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The missing toxic link: Exposure of non-target native marsupials to second-generation anticoagulant rodenticides (SGARs) suggest a potential route of transfer into apex predators

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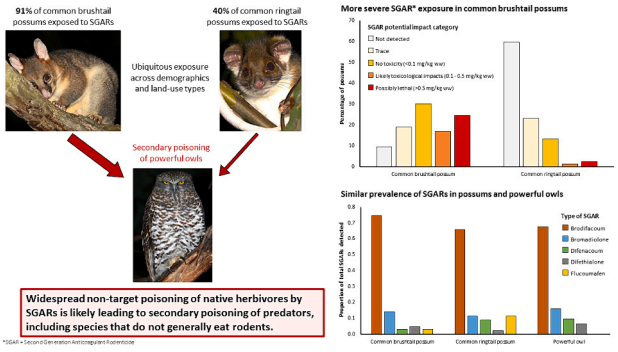
HIGHLIGHTS

- SGARs were found in 91 % of brushtail possums and 40 % of ringtail possums.
- SGARs in brushtail livers were substantially higher than in ringtail possums.
- Age and sex of possums showed similar exposure and was widespread across landscapes.
- Ratios of SGARs detected in powerful owls were similar to those detected in possums.
- Non-target exposure of possums to SGARs is likely poisoning their top-order predator.

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides (ARs) are used globally to control rodent pests. Second-generation anticoagulant rodenticides (SGARs) persist in the liver and pose a significant risk of bioaccumulation and secondary poisoning in predators, including species that do not generally consume rodents. As such, there is a clear need to understand the consumption of ARs, particularly SGARs, by non-target consumers to determine the movement of these anticoagulants through ecosystems. We collected and analysed the livers from deceased common brushtail possums (*Trichosurus vulpecula*) and common ringtail possums (*Pseudocheirus peregrinus*), native Australian marsupials that constitute the main diet of the powerful owl (*Ninox strenua*), an Australian apex predator significantly exposed to SGAR poisoning. ARs were detected in 91 % of brushtail possums and 40 % of ringtail possums. Most of the detections were attributed to SGARs, while first-generation anticoagulant rodenticides (FGARs) were rarely detected. SGAR concentrations were likely lethal or toxic in 42 % of brushtail possums and 4 % of ringtail possums with no effect of age, sex, or weight detected in either species. There was also no effect of the landscape type possums were from, suggesting SGAR exposure is ubiquitous across landscapes. The rate of exposure detected in these possums provides insight into the pathway through which ARs are transferred to one

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of their key predators, the powerful owl. With SGARs entering food-webs through non-target species, the potential for bioaccumulation and broader secondary poisoning of predators is significantly greater and highlights an urgent need for routine rodenticide testing in non-target consumers that present as ill or found deceased. To limit their impact on ecosystem stability the use of SGARs should be significantly regulated by governing agencies.

1. Introduction

The continued growth of the human population, and the resulting urban sprawl, has caused humans and wildlife to increasingly co-exist (Soulsbury and White, 2015). The global human population recently surpassed 8 billion (UN, 2022), with more people than ever now residing in cities and urban areas (McKinney, 2002). For many species, survival in these human-modified landscapes is challenging due to a range of threats not typically present in natural landscapes (Isaac et al., 2014; McDonald et al., 2013). One such threat is exposure to chemical compounds such as pesticides. With increased human occupation of landscapes there has been a notable rise in pesticide use (Sharma et al., 2019), with these chemical agents used to target invertebrate species that pose a threat to human livelihoods. The management of rodents is also increasing globally (Jacob and Buckle, 2018; Morzillo and Mertig, 2011) with the use of rodenticides rapidly increasing, particularly in urban and agricultural settings where rodents can cause considerable economic harm or are considered a nuisance (Jacob and Buckle, 2018; Watt et al., 2005).

