Interspecies comparisons of brominated flame retardants in relation to foraging ecology and behaviour of gulls frequenting a UK landfill

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Abstract

This study quantifies and compares concentrations and profiles of legacy and alternative (alt-) brominated flame retardants (BFRs) in the eggs of three gull (Laridae) species of international/UK conservation concern – great black-backed gulls (*Larus marinus; n* = 7), European herring gulls (*L. argentatus; n* = 16) and lesser black-backed gulls (*L. fuscus; n* = 11) in relation to their foraging ecology and behaviour in order to investigate potential exposure pathways at a remote landfill in western Scotland, UK. Egg concentrations of sum ($\sum$) polybrominated diphenyl ethers ($\sum_8$PBDEs) in all three species exceeded those for most reported avian species using landfill, except for those in North America. Despite relatively high detection frequencies of $\sum$hexabromocyclododecanes ($\sum_3$HBCDDs) (94–100%), concentrations of $\sum_8$PBDEs exceeded $\sum_3$HBCDDs and $\sum_5$alt-BFRs, with $\sum_8$PBDE levels similar in all three species. Egg carbon isotopic (δ^{13}C) values highlighted a greater marine dietary input in great black-backed gulls that was consistent with their higher BDE-47 levels; otherwise, dietary tracers were minimally correlated with measured BFRs. $\sum_3$HBCDD egg concentrations of herring gulls markedly exceeded those reported elsewhere in Europe. Decabromodiphenylethane (DBDPE) was the only alt-BFR detected (6–14% detection rate), in a single egg of each species. The great black-backed gull egg contained the highest concentration of DBDPE measured in biota to date globally and provides strong evidence for its emerging environmental presence as a BDE-209 replacement in UK wildlife. Correlations between δ^{13}C (dietary source) and some measured BFRs in eggs suggest multiple routes of BFR exposure for gulls frequenting landfill through their diet, behaviour, preening, dermal exposure and likely inhalation. The frequent use of landfill by herring gulls and their

44 increased egg FR burdens suggest that this species may be an important bioindicator of BFR

45 emissions from such sites.

46 **Keywords**

47 Birds, PBDEs, HBCDD, DBDPE, Stable isotopes, Behaviour

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49 **1. Introduction**

50   Brominated flame retardants (BFRs) are synthetic organohalogens that have been

51 applied to many manufactured polymeric products to impart fire retardancy (De Wit, 2002;

52 Jenssen et al., 2007). Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane

53 (HBCDD) were widely used BFRs historically but are now strictly regulated given their

54 toxicity, persistence and capacity for bioaccumulation, biomagnification, and long-range

55 atmospheric transportation. As a result, both of these 'legacy' BFRs are classed as persistent

56 organic pollutants (POPs) under the Stockholm Convention (Stockholm Convention, 2019).

57 In addition, and often in response to the regulation of legacy BFRs, manufacturers have

58 developed alternative BFRs (alt-BFRs), which have been subject to recent research into their

59 environmental presence and effects on biota (e.g., Marteinson and Fernie, 2019; Marteinson

60 et al., 2017; Verreault et al., 2018).

61   Given that they are repositories of obsolete consumer products, waste management

62 facilities such as municipal solid waste landfill/dump sites (hereafter 'landfill') often contain

63 elevated concentrations of PBDEs, HBCDD, and alt-BFRs in abiotic media such as air, soil,

64 and leachate (Eguchi et al., 2013; Harrad et al., 2019a; Morin et al., 2017) as a result of

65 weathering and abrasion of polymeric items (Stubbings and Harrad, 2014). Consequently,

66 landfills are well documented as major sources of these contaminants. Because of the large

67 quantities of human food refuse that can also enter landfill, various bird species routinely

68 forage at such sites (Oro et al., 2013; Plaza and Lambertucci, 2017). Increasingly, it has been

69 recognised that birds associated with landfill exhibit elevated legacy BFR and alt-BFR

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70 burdens (Tongue et al., 2019), which is of concern since legacy and some alt-BFRs have been

71 demonstrated to affect birds adversely through studies of reproductive success, behaviour and

72 growth of free-living and captive species (Guigueno and Fernie, 2017). Although attention is

73 now being paid to landfill as a source of organohalogen exposure in wild birds (Chen et al.,

74 2013; Tongue et al., 2019), ours is the first study to examine such exposure in the UK and

75 among different bird species using landfill.

76   The exposure of free-living birds to flame retardants (FRs) and other contaminants

77 can occur through multiple routes, including their diet, feather preening, dermal exposure and

78 inhalation. In studies of the avian diet, nitrogen (δ^{15}N) stable isotope values have been

79 examined to characterise trophic position (Bearhop et al., 2002; Hobson et al., 1994). By

80 comparison, carbon (δ^{13}C) tracers determine relative contributions of marine and terrestrial

81 food sources (Hobson, 1987; Inger and Bearhop, 2008), while sulphur (δ^{34}S) values allow

82 more precise determination of these nutrient sources in the same marine *vs*. terrestrial

83 environments (Eulaers et al., 2014; Hobson et al., 1997). Chen et al. (2012) and Roscales et

84 al. (2016) successfully used these dietary tracers in studying avian trophodynamics of

85 environmental contaminants. Direct ingestion of FR-treated items and their abraded particles

86 may be an important route of exposure and uptake by birds (Seif, 2017) that is rarely

87 explored (Brusseau et al., 2019), including in birds utilising landfill (Tongue et al., 2019).

