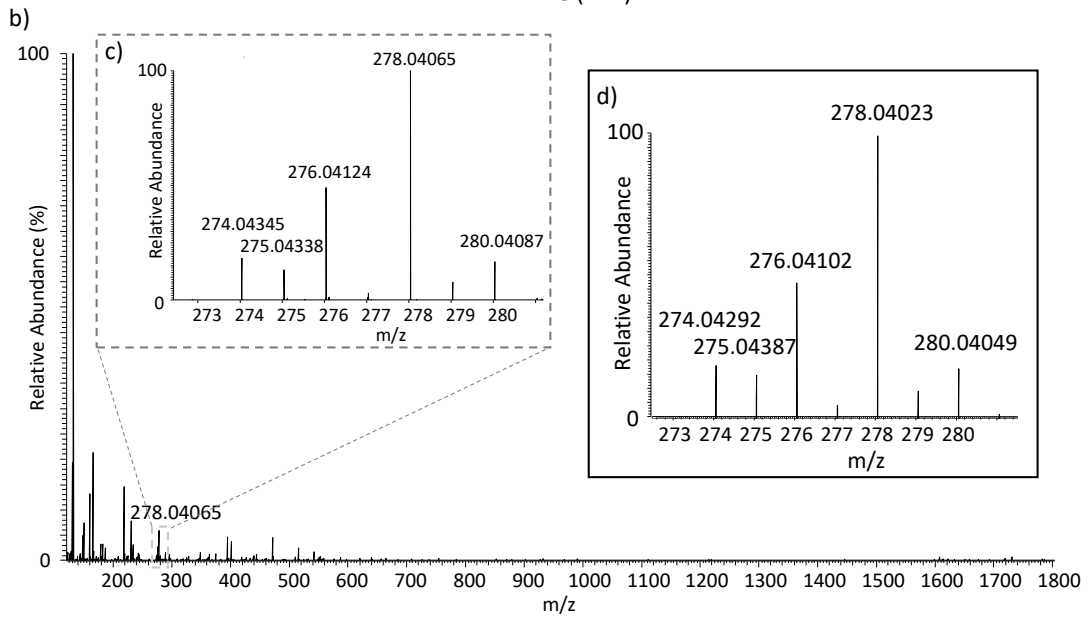
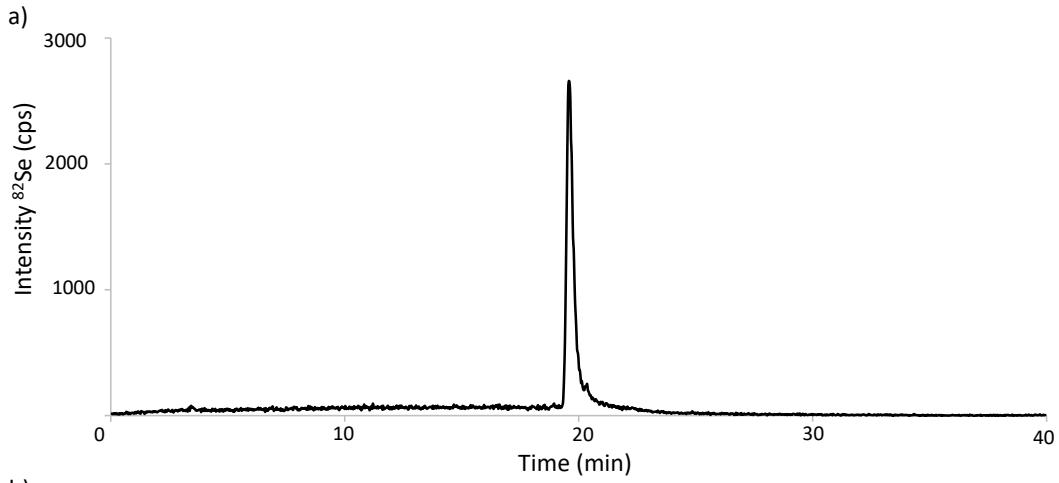
207 **Figure 1.** Typical SEC₂₀₀-ICP-MS chromatograms of ⁸²Se (solid line) and ²⁰²Hg (dotted line)
 208 corresponding to the water-soluble fraction of a) blood, b) brain, c) liver, d) kidneys and e) muscles of
 209 adult giant petrels. Insert corresponds to a zoom.

