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Long-term increase in secondary exposure to anticoagulant rodenticides in European polecats *Mustela putorius* in Great Britain

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ABSTRACT

As a result of legal protection and population recovery in Great Britain, European polecats (*Mustela putorius*) are expanding into areas associated with greater usage of second-generation anticoagulant rodenticides (SGARs). We analysed polecat livers collected from road casualties from 2013 to 2016 for residues of five SGARs. We related variation in residues to polecat traits and potential exposure pathways, by analysing stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in their whiskers. In all, 54 of 68 (79%) polecats had detectable residues of at least one SGAR. Bromadiolone (71%) was the most commonly detected compound, followed by difenacoum (53%) and brodifacoum (35%). Applying historical limits of detection to allow comparison between these new data and previous assessments, we show that in the 25 years from 1992 to 2016 inclusive, the rate of detection of SGARs in polecats in Britain has increased by a factor of 1.7. The probability of SGAR detection was positively related to increasing values of $\delta^{15}N$, suggesting that polecats feeding at a higher trophic level were more likely to be exposed. Total concentrations of SGARs in polecats with detectable residues were higher in polecats collected in arable compared to pastoral habitats, and in the west compared to the east of Britain. The number of compounds detected and total concentrations of SGARs increased with polecat age. There was no evidence of regional or seasonal variation in the probability of detecting SGARs, suggesting that the current risk of exposure to SGARs does not vary seasonally and has increased (from that in the 1990s) throughout the polecat's range. We recommend quantification of current practices in rodenticide usage, particularly in the light of recent regulatory changes, to enable assessment and mitigation of the risks of secondary exposure to rodenticides in non-target wildlife.

Keywords: rodenticides; polecat; secondary exposure; bromadiolone; difenacoum; brodifacoum

Capsule:

79% of polecats in Great Britain were found to have been exposed to rodenticides from 2013 to 2016 and exposure has increased by a factor of 1.7 since the 1990s.

INTRODUCTION

Rodents, primarily brown rats *(Rattus norvegicus),* are estimated to cost the UK economy between £60 and £200 million a year, arising primarily from spoiling of food and from disease transmission (Battersby, 2004). Anticoagulant rodenticides dispensed in baits are the primary means of reducing this damage. They function by interrupting the blood clotting mechanism by inhibiting the action of Vitamin K epoxide reductase (Watt et al., 2005) and lethal exposure leads to death by internal haemorrhaging (Watt et al., 2005; Rattner et al., 2014). In response to the emergence of resistance in rats to warfarin and other first generation rodenticides, second generation anticoagulant rodenticides (SGARs) with higher acute toxicity were developed (Buckle et al., 1994; WHO, 1995) and are now used routinely worldwide to control rodent infestations (Stone et al., 2003; Buckle and Smith, 2015).

The extensive use of SGARs has led to secondary exposure in a range of mustelids including stoats *(Mustela erminea)* and weasels *(Mustela nivalis)* (McDonald et al., 1998; Elmeros et al., 2011), European polecats *(Mustela putorius)* (Shore et al., 2003; Elmeros et al., 2018), American mink *(Neovison vison)* (Ruiz-Suárez et al., 2016), stone martens *(Martes foina)* (Elmeros et al., 2018) and fishers *(Pekania pennanti)* (Gabriel et al., 2012; Thompson et al., 2014). There is also evidence of widespread exposure in other predators such as red foxes (*Vulpes vulpes)* (Tosh et al., 2011; Geduhn et al., 2015), San Joaquin kit foxes *(Vulpes macrotis mutica)* (Cypher et al., 2014), mountain lions *(Puma concolor)* and bobcats *(Lynx rufus)* (Riley et al., 2007; Serieys et al., 2015), barn owls *(Tyto alba)* (Geduhn et al., 2016; Shore et al., 2016), sparrowhawks *(Accipiter nisus)* (Hughes et al., 2013; Hughes et al., 2014; Walker et al., 2015) tawny owls *(Strix aluco)* (Walker et al., 2008) and red kites *(Milvus milvus)* (Walker et al., 2017). Secondary exposure occurs via the consumption of exposed prey (Smith et al., 1990; Smith et al., 2007; Rattner et al. 2014). These may be target species that are the subject of control measures, such as the brown rat and house mouse (*Mus*

69 *domesticus*), or non-target species that feed on bait and are inadvertently contaminated during
70 control campaigns targeted at commensal rodents (Tosh et al., 2012; Elliott et al., 2014). The
71 scale of secondary exposure in predators can vary with habitat (Geduhn et al., 2014; Nogeire
72 et al., 2015), sex (McDonald et al., 1998) and time of year (Shore et al., 2003). In some
73 species the magnitude of residues is greater in older animals (Ruiz-Suárez et al., 2016),
74 arising from the cumulative effect of multiple sub-lethal exposures and the relatively long
75 tissue half-lives of these compounds (Vandenbroucke et al., 2008; EPA, 2008).

76 There is concern that secondary exposure may lead to significant impacts on predators, many
77 of which are species of conservation interest. The extent of any mortality is likely to be
78 species-dependent as tolerance varies by several orders of magnitude (WHO, 1995; Erickson
79 and Urban, 2004; Thomas et al., 2011; Berny et al., 2010). Relatively few poisoned animals
80 are reported in national surveillance schemes, when compared to the numbers known to be
81 exposed (e.g. Barnett et al., 2004; Barnett et al., 2005). The likelihood that exposed
82 individuals die out of sight (Newton et al., 1999), combined with limited external signs of
83 toxicosis (Murray, 2011) and difficulties with using liver residues as a diagnostic of mortality
84 (Thomas et al., 2011), mean that the true extent of secondary poisoning may be
85 underestimated. There may also be sub-lethal effects such as increased susceptibility to
86 natural and anthropogenic stressors (Albert et al., 2010), reduced body condition (Elmeros et
87 al., 2011) and less resistance to pathogens mediated through impairment of the immune
88 system (Riley et al., 2007; Serieys et al., 2015). However, the mechanisms by which any sub-
89 lethal effects occur and their possible impacts on long-term survival and reproductive output
90 remain unclear.

91 Species that consume rats and other target species may be at particular risk of secondary
92 exposure and poisoning by SGARs (Eason and Spurr, 1995; Brakes and Smith, 2005). The
93 European polecat, a medium-sized carnivore that occurs across Europe, is one such species.

