Endangered Australian top predator is frequently exposed to anticoagulant rodenticides

ABSTRACT

Anticoagulant rodenticides (ARs) used to control mammalian pest populations cause secondary exposure of predatory species throughout much of the world. It is important to understand the drivers of non-target AR exposure patterns as context for assessing long-term effects and developing effective mitigation for these toxicants. In Australia, however, little is known about exposure and effects of ARs on predators. We detected AR residues in 74% of 50 opportunistically collected carcasses of the Tasmanian wedge-tailed eagle (*Aquila audax fleayi*), an endangered apex predator. In 22% of birds tested, or 31% of those exposed, liver concentrations of second generation ARs (SGARs) were > 0.1 mg/kg ww. Eagles were exposed to flocoumafen, a toxicant only available from agricultural suppliers, at an exceptionally high rate (40% of birds tested). Liver SGAR concentrations were positively associated with the proportion of agricultural habitat and human population density in the area around where each eagle died. The high exposure rate, in a species not known to regularly prey upon synanthropic rodents, supports the hypothesis that apex predators are vulnerable to SGARs. Our results indicate that AR exposure constitutes a previously unrecognized threat to Tasmanian wedge-tailed eagles and highlight the importance of efforts to address non-target AR exposure in Australia.

Key words: Rodenticide, Environmental contamination, SGARs, Secondary poisoning, Predator

1. Introduction

Anticoagulant rodenticides (ARs) are used worldwide to control mammalian pest populations. These compounds function by inhibiting blood clotting mechanisms in vertebrates, resulting in internal hemorrhaging (Rattner et al., 2014). The discovery of resistance to the first-generation of ARs (FGARs) led to the development of second-generation ARs (SGARs) in the 1970s (van den Brink et al., 2018). To be lethal, FGARs generally require consecutive intake over several days to accumulate sufficiently high concentrations (Erickson and Urban, 2004). Conversely, SGARs are usually lethal from a single exposure and persist longer in the environment (Erickson and Urban, 2004; van den Brink et al., 2018). The persistence of AR compounds (Horak et al., 2018), the delay in mortality after bait consumption (Lee et al., 2006) and the behavioral changes that occur as a symptom of poisoning (Brakes and Smith, 2005; Mooney, 2017) can make poisoned rodents AR vectors to non-target predatory species.

Detrimental non-target exposure to ARs has been shown in numerous populations of predators in Europe and North America (Christensen et al., 2012; López-Perea et al., 2015; Riley et al., 2007; Shore et al., 2003; Thomas et al., 2017). These effects can be significant, with population-level effects from non-target exposure documented for mammals (Jacquot et al., 2013) and raptors (Thomas et al., 2011). It is thought that species that regularly prey upon small rodents are at higher risk of poisoning, due to the likelihood of consuming AR targeted species (Hindmarch and Elliott, 2018). However, the primary consumption of AR baits by non-target species, as well as the potential for SGARs to move through trophic levels, may lead to wider contamination of terrestrial food chains (Hindmarch and Elliott, 2018; Thomas et al., 2011). If such broadscale contamination is apparent, species at higher trophic levels may be at increased risk of AR exposure (Riley et al., 2007; Thomas et al., 2011).

It is necessary to understand the drivers of patterns in non-target AR exposure in order to assess long-term effects and to develop effective mitigation. There are documented differences in AR exposure of predators between the sexes (Mcdonald et al., 1998) and among age groups (Christensen et al., 2012; Ruiz-Suárez et al., 2016). That said, local anthropogenic factors are likely the most significant drivers of overall risk of non-target exposure. For example, human population density and developed surface area have been linked to the probability and level of AR exposure of numerous predators (Lohr, 2018; Lopez-Perea and Mateo, 2018; Nogeire et al., 2015; Serieys et al., 2015). Agricultural AR use has also been suggested as the cause of non-target poisoning of predators (Birks, 1998; Fourel et al., 2018; Hindmarch et al., 2017; Hughes et al., 2013), but only a few recent studies have found empirical evidence of this relationship (Coeurdassier et al., 2019; López-Perea et al., 2018; Rial-Berriel et al., 2021; Sainsbury et al., 2018).

AR use is largely unmonitored in Australia and recent work has highlighted the need for the evaluation of its effects on Australasian taxa (Lohr, 2018; Lohr and Davis, 2018). The Tasmanian wedge-tailed eagle (*Aquila audax fleayi*) is a subspecies of wedge-tailed eagle endemic to the Australian island of Tasmania (Commonwealth of Australia, 1999). With the loss of the thylacine (*Thylacinus cynocephalus*) and recent declines in populations of Tasmanian devils (*Sarcophilus harrisii*), the wedge-tailed eagle serves a particularly important ecological function as one of the few remaining top predators in Tasmanian ecosystems. The subspecies is listed as endangered (Commonwealth of Australia, 1999; State Government of Tasmania, 1995), with conservation concern based upon a series of threats, including nest failures caused by anthropogenic disturbance, low breeding success rates, habitat loss, collisions with

anthropogenic structures, lead poisoning, and illegal persecution (Bell and Mooney, 1998; Mooney and Holdsworth, 1991; Pay et al., 2020; Threatened Species Section, 2006).