Anticoagulant rodenticides (ARs) are the most extensively used rodenticides across diverse settings globally (Jacob and Buckle, 2018; Watt et al., 2005). Once consumed, ARs function by disrupting the vitamin K cycle, resulting in inhibited blood clotting capabilities and eventually, if a high enough dose is consumed, inducing fatal haemorrhaging (Murphy, 2018; Rattner et al., 2014). There are two types of ARs currently available globally. First-generation anticoagulant rodenticides (FGARs), e.g., warfarin, require multiple feeds to cause death in rodents but their use led to genetic and behavioural resistance (Rattner et al., 2014), which necessitated the development of the more potent second-generation anticoagulant rodenticides (SGARs). SGARs, such as brodifacoum and bromadiolone, generally require a single feed to cause death and have a longer half-life in the body (Rattner et al., 2014). Death however can take up to two weeks in rodents, during which time the individuals can consume additional doses of the poison, which accumulates in their body (Mason and Littin, 2003). Furthermore, individuals that have consumed sub-lethal and lethal doses of SGARs often show behavioural changes that make them more vulnerable to predation (Brakes and Smith, 2005). As such, SGARs pose a significant risk of bioaccumulation and secondary poisoning in predators (Erickson and Urban, 2004; Herring et al., 2017; Thomas et al., 2011). Due to their non-specific nature, the use of ARs is becoming increasingly regulated in many regions and countries in Europe (Elmeros et al., 2018) and North America (British Columbia, 2023; USEPA, 2022); however, their use remains relatively unregulated in a large portion of the world, including Australia (APVMA, 2023; Pay et al., 2021). While there are significant concerns about the use of ARs and particularly SGARs, they are highly effective at managing rodent populations. Limited evidence suggests genetic resistance to SGARs, and their long latency period allows for the administration of antidotes to prevent death.

Much of the research investigating the impact of ARs on non-target species, including predators, has been undertaken in Europe and North America (Elliott et al., 2022; Oliva-Vidal et al., 2022; Sainsbury et al., 2018; Thomas et al., 2011). In north-east Spain, López-Perea et al. (2015) found that 62.8 % out of 344 individuals from 11 predatory species of birds and mammals had detectable levels of rodenticides in their livers, with 23.3 % of individuals exposed to lethal concentrations of these chemicals. Within predatory birds, Christensen et al. (2012) found that 92 % of 430 individuals across 11 different species were exposed to ARs in Denmark, and Elliott et al. (2022) found AR residues in 74 % of individuals from 12 species in British Columbia, Canada.

Within Australia, the unintended effects of ARs are poorly understood, but there has been a focus on this issue in recent years with studies detecting the bioaccumulation of SGARs in predatory birds. For instance, Lohr (2018) found SGAR accumulation in southern boobooks (*Ninox boobook*), with this accumulation likely occurring through the owls consuming poisoned rodents, as this species is known to consume rats and mice (McDonald and Pavey, 2014). Three other Australian studies, however, found SGAR accumulation in species that do not typically consume rodents. Pay et al. (2021) found SGAR exposure in Tasmanian wedge-tailed eagles (*Aquila audax fleayi*), and Cooke et al. (2022, 2023) found SGAR accumulation in powerful owls (*Ninox strenua*). These species do not primarily consume rodents (Bilney, 2013; Brun et al., 2022), and it is likely that these predators were exposed to SGARs through other, non-rodent prey species that had consumed rodenticide baits.

Powerful owls prey on two main species, the common brushtail possum (*Trichosurus vulpecula*) and the common ringtail possum (*Pseudocheirus peregrinus*) (Cooke et al., 2006). These native possums are prevalent in eastern Australia (Harper et al., 2008; Kerle, 1984), coinciding with the distribution of powerful owls (Carter et al., 2019). Common brushtail possums (hereafter 'brushtails') are the larger of the two species, typically weighing between 1 and 4.5 kg (Kerle, 2001). They exhibit a flexible generalist herbivorous/omnivorous diet, mostly consuming fruits, flowers, and leaves from various plant species (Marsh et al., 2006). Common ringtail possums (hereafter 'ringtails') are smaller, with an average weight of 1 kg (Kerle, 2001), and are primarily folivorous (Hermsen et al., 2015). Both species have successfully adapted to human-modified landscapes, with high population densities observed in many urban areas. These high densities have resulted in significant foliage destruction in some regions (Miller et al., 2008), and consequently they are considered pests by some people (Hill et al., 2007). