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Individual variability in contaminants and physiological status in a resident Arctic seabird species

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Abstract :

While migratory seabirds dominate ecotoxicological studies within the Arctic, there is limited knowledge about exposure and potential effects from circulating legacy and emerging contaminants in species who reside in the high-Arctic all year round. Here, we focus on the case of the Mandt's Black guillemot (Cepphus grylle mandtii) breeding at Kongsfjorden, Svalbard (79.00°N, 11.66°E) and investigate exposure to legacy and emerging contaminants in relation to individual physiological status, i.e. body condition, oxidative stress and relative telomere length. Despite its benthic-inshore foraging strategy, the Black guillemot displayed overall similar contaminant concentrations in blood during incubation (∑PCB11 (15.7 ng/g w.w.) > ∑PFAS5 (9.9 ng/g w.w.) > ∑Pesticides9 (6.7 ng/g w.w.) > ∑PBDE4 (2.7 ng/g w.w.), and Hg (0.3 µg/g d.w.) compared to an Arctic migratory seabird in which several contaminantrelated stress responses have been observed. Black guillemots in poorer condition tended to display higher levels of contaminants, higher levels of reactive oxygen metabolites, lower plasmatic antioxidant capacity, and shorter telomere lengths; however the low sample size restrict any strong conclusions. Nevertheless, our data suggests that nonlinear relationships with a threshold may exist between accumulated contaminant concentrations and physiological status of the birds. These findings were used to build a hypothesis to be applied in future modelling for describing how chronic exposure to contaminants may be linked to telomere dynamics.

Graphical abstract



Highlights

► The Arctic resident Black guillemot showed overall similar exposure to contaminants during incubation compared to an Arctic migratory seabird in which several contaminant-related stress responses have been observed. ► Black guillemots in poorer condition tended to display higher levels of contaminants and shorter telomere lengths. ► Our data suggests that nonlinear relationships with a threshold may exist between accumulated contaminant concentrations and physiological status of the birds.

Keywords : black guillemot, pollutants, Polar Regions, oxidative stress, seabirds, telomeres

54 **1. Introduction**

55 A seabird's contaminant burden at a given time can be viewed as the product of cumulative 56 processes driven by small or large differences in foraging strategies, physiology and life history. 57 As a result, individuals foraging high in the food web, on lipid-rich prey - or in areas with higher 58 inputs of contaminants often present the highest concentrations of contaminants (Muir et al., 59 1992; de March et al., 1998; Dietz et al., 2000). The growing body of research on Arctic seabirds 60 demonstrates that species with higher concentrations of contaminants experience sub-lethal 61 effects, such as lower breeding success, reduced return rate and higher stress responses (e.g. 62 Erikstad et al., 2013; Tartu et al., 2014a; Bustnes et al., 2015). Yet, the fact that the toxicological 63 effects do not depend solely on contaminant exposure, but are also influenced by life history, physiology, and other environmental stressors (Bourgeon et al., 2012; Goutte et al., 2014) often 64 obscure the relationships between contaminant exposure and health impacts. Consequently, two 65 populations - or species - may exhibit different negative effects of contaminants, despite having 66 67 similar contaminant body burdens (Bustnes et al., 2015). Most seabirds nesting in the Arctic migrate to lower latitudes after breeding and experience different contaminant exposure at their 68 69 overwintering sites compared to their Arctic breeding grounds (Baert et al., 2013; Fort et al., 70 2014); however species that reside in the high Arctic all year round remain understudied and so 71 does their response to the current multi-stress scenarios in Polar Regions.

72 The Mandt's Black guillemot (*Cepphus grylle mandtii*) is known to be one of the few 73 seabird species that overwinter in the Arctic, as it's movements are limited to open water near 74 the sea ice margins close to its breeding site (Brown, 1985; Divoky et al., 2016). While this 75 seabird's life history exposes it to various contaminants that have deposited in the Arctic via 76 long-range transport/deposition mechanisms (Vorkamp et al., 2004 & 2015; Braune et al., 2015 77 & 2016; Peck et al., 2016), the Black guillemot is considered to be sensitive to current and future 78 climate alterations due to its dependency on the sea ice marginal zone in both the breeding 79 (Divoky et al., 2015) and non-breeding seasons (Divoky et al., 2016). Typically, the Black 80 guillemot will alternate between the use of nearshore waters in the breeding season and offshore 81 waters during non-breeding season foraging mostly on Arctic cod (Hobson, 1993), which could 82 differ in contamination and quality of prey (i.e. lipid content and antioxidants). During the 83 breeding season, it rarely feeds more than ten kilometres away from the nest site; while chicks

are fed almost exclusively on benthic fish, adults have a mixed diet of both benthic fish and
invertebrates (Cairns, 1987a & b; Barrett *et al.*, 2002).

The few exposure studies on black guillemots have used invasive and/or lethal sampling matrices or tissues that reflect time-interval of contaminants exposure outside the breeding seasons (e.g. liver, muscles, eggs, feathers) (e.g. Koistinen *et al.*, 1995; Appelquist *et al.*, 1983; Borgå *et al.*, 2001 & 2005 & 2007; Olafsdottir *et al.*, 2005; Vorkamp *et al.*, 2015). Thus, the impact of exposure from blood circulating contaminants on health parameters during sensitive life history events (e.g. breeding season) in this resident, nearshore Arctic species remains unknown.

Exposure to contaminants has the potential to cause an imbalance in the anti-oxidant 93 94 defence system through increased levels of oxidative stress (Constantini et al., 2014; Wielsøe et al., 2015). Here contaminants may play two different roles: a) as an actual threat through energy 95 96 competition resulting from biotransformation and maintenance (e.g. Durant et al., 2007), and/or 97 b) as a "blurring" factor by triggering the hypothalamic-pituitary-adrenalin axis (HPA) (Tartu et 98 al., 2014a), *i.e.* due to their endocrine disruptive properties, or by interfering with the antioxidant 99 defence system itself. Both mechanisms may result in increased oxidative stress and ultimately 100 reduced health status over time.