88 Avian behaviours can influence exposure to FRs, including feather preening, when preen oil

89 traps contaminant particulates on the plumage which is inadvertently ingested during feather

90 maintenance (Jaspers et al., 2007). Exposure may also result from inhalation of airborne

91 contaminants by birds because of their specialised respiratory system (Brown et al., 1997).

92 Finally, dermal contact may also be a major exposure route (Mineau, 2011), particularly in

93 gulls that forage on landfill substrate, thereby potentially exposing the skin of their webbed

94 feet, legs, and facial bare parts to FRs.

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95   In north-western Europe, several gull species are associated with landfill, including

96   great black-backed gulls (*Larus marinus*), European herring gulls (*L. argentatus*) (hereafter

97   'herring gulls') and lesser black-backed gulls (*L. fuscus*) (Coulson, 2019; Greig et al., 1986;

98   Horton et al., 1983) (Table S1). If landfill is a considerable source of exposure for such gulls

99   to accumulate FRs (and other environmental pollutants), this may potentially be of

100   conservation significance. Herring gulls are designated as 'Near Threatened' in the EU

101   (BirdLife International, 2015), while great black-backed gulls and lesser black-backed gulls

102   are of conservation concern in the UK (Eaton et al., 2015). The exposure and accumulation of

103   BFRs by these species while frequenting landfill have previously not been considered in a

104   conservation context, nor has any study to date examined BFR profiles and concentrations

105   across an avian species assemblage associated with landfill. Here, we address the following

106   objectives: (i) to assess and compare the concentrations and profiles of PBDEs, HBCDD, and

107   five alt-BFRs accumulated in eggs laid by great black-backed gulls, herring gulls, and lesser

108   black-backed gulls breeding adjacent to a landfill frequented by gulls; (ii) to investigate BFR

109   trophodynamics using stable isotope analyses of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ in egg contents; and (iii)

110   to identify and characterise possible (behavioural) routes of BFR contamination in birds using

111   landfill.

112

113   **2. Materials and methods**

114   This study was conducted with the necessary authorisation from Scottish Natural

115   Heritage (SNH; licence numbers 77830, 92331 & 112381) for egg collection; birds were not

116   directly handled and so no other licences or permits were required.

117

118   *2.1. Study site*

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119   The study was conducted at an active landfill (behavioural observations) with eggs

120   collected from a nearby (~2 km distant) mixed breeding colony of herring gulls, great black-

121   backed gulls and lesser black-backed gulls, located in western Scotland, UK (Fig. 1). During

122   2016–2018, the landfill received on average 16,000 metric tonnes of household and

123   commercial waste annually from a human population of ~20,000 (Scottish Environmental

124   Protection Agency information request response).

125

126   *2.2. Egg sampling*

127   Locally breeding gulls are known to begin egg laying in late April (Kim et al., 2010)

128   when maternal deposition of BFR burdens into eggs occurs, since gulls rely on exogenous

129   nutrients gathered immediately prior to egg formation (Drent and Daan, 1980). Table S1

130   details the number of eggs of each species collected during our study and provides general

131   information about diet and local movements of each species. Eggs were collected between

132   April and May 2016 from a mixed colony of gulls containing approximately 250 pairs of

133   herring gulls, 30 pairs of lesser black-backed gulls, and three pairs of great black-backed

134   gulls. Due to the comparatively low number of great black-backed gull eggs collected in 2016

135   ($n = 4$), an additional three eggs of this species were collected from the same colony in 2017

136   ($n = 2$) and 2018 ($n = 1$) and analysed. From randomly-selected individual nests containing

137   full clutches of three eggs, the estimated largest egg was collected between ~1–6 d following

138   incubation onset as assessed via flotation testing (OSPAR, 2000) and individually identified.

139   Egg length and breadth were recorded to the nearest 0.1 mm using digital Vernier calipers

140   (MachineMart, Nottingham, UK), allowing the calculation of volume (Narushin, 2005). Eggs

141   were weighed (to the nearest 0.1 g) (On Balance, Liverpool, UK), wrapped in aluminium foil,

142   placed into individually-labelled Whirlpak™ sample bags (Nasco, Fort Atkinson, WI, USA),

143   and stored securely in foam-lined Peli Storm iM2300™ cases (Pelican Products, Torrance,

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144   CA, USA) until transportation to the University of Birmingham, UK for laboratory analysis.

145   Egg contents were collected, homogenised and frozen at -70°C until chemical analysis.

146

147   *2.3. Sample extraction and clean-up*

148   Egg samples underwent a combined pressurised liquid extraction (PLE) and clean-up

149   process for all target BFRs, which comprised the following PBDE congeners: BDEs -28, -47,

150   -99, -100, 153, -154, -183 and -209, α-, β- and γ- HBCDD diastereomers, and five alt-BFRs:

151   (1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenylethane (DBDPE), 2-

152   ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), pentabromobenzene (PBB), and

153   pentabromoethylbenzene (PBEB). Aliquots (~1 g wet weight) of homogenised egg sample

154   were accurately weighed and loaded into a pre-cleaned 66 mL Dionium™ extraction cell pre-

155   packed from the bottom upwards with: two glass fibre filters (GFFs), 3 g of pre-cleaned

156   hydromatrix, 2 g of 1% deactivated silica, 1 GFF, 10 g of 44% acid impregnated silica, and 4

157   g of Florisil. Cells were then spiked with known quantities of internal standards (BDE-77,