It is protected in England and Wales under the Wildlife and Countryside Act (1981) and is currently expanding its distribution, having been extirpated (through predator control) from most of its range in Great Britain during the nineteenth century (Birks, 2015; Croose, 2016). Although the polecat is a generalist feeder with a diverse diet that varies across its European range (Blandford, 1987; Lodé, 1996, 1997; Birks and Kitchener, 1999; Baghli et al., 2002; Hammershøj et al., 2004; Rysava-Novakova and Koubek, 2009; Santos et al., 2009; Malecha and Antczak, 2013), in England and Wales rabbits (*Oryctolagus cuniculus*) and rats are the primary prey (Birks and Kitchener, 1999).

A study of rodenticide residues in polecats in Great Britain that died between 1992 and 1999 established that 31 out of a sample of 100 animals had detectable residues of at least one SGAR (Shore et al., 2003). Detection rates were slightly higher (40%) in animals that died in the first half of the year. It was speculated that this may have been a result of the predominance of rats in the diet during the winter, since rats may comprise up to 65% of polecat diet in the winter months (Birks, 1998). However, SGAR exposure in polecats has not specifically been linked to any contemporary dietary analysis. Stable isotope analysis offers the opportunity to explore such links. $\delta^{15}N$ and $\delta^{13}C$ are measures of the ratio of heavier to lighter stable isotopes of nitrogen (^{15}N to ^{14}N) and carbon (^{13}C to ^{12}C) relative to a standard (DeNiro and Epstein, 1981). As the lighter ^{14}N is preferentially excreted during metabolic processes, ^{15}N enrichment from prey item to predator occurs (DeNiro and Epstein, 1981). Variation in $\delta^{13}C$ reflects diversity in basal resources consumed, e.g. between marine and terrestrial, and plants with C3 or C4 photosynthetic pathways (Smith and Epstein, 1971, DeNiro and Epstein, 1978). Analysis of $\delta^{15}N$ has been widely used for developing understanding of biomagnification of contaminants with increasing trophic level in fresh-water and marine environments (Spies et al., 1989; Cabana and Rasmussen, 1994; Kidd et al., 1995; Jarman et al., 1996; Bearhop et al., 2000; Hobson et al., 2002), and can be applied to

119 examine secondary exposure to rodenticides. Rats are omnivorous opportunistic feeders and
120 their diets vary with location (Major et al., 2007; Dammhahn et al., 2017), so polecats feeding
121 on rats might be expected to have enriched $\delta^{15}N$ signatures compared to those eating a greater
122 proportion of rabbits, which are herbivorous (Southern, 1940). If rats are the main trophic
123 pathway through which polecats are secondarily exposed to SGARs, it would be expected
124 that there might be a positive association between liver SGARs and enriched $\delta^{15}N$ signatures.

125 In the 20-25 years since the last quantification of the exposure of polecats in Great Britain to
126 SGARs (Shore et al., 2003), populations of this species have undergone a substantial
127 recovery and have expanded their range into areas of the country associated with higher
128 usage of SGARs (Packer and Birks, 1999; Birks, 2000; Dawson et al., 2003; Dawson and
129 Garthwaite, 2004). It might therefore be predicted that overall exposure in the polecat
130 population is likely to have increased, if animals in newly recolonised areas subject to greater
131 SGAR usage also feed on rats. Furthermore, the methods of chemical analysis for
132 rodenticides have become more sensitive (lower limits of detection) and so earlier studies in
133 any case are likely to have underestimated levels of exposure (Dowding et al., 2010). The
134 current extent of exposure of polecats to SGARs, and how and why this varies between
135 individuals, is therefore unknown. Using polecat carcasses collected from across their range
136 in Great Britain between 2013 and 2016, our aims in the present study were to: (i) determine
137 the current extent of SGAR exposure in polecats (via measurement of liver residues) and
138 whether this has changed over the last 20-25 years; (ii) identify any spatial and temporal
139 patterns in exposure; (iii) elucidate trophic correlates of exposure through stable isotope
140 analysis of whiskers, and (iv) explore the effect of age on rodenticide accumulation in
141 polecats, a factor not examined by Shore et al. (2003), but recently found to be important in
142 other mustelids (Ruiz-Suárez et al., 2016).

143

METHODS

Carcass collection and sample preparation

Polecat carcasses were collected as part of a national monitoring survey carried out by The Vincent Wildlife Trust between December 2013 and March 2016 (Croose, 2016). Sixty-eight carcasses were selected for rodenticide analysis, based on stratification by sex, location and collection date. Of the animals selected, 82% (n = 56) were road traffic casualties; the remainder were found dead in fields, killed by dogs, trapped or the cause of death was unknown.

Collection date and location were recorded for all carcasses, which were stored frozen until necropsy examination at the National Museum of Scotland. The poor condition of the majority of the carcasses precluded assessment of clinical signs of exposure to rodenticides. Where carcass condition allowed, gross necropsy examination included recording of sex, head and body length (nose to tip of tail), mass and internal fat, scored on a five-point scale (McDonald et al., 1998). A body condition score (e.g. Schulte-Hostedde et al., 2005) was not calculated because many carcasses were too damaged or incomplete. Teeth (for ageing), whiskers (for stable isotope analysis) and liver tissue (for rodenticide analysis) were collected. Liver samples were frozen and transferred to the Centre for Ecology & Hydrology (CEH) for rodenticide analysis. Whiskers were prepared for analysis at the University of Exeter and analysed at Elemtex, UK and teeth were sent to Matson's Lab LLC, USA for aging by analysis of cementum layers.