ARs are not recognized as a significant threat to the Tasmanian wedge-tailed eagle population, as the species generally avoids areas of high human population density, and rodents represent a very small portion of their diet (Marchant and Higgins, 1993). That said, wedge-tailed eagles show a high sensitivity to pindone (Martin et al., 1994); this AR is used to control European rabbit (*Oryctolagus cuniculus*) populations, a primary prey species of wedge-tailed eagles (Debus et al., 2007; Marchant and Higgins, 1993). Furthermore, if ARs are moving through Tasmania's food chains, then the high trophic position of the wedge-tailed eagle may increase their susceptibility to exposure to various AR compounds. Finally, because of the long-lived and slow breeding life history strategy of this species, it is likely highly vulnerable to increased mortality rates brought on by toxicants such as ARs.

Our aims in this research were to determine to what extent Tasmanian wedge-tailed eagles are exposed to ARs, and to investigate the factors that influence AR exposure in the population. Specifically, we evaluated (1) liver tissue concentrations of individual ARs known to be used in Tasmania and the total SGAR concentration of each individual eagle; and the relationship between both (2) total liver SGAR concentration and (3) probability of exposure with intrinsic (age and sex) and extrinsic (human population density, agricultural land use, and year of death) factors.

2. Methods

2.1. Study area

This study was conducted on mainland Tasmania, an island state located 240 km south of continental Australia. Tasmania covers an area of 68,150 km^2, with an estimated human population of 520,830 (Australian Bureau of Statistics, 2018; Figure 1b). Areas of minimal land use, nature conservation and other protected areas account for 50.3% (34,280 km^2) of the Tasmanian land area (DPIPWE, 2015). Agriculture occupies 18,900 km^2 (27.7%; DPIPWE, 2015) mostly in the north and east of the state (Figure 1c). The Tasmanian agricultural land area is comprised of modified pastures (75.4%), native vegetation pastures (14.5%), irrigated crops (8.8%), and non-irrigated crops (1%; DPIPWE, 2015).

2.2. Sample collection

Eagles were collected as carcasses found opportunistically throughout Tasmania (Figure 1a) between 1996 and 2018, by government departments, various industries, and volunteers. All carcasses were placed in -20˚C freezer storage by the Department of Primary Industries, Parks, Water and the Environment (DPIPWE, Threatened Species Section, Hobart, Tasmania) and the Tasmanian Museum and Art Gallery (TMAG, Collection and Research Facility, Rosny, Tasmania). Data recorded for each carcass included location and the date the carcass was found. We thawed the carcasses and harvested tissues from them between May 2017 and March 2018. We collected a whole liver lobe and a muscle sample from each carcass. The tissue samples were stored at -20˚C until sample preparation, when we thawed them at room temperature. We weighed out a 4 g (± 0.1 g) wet weight sample from the middle of each liver lobe using a digital

balance (precision ± 0.0001 g (Mettler Toledo, US). New scalpel blades and gloves were used between samples during collection and preparation to prevent cross contamination.

2.3. Residue analysis

2.3.1. Sample preparation

All toxicological analyses were carried out at Edith Cowan University Analytical Facility (Joondalup, Western Australia). Each liver sample was freeze-dried and homogenized. Homogenized samples were transferred into centrifuge plastic tubes (15 ml) and 10 ml of acetonitrile was added to the tubes with a 10 µl (10 ng/µl) solution containing deuterated surrogates. Analytes were extracted using a sonication bath (15 minutes sonication for each aliquot). After extraction, samples were centrifuged at 3,247 relative centrifugal force (rcf) for 5 minutes, transferred to a new centrifuge tube with 2 ml of hexane, vortexed for 5 minutes and centrifuged at 3,247 rcf for a further 5 minutes. Each sample was then evaporated and reconstituted in 400 µl of 50:50 ACN/H20 solution. The final extracts were transferred to 2 ml Teflon-lined vials and stored at 0–4˚C until analysis.

2.3.2. LC-MS analysis

Liver samples were analyzed for ARs registered for use in Australia (Australian Pesticides and Veterinary Medicines Authority, 2019). Concentrations of five SGARs (brodifacoum, bromadiolone, difethialone, difenacoum and flocoumafen) and three FGARs (coumatetralyl, pindone and warfarin) (see Appendix Table A.1 for the manufacturers of the analytical standards and surrogates) were evaluated using a TSQ Quantiva triple quadrupole Mass Spectrometer (LC-MS) from Thermo Fisher (Thermo Fisher Scientific Corporation, US) (see Appendix B for details of the chromatographic method). Calibration curves and recovery

rates for each analytical run were calculated using organic chicken livers spiked with three working solutions of each analytical standard. Recovery rates for the target ARs averaged 96.75 %, whilst limits of detection (LOD) and limits of quantification (LOQ) ranged from 0.0005–0.0125 mg/kg and 0.001–0.025 mg/kg respectively (Appendix Table A.2). All detections that were > LOD but < LOQ were reported as present at trace levels. Three organic chicken liver blanks were included in each run to monitor cross-contamination. Every 10th sample was reinjected for a duplicate read (average percentage relative standard deviation of recoveries (RSD) 4.1%) and duplicate blind sample extractions were carried out for five randomly selected samples (average RSD 4.1%). Concentrations were reported on a dry weight basis (mg/kg dw).

2.4. Potential drivers of AR exposure

We evaluated potential drivers of AR exposure as a response to a suite of intrinsic and extrinsic explanatory variables. The intrinsic variables we considered were the sex of the bird (determined genetically using muscle tissue; Appendix C) and its age (broadly characterized into adults and pre-adults based on plumage; Appendix D). Extrinsic explanatory variables were the year in which the carcass was found, and both the mean human population density per km^2 (Australian Bureau of Statistics, 2018) and the proportion of agricultural area (DPIPWE, 2015) in the area surrounding where each carcass was found. Areas we categorized as agricultural included all types of animal production (intensive animal production, native vegetation grazing, and modified pastures grazing) and all types of horticulture (both non-irrigated, and irrigated cropping; see Appendix E). We defined the area around where each carcass was found based on the size of the estimated home range of adult and pre-adult eagles (25 km^2 for adults and 420 km^2 for pre-adults; see Appendix F). We buffered each carcass location by an area corresponding

to the age-specific home range and calculated the mean human population density per km^2 and the proportion of agricultural land within the buffered area. To maximize accuracy in estimates of spatial predictor variables, both human population density and agricultural land use area were calculated from data as close to the year the carcass was found as possible (maximum differences between year of death and spatial data were six years for human population and five years for agricultural land use).

2.5. Data analysis

We performed all statistical analyses in R, version 3.2.0 (R Core Team, 2016). We analyzed the data using censored data techniques (R packages NADA; Lee, 2017, and Survival; Therneau, 2018) as some AR concentrations were below the LOD of the LC-MS.

2.5.1. Individual AR and total SGAR concentration

We used a Kaplan-Meier cumulative probability distribution (NADA function 'cenfit') to calculate censored summary statistics (mean, median and standard error) of each AR compound and the total SGAR concentration for each individual eagle. We also calculated analogous standard (non-censored) summary statistics for only the eagles in which ARs were detected. Creating these analogous summary statistics facilitated comparisons among our study and prior work as other studies have used this approach (e.g. Hughes *et al.*, 2013; Lopez-Perea and Mateo, 2018). To facilitate comparisons to other studies, we calculated summary statistics on a wet weight basis. To do this we converted dry weight concentrations (provided in mg/kg dw) to wet weight (mg/kg ww) by multiplying the dry weight concentrations by the dry to wet weight ratios of each sample.

We used total SGAR concentrations to estimate the effects of over-all SGAR contamination due to their similar mode of action (Rattner and Harvey, 2021). FGARs were not included in the summed concentrations due to large differences in molecular weight, potency and half-life compared to SGARs (Rattner and Harvey, 2021). To estimate potential toxicological effects of the total SGAR concentrations detected, we used published contamination thresholds (see Lohr, 2018) as follows: (i) 0.001–0.01 mg/kg ww, probably no toxicity; (ii) 0.01–0.1 mg/kg ww, unlikely lethal / possible toxicity; (iii) 0.1–0.5 mg/kg ww, possibly lethal / likely toxicity; (iv) 0.5–0.7 ww, probably lethal; (v) > 0.7 mg/kg ww, lethal. We used the converted wet weight concentrations for this evaluation as the thresholds were based on wet weight concentrations.

2.5.2. Correlates of degree and likelihood of exposure

We explored relationships between the extrinsic and intrinsic explanatory variables (age, sex, year of death, human population density, and proportion of agricultural area) and total SGAR concentration with left-censored regression models (Helsel, 2012; Survival function 'survreg'). We assigned censored data the corresponding LOD value with an indicator variable denoting the observation as below the LOD. Uncensored data were assigned the total liver SGAR concentration measured by the LC-MS and an indicator variable denoting the observation as not censored. The correlation of predictor variables was checked before inclusion in the models, and any correlated predictors (Pearson's $r > 0.3$) were not included in the same model. The dependent variable in these models was the total liver SGAR concentration (mg/kg dw) for each sample. Our initial model set included all possible combinations of submodels. We used corrected Akaike's Information Criterion (AICc) to rank model performance. We excluded models in the candidate set if they had an AICc value greater than six ΔAICc (Richards, 2005). The use of AICc as the sole selection criterion may select overly complex models, thus we

considered only those models that had AICc values smaller than all the simpler models within which they were nested (Richards, 2008). We based biological inferences on the coefficients of the top-performing model and considered a parameter to have strong support if it was included in all candidate models.

We also explored the relationship between the same suite of extrinsic and intrinsic predictor variables with the probability of AR residues (both of SGARs and FGARs) being detected using a binomial generalized linear model with logit link function. The dependent variable in these models was whether the eagles were exposed (AR concentrations > LOD) or unexposed (AR concentrations < LOD). We again considered all possible parameter combinations and retained models in the candidate set that were both within six ΔAICc and had AICc values smaller than all the simpler models within which they were nested.

3. Results

We analyzed tissue from 50 eagle carcasses that were collected between 1996 and 2018, although most were collected after 2006 (n = 37). All birds were successfully sexed and aged, with 41 eagles identified as pre-adult, and 22 as female. Data available for the sampled carcasses included location (n = 50; Figure 1a) and year the carcass was found (n = 50).