101 A new and promising cellular biomarker in ecotoxicology is telomere dynamics. 102 Telomeres are repetitive, non-coding DNA sequences located at the end of each chromosome, 103 whose length is reduced during cell division (i.e. through aging) - and through oxidative stress 104 (see Blackburn, 2000; Von Zglinicki, 2002; Epel et al., 2004; Monaghan & Haussmann, 2006, 105 Reichert & Stier, 2017). Thus, telomere length depends on hereditary factors, early-life 106 conditions and life-history trade-offs (Beaulieu et al., 2011; Schull et al., 2016) and should be 107 informative as to integrated stress during an individual's lifetime (Horn et al., 2010). In addition, 108 telomere length is a strong predictor of longevity (Barrett et al., 2013). Long-lived species, such 109 as seabirds, are able to decrease the rate of telomere reduction through enzymatic activity 110 (Blackburn, 2005; Haussmann et al., 2007), and telomere length is proposed to be a predictor of 111 intrinsic individual quality (Bauch et al., 2013; Young et al., 2017). Recently, Blévin et al. (2016

- 112 & 2017a) found associations between contaminants (i.e. perfluorinated compounds, oxy-
- 113 chlordane) and relative telomere length in breeding black-legged kittiwakes (*Rissa tridactyla*).
- 114 The main objective of the present study is to investigate how exposure to both legacy and
- emerging contaminants may influence physiological status in an understudied resident Arctic
- 116 seabird species. This is attempted by relating circulating concentrations of both legacy and
- 117 emerging contaminants in blood to individual physiological status defined by body condition,
- 118 stress levels and relative telomere length in black guillemots.

119 **2. Materials and methods**

120 2.1 Sampling

121 Our study was carried out in Kongsfjorden, Svalbard (79.00°N, 11.66°E), on three different 122 black guillemot nest sites (Juttaholmen, Midtholmen, Observasjonsholmen) during the breeding 123 season of 2015 (June-July), with the necessary permissions from the Governor of Svalbard (RIS 124 ID 10290) and Norwegian Animal Welfare authorities (FOTS ID 7666). We used snares to catch 125 black guillemots (n = 15) resting on rocks outside of their nest during incubation phase. Of these, 126 11 birds were successfully sampled for blood (i.e. minimum 2 mL, maximum < 10 % of body 127 mass) from their brachial wing vein, using heparinized syringes. Blood samples were kept cold $(< 4^{\circ}C)$ and in the dark, before being centrifuged and stored at -20°C. All captured birds were 128 129 biometrically measured (i.e. body mass, wing length, bill and skull size) and inspected for brood

130 patch.

131 2.2 Contaminant analyses

132 We measured mercury (Hg) in red blood cells (~ 0.5 mL) of 11 black guillemots. Hg was

determined using in-house methods: NILU-U-117 "Procedure for sample digestion by use of

- 134 high performance microwave reactor (UltraClave)" and NILU-U-65 "Procedure for
- 135 determination of Hg in biological samples using Could Vapor Atomic Fluorescence
- 136 Spectrophotometry (CV-AFS)". Aliquots of 0.20-0.35 g of red blood cells were added 5 mL

137 concentrated supra pure HNO₃ and 3 mL deionized water (MilliQ) and digested at 250°C for 15

- 138 minutes, using UltraClave (Milestone, Italy). Blank samples and certified reference material
- 139 (CRM) were digested in the same run as the blood samples. After digestion, samples, blank
- 140 samples and CRM were diluted to 50 mL. From the extracts, subsamples of 25 mL were further
- 141 diluted to 50 mL before 5 ml BrCl were added. Determination of Hg was performed using CV-
- 142 AFS from Tekran, Canada according to method US-EPA-1631.
- 143 In the statistical analysis, we also included published contaminant data from reported in
- 144 Haarr et al. (2017) on the same black guillemots individual as in present study. These
- 145 contaminant data include concentrations of polychlorinated biphenyls (PCBs),
- 146 dichlorodiphenyltrichloroethane (DDTs) and its metabolites, isomers of hexachlorocyclohexane

- 147 (HCH), hexachlorobenzene (HCB), chlordanes, polybrominated diphenyl ethers (PBDEs),
- 148 perfluorinated compounds (PFAS) that was extracted from plasma (1 mL) using protocols
- 149 described in detail in Blévin et al. (2017 a & b) and Haarr et al. (2017).

150 **2.3 Isotopic signatures in black guillemots**

- 151 Signatures of stable isotopes are used to estimate individual foraging behaviour in two-
- 152 dimensions: pelagic-benthic and low-high trophic level feeding. The use of carbon and nitrogen
- 153 stable isotope ratios (∂^{13} C and ∂^{15} N in ‰) is an established practice for assessing carbon source
- 154 (e.g. pelagic vs. benthic) and relative positions in the food web (i.e. high or low trophic levels)
- 155 (Hobson *et al.*, 1995). Red blood cells (~200 μ L) from black guillemots (n = 11) were freeze-
- dried and analysed for heavy and light isotopes of nitrogen and carbon at the University of
- 157 Windsor, Canada; as described in Fisk *et al.* (2001).

158 **2.4 Oxidative stress, telomere length and body condition in black guillemots**

- 159 We estimated oxidative stress levels in plasma of black guillemots using well-established
- 160 methods on derivatives of reactive oxygen metabolites (d-ROM) in plasma and total plasma
- 161 antioxidant capacity (Oxy), described in detail by Schull et al. (2016). In short, we measured the
- amount of hyperoxides (d-ROM test, Diacron International) in 8 µl of plasma for each bird,
- 163 (expressed as mg H_2O_2 equivalent dl⁻¹) with duplicates for each sample. We used the Oxy
- adsorbent test (Diacron International, Grosseto, Italy) to quantify the ability to buffer massive
- 165 oxidation (i.e. hydroperoxide acid) in 5 µl of 1:100 diluted plasma, for each bird with duplicates
- 166 for each sample. The variation between individuals was 5.5% and 5.5% for d-ROM and Oxy,
- 167 respectively (only one plate was run, thus no inter-plate repeatability was assessed).
- 168 We extracted DNA using Nucleospin Blood QuickPure Kit (Macherey-Nagel, Düren
- 169 Germany) and estimated the relative length of telomeres by qPCR using a well-established
- 170 protocol for King penguin (*Aptenodytes patagonicus*) (Stier *et al.*, 2014; Le Vaillant *et al.*, 2015;
- 171 Reichert *et al.*, 2015). Chromosomic sex of individuals was determined from red blood cells
- 172 extracted DNA following Sambrook et al. (1989) protocol.
- In order to understand how successfully a bird interacts with its environment, we used a
 body condition measure to estimate its supply of energy (i.e. fat and protein content) (Labocha &

175 Hayes, 2012). Since larger birds are obviously heavier than smaller birds, the variation in mass

176 generally correlates with size, while departures from this pattern are explained by inter-

177 individual differences in body condition. Therefore, we calculated a Scale Mass Index - SMI,

178 using the biometric measure with the highest correlation coefficient with body mass in our data

179 (i.e. bill length) as length measure to scale the mass accordingly (see Peig & Green 2009):

180 $SMI_{individual} = body mass_{individual} x (bill_{mean}/bill_{individual}) ^ coef_{body mass~beak}$

181 **2.5 Statistical analyses**

Statistical analysis was conducted in R 3.4.2 (R Development Core Team, 2016). One black
guillemot individual was excluded due to issues during chemical analyses, thus leaving us with a
final dataset of ten birds.

185 The Limit of Detection for contaminants (LOD) was set as the mean of the blanks added 186 by three times the standard deviation of the blanks (Armbruster & Pry, 2008). Values below 187 LOD – or non-detects – were treated using distributional methods and imputation by maximum likelihood (Baccarelli et al., 2005). For each contaminant, we assumed a log-normal distribution. 188 189 Non-detects were treated using three different steps: 1) the most likely distribution was fitted 190 based on both censored and non-censored data using a simple Expectation-Maximisation 191 algorithm; 2) the minimum number of non-censored points (i.e. cut-off) necessary for a robust 192 inference of the log-normal distribution parameters was estimated by simulations, and filtered 193 accordingly; 3) for contaminants with a sufficiently low number of non-detects, multiple 194 imputation by maximum likelihood was performed using a specified interval (i.e. zero to LOD).

195 Contaminant profiles are mixtures of many contaminants so to extract robust indicators 196 that reliably compare individuals in regression analyses, we need to reduce variation to a lower 197 number of dimensions. We thus first collapsed contaminants into groups depending on their 198 chemical structure, in order to account for differences in weights (i.e. functional relationships 199 with response variables) between different chemical groups. Secondly, since contaminants within 200 the same chemical group may have different dose-dependency (i.e. toxic potential), we need to 201 scale their relative contribution to focus on the shape and not the size of the data (Greenacre, 202 2017). Hence, we preferred to use an equally weighted index that rank individuals based on their

203 exposure to different chemical groups and total contaminants load. This approach does not 204 account for differences in toxic potency and functional relationships within chemical groupings; 205 however we consider it to address more correctly the underlying theoretical assumptions, and the 206 approach itself produces an index that provide a more transparent interpretation when used as a 207 predictor, compared to principle components for example. Thus, we created a score index for 208 each chemical group (i.e. iPCB, iPesticides, iPBDE, iPFAS and iHg) and one for total 209 contaminant load (iCON), where each individual is given a summed score relative to the mean of 210 the sample population for each contaminant:

211 Index Contaminant group $X = \sum (x_i / \overline{x})$

where x_i are the values of each of the individual chemical groups in individual *i*, \overline{x} represents the mean concentration of the individual chemical over the entire sample population within the given chemical group (X).

215 We applied different generalized linear models (GLMs) to test potential relationships 216 between the contaminant groups and the response variables as well as residual plot analysis to 217 assess the validity of the model. If the residuals showed non-random patterns, we tried different 218 families of transformations, g(y), until the residuals plots of the regression g(y) = beta_0 + Σ 219 beta X were consistent with independent Gaussian error.

3. Results

221 **3.1** Contaminants and isotopic signatures in black guillemots

222 Concentrations of Hg in red blood cells of black guillemots was measured to 0.32 ± 0.8 ug/g d.w. 223 Treatment of LOD censored values resulted in detection of all 11 targeted PCB congeners, but

only 10 of the 17 organochlorine pesticides, 5 of 20 perfluorinated compounds, and 4 of 10

225 PBDEs, in plasma of black guillemots (see Supplementary Table 1). ∑PCBs was the dominating

- compound group (dominated by PCB153 > PCB138 > PCB180), followed by Σ PFAS (mainly
- 227 $PFOS > PFUnA) > \Sigma Pesticides (mainly HCB > p,p'-DDE) > \Sigma PBDEs (mainly PBDE47) (see$
- 228 Fig. 1).

The black guillemots show a high dispersion in trophic positions ($\partial 15N$ ranging from 12.0 - 13.5‰), while carbon isotopic signatures show a clear benthic signal and overall low dispersion - except one extreme individual ($\partial^{13}C = -21.5\%$). This female presented also higher lipid content and was one the two individuals with highest contaminant concentrations, highest stress levels and shortest telomeres. As we did not have the sufficient information (unknown breeding status) to exclude her from the statistical analysis, we kept this individual in further analysis (see Discussion).

236

3.2 Relationships among contaminants, isotopic signatures and physiological status in black guillemots

We created four score indices based on the chemical groupings where each bird was given a score relative to the mean of the population per chemical group (i.e. iPCB, iPesticides, iPBDE, iPFAS and iHg) and one for total contaminant load (i.e. iCON). Correlation tests (Spearman's test) between the indices showed that birds with high scores on PCB also had high scores on the other contaminants. However, we found no significant correlations among iPBDE, iPFAS and iHg, or between iHg and iPesticides (see Supplementary Fig 2.). All score indices, except iHg, correlated significantly with iCON.

No significant relationship among isotopic signatures, lipid content and physiological
status measures (for p-values see results from Spearman's correlation test in Supplementary Fig.
1) was detected. We found a significant positive correlation between body condition (SMI) and
telomere length (RTL) and a significant negative correlation among iPesticides and SMI and
RTL (see p-values from Spearman's correlation test in Supplementary Fig 1).

Our low sample size prevented us from conducting a thorough evaluation of the relationship between contaminants and physiological status in black guillemots. However, the data provided insight into these relationships and showed a consistent tendency with several variables driven by the two most contaminated individuals (see Fig 2). It should be noted that one individual (female) obscured this tendency; the individual with the shortest telomeres was in the modal of lower contaminant concentrations.

3.3 From contaminants to telomere length

Initial evaluation of our data through the residual plot analysis revealed a nonlinear relationship between iCON, SMI, Oxy, d-ROM and RTL (see Fig 2). The observed patterns imply that the average effect of the predictor on the response variable increases for moderate contaminant concentrations, but after a certain value is reached, the response approaches a saturation level (K) that needs to be determined. This suggests that an S-shaped function would be appropriate and we opted for a regression model of the form:

$$Y_K = K \frac{e^{(\beta_0 + \beta_1 X)}}{1 + e^{(\beta_0 + \beta_1 X)}}$$

This function describes all nonlinear effects found in the data better than other simple nonlinear functions such as quadratic or exponential. This parametric family of functions describes all nonlinear effects found in our variables and is flexible enough to provide a good fit in all situations in our dataset. In this equation, Y represents the response (i.e. SMI, d-ROM, Oxy, RTL), and X represents each of the contaminant score indices (i.e. iCON, iPCB, iPesticides, iPFAS, iHg) and physiological status variables (i.e. SMI, d-ROM, Oxy) (see Table 1 for parameter estimates and p-values).

271 K is a constant determined by minimising the mean squared error of the regression fit, that is:

272
$$\mathbf{K} = \arg\min\sum (\mathbf{Y}_{\mathbf{K}} - \mathbf{Y}_{\mathbf{K}}^*)^2$$

273 where Y_K is the value of the function using the measured data, and Y_K^* is the predicted value

274 under a logistic model fitted with value K as scaling factor, that is:

$$\operatorname{logit}\left(\frac{Y^*}{K}\right) = \beta_0 + \beta_1 X$$

We found a significant relationship between RTL and iPesticides (p-value = 0.022) RTL and iCON (p-value = 0.047), RTL and SMI (p-value = 0.002), and between SMI and iPesticides (pvalue = 0.039). There were no significant relationships between oxidative stress and any of the other variables.

4. Discussion

4.1 Contaminant concentrations and profiles in black guillemots during the breeding season

282 To our knowledge, the present study is the first to report concentrations of circulating 283 contaminants in blood, a less-invasive matrix, in breeding Mandt's Black guillemot. Blood 284 reflect contaminant concentrations from recently consumed prey at a relatively short time-frame 285 (< 10 hours, Drouillard & Norstrom, 2000) and thus our study is restricted to reflect the 286 contamination situation for black guillemots during incubation period. Our results shed light on 287 different aspect of the black guillemots interactions with contaminants: i) black guillemots seem 288 to experience different contaminant exposure depending on the Arctic region and 289 season/foraging areas, and ii) black guillemots during breeding season appear to experience 290 similar exposure to circulating contaminants compared to another seabird species in which 291 several studies have reported increased stress-response, decreased physiological condition and 292 adult survival related to contaminants (e.g. Tartu et al. 2013; Goutte et al., 2015; Blevin et al., 293 2017 a & b).

294 Black guillemots are diving, subsurface feeders that switch from offshore Arctic cod 295 (Boreogadus saida) to nearshore and near-nest-site benthic prey such as sculpin (Myoxocephalus 296 scorpioides) or different invertebrates during their breeding season (Cairns, 1987a & b; Mehlum 297 & Gabrielsen, 1993; Byers et al., 2010). While non-breeding guillemots and guillemots from 298 other Arctic regions share similar contaminant patterns, black guillemots present during the 299 breeding season in Kongsfjorden different patterns of POPs in the plasma ($\Sigma DDTs > \Sigma PCBs >$ 300 $HCB > \Sigma CHL > Mirex > \Sigma HCHs)$ compared to what has been previously reported for liver and 301 muscle of black guillemots from the Barents Sea and in eggs from the Canadian Arctic (\sum PCBs 302 > Σ DDTs >HCB > Σ CHL > Σ HCHs >Mirex) (Borgå *et al.*, 2001; Braune *et al.*, 2018). We also 303 found a higher number of congeners of PFASs than earlier studies investigating liver and eggs of 304 black guillemots in the West Arctic (Martin et al., 2004; Peck et al., 2016). The black guillemots in our study show similar mean $\partial^{13}C$ signal as earlier studies on non-

The black guillemots in our study show similar mean ∂^{13} C signal as earlier studies on nonbreeding black guillemots from Greenland which indicate that black guillemots breeding in Kongsfjorden may forage less benthic at least during incubation (Linnebjerg *et al.*, 2016). One

female, however, has a more pelagic $\partial^{13}C$ signal similar to juvenile black guillemots during non-308 breeding season in the central Barents Sea (Borgå et al., 2005). This specific female also displays 309 310 higher contaminant concentrations, especially HCB, trans-Nonachlor, PBDE-47, Hg and certain 311 PFASs which support a different exposure scenario depending on either breeding state or 312 foraging grounds. Unfortunately, although there was no apparent brood patch, we do not have 313 sufficient information to determine the breeding status (e.g. juvenile or inexperienced/late/failed 314 breeder). Implications of differences in breeding status on physiological status is further discussed in chap. 4.2. ∂^{15} N signals range from 11.9 to 13.5‰, which is similar to the isotopic 315 316 range of juveniles reported by Borgå et al. (2005). At best, our results suggest that black 317 guillemots experience different contaminant situation at different foraging grounds (offshore-318 nearshore/benthic-pelagic). Whether foraging offshore or pelagic lead to higher exposure remains unclear as the most contaminated bird (a male with especially high level of PFASs) 319 displayed a ∂^{13} C signal similar to the rest of the sample population. 320

321 Compared to other seabird species breeding in the same fjord, black guillemots i) have 322 lower Hg concentrations (0.32 ug/g d.w.) than previously reported for black-legged kittiwakes 323 (Rissa tridactyla; 1.8 ug/g d.w. red blood cells, Tartu et al., 2013) and ii) show similar wet 324 weight plasma of Σ PCBs, Σ Pesticides, Σ PBDEs and Σ PFAS compared to black-legged 325 kittiwake sampled the same year (Haarr et al., 2017). However, plasma concentrations are often 326 used as proxy for body burden, expressed as lipid weight concentrations to control for the 327 varying lipid content in blood within and between species (Bustnes et al., 2001; Hebert & 328 Keenleyside, 1995). The two species differed in lipid weight contaminant concentrations, thus, 329 we cannot exclude that the two species may still differ in total body burden, i.e. stored 330 contaminants in other organs and tissues accumulated outside breeding season. 331 Seabirds can go through drastic changes in body mass during breeding season, resulting in 332 lipid-mobilisation and subsequently re-mobilise of contaminants into the bloodstream (Henriksen 333 et al., 1998; Bustnes et al., 2012 & 2017; Fenstad et al., 2016). This seems unrealistic as black 334 guillemots appear rather to increase their body mass during incubation (Aevar Petersen, 335 *pers.com.*) and we find no relationship between body condition and blood lipid content. It is

- more plausible that black guillemots have a higher lipid content than other seabird species as
- reported in Harr et al. (2017) due to frequent inputs from more lipid-rich prey during incubation.