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

235

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

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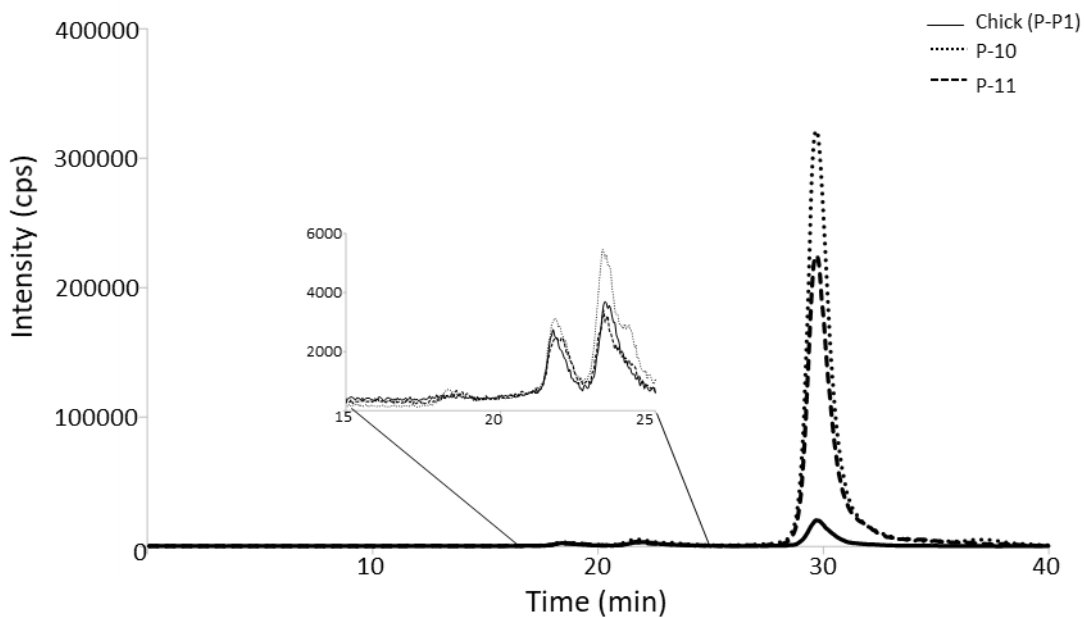
244 Considering that a single Se fraction was found in both size exclusion chromatographic columns
245 (Figure 1 and Figure S1) and also in the specific analyses by HILIC-ICP/ESI-MS/MS (Figure
246 2), the proportion of Se present as selenoneine (Table 1) was estimated by calculating the
247 percentage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

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

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

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286

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

289

290 The studies that report the presence of selenoneine in biota are reduced to a few examples and
 291 there is a lack of information about the sources of this selenocompound. Dietary exposure seems
 292 to be the principal source of selenoneine for animals.^{40,56,62} The bioaccumulation pathway of
 293 selenoneine is expected to be similar to ergothioneine (its sulfur analog) based on their chemical
 294 similarity.⁶⁸ Ergothioneine is primarily synthesized by fungal and bacterial species⁶³⁻⁶⁵ and
 295 largely accumulated in animals through the food chain.⁶⁶

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

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

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

312 Brain exhibits the highest fraction of Se as selenoneine of all internal organs. The percentage
313 of selenoneine, comprising the chick and two adults, is quite homogeneous in this organ (78-
314 88%), suggesting that selenoneine plays a crucial role in the (protection/functioning) nervous
315 system. The functions of Se in brain are considered vital⁷³⁻⁷⁵ and its homeostasis in this organ
316 evidenced in several studies^{73,75} The deficiency of this element leads among others, to
317 irreversible neuronal tissue injury causing several diseases.⁷⁴⁻⁷⁶ The transfer of selenoneine in
318 a well-established *in vitro* model of the blood–brain barrier has been recently demonstrated⁷⁷
319 and our study reveals its presence in this organ for the first time.