3.1. Individual AR and total SGAR concentration

AR residues were detected in 74% of wedge-tailed eagles included in the study (Table 1). Residues of more than one AR compound were detected in 38% of the birds, and 12% of birds had three different compounds detected. The mean total SGAR concentrations of birds in which

219 SGARs were detected was 0.143 mg/kg ww (±SE 0.031) and the censored mean of the entire
220 study sample was 0.100 mg/kg ww (±SE 0.023). The majority of AR residues were SGARs.
221 Brodifacoum (56% of birds), flocoumafen (40%) and bromadiolone (22%) were the most
222 predominant SGARs detected. FGARs were only detected in three individuals (one of these
223 individuals was also exposed to the SGAR flocoumafen). Warfarin was detected at very low
224 concentrations (< 0.01 mg/kg ww) in two birds and coumatetralyl was detected in one bird.

225 We recorded a potentially lethal total liver SGAR concentration (> 0.1 mg/kg ww;
226 Newton *et al.*, 1999) in 11 of the wedge-tailed eagles sampled (22%; 31% of those in which
227 SGARs were detected; Figure 2). Furthermore, concentrations were above probably lethal levels
228 of > 0.5 mg/kg ww in 4% of the eagles (6% of those in which SGARs were detected). That said,
229 liver AR concentrations do not allow the confirmation of lethality without a necropsy of the
230 animal identifying signs of toxicity.

231

232 *3.2. Correlates of degree of exposure*

233 The top-performing censored regression model suggested that total liver SGAR
234 concentration (mg/kg dw) was driven most strongly by the year the carcass was found, the
235 amount of agricultural area, and the human population density in the area around where the
236 carcass was found (see Appendix Table G.1). This model was 42.83 times more likely than the
237 null model. A simpler model that excluded human population density was also retained in the
238 candidate model set (Table 2). The year the carcass was found and agricultural area were
239 included in both candidate models, suggesting that these variables were the most important to
240 explaining total liver AR concentration. Coefficients of the best performing model indicated that
241 year of death, agricultural area, and human population density were all positively associated with

total AR concentration (Table 3, Figure 3a). The model suggested that a 10% increase in agricultural habitat in the area around where the bird died would result in an increase in liver AR concentrations by a factor of 2.11. Likewise, each later year in the study was estimated to increase AR concentrations by a factor of 1.40. The relationship between total AR concentration and human population density suggested an increase in 100 habitants per km^2 would increase total AR concentration by a factor of 7.23.

3.3. Correlates of likelihood of exposure

The top-performing binomial model to explain the probability of an eagle being exposed to ARs included the year the carcass was found and the proportion of agricultural area within the area around where the carcass was found (see Appendix Table G.2). The candidate model set included two simpler models, including the null model (Table 2), although the top-performing model was 8.9 times more likely than the null model based on AICc weight. Coefficients of the top-performing model indicated that the probability of ARs being detected increased with carcasses found more recently and in areas with higher proportions of agricultural area (Table 3). The odds of ARs being detected in a carcass were 1.46 times greater for each 10% increase in agricultural habitat proportion in the area around where the bird died and 1.21 times greater for each advancing year of the study period (Figure 3b, Appendix Figure G.1).

4. Discussion

The frequency and magnitude of AR exposure in Tasmanian wedge-tailed eagles, and their correlation to agricultural areas and human population density, have several implications for our understanding of rodenticide exposure and the Tasmanian ecosystem. First, rodenticide

exposure is high among these birds, suggesting that rodenticides are frequently finding their way into top predators in the ecosystem. Furthermore, extrinsic (i.e. agricultural area, human population density, and year of death) rather than intrinsic factors (i.e. age, sex) influence the probability of exposure to ARs and total SGAR concentration. These findings illustrate how AR exposure of the Tasmanian wedge-tailed eagle is driven by anthropogenic processes and thus identify directions to solve this conservation problem.

4.1. Individual AR and total SGAR concentration

The high prevalence of SGARs detected in our study is consistent with research implicating SGARs as the predominant cause of non-target AR exposure of predators (Lohr, 2018; López-Perea et al., 2015). SGARs brodifacoum, bromadiolone, and flocoumafen accounted for 99.6% of the total AR concentrations observed in the Tasmanian wedge-tailed eagle. The first two of these are the AR compounds most commonly identified in non-target predators in numerous ecosystems worldwide (Hosea, 2000; Koivisto et al., 2016; Langford et al., 2013; Ruiz-Suárez et al., 2014; Sharp et al., 2005). The extent of the flocoumafen contamination we detected is more surprising and represents one of the highest exposure rates documented globally (see Appendix Table G.3). Flocoumafen is only available through wholesale outlets in Tasmania, suggesting that agricultural asset protection and professional pest controllers could be important sources of non-target AR exposure in Australia.

The low concentrations and frequency of detections of FGARs also corresponds with findings for other species, both in Australia and globally (Cypher et al., 2014; Lohr, 2018; Murray, 2020, 2017). This was of particular interest in the case of the FGAR pindone. We expected pindone to be the AR most frequently detected in wedge-tailed eagles, since it targets a

287 common prey item for the species (the European rabbit). Our low detection of FGARs could be
288 due to their characteristics - their shorter half-life and lower toxicity - and, in the case of
289 pindone, its localized use in targeted control efforts (Lohr, 2018). Although this low rate of
290 detection may suggest FGARs pose a lower risk of non-target exposure, their characteristics may
291 also impede their detection in studies using opportunistic sampling and prolonged tissue storage
292 (Herring et al., 2017; Rattner et al., 2014), consequently underestimating their true prevalence in
293 the Australian environment.

294 The frequency and magnitude of AR exposure in the Tasmanian wedge-tailed eagle was
295 high for an *Aquila* species. Raptor studies showing comparable AR detection rates typically
296 involve smaller species known to be at risk due to their dietary specialization on rodents
297 (Christensen et al., 2012; López-Perea et al., 2015; Walker et al., 2011). The proportion of birds
298 we observed with concentrations > 0.2 mg/kg ww (16%) is substantially higher than that found
299 in congeners (0–6%; Hosea, 2000; Langford et al., 2013; Sánchez-Barbudo et al., 2012), and the
300 highest concentration of an SGAR we detected in an individual (0.635 mg/kg ww of
301 Brodifacoum) is substantially higher than the highest concentration of an AR previously reported
302 in an *Aquila* species (0.154 mg/kg ww of Bromadiolone; Langford et al., 2013). Both the
303 censored mean SGAR concentrations of all eagles sampled (0.100 mg/kg ww) and the mean only
304 of those with detected SGAR levels (0.143 mg/kg ww) were higher than mean concentrations
305 reported for congeners (0.006–0.073 mg/kg ww; Langford et al., 2013; Sánchez-Barbudo et al.,
306 2012), but lie within the range of values reported for other raptors exposed to SGARs (0.005–
307 0.413 mg/kg ww; Thomas et al. 2011; Christensen 2012; Lohr 2018).

308 The high exposure to SGARs in the Tasmanian wedge-tailed eagle, a species not known
309 to regularly prey upon synanthropic rodents, supports the suggestion that apex predators are

vulnerable to SGARs (López-Perea et al., 2015; Riley et al., 2007). The long half-life and persistence of SGARs gives these compounds the capacity to move through food chains (López-Perea et al., 2015), a theory evidenced by the presence of ARs in apex predators (Riley et al., 2007). The wedge-tailed eagle preys upon several carnivorous species that are known to consume synanthropic rodents. For example predatory and scavenging species such as forest ravens (*Corvus tasmanicus*), kookaburras (*Dacelo novaeguineae*), common brush-tail possums (*Trichosurus vulpecula*), cats (*Felis catus*), and other raptors have been recorded in wedge-tailed eagle diets (Marchant and Higgins, 1993). The potential for SGARs to move through multiple trophic levels may therefore be causing extensive contamination of Tasmania's terrestrial food chains (Thomas et al., 2011). If this is the case, then numerous other predatory species may be at risk in the region, including the endangered Tasmanian devil and eastern quoll (*Dasyurus viverrinus*; IUCN, 2020).

The high exposure we detected may also be driven by the improper use of ARs and non-target AR vectors. The use of SGARs in Australia does not require a license, products can be easily purchased in large quantities, and awareness of use guidelines may be low. If SGARs are not being used as directed, numerous non-rodent species may consume the poisons and act as AR vectors to predators. Furthermore, ARs have recently been detected in Australian reptile species; this exposure could be through direct consumption of ARs used correctly, since these species are small enough to enter AR bait boxes (Lettoof et al., 2020). Reptiles are prey for wedge-tailed eagles, as well as other predators, and may therefore have a role as AR vectors in Australia.

4.2. Correlates of AR exposure

The positive association between hepatic AR concentrations and human population density and agricultural land use may indicate localized use that is having wider scale effects. AR residues in predators have been linked to human population density (López-Perea et al., 2018, 2015), the amount of urbanized area (Coeurdassier et al., 2019; Lohr, 2018; Serieys et al., 2015), and the amount of both arable and pastoral agriculture (Coeurdassier et al., 2019; López-Perea et al., 2018; Sainsbury et al., 2018). These relationships are unsurprising in study species known to use urban and agricultural habitats. However, Tasmanian wedge-tailed eagles are less associated with densely populated areas. Although human population growth has been relatively low in Tasmania for the past two decades, there has been an increase in the number of residences built in more rural and natural areas and agricultural development has expanded (Australian Bureau of Statistics, 2018). Such practices may introduce ARs into more natural areas. Furthermore, if ARs are passing through multiple trophic levels, they will spread more widely from the initial bait. The effects of these more remote developments and agricultural activities may therefore have incommensurately greater effects on predatory species than suggested by the landscape footprint.

4.3. Recent increases in AR exposure

The higher total SGAR concentrations and probability of AR exposure of the birds that had died more recently could be due to either the increased exposure to ARs over time or the degradation of the compounds with prolonged storage. Although SGAR residues are stable within tissues over the short-term (Gallocchio et al., 2014; Jin and Chen, 2006), the effects of long-term -20˚C freezer storage on tissue residues is not well understood, with studies

documenting various rates of degradation (e.g. 6–41% over 0.5–3 years; P. Fisher unpublished data; Vindenes *et al.,* 2008). Despite this, patterns in the AR concentrations we detected are consistent with increased probability of exposure over time. There would need to be a substantial reduction in AR residues (much greater than the degradation rates documented) for an AR-exposed bird to be considered unexposed in our study, as the lowest AR concentration we detected was still 200% greater than the associated LOD. Consequently, the increased AR concentrations in Tasmanian wedge-tailed eagles that had died more recently is more likely due to increases or changes in AR use in Tasmania than to sample degradation. However, there is no information available on the volume of ARs used in Australia (Lohr and Davis, 2018), which impedes our quantification of the relationship between AR application and non-target AR exposure.