338 Black-legged kittiwakes are subsurface feeders foraging on fish, invertebrates and 339 zooplankton in the pelagic part of the ecosystem, with an offshore foraging distribution during 340 winter. During the breeding season, the black-legged kittiwakes of Kongsfjorden forage within 341 the same fjord system as the black guillemots in this study (nearshore) but in different parts 342 (benthic vs. pelagic) (Vihtakari et al., 2018). Our results also support that auks have a lower 343 biotransformation capacity compared to other gulls (Borgå et al., 2005); as plasma 344 concentrations mirror the same high proportion of cis-Chlordane in Σ CHL as found in internal-345 tissue samples of non-breeders and black guillemot eggs (Borgå et al., 2001; Braune et al., 346 2018). Thus, it may seem that even though these two seabirds species have different foraging 347 strategies and biotransformation capacities, they end up with the same exposure to circulating 348 contaminants (of the ones we targeted in this study, except Hg). Furthermore, even though blood 349 lipid content and isotopic signatures fall short to explain contaminants concentrations in the 350 Black guillemot of our study, foraging in less contaminated areas but on prey with higher lipid 351 could lead to the same exposure as the opposite. This aspect may only be understood through 352 integrative methods combined with individual tracking data, as demonstrated by Fort et al. 353 (2014).

354

355 4.2 The relationships between contaminants and physiological status

356 Telomere length is a time-integrated measure of life history processes, including age and 357 experienced stress (Young et al., 2013). It has also been proposed as a predictor for *intrinsic* 358 quality (see discussions on different definitions and use of quality in Wilson & Nussey (2010) 359 and Bergeron et al. (2011)). In this case, lower quality individuals are expected to present a 360 lower physiological status (i.e. poorer condition) experience more stress and thus greater 361 telomere attrition. Our results show that birds in poor condition had shorter telomeres than birds 362 in better condition, which is consistent with findings in other seabird species showing that relative telomere length and body condition are positively related (Le Vaillant et al., 2015). In 363 364 addition, birds with higher contaminant scores also tended to be in poorer condition and have 365 shorter telomeres (only significant for total contaminant load and pesticides). This is consistent

with what has been observed for oxychlordane and telomere length in the chick-rearing blacklegged kittiwakes by Blévin *et al.* (2016), despite that concentrations were lower in our study.

368 The two most contaminated individuals (one female and one male) separating the dataset 369 into two groups dictated the significance in the correlations observed in our data. Although 370 observed at the nest site together with other breeding individuals, we were not able to determine 371 breeding status for the female (i.e. non-breeder or failed or late breeder). Moreover, we lack 372 information about the age of these birds, thus it is difficult to conclude whether or not this female 373 was a juvenile visitor that did not try to breed at all or an unexperienced breeder attempting and 374 failing to reproduce, returning to off-shore foraging ground to eat and coming back to the colony. 375 This bird could also have been a late breeder that recently arrived from off-shore foraging 376 grounds: in the closely related Pigeon guillemot (*Cepphus columba*), experienced breeders arrive 377 earlier than unexperienced, and during egg-formation, females have shown reduced nest 378 attendance compared to males (Nelson, 1987). This female may also have failed because of a 379 poor condition as indicated, by her low body condition, high stress levels and short telomeres. 380 Only in the first situation (juvenile), would it be statistically sound to exclude the individual from 381 the analysis as an outlier; however immature individuals tend to have smaller body size measures 382 (i.e. wing length, bill depth) and this was not the case for this specific individual compared to the 383 rest of the sample population. Thus, with noted caution, we consider it more plausible that the 384 female reflect natural variation of contaminant concentrations and physiological condition 385 related to individual foraging tactics within breeding black guillemots.

386 Another aspect is the sample size: an increase of sample size could well have distinguished 387 these two individuals as clear outliers; on the other hand, one should also take caution being-388 blinded by the mean-response of the population (Bennett, 1987) as toxic effects can also 389 influence the variance of the physiological response (Forbes & Depledge, 1999). The distribution 390 in our contaminant data may also be the product of cumulative processes: some birds end up as 391 extremes due to individual strategies over time (e.g. individual foraging specialisation as 392 observed for diving seabirds by Bearhop et al. (2006)). Such extreme values induce non-linearity 393 in the data (i.e. S-shaped function) with 1) lower contamination score with little or no effect 394 observed on response variables measured, and 2) high contamination score and higher effect on 395 response variables measured. This is also what we see as the main tendency in our data. Yet iHg

did not correlate with the other score indices but showed a more linear functional relationshipwith the physiological status responses.

398 The same tendency of relationship with contaminants was also observed in respect to 399 stress: although insignificant, the reactive oxygen metabolites (d-ROM) levels seemed to 400 increase with increasing contaminant score, while for the variance, observations indicate a 401 decrease. The individuals with higher d-ROM had also lower global plasmatic antioxidant 402 defence (Oxy). This is consistent with previous findings in birds (Hegseth et al., 2011; Bourgeon 403 et al., 2012; Sletten et al., 2016; Abbasi et al., 2017) that support the hypothesis that contaminant 404 exposure may contribute to increased oxidative stress (Isaksson et al., 2010; Constantini et al., 405 2014). The lack of relationship between d-ROM and telomeres may be due to a mismatch of 406 tissue (Constantini et al., 2014) or time scales. Telomere length is commonly understood as an 407 integrative measure of accumulated oxidative stress, while d-ROM is an indicator of 408 instantaneous oxidative stress (Urvik et al., 2016).

409 **4.3** Theoretical response pathway for contaminant exposure and telomere length

The low sample size restricted us from evaluating our proposed response pathway in regards to contaminant loading and telomere length. However, the tendency in the data allowed us to build a hypothetical scenario for future investigation in which oxidative stress and telomere length will be dictated by accumulated contaminant burden and body condition above and below certain thresholds.

415 The threshold idea is based on inter-individual physiological variability (Depledge, 1990)

416 resulting in different abilities to perform across different environmental conditions (e.g., low and

417 high food availability, low and high contamination), without increasing the cost (i.e. stress,

418 condition) – or *intrinsic quality* (Bergeron *et al.*, 2011). Furthermore, at some point, contaminant

419 exposure may start influencing these abilities. A central question is then if birds with higher

420 contaminant concentrations are a result of lower body condition, as observed in Wayland *et al.*

- 421 (2002) or vice versa if higher concentrations of contaminants have led to lower body
- 422 condition. In the former, body condition may determine physiological status while the correlating

423 contaminants play a co-founding role; the effect of weight loss is more important for the overall

424 stress situation compared to the contribution from contaminants (Fenstad *et al.*, 2014).

- 425 However, black guillemots do not experience significant weight loss during incubation due to
- 426 shared parental effort and strategies of short-distance trips (both partners forage every day)
- 427 (Aevar Petersen & George Divoky, *pers.com*.). This is supported by our findings, where no
- 428 relationship between lipid content, body condition and contaminants was observed. An
- 429 alternative explanation is that the most contaminated birds may be in poorer condition due to the
- 430 presence of high contaminant concentrations over time (i.e. strategy driven cumulative
- 431 processes). In this case, we may expect a shift between physiological states where an individual
- 432 with contaminant concentrations above a certain threshold value is bound to have a lower
- 433 physiological status over time.

434 **5.** Conclusions

In conclusion, our results demonstrate that, during the breeding season, black guillemots 435 436 experience the same circulating contaminant concentrations as a pelagic migratory seabirds, the 437 Black-legged kittiwake, for which several clear associations between contaminants, stress 438 response and breeding success have been reported. Our study also emphasize the importance of 439 individual tracking datasets and information on breeding and foraging status in wildlife 440 ecotoxicology. Despite non-significant relationships, our data shows a consistent tendency that 441 black guillemots in poorer condition had shorter telomeres, higher concentrations of 442 contaminants and higher levels of oxidative stress. Our threshold hypothesis should be tested 443 with a larger dataset, coupled with life history data, in the future: if a threshold for effects exists, 444 it will likely both depend on breeding behaviour (e.g. fasting during incubation shift) and 445 environmental stressors (e.g. food availability, habitat quality acting on body condition), and a 446 species' behavioural and physiological traits (e.g. life history, biotransformation). Thus, we 447 should expect the same non-linear relationship between telomere length and contaminants and 448 body condition, with species-specific thresholds, in other seabirds as well.

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458 Data availability

- 459 Data is publicly available on FigShare (DOI: 10.6084/m9.figshare.6225071). [During the review
- 460 process these data can be accessed using this temporary URL:
- 461 <u>https://figshare.com/s/8d10a917a3274aba1d6b]</u>.

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695 Figure legends

- 696 **Figure 1. Contaminant profiles in the Black guillemot.** Radar plot showing concentrations
- 697 (ng/g w.w.) and profile signatures of PCB, pesticides, PBDE and PFAS congeners detected in
- 698 incubating black guillemots (points, with 'extreme' male marked in red colour) and line (black)
- 699 indicating the mean.
- 700 Figure 2. Scatterplots of relations between contaminant score indices, body condition,
- 701 oxidative stress, anti-oxidant capacity, and relative telomere length, in females (red) and
- 702 males (blue). (a) iCON and SMI with RTL indicated by the size of the points. (b) iCON and d-
- ROM with RTL indicated by the size of the points. (c) d-ROM and Oxy with iCON indicated by
- the size of the points. (d) iPCB and SMI with RTL indicated by the size of the points. (e)
- iPesticides and SMI with RLT indicated by the size of the points. (f) iHg and body SMI with
- 706 RTL indicated by the size of the points. (g) iPBDE and body SMI with RTL indicated by the size
- 707 of the points. (**h**) iPFAS and SMI with RTL indicated by the size of the points.
- 708 Table 1: Parameter estimates for modelling the relationships among contaminants (iCON)
- 709 and physiological status (SMI, d-ROM, RTL) in incubating black guillemots. Bold
- 710 characters refer to significant relationships under alpha < 0.05.
- 711





Table 1.

Dependent	Independent	K	Beta0	Beta1	Df	t-value	P-value
variable	variable						
RTL	iCON	1.5	0.77	-0.02	8	-2.34	0.047
	iPCB	1.5	0.77	-0.06	8	-2.12	0.067
	iPesticides	1.5	0.88	-0.08	8	-2.85	0.022
	iPBDE	1.5	0.48	-0.09	8	-1.04	0.328
	iPFAS	1.54	0.33	-0.05	8	-1.79	0.112
	iHg	1.5	0.85	-0.73	8	-1.16	0.279
	SMI	1.5	-8.70	23.00	8	4.69	0.002
	d-ROM	1.5	1.55	-0.01	8	-1.34	0.218
	Оху	1.64	-0.31	0.004	8	0.64	0.538
SMI	iCON	2.04	-1.40	-0.002	8	-1.95	0.086
	iPCB	0.61	0.65	-0.012	8	-1.51	0.168
	iPesticides	2.04	-1.38	-0.009	8	-2.47	0.039
	iPBDE	0.63	0.57	-0.04	8	-1.90	0.094
	iPFAS	2.04	-1.44	-0.005	8	-1.35	0.216
	iHg	2.04	-1.37	-0.098	8	-1.33	0.219
d-ROM	iCON	800.95	-1.80	0.005	8	1.17	0.277
	SMI	257.19	0.27	-0.557	8	-0.09	0.928
Oxy	iCON	141.27	0.01	-0.018	8	-0.98	0.355
	SMI	141.27	-1.91	3.616	8	0.236	0.819

724 SUPPLEMENTARY MATERIAL

Supplementary Table 1. Concentrations of contaminants (pg/g w.w., Hg given as ng/g d.w.),
lipid content, dietary signatures (‰), oxidative stress, and relative telomere length in blood from
black guillemots. Values are given as detection frequency, mean, standard deviation, and range
(min-max).

* Due to poor extraction recoveries with two of the three method blanks of the internal standard for oxychlordane, we calculated a conservative detection limit by multiplying the blank response in the blank sample which acceptable extraction recoveries were obtained by a factor of three to avoid the potential of reporting false positives. In addition, a minor isobaric interference coeluted with oxychlordane. As blank substraction was not performed, concentrations approaching detection limits will be heavily influenced by co-eluting interference and should be treated with caution. We decided on a conservative, cautionary approach and excluded oxychlordane from

further analyses.

		Detection ACCEP	Mean (median) TED MANUS	Standard CRIPT deviation	Min	Max
		(%)		ut fintion		
\sum Pesticides ₁₇	PeCB	100	109 (92.8)	60.5	45.1	274.5
	аНСН	60	14.8 (15.5)	3.7	9.1	19.2
	ьнсн	100	186.2 (180.6)	94.8	59.0	382
	үНСН	10	<lod< td=""><td></td><td></td><td>~</td></lod<>			~
	НСВ	100	2227 (1900)	925	929	4300
	Oxychlordane*	50	900 (778)	248	699	1246
	Trans-chlordane	0	<lod< td=""><td></td><td>Q-</td><td></td></lod<>		Q-	
	Cis-chlordane	100	16.1 (16.5)	2.4	12.1	19.5
	Trans-nonachlor	100	85.0 (61.3)	81.6	24.7	297
	Cis-nonachlor	100	605 (452)	456	278	1491
	Mirex	100	352 (327)	214	131	877
	o,p DDE	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	p,pDDE	100	3188 (2259)	2235	1338	8653
	o,p DDD	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	p,p DDD	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	o,p DDT	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	p,p DDT	0	<lod< td=""><td></td><td></td><td></td></lod<>			
$\sum PCB_{11}$	PCB28	60	118 (90.6)	69.6	74.2	258
	PCB47	100	173 (147)	83.8	101	328
	PCB52	100	184 (145)	128	86.0	442
	PCB66	100	337 (302)	191	111	612
	PCB71	100	231 (196)	115	82.0	478
	PCB99	100	1390 (1179)	757	625	3033
	PCB101	100	479 (360)	349	147	1277
	PCB118	100	1977 (1779)	1145	577	4529

	PCB138	100	4689 (3793)	2646	2378	11156
	PCB153	100	6897	4143	3169	17303
	PCB180	100	2474 (2076)	1710	1034	6922
$\sum PBDE_{10}$	PBDE28	20	<lod< td=""><td></td><td></td><td></td></lod<>			
	PBDE71	0	<lod< td=""><td></td><td></td><td>2</td></lod<>			2
	PBDE47	100	145 (127)	80.4	72.0	322
	PBDE66	0	<lod< td=""><td>Č</td><td></td><td></td></lod<>	Č		
	PBDE100	100	48.1 (37.0)	24.2	24.0	88.0
	PBDE99	90	35.9 (32.2)	20.2	12.0	79.0
	PBDE154	90	43.1 (38.0)	19.4	24.0	70.0
	PBDE153	20	<lod< td=""><td>></td><td></td><td></td></lod<>	>		
	PBDE183	10	<lod< td=""><td>Ŷ</td><td></td><td></td></lod<>	Ŷ		
	PBDE209	0	<lod< td=""><td></td><td></td><td></td></lod<>			
$\sum PFAS_{20}$	6:2FTS	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	8:2FTS	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	PFOSA	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	PFBS	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	PFPS	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	PFHxS	40	<lod< td=""><td></td><td></td><td></td></lod<>			
	PFHpS	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	brPFOS	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	linPFOS	100	3892 (1984)	4923	630	17162
	PFNS	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	PFDcS	0	<lod< td=""><td></td><td></td><td></td></lod<>			

	PFHxA	0	<lod< th=""><th></th><th></th><th></th></lod<>			
	PFHpA	0	<lod< td=""><td></td><td></td><td></td></lod<>			
	PFOA	20	<lod< td=""><td></td><td></td><td></td></lod<>			
	PFNA	100	1067 (807)	876	139	3247
	PFDcA	100	568 (393)	581	44.4	2048
	PFUnA	100	2718 (2086)	2033	926	7450
	PFDoA	20	<lod< td=""><td>Č</td><td>~~~</td><td></td></lod<>	Č	~~~	
	PFTriA	90	1781 (872)	2457	577	8286
	PFTeA	20	<lod< td=""><td>1</td><td></td><td></td></lod<>	1		
∑Hg	totalHg	100	323 (330)	83.2	175	469
%Lipid	ЕОМ	100	1.2 (1.34)	0.5	0.5	1.9
Trophic position	∂15N (‰)	100	12.7 (12.9)	0.6	11.9	13.5
Carbon source	∂13C (‰)	100	-19.9 (-19.7)	0.7	-21.5	-19.2
Baseline- corrected trophic level	TL	100	4.5	0.2	5.0	4.3
Derivatives of reactive oxygen metabolites	d-ROM	100	132 (130.2)	20.5	91.8	160
Plasmatic antioxidant barrier	Oxy	100	55.3 (53.9)	23.7	13.9	94.2
Relative telomere length	RTL	100	0.80 (0.87)	0.2	0.5	1.0





741 Supplementary Figure 1. Left: Correlations among the contaminant score indices (iCON, iPCB,

742 iPesticides, iHg, iPBDE, iPFAS), dietary descriptors (215N, 213C, lipid content) and

743 physiological status measures (SMI, d-ROM, Oxy, RTL) in the Black guillemot. Significant

relations (Spearman's test) indicated by *** (p<0.001), ** (p<0.05) and * (p<0.1). Right: 744

Correlation network graph between the contaminant score indices, dietary descriptors and 745

physiological status. 746

747

Chemical analysis: Details of instrument 748

749

750 **Analysis of PCBs**

751

752 Analysis of PCBs was performed by gas chromatography mass spectrometry (GC/MS) using an

753 Agilent 7890A gas chromatograph (Agilent Technologies, Böblingen, Germany) coupled to a

754 Quattro Micro triple quadrupole mass spectrometer (Waters Coporation, Manchester, United

755 Kingdom). Separation was carried out using a DB-5 MS column (30 m x 250 µm inner diameter

756 (id), 0.25µm film thickness, J&W, Folsom, USA) equipped with a 5 m guard column (Restek