Determination of rodenticides in liver using liquid chromatography tandem mass spectrometry

166 Concentrations of the five SGARs licensed for use in Great Britain (bromadiolone,
167 difenacoum, brodifacoum, flocoumafen and difethialone) were determined in the polecat
168 livers. The analytical method is summarised here. A detailed description is available in
169 Walker et al. (2017). A 0.25 g sub-sample of each liver was thawed, weighed accurately,
170 ground and dried with anhydrous sodium sulphate. Labelled standard (d^5- Bromadiolone,
171 QMx) was added to each sample for quality control purposes and determination of analyte
172 recovery. Each liver sub-sample was solvent-extracted and then cleaned-up using size
173 exclusion chromatography followed by elution through solid-phase cartridges. Extraction was
174 carried out twice with clean solvent. Each extraction involved vortex mixing of the sample
175 with 1:1 v/v chloroform:acetone, mechanical shaking and centrifugation. The resultant
176 supernatants from the two extraction runs were combined, solvent-exchanged into (1:1; v/v)
177 chloroform:acetone, filtered (0.2 mm PTFE filter), subjected to a further solvent exchange
178 into (1:23; v/v) acetone:DCM, filtered again, and cleaned-up by size-exclusion
179 chromatography (Agilent 1200 HPLC). The cleaned extract was solvent-exchanged into
180 1:1:8; v/v. chloroform:acetone:acetonitrile and underwent a second clean-up using solid
181 phase, methanol-washed, acetonitrile-activated extraction cartridges (ISOLUTE® SI 500 mg,
182 6 ml). The cartridges were eluted with the same solvent and the eluate exchanged for the
183 mobile phase.

184 Liver SGAR residues were quantified by HPLC linked to a triple quadrupole mass
185 spectrometer interfaced with an ion max source in Atmospheric Pressure Chemical Ionisation
186 mode (APCI) with negative polarity. Full details of the operational parameters used are as
187 given by Walker et al. (2017). All rodenticide standards (Dr Ehrenstorfer) were matrix
188 matched and linear calibration curves were defined such that R^2>0.99. A blank was run with
189 each batch of unknowns. The mean method limit of detection (LOD) across batches for each
190 compound was 0.0014 µg/g, except for difethialone which was 0.0022 µg/g. The mean (±

SE) recovery for the total procedure was calculated from the labelled bromadiolone standard applied to each sample and was 68.0 ±2.1%. Liver SGAR concentrations were not recovery corrected and are expressed on a wet weight basis. Summed (Σ) SGAR liver concentrations in individual animals were calculated by summing the concentrations of the five different SGARs, a zero concentration being assigned to individual compounds that were not detected.

Stable isotope analysis

Whiskers were gently rinsed in distilled water and then freeze dried for 24 hours. One whisker per animal was cut into ~1mm segments using a scalpel, starting at the proximal end of the whisker. Consecutive segments were pooled until the summed sample weight was ~0.7 mg (mean ± SE sample weight 0.68±0.01 mg). The sample was enclosed in a tin cup and put into a tray for analysis. The next segment was prepared in the same way and the process was further repeated until either the whole whisker was used, or less than 0.2 mg was remaining. Samples were analysed on a Thermoquest EA1110 elemental analyser linked to a Europa Scientific 2020 isotope ratio mass spectrometer at Elemtex Ltd (Cornwall, UK) for $\delta^{15}N$ and $\delta^{13}C$. $\delta^{15}N$ and $\delta^{13}C$ abundance are reported as δ-values and expressed as a per mil (‰) deviation from the international reference standards (PDB for carbon and AIR for nitrogen) (Mariotti, 1983):

$$\partial^{15}X‰ = \left[\frac{\left({}^{15}X/{}^{14}X\right)sample}{\left({}^{15}X/{}^{14}X\right)standard - 1]}\right] \times 1{,}000$$

Replicate analysis of standards (USGS 40, USGS 41 and an in-house bovine liver standard) yielded standard deviations of 0.05 – 0.29 for $\delta^{15}N$ and 0.05 – 0.22 for $\delta^{13}C$.

Cementum aging

212 Cementum ageing was undertaken by Matson's Lab LLC (Manhattan, MT, USA) following a
213 standard protocol (Matson et al., 1993). In brief, after decalcification in a weak hydrochloric
214 acid solution, teeth were sectioned sagittally and mounted on glass slides. The sections were
215 stained to allow visual differentiation of annual cementum growth layers. These layers
216 (annuli) were examined microscopically for age estimation at time of death. Birth date was
217 set to 1 May for the purpose of estimating age in months.

218 *Data analysis*

219 All data were analysed using R [version 3.4.1] and R Studio [version number 0.99.896].
220 Generalised linear models were built using a) the 2013-16 data (henceforth "new data") and
221 b) a combination of new data and the historical polecat rodenticides data from Shore et al.
222 (2003) (henceforth "combined data"). Combination of new and historical data involved
223 applying the limits of detection (LOD) for each compound from Shore et al. (2003), which
224 were higher than those in the present study, to eliminate biases caused by changes in
225 analytical sensitivity.

226 We modelled exposure in three ways: i) probability of detecting at least one SGAR; ii)
227 number of SGARs detected; and iii) of those polecats with detectable residues, total
228 concentration levels of all SGARs detected. Total SGAR concentration data were log-
229 transformed before building models so that they were normally distributed. Polecats with no
230 SGARs detected were excluded from the total SGAR concentration models to allow us to
231 explore the variables related to differences in concentration levels.

232 Explanatory variables included in the three "new data" models were: age (months), sex (male
233 / female), half of year in which the carcass was collected (first / second), region (North /
234 South / East / West), land class (arable / pastoral), fat score, $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰).
235 Carcasses collected between January– June were categorised as "first" half of the year, those

collected between July – December were categorised as “second”. Regions were defined using U.K. Government Office Regions. North comprised North East, North West, Yorkshire and the Humber; South comprised London, South East and South West; East comprised Eastern and East Midlands and West comprised Wales and West Midlands. No animals were analysed from Scotland. Quantum GIS [version 2.12.3] was used to generate land class classifications. Carcass collection locations were overlaid onto the CEH Land Cover map (2007 https://www.ceh.ac.uk/services/land-cover-map-2007), 1 km buffers were applied around each carcass coordinate and the majority land class calculated for each point, for whichever was largest between “arable” or “pastoral”, i.e. improved grasslands. Models included the mean $\delta^{15}N$ and $\delta^{13}C$ for each whisker. We also modelled the maximum $\delta^{15}N$ value for each whisker in place of the mean $\delta^{15}N$, as it was considered that it may only take one contaminated meal to cause secondary exposure and maximum $\delta^{15}N$ might better reflect such episodic incidents than the mean value for the whole whisker. However models with the maximum $\delta^{15}N$ did not differ markedly from the models with the mean $\delta^{15}N$ and hence analysis of maximum values is not reported.

The “combined” data models, adjusted for limits of detection, included two categorical explanatory variables: collection period (1992 – 1995, 1996 – 1999, 2013 – 2016) and location (in or outside of the 1990s polecat range as determined by Birks & Kitchener (1999)). The first two carcass collection periods were 1992 – 1995 and 1996 – 1999, and represent an approximately even split (in calendar years and numbers) of the 100 polecats analysed by Shore et al. (2003). The third collection period was the “new data” carcasses collected in 2013 – 2016. Location was included with the aim of assessing whether polecat expansion into new areas where SGAR use may have been greater was a factor that might enhance SGAR exposure.

Models were built using lme4, MuMIn and car packages in R. Models were checked for collinearity (none was evident). Model fit was assessed using QQ plots. Models were mean centred and standardised using two standard deviations to facilitate comparisons between effect sizes (Gelman, 2008). Top models were then selected using Akaike's Information Criterion (AIC) values less than two different from the best model. Averaged models were created using the top models as none of the top models was weighted >0.9 (Grueber et al., 2011). Interaction effects between parameters were not significant and did not appear in any of the top models when added, and so were removed for simplicity. Standardised conditional average model outputs were summarised. Model predictions were drawn using the ggplot2 package in R.

RESULTS

The 68 polecats analysed for SGARs came from throughout England and Wales (Figure 1); 29 were female, 38 male and the sex of one could not be determined. The age of the polecats in our sample ranged from one month to six years. The youngest polecats with detectable residues of SGARs were two months old while the oldest polecat without detectable SGARs was three years old. Mean $\delta^{15}N$ values for polecat whiskers ranged between 7.2 and 13.2‰. Mean $\delta^{13}C$ values ranged from -27.98 to -21.41‰. In all, 54 (79%) polecats had detectable liver residues of at least one SGAR compound (Table 1). The number of polecats with one, two, three or four compounds in the liver were 19 (27.9%), 16 (23.5%), 16 (23.5%) and 3 (4.4%) respectively. The median number of compounds detected in polecat livers was 2.

The rate of detection of liver SGARs differed significantly between compounds ($\chi^2 = 77.5$, df = 4, $p < 0.0001$), with bromadiolone most frequently detected, followed by difenacoum and brodifacoum (Table 1). Difethialone was only detected in livers that contained residues of all three commonly detected SGARs. Flocoumafen was never detected. There was no significant

difference between compounds in the median concentrations of residues in those animals with detected residues (KW = 2, df = 2, p = 0.37).

Probability of detecting at least one SGAR in the liver

The probability of detecting liver SGAR residues could be explained by set of top models that included age, $\delta^{15}N$, $\delta^{13}C$, fat score and land class; age and $\delta^{15}N$ appeared in all the top models (Table 2a). In the resultant average model (Table 2b), there was a positive effect of enriched $\delta^{15}N$ signatures on the likelihood of SGAR detection in liver residues. The model predicted that at the mean level of $\delta^{15}N$ (9.9 ‰), the probability of detecting SGARs was 89% (95% confidence limits: 68% - 97%, Figure 2). Although age, $\delta^{13}C$, fat score and land class also featured in the average model, the confidence intervals for the effects of these parameters overlapped 0, indicating that they had no significant effect on the probability of detecting liver SGAR residues.

Number of SGARs detected in the liver

Age, $\delta^{13}C$, $\delta^{15}N$ and half of year were selected in the top models of the number of liver SGARs detected in individuals (Table 2a). Age appeared in all of the top models and, in the average model (Table 2b), was positively associated with the number of compounds detected. The effects of $\delta^{15}N$, $\delta^{13}C$ and time of year were also included in the average model but had no clear effect on the number of SGARs detected. Overall, the model predicted that by thirty-six months old, polecats will on average have accumulated detectable concentrations of 2.1 SGARs (95% confidence limits: 1.5 - 2.7) in their livers, assuming mean $\delta^{15}N$, mean $\delta^{13}C$ and first half of year values.

Total SGAR concentrations

There were five top models for total SGAR concentrations and these contained age, land class, region, $\delta^{13}C$ and fat score as variables (Table 2a). Age was positively associated with total SGAR concentrations in the average model. Total SGAR concentrations were also significantly higher in polecats collected from arable compared with pastoral landscapes and in animals in the west compared with those in the east (Table 2b). There was no clear effect of $\delta^{13}C$ or fat score on total SGAR concentrations.

Comparison of exposure in polecats from 1992-9 and from 2013-16

When historical limits of detection (0.027, 0.010 and 0.005 µg/g for bromadiolone, difenacoum, and brodifacoum respectively) from less sensitive analytical techniques as used in the earlier study by Shore et al. (2003) were applied to our "new data" for animals that died in 2013-16, the rates of detection in the "new data" were reduced to 40% (bromadiolone), 35% (difenacoum), 21% (brodifacoum) and 54% (any SGAR). As flocoumafen was not detected in any animals in either study and difethialone was not tested for in the 1990s, these compounds were excluded from this part of the analysis. These compare to detection rates of 12%, 22%, 3% and 31% respectively in Shore *et al.* (2003). The change in prevalence from 31% to 54% of polecats with one or more SGAR detected equates to an increase in the rate of detection by a factor of 1.7 between the two studies. A greater proportion of animals in the "new data" had two (24%) and three compounds (9%) than those recorded by Shore *et al.* (2003), who found that only 2% of polecats had liver residues of two compounds and a further 2% had detectable liver residues of three compounds.

Survey period and location appeared in all top model sets (Table 3a). In the average models of the probability of detecting SGARs residues and the number of SGARs detected, the period 2013 – 2016 was associated with higher rates of detection of rodenticides than the period 1992 – 1995 (Table 3b). There was also an increase in the rate of detection between

330 the 2013 – 2016 when compared to polecats collected in the period 1996 – 1999, but this was
331 a smaller effect. The number of compounds detected in the most recent survey was higher in
332 the most recent survey period than both of the previous collection periods. Survey period did
333 not have a consistent effect on the total concentrations of SGARs detected. Location (animals
334 in 1990s range vs animals in areas colonised post 1990s) did not have a consistent effect in
335 any of the average models.

336

337 **DISCUSSION**

338 The detection of SGARs in 79% of the polecats collected 2013-16 was comparable with the
339 findings of recent studies of other mustelids from elsewhere. Detection rates of ~79% were
340 reported for American mink in Scotland (Ruiz-Suárez et al., 2016), 78% were reported for
341 fishers in California (Gabriel et al., 2012) and 95% for stoats and weasels in Denmark
342 (Elmeros et al., 2011). A recent study of exposure of polecats and stone marten *(Martes*
343 *foina*) in Denmark detected SGARs in 94% and 99% of animals respectively (Elmeros et al.,
344 2018). Similarly high prevalence of residues has been found in birds of prey in Britain, with
345 94% of barn owls (a generalist small mammal predator) with detectable residues of one or
346 more SGARs (Shore et al., 2016) and 100% of a sample of 18 red kites, a scavenger that
347 often feeds on rats, with detectable liver SGAR residues (Walker et al., 2017).

348 Overall, the prevalence of residues in the present study is greater than that reported for
349 polecats that were collected in the 1990s in Britain (Shore et al., 2003). This is in part due to
350 improvements in analytical sensitivity, but even when this methodological difference is
351 accounted for (by applying common limits of detection), we identified an increase by a factor
352 of 1.7 in the prevalence of SGAR residues over the 25 years from 1992 to 2016 inclusive. We
353 found no evidence of differences in rates of detection between polecats within and beyond the

limits of their 1990s range, suggesting that the increase in exposure over time has occurred throughout the polecat's current range in Britain, and has not been caused simply by expansion into areas where SGAR use has traditionally been considered to be higher (Dawson et al., 2003; Dawson and Garthwaite, 2004).

SGAR detection in polecats may have increased owing to more widespread use of SGARs and / or changes in polecat diet. There is some evidence of an increase over time in SGAR usage. In a nationwide survey of rodenticide usage, Dawson et al. (2003) found that between 1992 and 2000 the proportion of farms in Britain using SGARs changed from 74% to 89%. Furthermore, rabbit populations have declined since 1995 (Aebischer et al., 2011; Battersby, 2005), which may have increased the reliance of polecats on rats and other rodents as prey. In our study, the increased prevalence of brodifacoum from 3% in Shore et al. (2003) to 35% in our most recent survey (21% using historical LODs) was particularly notable and may reflect growing resistance in rats to bromadiolone and difenacoum in England and Wales (Buckle, 2013) and a consequent attempt to control resistant populations through use of brodifacoum. The proportion of American mink in Scotland recently found with liver residues of brodifacoum and flocoumafen was only 10% (Ruiz-Suárez et al., 2016) but resistance to bromadiolone and difenacoum is not widely documented in Scotland (Buckle and Prescott, 2012) and so there may be less pressure to use compounds, such as brodifacoum, when there is no or little known resistance in rats.

The positive relationship between more enriched values of $\delta^{15}N$ and the presence of rodenticide residues (Figure 2) was consistent with our hypothesis that polecats would be more likely to be exposed to SGARs due to their consumption of contaminated target prey, primarily rats, which are likely to have higher $\delta^{15}N$ signatures than herbivorous rabbits. Other studies have found that detection of SGAR residues in predators varies with available food sources (Hegdal and Blaskiewicz, 1984; Tosh et al., 2011; Geduhn et al., 2016) and while it

seems most likely that the elevated $\delta^{15}N$ signatures reflect polecats feeding at higher trophic level, we cannot be certain whether the sources of contamination are rats as the target species, or other non-target omnivorous rodents. Alternatively, enriched $\delta^{15}N$ signatures might distinguish polecats that had been living and feeding in landscapes exposed to anthropogenic enrichment of soil ^{15}N, perhaps associated with practices associated with agricultural intensification (Rubenstein and Hobson, 2004; Crawford et al., 2008). It was notable that there was no significant relationship between $\delta^{15}N$ and total SGAR concentrations and this suggests that dietary preferences may have the greatest effect on whether exposure takes place at all, rather than influencing the magnitude of exposure. The frequency of exposure and resultant residue accumulation is likely to be driven more by patterns that influence the extent of exposure in the prey and the numbers of those prey that are eaten over time.

Age was positively related to number of SGARs detected in the liver and to total SGAR concentrations in polecats that died between 2013 and 2016. This reflects the greater time period over which older polecats can encounter and eat contaminated prey, together with the persistence of SGAR residues in liver tissues. Similar positive associations between age and exposure have been found in birds (Christensen et al., 2012; Walker et al., 2015) and mustelids (Gabriel et al., 2012; Ruiz-Suárez et al., 2016).

We found that total SGAR concentrations in the 2013-16 polecats varied with the predominant land-use in the area in which they died. Geduhn et al. (2015) found a significant difference in contamination between urban areas and areas with high livestock density. Total SGAR concentrations were higher in polecats from arable than pastoral areas, which may indicate heavier SGAR usage on arable farms. This is in line with findings from previous national rodenticide usage surveys on arable farms compared to farms growing grass and fodder (De'Ath et al., 1999; Garthwaite et al., 1999). The higher total SGAR concentrations in polecats collected in the west compared to the east was surprising, as we might have

expected rodenticide usage to be higher in the east of England where there is a greater density of arable farms (Dawson et al., 2003). However, this finding is consistent with those of Shore et al. (2003), in which bromadiolone residues were higher in polecats in Wales, Midlands and West England than in animals in the East and the South-East of England, and difenacoum residues were higher in Wales than in the East and South-East of England. We did not detect significant variation between exposure at different times of year in the polecats that died in 2013-16, contrary to the earlier polecat surveys (Shore et al., 1999; Shore et al., 2003). Thus we have no evidence that current exposure in polecats is greatest in the autumn and winter, as previously thought, and may indicate that exposure is now similar year-round.

In conclusion, we have determined that SGAR contamination in polecats in Britain is likely to be greatest in older animals that eat rodents, live in the west of the country and inhabit arable areas; these individuals may therefore be at greater risk of adverse effects. We have also demonstrated that exposure has increased in scale (proportion of animals exposed, number of residues accumulated) since the 1990s and that this increase appears to have occurred throughout the polecat's range. The implications for polecats arising from this widespread exposure to SGARs is a key question arising from this study. Diagnosis of mortality caused by rodenticides would ideally draw upon ante-mortem observations, post-mortem detection of non-trauma related haemorrhaging and quantification of liver AR residues (Murray, 2018). Although liver concentrations >0.2 µg/g wet weight have elsewhere been considered to be potentially lethal (in barn owls; Newton et al. 1999), liver residues alone cannot be used as clear indicators of lethal poisoning, as the relationship between residue magnitude and likelihood of mortality is variable (Thomas, 2011). We have identified high liver SGAR residues in some polecats but all of these animals were killed on the road and the resultant trauma precluded clinical detection of any rodenticide-related haemorrhaging. It is nevertheless conceivable that SGAR exposure may have contributed to

429 their mortality if such exposure affected the likelihood of animals being run-over and/or if it
430 exacerbated trauma. It is also possible that these animals may ultimately have succumbed to
431 SGAR poisoning, had they not been run-over. We did not find any evidence of sub-lethal
432 effects, such as reduced kidney fat levels, in animals with detectable liver residues, which
433 may have been expected given that reduced body condition has been observed in other
434 studies of secondary exposure in mustelids (Elmeros et al. 2011). Overall, whilst we have
435 shown that the rate of detection of SGARs and the number of compounds detected per animal
436 have both increased over time, polecats have continued to recolonise Great Britain over the
437 same period (Birks and Kitchener, 1999; Birks, 2008; Croose, 2016). They are now
438 widespread in central, eastern and southern England, but are yet to re-establish themselves in
439 parts of northern England and Scotland. Research exploring polecat survival and productivity
440 in relation to varying degrees of exposure to SGARs would help inform our understanding of
441 the impacts that SGARs may have on polecat populations and rates of recolonisation.

442 The regulatory framework concerning SGAR deployment in Britain changed in July 2016,
443 with a relaxation of restrictions on the use brodifacoum, flocoumafen and difethialone, but
444 there has been a concomitant introduction of a stewardship scheme designed to promote best
445 practice in use and thereby reduce non-target primary and secondary exposure
446 (http://www.thinkwildlife.org/stewardship-regime/Stewardship). The effect of these
447 regulatory changes for primary consumers of SGAR target species, such as polecats, is
448 uncertain. The outcome could be less prolonged use of difenacoum and bromadiolone in
449 areas where resistance in rats to these two compounds is a problem, while at the same time
450 there may be an increase in the use of more acutely toxic, "resistance-busting" SGARs, such
451 as brodifacoum and flocoumafen.

452 One of the biggest gaps in our understanding of the risk posed by SGARs to polecats and
453 other non-target wildlife, concerns usage patterns and rodent control practices. There is a

need to determine how much and how frequently SGARs are used and how usage varies between different types of landowners in different parts of the country. Contemporary research into predator diets, including fine-scale application of stable isotope approaches to predators and their prey, will also improve understanding of pathways of exposure. Exploring user practices and how these may change following the introduction of stewardship is critical to inform our understanding of the current and likely future scale of the risks presented to non-target wildlife by anticoagulant rodenticides.

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724

TABLES AND FIGURES

Table 1. Prevalence and concentrations of residues of second generation anticoagulant rodenticides (SGARs) in the livers of 68 polecats collected in England and Wales, 2013-2016. Totals are the prevalence of residues of any SGAR and the median of the summed SGAR concentrations.

Compound	Number (% of total sample) of polecats with detected residues	Median (range) concentration (µg/g wet weight)
Bromadiolone	48 (71%)	0.0581 (0.0014 – 3.0833)
Difenacoum	36 (53%)	0.0587 (0.0021 – 0.5125)
Brodifacoum	24 (35%)	0.0080 (0.0016 – 0.7298)
Difethialone	3 (4%)	0.0193 (0.0035 – 0.0505)
Flocoumafen	0 (0%)	N/A
Total	54 (79%)	0.1204 (0.0014 – 3.1628)

Table 2a: Summary of statistical models of variation in second generation anticoagulant rodenticide (SGAR) residues in polecat livers collected from 2013 to 2016. Top models are from analyses of i) probability of detecting residues ii) number of compounds for which residues were detected and iii) total concentrations. AIC is Akaike's Information Criterion and ΔAIC is the difference in AIC from the best model. Only models with ΔAIC < 2 are included in the top model set. Weight is the weighting given to that model when the average model is calculated. Sample sizes vary because of missing variables and the exclusion of animals with no residues detected in models of total concentrations.

Model	Covariates	df	Log likelihood	AIC	Δ AIC	Weight
i) Probability of detecting ≥ 1 liver SGAR residue (n = 59)						
1	Age + $\delta^{15}N$	3	-24.72	55.87	0.00	0.24
2	Age + $\delta^{15}N$ + land class	4	-23.76	56.26	0.39	0.20
3	Age + $\delta^{15}N$ + $\delta^{13}C$ + land class	5	-22.70	56.53	0.66	0.17
4	Age + $\delta^{15}N$ + $\delta^{13}C$	4	-24.04	56.83	0.96	0.15
5	Age + $\delta^{15}N$ + fat score + land class	5	-23.04	57.21	1.34	0.12
6	Age + $\delta^{15}N$ + fat score	4	-24.34	57.41	1.54	0.11
ii) Number of SGARs detected (n = 59)						
1	Age + $\delta^{13}C$ + $\delta^{15}N$	4	-85.54	179.82	0.00	0.27
2	Age	2	-88.31	180.82	1.01	0.16
3	Age + $\delta^{13}C$	3	-87.24	180.92	1.10	0.15
4	Age + $\delta^{15}N$	3	-87.33	181.10	1.28	0.14
5	Age + half of year + $\delta^{13}C$	4	-86.20	181.14	1.33	0.14
6	Age + half of year + $\delta^{13}C$ + $\delta^{15}N$	5	-85.04	181.21	1.40	0.13
iii) Total SGAR concentration (n = 46)						
1	Age + land class + region	7	-87.51	191.97	0.00	0.33
2	Age + land class	4	-92.11	193.19	1.22	0.18
3	Age + land class + $\delta^{13}C$	5	-90.86	193.22	1.25	0.18
4	Age + land class + $\delta^{13}C$ + region	8	-86.71	193.31	1.34	0.17
5	Age + land class + region + fat score	8	-86.92	193.72	1.75	0.14

Table 2b: Standardised conditional average model coefficients and relative importance of variables include in top model sets (AIC < 2) of variation in second generation anticoagulant rodenticide residues in polecat livers for i) probability of detecting residues; ii) number of compounds for which rodenticides were detected; and iii) total concentrations. Parameter names with brackets show the effect of that parameter category against the reference category (half of year = first, land class = arable, region = east). Parameters highlighted in bold are those where the confidence intervals do not span zero on the model scale, indicating a consistent directional effect. Coefficient estimates, standard errors and confidence intervals are presented on the model scales. Importance reflects the number of models that the parameter appears in and its importance to the average model.

Parameter	**Coefficient estimate**	**SE**	**2.5% CI**	**97.5% CI**	**Importance**
i) Probability of detecting ≥ 1 liver SGAR residue (binomial regression, logistic scale)					
(intercept)	1.54	0.55	0.44	2.65	-
Age	2.20	1.18	-0.17	4.57	1.00 (6)
$\delta^{15}N$	**2.53**	**0.92**	**0.68**	**4.37**	**1.00 (6)**
Land class (pastoral)	1.16	0.80	-0.43	2.76	0.50 (3)
$\delta^{13}C$	1.10	0.88	-0.66	2.86	0.32 (2)
Fat score	-0.78	0.78	-2.34	0.78	0.24 (2)
ii) Number of SGARs detected (Poisson regression, log scale)					
(intercept)	0.46	0.13	0.20	0.73	-
Age	**0.47**	**0.17**	**0.13**	**0.81**	**1.00 (6)**
$\delta^{13}C$	0.40	0.22	-0.05	0.84	0.70 (4)
$\delta^{15}N$	0.36	0.22	-0.09	0.81	0.54 (3)
Half of year (second)	-0.28	0.24	-0.76	0.19	0.27 (2)
iii) Total SGAR concentration (linear regression, log scale)					
(intercept)	-1.97	0.52	-3.03	-0.92	-
Age	**1.44**	**0.56**	**0.30**	**2.57**	**1.00 (5)**
Land class (pastoral)	**-1.98**	**0.67**	**-3.33**	**-0.62**	**1.00 (5)**
Region (north)	0.29	0.97	-1.67	2.25	0.64 (3)
Region (south)	0.37	0.79	-1.22	1.97	0.64 (3)
Region (west)	**1.97**	**0.82**	**0.32**	**3.63**	**0.64 (3)**
$\delta^{13}C$	0.74	0.55	-0.38	1.85	0.35 (2)
Fat score	0.56	0.56	-0.56	1.69	0.14 (1)

Table 3a: Summary of statistical models of variations in second generation anticoagulant rodenticide (SGAR) residues in polecat livers. Top models from analysis of i) probability of detecting residues; ii) number of rodenticides detected and iii) total concentrations using "combined" Shore et al. (2003) and new rodenticide data. AIC is Akaike's Information Criterion and ΔAIC is the difference in AIC from the best model. Only models with ΔAIC < 2 are included in the top model set. Weight is the weighting given to that model when the average model is calculated. Sample sizes vary because of the exclusion of animals with no residues detected in models of total concentrations.

Model rank	Covariates	df	Log likelihood	AIC	ΔAIC	Weight
i) Probability of detecting ≥ 1 liver SGAR residue (n = 168)						
1	Survey	3	-107.70	221.55	0.00	0.72
2	Survey + location	4	-107.59	223.43	1.88	0.28
ii) Number of SGARs detected (n = 168)						
1	Survey	3	-168.05	342.26	0.00	0.52
2	Survey + location	4	-167.10	342.45	0.19	0.48
iii) Total SGAR concentrations (n = 68)						
1	Null	2	-104.13	212.44	0.00	0.43
2	Location	3	-103.26	212.90	0.46	0.34
3	Survey	4	-102.53	213.69	1.25	0.23

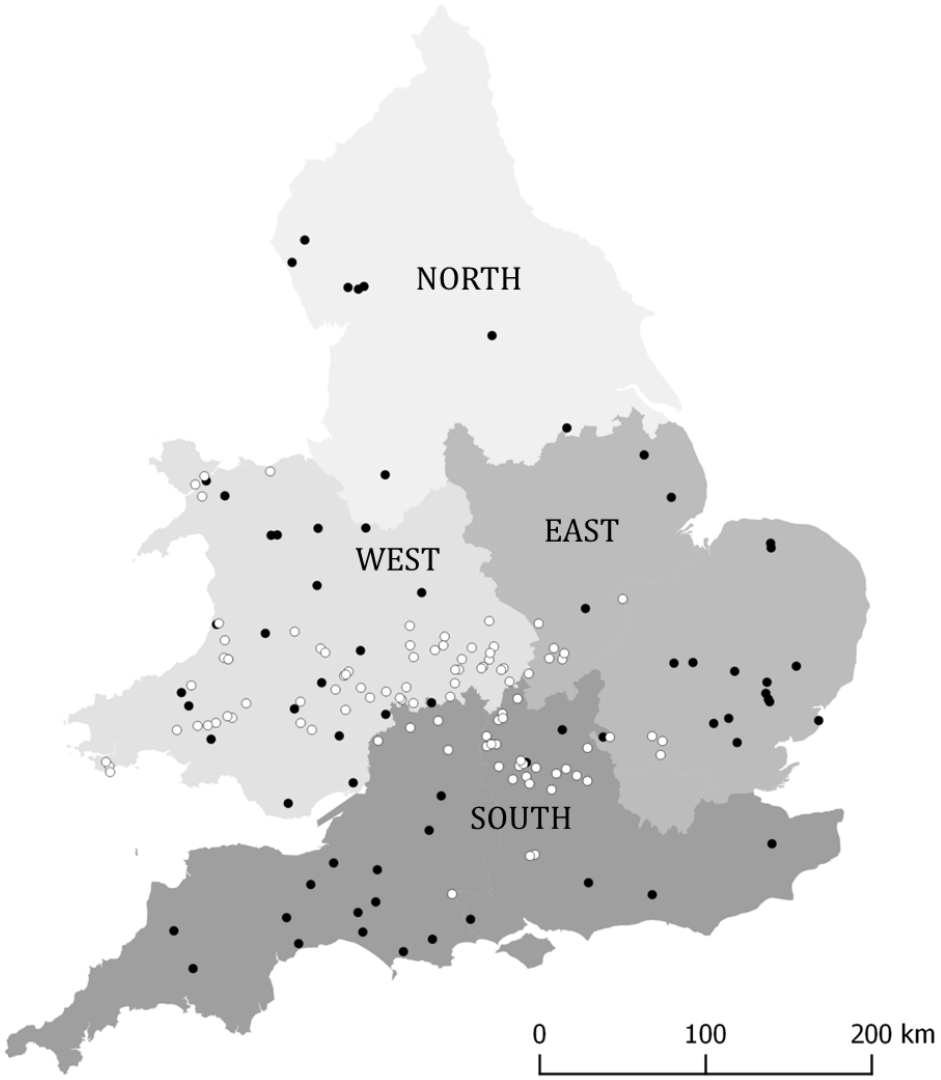
Table 3b: Standardised conditional average model coefficients and relative importance of variables include in top model sets (AIC < 2) of variation in second generation anticoagulant rodenticide (SGAR) residues in polecat livers for i) probability of detecting residues; ii) number of compounds for which rodenticides were detected; and iii) total concentrations using "combined" Shore et al. (2003) and new rodenticide data. Parameter names with brackets show the effect of that parameter category against the reference category (survey = "2013-2016", location = "inside 1990s range"). Parameters highlighted in bold are those where the confidence intervals do not span zero on the model scale, indicating a consistent directional effect. Coefficient estimates, standard errors and confidence intervals are presented on the model scales. Importance reflects the number of models that the parameter appears in and its importance to the average model.