4.4. Conservation implications

Our results indicate that AR exposure is likely a significant factor to consider in the conservation management for the Tasmanian wedge-tailed eagle. This is true even given the potential biases inherent to the non-random carcass collection we relied on to gather samples. AR studies using opportunistic samples may inflate the proportion of animals with sub-lethal AR concentrations detected and underestimate the proportion of birds detected with fatal levels (Lohr, 2018; Newton et al., 1990). We found exposure at rates that are high compared to other studies using similar sampling methods.

The use of AR concentration thresholds to interpret the likely physiological result has limitations due to inter- and intra-specific variation in susceptibility to toxicity (Rattner and Harvey, 2021 ; Thomas et al., 2011). That said, concentrations in 22% of the birds we studied

were well above the potentially lethal range reported for European barn owls (*Tyto alba*; > 0.1 mg/kg; Newton et al. 1999). Furthermore, 56% had levels that can cause symptoms of toxicity (> 0.01 mg/kg; (Lohr, 2018; Murray, 2018);. These comparative data therefore suggest that the level of exposure we detected indicates that AR exposure could be influential to survival and possibly conservation of these birds.

Our findings underscore the importance of efforts to address non-target AR exposure in Australian wildlife. SGARs are currently registered for domestic (non-professional) use in Australia (Australian Pesticides and Veterinary Medicines Authority, 2019), despite increasing regulation and monitoring in other countries (USEPA, 2008). Increased legislative control of SGARs and removal from public retail have been suggested as steps to reduce the ecological effects of SGAR use in Australia (Lohr and Davis, 2018). However, our findings of an association between agriculture and AR concentrations in the Tasmanian wedge-tailed eagle, as well as widespread contamination of an AR not readily available for residential use (flocoumafen), suggests that professional pest control may also be an important cause of non-target AR exposure. Addressing mechanisms of spread from both professional and non-professional application of SGARs may therefore be important to reducing AR exposure in Tasmanian wedge-tailed eagles and other Australian wildlife.

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603

604 Table 1. Summary statistics describing liver AR concentrations of each AR assessed and total liver SGAR concentration of Tasmanian
605 wedge-tailed eagle carcasses collected between 1996 and 2018. Non-censored and censored summary statistics are presented. Non-
606 censored statistics were calculated using only the eagles with detected AR concentrations. Censored summary statistics consider all
607 individuals and account for unknown values below the corresponding limit of quantification (LOQ). All summary statistics are
608 reported on a wet weight basis.

	Brodifacoum	Bromadiolone	Coumatetralyl	Difenacoum	Difethialone	Flocoumafen	Pindone	Warfarin	Total SGAR
Not censored									
LOQ (mg/kg)	0.005	0.001	0.002	0.0025	0.010	0.0025	0.025	0.002	NA
Birds exposed (%)	28/50 (56%)	11/50 (22%)	1/50 (2%)	0/50 (0%)	0/50 (0%)	20/50 (40%)	0/50 (0%)	2/50 (4%)	37/50 (74%)
Max (mg/kg ww)	0.635	0.241	0.014	0.000	0.000	0.348	0.000	0.002	0.651
Min (mg/kg ww)	0.003[a]	0.003	0.014[a]	0.000	0.000	0.002[a]	0.000	0.001[a]	0.002
Mean (mg/kg ww)	0.136	0.045	0.014	0.000	0.000	0.035	0.000	0.002	0.143
Median (mg/kg ww)	0.072	0.023	0.014	0.000	0.000	0.003	0.000	0.002	0.074
SE (mg/kg ww)	0.030	0.021	0.000	0.000	0.000	0.022	0.000	0.001	0.031
Censored									
Mean (mg/kg ww)	0.077	0.012	0.014	NA	NA	0.015	NA	0.002	0.100
Median (mg/kg ww)	0.011	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.017
SE (mg/kg ww)	0.019	0.005	NA	NA	NA	0.009	NA	0.000	0.023

609 [a] Trace value

610

611 Table 2. Candidate models relating total liver SGAR concentrations and probability of ARs being detected in Tasmanian wedge-tailed
612 eagle carcasses collected between 1996 and 2018 to intrinsic and extrinsic factors [a].