757 Corporation, Bellefonte, PA, USA) at 1ml/min with Helium as a carrier gas. Samples were 758 injected at 250°C using a split/splitless injector equipped with a 4 mm id packed (wool) glass 759 liner operated in splitless overflow mode with an injection of 5 µL injection volume. Oven 760 temperature program started at an initial temperature of 67°C (1.5 min hold), ramped at 761 15°C/min to 180°C, followed by a second temperature ramp of 5°C/min to 280°C (hold 3 min). 762 Single ion monitoring was used for the detection of PCB congeners using mass spectrometry in 763 electron impact mode (70 eV) with the ion source temperature of 250°C. Ions monitored for both 764 parent and 13C12 mass-labelled internal standards are outlined in Supplementary Table 2. 765 766 **Analysis of DDT/DDE** 767 768 Analysis of DDT and DDE isomers was performed by GC/MS) using an Agilent 7890A gas 769 chromatograph (Agilent Technologies, Böblingen, Germany) coupled to a Quattro Micro triple 770 quadrupole mass spectrometer (Waters Coporation, Manchester, United Kingdom). Separation 771 was performed using a Zebron multi-residue1 column (30 m x 250 µm id, 0.25µm film 772 thickness, Phenomenex, Denmark) equipped with a 5 m guard column (Restek Corporation, 773 Bellefonte, PA, USA) at 1ml/min with Helium as a carrier gas. Similar to PCBs, samples 774 injected using a split/splitless injector equipped with a 4 mm id packed (wool) Sky liner (Restek 775 Corporation, Bellefonte, PA, USA) in splitless overflow mode using a 5 µL injection volume at 776 220°C. Oven temperature program started at an initial temperature of 85°C (1.0 min hold), 777 ramped at 25°C/min to 280°C (hold 2 min), followed by a second temperature ramp of 40°C/min 778 to 300°C (hold 5 min). Detection of DDT and DDE isomers my mass spectrometry used 779 conditions as described for PCB analysis. Parent and 13C12 mass-labelled internal standards are 780 outlined in Supplementary Table 2. 781 782 Analysis of organochlorine pesticides

783

Analysis of organochlorine pesticides was performed by GC/MS using an Agilent 7890A gas

- chromatograph equipped with a 5975C inert XL mass spectrometer (Agilent Technologies,
- Böblingen, Germany). Separation was carried out using a DB-Ultra2 column (25 m x 200 µm id,
- 787 0.11µm film thickness, J&W, Folsom, USA) equipped with a 5 m guard column (Restek

- 788 Corporation, Bellefonte, PA, USA) at 1ml/min with Helium as a carrier gas. Injection of samples
- 789 was carried out under similar conditions as described for DDT/DDE isomers. Oven temperature
- 790 program started at an initial temperature of 80°C (2 min hold), ramped at 20°C/min to 100°C
- (hold 5 min), 20°C/min to 170°C (hold 3 min), 5°C/min to 200°C, and a final ramp of 20°C/min
- to 300 (hold 2 min). Detection of organochlorine pesticides was performed using chemical
- ionization with methane as a reagent gas at a flow rate of 0.4 ml/min with a source temperature
- of 160°C. Parent and 13C12 mass-labelled internal standards are outlined in Supplementary
- 795 Table 2.
- 796
- 797 Supplementary Table 2. Quantification and qualifier ions for targeted compounds and their
- 798 13C-mass labelled internal standards.

Compound	Quantification/Qualifier ions
PCBs	
Tri-chlorinated PCB	256/258
13C12- Tri-chlorinated PCB	268
Tetra-chlorinated PCB	290/292
13C12- Tetra-chlorinated PCB	302
Penta-chlorinated PCB	324/326
13C12- Penta-chlorinated PCB	336
Hexa-chlorinated PCB	360/362
13C12- Hexa-chlorinated PCB	370
Hepta-chlorinated PCB	392,394
13C12- Hepta-chlorinated PCB	404
DDT/DDE isomers	
o,p-DDE	246/248
p,p-DDE	246/248
13C12-p,p-DDE	258
o,p-DDD	235/237
p,p-DDD	235/237
o,p-DDT	235/237
p,p-DDT	235/237
13C12 p,p-DDT	247
Organochlorine pesticides	
HCH isomers	253/255

13C6-HCH isomers	260	799
НСВ	284/286	
13С6 -НСВ	290	
Oxychlordane	350/352	
trans-chlordane	408/410	
cis-chlordane	408/410	
13C12 –cis-chlordane	418	
trans-nonachlor	442/444	
cis-nonachlor	442/444	
13C12-cis-nonachlor	452	
Mirex	441/439	
13C12-Mirex	449	<u> </u>

801 Quality Assurance

- To avoid the reporting of false positives, concentrations were only reported for targeted analytes
 in which both quantification and qualifier ions were detected. In addition, target compounds
 detected in sample extracts must possess ion ratio between quantification and qualifier within
 20% to the ion ration observed within the quantification standard. A minimum of three method
 blanks were extracted with each sample batch to calculate limits of detection per extraction
 batch. Extraction recoveries for internal standards from sample extracts are listed in
 Supplementary Table 3.

Supplementary Table 3. Internal standard recoveries (%) and variation within sample extracts.

Compound	Average Recovery (%) and variation
13C12-PCB 28	94 ± 19
13C12-PCB 52	93 ± 16
13C12-PCB 101	90 ± 16
13C12-PCB 118	90 ± 15
13C12-PCB 138	86 ± 16
13C12-PCB 153	88 ± 17
13C12-PCB 180	90 ± 18
13C12-p,p'-DDE	92 ± 14
13C12-p,p'-DDT	59 ± 23

13С6-αНСН	81 ± 17	
13С6-βНСН	75 ± 24	
13С6-үНСН	85 ± 19	
13C6-HCB	80 ± 19	
13C12-trans-chlordane	99 ± 22	
13C12-cis-chlordane	96 ± 21	
13C12-trans-nonachlor	101 ± 24	
13C12-cis-nonachlor	84 ± 14	
13C12-trans-nonachlor	94 ± 20	