Parameter	**Coefficient estimate**	**SE**	**2.5% CI**	**97.5% CI**	**Importance**
i) Probability of detecting ≥ 1 liver SGAR residue (binomial regression, logistic scale)					
(intercept)	0.21	0.28	-0.34	0.76	-
Survey (1992 - 1995)	**-1.40**	**0.46**	**-2.30**	**-0.50**	**1.00 (2)**
Survey (1996 - 1999)	-0.75	0.39	-1.52	0.03	1.00 (2)
Location (outside 1990s range)	-0.23	0.49	-1.19	0.74	0.28 (1)
ii) Number of SGARs detected (Poisson regression, log scale)					
(intercept)	0.03	0.16	-0.29	0.35	-
Survey (1992 - 1995)	**-1.22**	**0.32**	**-1.86**	**-0.59**	**1.00 (2)**
Survey (1996 - 1999)	**-0.89**	**0.26**	**-1.41**	**-0.38**	**1.00 (2)**
Location (outside 1990s range)	-0.35	0.25	-0.85	0.15	0.48 (1)
iii) Total SGAR concentrations (linear regression, log scale)					
(intercept)	-1.93	0.20	-2.32	-1.54	-
Survey (1992 - 1995)	-0.49	0.40	-1.28	0.31	0.23 (1)
Survey (1996 - 1999)	-0.48	0.31	-1.09	0.13	0.23 (1)
Location (outside 1990s range)	0.41	0.31	-0.22	1.04	0.34 (1)

Figure captions

Figure 1: Collection locations of polecat carcasses used for analysis of second generation anticoagulant rodenticides. Black points are carcasses collected and analysed in this survey while white points are carcasses collected and analysed in Shore *et al.* (2003).

Figure 2: Predictions based on output of the averaged model for the probability of detecting second generation anticoagulant rodenticide residues in polecat livers. Figure 2a shows the probability of detecting SGARs at different levels of $\delta^{15}N$ in pastoral landscapes, when polecat age, $\delta^{13}C$ and fat score are kept constant at their mean values (16.2 months, -25.54 ‰ and 2.6, respectively).



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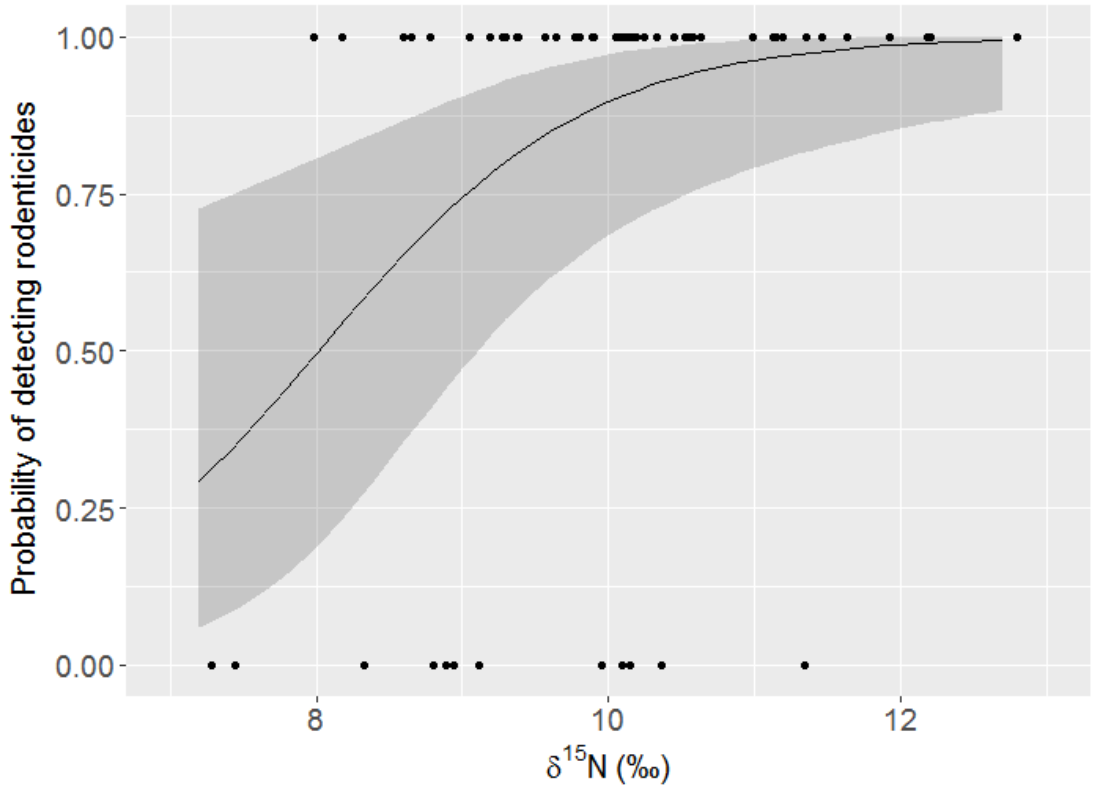

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