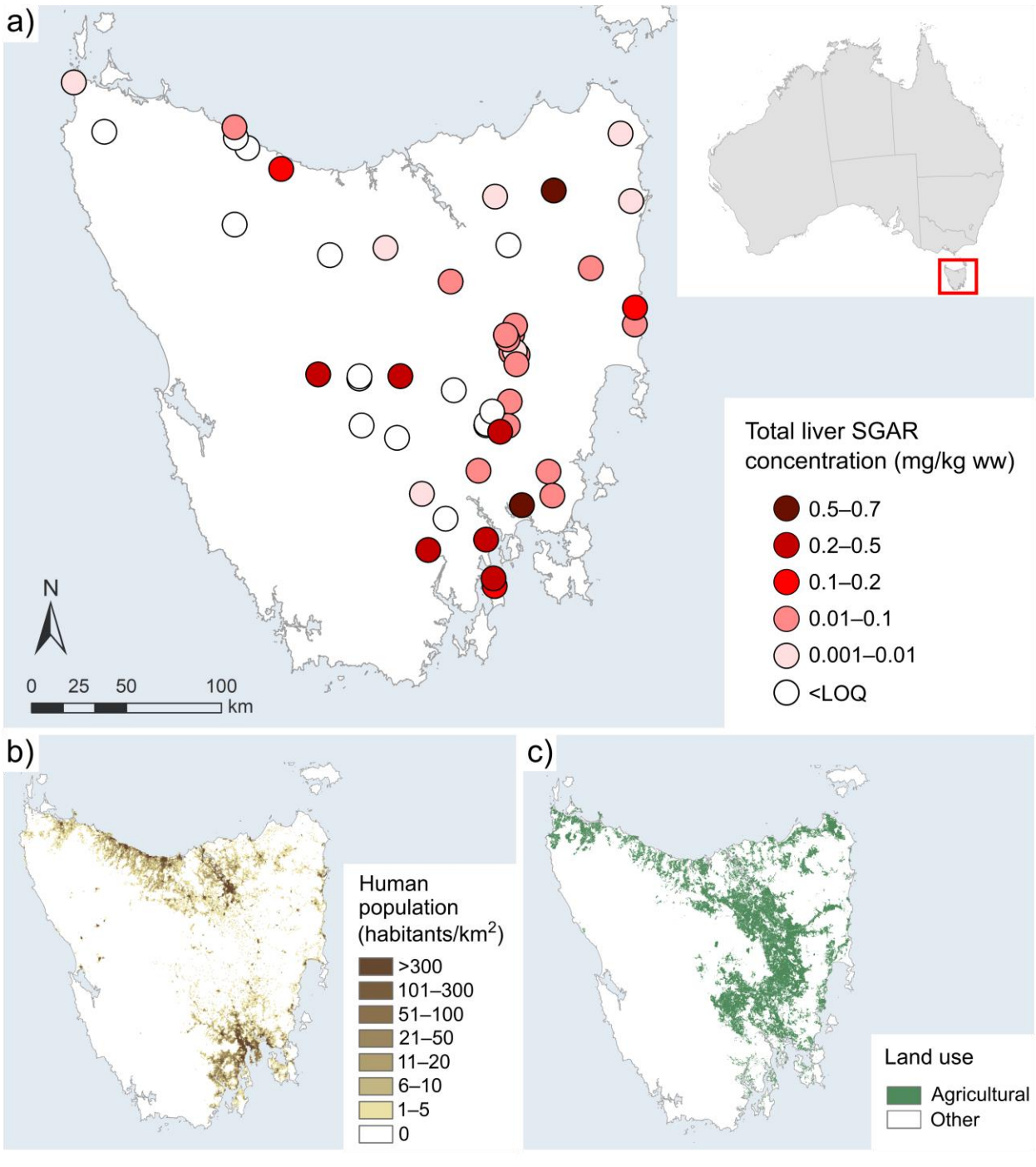
Rank	Model variables	df	AICc	AICc weight
Total liver AR concentration				
1	Year of death (+); Agricultural area (+); Human population density (+)	5	93.170	0.795
2	Year of death (+); Agricultural area (+)	4	95.885	0.205
Probability of exposure				
1	Year of death (+); Agricultural area (+)	3	55.010	0.712
2	Year of death (+)	2	57.471	0.208
3	*Null model*	1	59.389	0.079

613 [a] Only models that were both within six ΔAICc and had AICc values smaller than all the simpler models within which they were nested were
614 retained in in the candidate model set (Richards, 2008). For year of death, "+" indicates association to carcasses found more recently.

615

Table 3. Model coefficients for top performing models describing the estimated effect of each variable on total liver SGAR concentrations (censored regression) and the probability of an eagle being exposed to ARs (binomial probability).

Model	Parameter	Estimate	95% CI		*z*
			Lower	Upper	
Total liver AR concentration	Intercept	-12.105	-16.669	-7.542	-5.20
	Year of death	0.337	0.137	0.536	3.31
	Agricultural area	0.749	0.248	1.249	2.93
	Human population density	1.978	0.306	3.650	2.32
Probability of exposure	Intercept	-2.945	-6.313	-0.165	-1.93
	Year of death	0.193	0.053	0.365	2.48
	Agricultural area	0.375	0.035	0.781	2.02



621

622 Figure 1. a) Location and liver total SGAR concentration threshold for 50 Tasmanian wedge-
623 tailed eagle carcasses collected between 1996 and 2018. Maps b) and c) indicate the spatial
624 distribution of the Tasmanian human population (2016 data; Australian Bureau of Statistics,
625 2018) and agricultural land use area (2015 data; DPIPWE, 2015) respectively.

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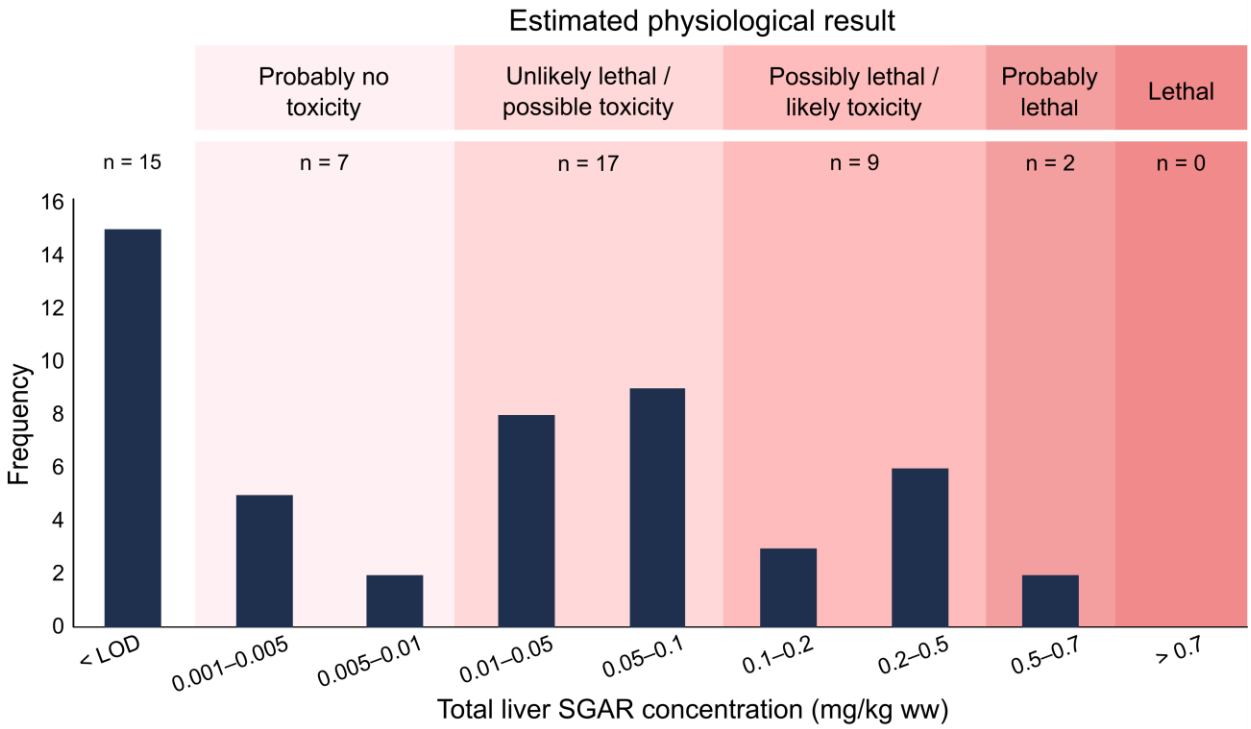

628

629 Figure 2. Total liver SGAR concentrations (mg/kg ww) for Tasmanian wedge-tailed eagles that
630 died between 1996 and 2018 (n = 50). The number of eagle carcasses with liver SGAR
631 concentrations within each toxicity threshold proposed by Lohr (2018) is presented.

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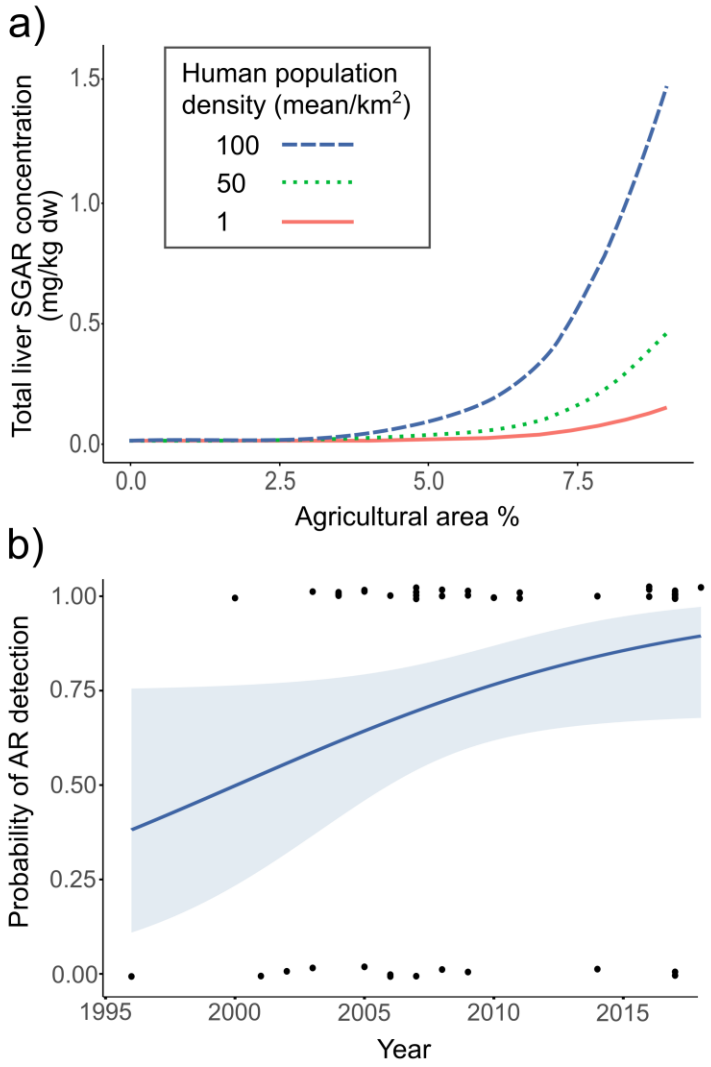

637

638 Figure 3. a) Predicted response of total liver SGAR concentrations (mg/kg dw) in Tasmanian
639 wedge-tailed eagle carcasses as a function of the proportion of agricultural land area and mean
640 human population density in the area around where the bird died. The three lines are the
641 estimated response of liver AR concentration with human population per km^2 held at three
642 levels. Year of carcass discovery is held at its mean. b) Logistic plot of the effect of year of death
643 on the probability of AR exposure. Agricultural land area is held at its mean.

644