158   BDE-128, $^{13}C_{12}$-BDE-209, $^{13}C_{12}$- α-, β- and γ-HBCDD, and $^{13}C_6$-BTBPE). Cells underwent

159   PLE on a Dionex Accelerated Solvent Extractor (ASE) 350 at a pressure of 1,500 psi with

160   hexane/dichloromethane ((DCM); 3:1, v/v ratio) as the extraction solvents. The oven

161   temperature was 90°C, with a heating time of 5 min. Each sample underwent three static

162   cycles with a static time of 4 min, purge time of 90 sec and flush volume of 40%. Clean

163   extracts were transferred to 200 mL tubes and concentrated to near-dryness at 40°C under a

164   gentle stream of nitrogen. The sample was reconstituted in 50 µL of toluene containing 10 ng

165   each of PCB-129 and d_{18}-γ-HBCDD as recovery determination standard (RDS), sonicated for

166   10 sec and transferred to a labelled glass-inserted vial prior to analysis.

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168   *2.4. Instrumental analysis*

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169   HBCDD levels in eggs were determined on a Shimadzu LC-20AB liquid

170   chromatograph (LC) (Shimadzu Corporation, Kyoto, Japan), coupled to an AB Sciex API

171   2000 triple quadropole mass spectrometer (MS/MS) (Applied Biosystems, Foster City, CA,

172   USA). Full details of the LC-MS/MS methodology have been published previously (Abdallah

173   et al., 2008). Concentrations of PBDEs and alt-BFRs were determined on a Thermo Scientific

174   Trace 1310 gas chromatograph (GC) coupled to a Thermo Scientific ISQ MS. Full GC/MS

175   parameters are provided in Abdallah et al. (2017).

176

177   *2.5. Lipid analysis of egg samples*

178   The lipid content of egg samples was determined gravimetrically. One gramm of

179   sample was weighed into an ASE cell containing clean hydromatrix (*W1*). The cell was then

180   extracted following the same conditions as for BFR extraction (see Section 2.3). An empty

181   Turbovap™ tube was weighed (*W2*) and the entire extract was transferred to the tube. The

182   extract was concentrated to dryness at 40°C under a gentle stream of nitrogen and the

183   Turbovap™ tube was re-weighed (*W3*). The lipid content was determined using the equation:

184    $Lipid\ content\ (\%) = \dfrac{(W3 - W2)}{W1} \times 100$

185

186   *2.6. Quality Assurance/Quality Control*

187   A reagent blank was analysed with every batch of nine samples. In the majority of

188   sample batches, none of the target compounds was measured above the limit of detection

189   (LOD). In these cases, the samples were assigned limits of quantification (LOQs) based on a

190   signal to noise ratio of 10:1. However, in three batches of samples, BDE-209 was detected in

191   the blank above the LOD (0.95, 0.90 and 0.95 ng/g). In these instances, the LOQ was

192   reported as the average blank plus three times its standard deviation (i.e., 1.0 ng/g). For the

193   three sample batches where BDE-209 was detected in reagent blank samples, the

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194 concentration of BDE-209 in each sample was corrected by subtracting from the

195 concentration in the sample the average blank concentration plus three times its standard

196 deviation. If the blank concentration was > 50% of the sample concentration (which occurred

197 in the case of four samples), the sample was reported as < 1.0 ng/g.

198   In the absence of an appropriate certified reference material, the full analytical

199 method was validated by replicate analysis ($n = 10$) of domestic chicken (*Gallus gallus*) egg

200 spiked with known concentrations of target compounds. Concentrations of target analytes

201 were then measured according to the above analytical protocols. All measured concentrations

202 were 80–120% of their spiked concentration, with a relative standard deviation of < 15%. For

203 ongoing accuracy and precision, a control sample ($n = 9$) spiked with target compounds was

204 analysed every twentieth sample and was required to be within 80–120% of the spiked

205 concentration for the sample batch to be accepted. Full details of the method validation and

206 ongoing accuracy and precision are provided in the Supplementary Information (SI) (Tables

207 S2 and S3).

208

209 *2.7. Stable isotope analysis (SIA)*

210   Analysis of homogenised egg (i.e., yolk and albumen) contents for $\delta^{13}C$, $\delta^{15}N$ and

211 $\delta^{34}S$ (using lipid extracted aliquots in the case of $\delta^{13}C$) was carried out at the Scottish

212 Universities Environmental Research Centre (SUERC) at East Kilbride, UK, using

213 standardised methods (Bell et al., 2017; Webb et al., 2017) for a subset of analysed eggs laid

214 by great black-backed gulls ($n = 5$; collected 2016–2018), herring gulls ($n = 14$; 2016) and

215 lesser black-backed gulls ($n = 7$; 2016). Prior to SIA, aliquots of egg (~2 mL) were freeze-

216 dried for 24 hours using a Christ Beta 1–8 LSCplus freeze-dryer (Martin Christ, Osterode am

217 Harz, Germany). Further details of SIA methodology are provided in the SI.

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219 *2.8. Behavioural observations*

220   Behavioural observations (undertaken only for gulls in adult plumage) coincided with

221 the likely period of yolk formation in large gulls (Roudybush, 1979) and further details are

222 provided in the SI. Briefly, data were obtained in 2018 by video recording of gull foraging

223 behaviour on the active tipping area of the landfill and observing the incidence of preening at

224 a separate area within the landfill where gulls gathered to loaf (i.e., rest). Data were collected

225 during landfill operational hours (i.e., Monday to Friday; 09.00–16.00hrs GMT; 9–20 April

226 2018). In addition, counts and observations of loafing birds were made at 30-minute intervals

227 and the following recorded: total numbers of birds of each species resting and the incidence

228 and duration of preening performed by individual adult (i.e., potentially breeding-age) gulls.

229 Given resource and time constraints, it was not possible to collect eggs from individuals

230 clearly identified and known to frequent the landfill, although the constant movement of birds

231 between the landfill and the colony suggested that the landfill was an important and

232 regularly-used site for these birds. Data were extracted from video footage following (Greig

233 et al., 1985, 1986). For foraging observations, an observation period of 15 sec per bird was

234 defined, during which the number of (i) pecks made into the substrate, (ii) swallowing events,

235 and (iii) paces across the substrate (scored from 0 to 3: 0 = 0 paces, 1 = 1–4 paces, 2 = 5–10

236 paces, and 3 => 10 paces) were recorded. The mean number of paces was estimated and

237 scored from 1 to 3: 1 = 2.5 paces, 2 = 7.5 paces, 3 = 15 paces (approx.). To provide further

238 insight into potential dermal exposure we also estimated and ranked the mean length of time

239 each bird stood stationary while foraging (0 = 0 sec, 1 = 1–5 sec, 2 = 6–10 sec, and 3 = 11–15

240 sec) or not foraging (1 = 2.5 sec, 2 = 7.5 sec, and 3 = 12.5 sec) within the 15 sec

241 observational period. These behaviours were identified as some of the most likely behaviours

242 that would result in birds being exposed to FRs (Table S5). We analysed both the number of

243 paces made and time spent stationary because either behaviour may potentially expose birds

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244 to FRs via dermal contact (Alharbi et al., 2016; Henderson et al., 1994; Mineau, 2011). Review

245 of video footage yielded 2,329 observations of foraging birds, consisting of 255 observations

246 of great black-backed gulls, 1,961 of herring gulls and 113 of lesser black-backed gulls. Over

247 the course of a given day, gulls regularly loafed (rested) on a plastic and gravel-covered

248 former waste pile on site, located ~400 m from the active tip face. Counts and observations of

249 loafing birds were made at 30-min intervals and recorded on pre-printed field datasheets.

250

251 *2.9. Data visualisation, manipulation and statistical methods*

252   For statistical purposes, in samples where the detection frequency (DF) was lower

253 than 50%, "zero" values were replaced with the DF multiplied by the LOQ. For samples

254 where the DF was $\geq 50\%$, values of zero were replaced with $0.5 \times$ LOQ. Those measured

255 compounds for which detection frequencies were $< 30\%$ for all three species were excluded

256 from statistical analyses (Table 1). All statistical analyses were undertaken using R (R Core

257 Team, 2018). Data were checked for normality via visual inspection and Shapiro-Wilk tests.

258 Homogeneity of variance was assessed using the Levene's test. The BFR data were not

259 normally distributed and could not be successfully transformed to normality. Therefore, the

260 non-parametric Kruskal-Wallis (K-W) test was used to compare egg BFR concentrations and

261 relative contributions between species with subsequent pairwise Mann-Whitney (M-W) post-

262 hoc tests using a Holm correction. Stable isotope data were normally distributed and therefore

263 interspecies comparisons of these data were made using one-way analysis of variance

264 (ANOVA), with post-hoc testing undertaken using the Games Howell test. The 'Corrplot'

265 package in R (Wei and Simko, 2017) with Spearman's correlation coefficient was used to

266 examine relationships between egg BFR concentrations and isotope values. Confidence limits

267 were set to 95% and an alpha threshold of 0.05 was used for statistical comparisons, except

268 for behavioural data when this was adjusted to 0.01 (after Grant and Grant, 2002; Portugal et

11

269   al., 2010) since statistical independence of behavioural data could not be guaranteed as gulls

270   were not individually identifiable.

271

272   **3. Results**

273   *3.1. Profiles and concentrations of PBDEs in gull eggs*

274   Detection frequencies of each of the eight measured PBDE congeners (i.e., BDEs -28,

275   -47, -99, -100, -153, -154, -183, and -209) varied amongst the three gull species observed on

276   the landfill and were most frequently detected in the eggs of great black-backed gulls (71–

277   100%), followed by lesser black-backed gulls (45–90%) and then herring gulls (62–88%)

278   (Table 1). The PBDEs with the highest detection rates were similar in the eggs of lesser

279   black-backed gulls (BDE -47 ≈ -153 ≈ -209) and herring gulls (BDE-47 ≈ -153 ≈ -154 ≈ -

280   209) in contrast to the eggs of great black-backed gulls (BDE-47 > -99 ≈ -153 ≈ -183) (Table

281   1).