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

327

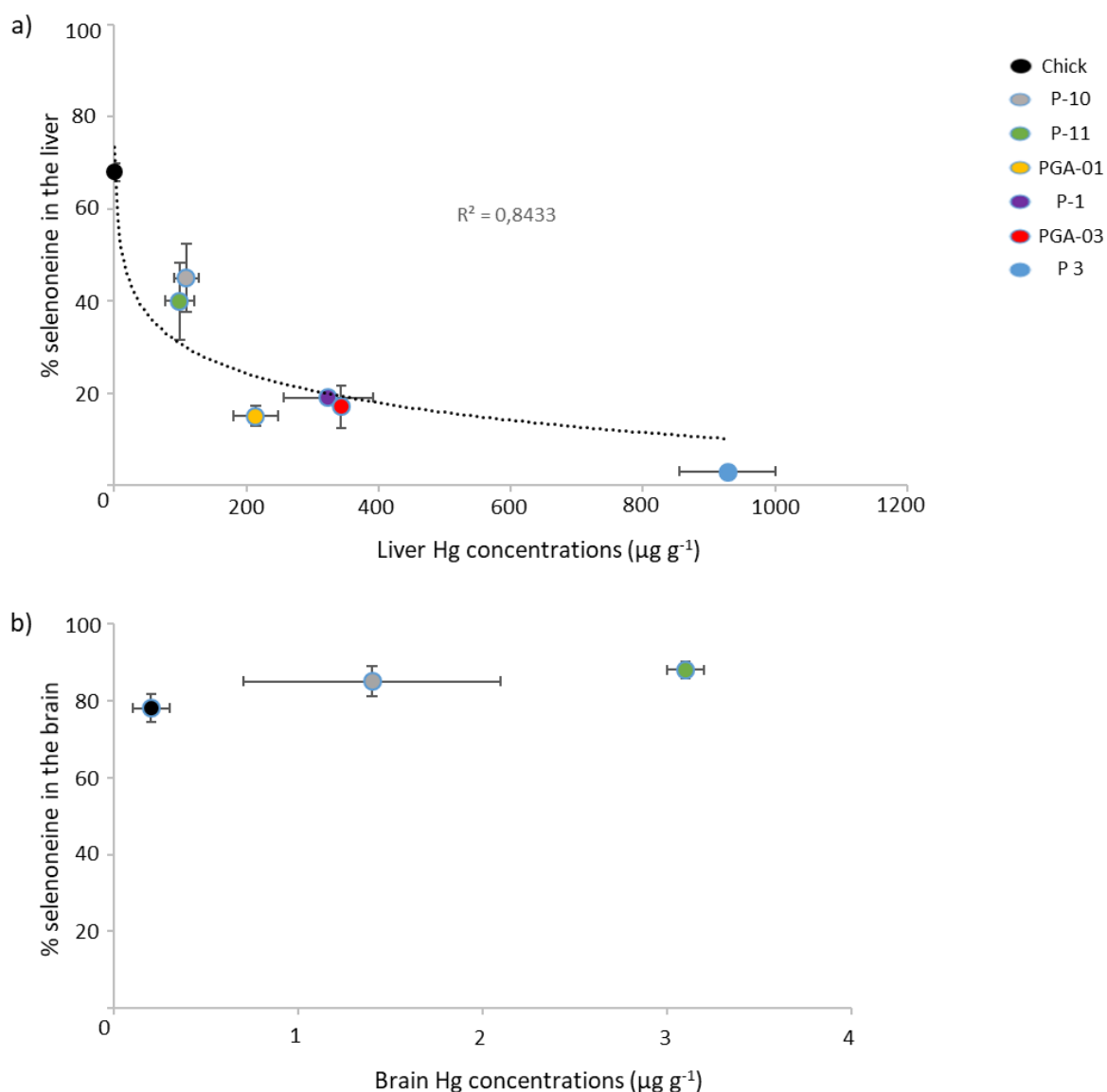
328 **Potential role of selenoneine in (methyl)mercury detoxification**

329

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

341 HgSe nanoparticles have been recently identified in several organs of giant petrels, including
342 liver, kidneys, muscles and brain, representing up to 95% of the total Hg in the latter tissue.²⁶
343 In addition to tiemannite, another Hg-Se complex, a tetraselenolate complex with a molar ratio
344 1:4 (Hg:Se) was reported in these samples.²⁶ According to Palmer et al.⁸² MeHg demethylation
345 could be mediated by a structural analogue of selenoneine (1-methyl-1,3-dihydro-2H-
346 benzimidazole-2-selone) through a Hg-Se complex exhibiting a similar Hg:Se ratio (1:4). The
347 selenoamino acid complexes readily promote MeHg induced protolytic cleavage of Hg-C
348 bonds.⁸² Therefore, considering that: *i.* selenoneine is the major water soluble Se species in
349 giant petrel samples and *ii.* the coordination number of Hg with selenoneine is four,⁸² we could
350 speculate that the identified selenoamino acid is directly involved on the MeHg demethylation
351 step prior to HgSe precipitation.

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



357
 358 **Figure 4.** Relationship between the selenoneine fraction and Hg concentration in a) the liver (with a
 359 logarithmic curve) and b) brain of giant petrels.

360
 361 The decrease of the selenoneine proportions (from 70 to 5%) with increasing Hg concentrations
 362 could be a consequence of the implication of this selenoamino acid in the demethylation of
 363 MeHg, leading to the co-degradation of MeHg and selenoneine resulting in HgSe formation,
 364 the main Hg compound (95%) in the liver.²⁶ In contrast, the percentage of selenoneine in brain
 365 remains stable irrespectively of the increment of Hg concentrations (Figure 4b). HgSe
 366 nanoparticles are also formed in the brain (approximately 35% of total Hg),²⁶ as the result of
 367 MeHg detoxification. Considering that this organ shows the highest Se:Hg molar ratio (33-77)
 368 and percentage of selenoneine (80%), we hypothesize that this novel selenocompound plays a
 369 crucial role in the nervous system, comprising probably a protection against Hg toxicity.

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

378

379 **Supporting Information**

380 Additional experimental details, including tables and figures.

381

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622 **Table caption:**

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

626 **Figure caption:**

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

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

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

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

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