282   There were no significant interspecies differences in egg concentrations of $\sum_8$PBDEs

283   (K-W test: $H = 0.2$, df = 2, $P = 0.91$) or of $\sum_7$PBDEs (i.e., excluding BDE-209; K-W test: $H =$

284   0.01, df = 2, $P = 0.99$) (Table 1). However, there was a significant interspecies difference for

285   egg concentrations of BDE-47 (K-W test: $H = 7.3$, df = 2, $P = 0.02$). Post-hoc (M-W) testing

286   showed BDE-47 concentrations to be significantly higher in great black-backed gulls

287   compared to the other two species (pairwise post-hoc tests: both Ps ≤ 0.03) that were

288   statistically similar to each other (Fig. 2). There were no other significant interspecies

289   differences for concentrations of the remaining BDE congeners (pairwise post-hoc tests: all

290   Ps ≥ 0.20). In terms of relative contributions to $\sum_8$PBDE burdens, BDE-99 followed by BDE-

291   209 were the most prevalent congeners in the eggs of the great black-backed gulls and lesser

292   black-backed gulls in contrast to BDE-209 and, to a lesser extent, BDE-99 in herring gulls

293   (Fig. 3). In addition, the eggs of great black-backed gulls showed a significantly greater

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294 relative mean contribution of BDE-47 to their $\sum_8$PBDE burdens compared to the eggs of

295 herring gulls and lesser black-backed gulls (K-W test: $H = 9.7$, df = 2, $P = 0.007$; pairwise

296 post-hoc M-W tests: Ps ≤ 0.03) (Fig. 3). The relative contributions to $\sum_8$PBDEs of the other

297 individual congeners were comparable across the three species (K-W tests: all Ps ≥ 0.10).

298

299 *3.2. Profiles and concentrations of HBCDD in gull eggs*

300 We detected α-HBCDD in all great black-backed gull and lesser black-backed gull

301 eggs and in 87% of herring gull eggs (Table 1). There was marked variation among the three

302 species in the detection frequency of γ-HBCDD, which was 100% in great black-backed gull

303 eggs, 87% in herring gull eggs, but only 27% in lesser black-backed gull eggs. There were

304 significant interspecies differences in egg concentrations of $\sum_3$HBCDDs (K-W test: $H = 6.4$,

305 df = 2, $P = 0.04$), with pairwise post-hoc M-W tests showing that great black-backed gull

306 eggs contained significantly higher concentrations than lesser black-backed gulls ($P = 0.05$)

307 (Table 1). This appeared to be driven by the much lower detection rate (27%) of γ-HBCDD

308 in lesser black-backed gull eggs compared to great black-backed gulls (100%). The mean

309 relative contribution of α-HBCDD to $\sum_3$HBCDD significantly varied among the three species

310 (K-W test: $H = 11.8$, df = 2, $P = 0.002$) and was significantly higher in lesser black-backed

311 gulls (90%) than in herring gulls (62%) or great black-backed gulls (49%) (pairwise M-W

312 post-hoc tests: Ps ≤ 0.01). Correspondingly, the mean relative contribution of γ-HBCDD to

313 $\sum_3$HBCDD varied among all three species (K-W test: $H = 13.5$, df = 2, $P = 0.001$) and was

314 significantly lower in lesser black-backed gulls (10%) compared to both great black-backed

315 gulls (51%) and herring gulls (37%) (pairwise M-W post-hoc tests: Ps ≤ 0.01).

316

317 *3.3. Alt-BFR concentrations in Eggs*

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318   DBDPE was detected in only three of the 34 eggs collected, being in one egg of each

319   species. Specifically, the DBDPE concentration in the great black-backed gull egg (7,700

320   ng/g lw) exceeded significantly that in the egg of the herring gull (57 ng/g lw) and in the egg

321   of the lesser black-backed gull (68 ng/g lw). This represents DBDPE detection frequencies of

322   only 14%, 6%, and 9%, respectively. None of the other targeted alt-BFRs was detected in any

323   of the eggs analysed.

324

325   *3.4. Stable isotope analysis*

326   The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ dietary tracer values measured in eggs laid by the three gull

327   species are shown in Table 1. The only isotopic value that demonstrated significant

328   interspecies differences was $\delta^{13}C$ (dietary source) (one-way ANOVA: $F_{2,23} = 8.0$, $P = 0.002$)

329   (Fig. 4), with no significant differences among the three species in egg $\delta^{15}N$ (trophic position:

330    $F_{2,23} = 0.5$, $P = 0.60$) and $\delta^{34}S$ (dietary source: $F_{2,23} = 1.6$, $P = 0.20$). Post-hoc tests revealed

331   that there were significantly higher $\delta^{13}C$ values in eggs of great black-backed gulls ($P = 0.01$)

332   and herring gulls ($P = 0.02$) than those of lesser black-backed gulls, with no significant

333   difference in $\delta^{13}C$ egg values between great black-backed gulls and herring gulls ($P = 0.61$)

334   (Table 1; Fig. 4). A significant negative correlation was found between egg $\delta^{15}N$ isotopic

335   values and BDE-28 egg concentrations of herring gulls ($r = -0.32$, df =12, $P = 0.02$) only

336   (Fig. 5), but no other significant correlations were found between isotopic values and other

337   measured BFR concentrations in any of the three species.

338

339   *3.5. Behavioural observations*

340   Vantage point surveys revealed a regular movement of all three gull species between

341   the breeding colony and the landfill (Fig. 1). At any one time we observed a maximum of

342   ~800 birds at the landfill, with herring gulls comprising ~90% of all birds, while great black-

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343 backed gulls and lesser black-backed gulls made up ~7% and ~3% of the total birds observed

344 on the landfill, respectively.

345   Foraging strategy differed among the three gull species, although data were zero-

346 inflated (i.e., containing a preponderance of zero observations) for many of the behaviours.

347 The frequency of pecks at the substrate differed significantly between species overall (K-W

348 test: $H = 58.3$, df = 2, $P < 0.001$; Fig. 6a). Pairwise post-hoc comparisons showed that there

349 were significant differences between great black-backed gulls and herring gulls and between

350 lesser black-backed gulls and herring gulls (M-W tests: Ps < 0.001). Herring gulls were

351 observed pecking the landfill substrate more often (i.e., the lowest percentage of zero pecks

352 per bird-observation) (23%) than lesser black-backed gulls (30%) or great black-backed gulls

353 (51%), and while highly zero inflated, the number of observed swallowing events was nearly

354 significantly different among species (K-W test: $H = 8.3$, df = 2, $P = 0.02$). There was also a

355 significant difference overall in the number of paces taken across the landfill substrate (K-W

356 test: $H = 46.3$, df = 2, $P < 0.001$; Fig. 6b), with lesser black-backed gulls pacing (median: 7.5

357 paces) significantly more than the other two species (medians: 2.5 for each species; pairwise

358 M-W post-hoc test: Ps < 0.0001). The time spent stationary when foraging on the landfill

359 differed significantly overall (K-W test: $H = 93.5$, df = 2, $P < 0.0001$; Fig. 6c), with great

360 black-backed gulls (median: 12.5 sec) significantly more stationary than herring gulls

361 (median: 7.5 sec) or lesser black-backed gulls (median: 2.5 sec) (pairwise M-W post-hoc

362 tests: Ps < 0.0001). Episodes of preening by birds on the landfill were rare across all species

363 (15% of 129 observations) and in each species (13–15%), with similar duration of preening

364 for each species (100–116 sec).

365

366 **4. Discussion**

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367   In this study we observed interspecies differences in *in ovo* detection rates, profiles

368 and concentrations of BDE-47 and $\sum_3$HBCDDs among three gull species frequenting a

369 Scottish landfill. Of the five alt-BFRs targeted, DBDPE was the only one detected and with

370 one of the three eggs having the highest concentration reported in biota globally to date. The

371 diets of great black-backed gulls and herring gulls had greater marine input (as reflected in

372 egg δ^{13}C isotopic values) compared to the more terrestrially-based diet of the lesser black-

373 backed gulls, although finer marine-terrestrial dietary details (δ^{34}S) and trophic position

374 (δ^{15}N) were similar among the three species. Interestingly, dietary trophic level (δ^{15}N) and

375 BDE-28 were negatively correlated in herring gulls. There were interspecies behavioural

376 differences for gulls foraging on landfill which are potentially relevant as possible FR

377 exposure pathways.

378

379 *4.1. PBDE and HBCDD concentrations in gull eggs*

380   Compared to BFR burdens reported in other birds known to frequent landfill,

381 $\sum_8$PBDE egg concentrations of our great black-backed gulls, herring gulls, and lesser black-

382 backed gulls (i.e., 35, 54 and 61 ng/g ww, respectively; Table 1) exceed those reported for

383 white storks (*Ciconia ciconia*; 4.4 ng/g ww) in Spain (Muñoz-Arnanz et al., 2011), and

384 African sacred ibis (13.2 ng/g ww) in South Africa (Polder et al., 2008). Furthermore,

385 $\sum_8$PBDE egg concentrations of herring gulls and lesser black-backed gulls in our study

386 exceeded $\sum_{10}$PBDE egg concentrations (also including BDE-209) of yellow-legged gulls (*L.*

387 *michahellis*) (i.e., 38 ng/g ww) in Spain (Roscales et al., 2016). Such differences are likely

388 influenced by various factors, including the history of FR use in the respective countries

389 concerned, as well as species- and congener-specific toxicokinetics and metabolism (Chen

390 and Hale, 2010). Our egg samples were obtained from a landfill in a relatively remote

391 location in rural Scotland where there were likely to be fewer alternative sources of

16

392   environmental exposure to FRs (Fig. 1). In contrast, Polder et al. (2008) and Roscales et al.

393   (2016) studied birds laying eggs in predominantly urban/industrial locations. In this context,

394   our findings emphasise the importance of landfill (however remote) as a source of BFR

395   exposure and accumulation by birds frequenting such locations. Moreover, the gull eggs in

396   our study contained ∑PBDE concentrations that exceeded the 29 ng/g ww effects-level

397   threshold set by the Canadian Federal Environmental Quality Guidelines for avian eggs

398   (Environment and Climate Change Canada, n.d.), suggesting that the birds in our study could

399   have been exposed to potentially deleterious effects following PBDE exposure; nevertheless,

400   species' differences in sensitivity to the toxicity of PBDEs must also be acknowledged.

401   Some studies have found higher ∑PBDE egg concentrations in birds associated with

402   landfill across Canada, with concentrations of 268 ng/g ww (excluding BDE-209) in eggs of

403   common starlings (*Sturnus vulgaris*) (Eens et al., 2013), and 230 ng/g ww (including BDE-

404   209) in those laid by American herring gulls (*L. smithsonianus*) (Chen et al., 2012). Great

405   black-backed gulls in the Gulf of St Lawrence (Canada) laid eggs containing comparatively

406   elevated $\sum_5$PBDE (BDEs -47, -99, -100, -153, and -154) concentrations (i.e., $1{,}643 \pm 234$

407   ng/g lw) (Lavoie et al., 2010), although this study did not reveal whether that population was

408   associated with landfill. Elevated PBDE egg concentrations in these Canadian gulls likely

409   reflect the greater and longer period of use of PBDEs (particularly lower-brominated

410   commercial mixtures) in North America compared with in Europe (BSEF, 2003). However,

411   *in ovo* BDE-209 concentrations of up to 137 ng/g ww were reported in herring gulls breeding

412   in Canada (Chen et al., 2012), and indeed some of the highest avian liver concentrations of

413   BDE-209 ever recorded were in ring-billed gulls that frequently fed on landfill in Montreal,

414   PQ, Canada (57.2 ± 12.2 ng/g ww; Gentes et al., 2012).In terms of individual PBDE

415   congeners, the comparatively broad foraging niche and elevated trophic position of the great

416   black-backed gulls in the present study likely explain the high concentrations and relative

17

417　contribution of BDE-47 to the total PBDE burden found in their eggs compared to those of

418　herring gulls and lesser black-backed gulls (Fig. 2). Certainly, the predominance of BDE-47

419　in the PBDE congener egg profile is commonly associated with birds feeding predominantly

420　in aquatic rather than terrestrial food webs (Chen and Hale, 2010). Similarly, high

421　concentrations of BDE-47 were reported in eggs laid by piscivorous Audouin's gulls

422　(*Ichthyaetus audouinii*) compared to those laid by yellow-legged gulls that are dietary

423　generalists (Roscales et al., 2016).

424　　　　In the present study, the mean $\sum_3$HBCDD egg concentrations of great black-backed

425　gulls and herring gulls (i.e., 9.3 and 30 ng/g ww, respectively) exceeded those of glaucous-

426　winged gulls (*L. glaucescens*) associated with landfill in Canada (i.e., 4.5 ng/g ww). Herring

427　gulls in our study also had higher $\sum_3$HBCDD concentrations compared to herring gulls (i.e.,

428　16.6 ng/g ww) using landfill in Canada (Chen et al., 2012). In comparison with Europe,

429　HBCDD was generally used to a lesser extent in North America (BSEF, 2003; Law et al.,

430　2014). There are relatively few studies of egg HBCDD concentrations for known populations

431　of our target species that use landfill, with which we can compare our findings. However, for

432　illustrative purposes only we found that the $\sum_3$HBCDD concentrations in eggs of lesser

433　black-backed gulls (i.e., 2.2 ng/g ww) were comparable with those reported in eggs of

434　African sacred ibises using landfill in South Africa (1.9 ng/g ww; Polder et al., 2008),

435　although these $\sum_3$HBCDD concentrations were both exceeded by those in our great black-

436　backed gull eggs (Table 1).

437　　　　As reported previously in other biota, α-HBCDD is the most frequently found

438　HBCDD diastereomer in avian tissues (Letcher et al., 2015). The substantial relative

439　contribution of γ-HBCDD to $\sum_3$HBCDD burdens in the eggs of herring gulls and especially

440　great black-backed gulls in our study, conflicts with other published findings. For example, in

441　great black-backed gulls and herring gulls breeding in Norway, α-HBCDD comprised 97%

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and 100% of egg ∑₃HBCDD concentrations, respectively (Haukås et al., 2009; Helgason et al., 2009), and > 90% of the egg burdens of herring gulls breeding in Germany (Esslinger et al., 2011). The three gull species frequenting landfill in our study may have been exposed to more recent sources of HBCDD such as waste materials treated with this BFR, with γ-HBCDD being the dominant diastereomer in the commercial formulation (Harrad et al., 2019b; Law et al., 2005). In addition, the lower relative contribution of γ-HBCDD in the lesser black-backed gull eggs may reflect species- or diastereomer-specific toxicokinetics (Head et al., 2008; de Wit et al., 2019). Dietary differences may also play a role, since lesser black-backed gulls appeared to have a more terrestrial diet compared with the other two species (Table S1; Fig. 4). Moreover, maternal transfer of HBCDD and other measured BFRs in our study may also account for differences in the respective profiles among the three gull species.

4.3. Alt-BFR concentrations in gull eggs

Of the five alt-BFRs measured in our study, we detected only DBDPE in one egg of each of the three species. This is consistent with other studies that have found its emerging use as a replacement for Deca-BDE (summarised by Betts, 2009). While the detection frequency of DBDPE in gull eggs in our study was low (i.e., 10% for all species combined), the maximum concentration of 7,700 ng/g lw in a single egg laid by a great black-backed gull (Table 1) is the highest recorded in biota worldwide. This is suggestive of an upward trend in DBDPE usage as also highlighted by its elevated environmental concentration in indoor air and dust in the Republic of Ireland (Wemken et al., 2019). The highest concentration of DBDPE previously reported by Gauthier et al. (2007) was 505 ng/g lw in herring gull eggs in the Great Lakes in a study that had a similar detection frequency (i.e., 9%) to ours. The apparent high concentrations of DBDPE measured in some eggs may warrant further research

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467   into the possible embryonic toxicity of this alt-FR (see also Egloff et al., 2011; Zheng et al.,

468   2014, 2015). Indeed, the findings of these previous studies and our study, collectively suggest

469   that further research concerning DBDPE is required (de Wit et al., 2019; Guo et al., 2019;

470   Stubbings et al., 2019; Wemken et al., 2019).

471   The lack of detection of the other four targeted alt-BFRs in our gull eggs is broadly

472   consistent with the existing literature (reviewed in Tongue et al., 2019). Most alt-BFRs are

473   detected in birds at orders of magnitude less than legacy BFRs, possibly reflecting their

474   limited usage over time and space and/or possibly reduced bioavailability (Chen et al., 2013;

475   Covaci et al., 2011). Nevertheless, compared with other birds known to use landfill, BTBPE

476   and PBEB have been detected in eggs of herring gulls in the Great Lakes at low

477   concentrations of up to 0.7 ng/g ww and 1.4 ng/g ww, respectively (Gauthier et al., 2007),

478   and white storks in urban areas of Spain laid eggs containing PBEB at concentrations of up to

479   9.79 ng/g ww (detection frequency: 20%) (Munoz-Arnanz et al., 2010). Detection rates of alt-

480   FRs were also low in eggs of tree swallows (*Tachycineta bicolor*) nesting downstream of

481   waste water treatment plants, but concentrations of six alt-FRs including BTBPE were greater

482   than HBCDD or BDE-209 (Fernie and Letcher, 2018). The production and use of both

483   BTBPE and PBEB appear to be greater in North America compared to Europe (Covaci et al.,

484   2011; Harju et al., 2009).

485

486   *4.4. Trophic ecology of gulls on landfill*

487   Lesser black-backed gulls are the most widespread breeding gull species in urban

488   centres in western Scotland (Forrester and Andrews, 2012). Consistent with that, the

489   decreased $\delta^{13}C$ egg isotope values of lesser black backed gulls compared with the other two

490   gull species (Fig. 4) indicate that lesser black-backed gulls may have consumed more food

491   from terrestrial than from marine sources, notwithstanding potential confounding effects from

20

492   the consumption of anthropogenic items *vs.* 'natural' terrestrial food which may also have

493   influenced $\delta^{13}C$ values (Caron-Beaudoin et al., 2013). In the current study, lesser black-

494   backed gull eggs contained comparatively higher $\sum_8 PBDE$ concentrations than the other two

495   gull species, and compared to great black-backed gull eggs, they also had a significantly

496   lower relative contribution of BDE-47 to $\sum PBDEs$, indicating a relatively limited marine

497   component in their diet. BDE-47 tends to dominate the PBDE egg profile of aquatic feeding

498   birds compared to more terrestrial species (Chen and Hale, 2010). The comparatively low

499     $\delta^{34}S$ isotope values in herring gull eggs (Table 1) provide evidence of the reliance of this

500   species on landfill for foraging. Ramos et al. (2009) found especially low $\delta^{34}S$ isotopic values

501   in regurgitant of yellow-legged gull nestlings whose parents were dietary specialists on

502   landfill. The negative relationship between $\delta^{15}N$ isotope values and BDE-28 concentrations in

503   herring gull eggs (Fig. 5) suggests that for those individuals foraging at higher trophic levels

504   and thus consuming less refuse, they may be less exposed to this congener and/or

505   differentially metabolize and transfer BDE-28 to eggs compared to the other gull species.

506   This hypothesis warrants further investigation since the current study appears to be the first to

507   report this negative congener-isotope association in free-living birds.

508

509   *4.5. Behavioural observations*

510   Our findings suggest that there were multiple routes of BFR exposure for these adult-

511   plumage gulls when frequenting the study landfill, including their diet (Section 4.4),

512   behaviour and potentially also inhalation, that likely contributed to the observed differences

513   amongst species in egg BFR concentrations and profiles.

514   Behavioural observations allowed us to identify four potential routes of BFR exposure

515   to gulls on landfill: pecking at the substrate, swallowing items, walking across the substrate

516   and/or standing stationary on the substrate. There were considerable variations among the

three gull species in their major behaviours on the landfill, i.e., number of pecks, number of

paces, time stationary. However, the extent to which these behavioural differences among the

gulls may have contributed to the variations in egg contaminant burdens warrants further

research, ideally with observations of individuals for which egg burdens are known. Such

future studies should also investigate other possible routes of exposure to environmental

chemicals for gulls, including preening of contaminated plumage while loafing (Jaspers et al.,

2007) and inhalation (Gentes et al., 2015; Sorais et al., 2020). Compared with mammals, the

avian respiratory system requires a larger air volume to be inhaled to supply a near-constant

airflow through the lungs and air sacs (Brown et al., 1997), potentially making birds

especially susceptible to inhaled gaseous/particulate phase BFRs and other contaminants.

We studied three gull species that are of national/international conservation concern

and that frequented a remote landfill in Scotland, UK. Their egg concentrations of BFRs,

reflecting maternal exposure, transfer and deposition, are at some of the highest levels of

PBDEs and HBCDDs for gull species compared to those in mainland Europe or North

America also using landfill. We also identified the highest concentration of DBDPE ever

reported in biota to date. Given previous avian studies demonstrating adverse reproductive

changes in relation to similar egg concentrations of some of these FRs (Guigueno and Fernie,

2017), it is important for future research to address possible reproductive and/or

physiological implications for these species as a result of their contaminant burdens and

association with landfill. Our results, in conjunction with those of previous studies, identify

landfill as an important point source of FRs (and other environmental contaminants) for birds.

Our study also highlights that further research is required to characterize and understand

better the relative importance of direct ingestion, inhalation, dermal contact and preening as

pathways of exposure to BFRs and other environmental contaminants that birds are exposed

22

542   to at landfill. Given the far greater numbers of herring gulls observed on landfill in the

543   present study, and the considerable FR concentrations measured in their eggs, we suggest that

544   herring gulls may be a highly appropriate bioindicator species for similar (landfill) studies.

545

558

559   **Appendix A. Supplementary Information**

560   Supplementary Information to this article can be found online at [PLEASE INSERT WEB

561   ADDRESS]

562

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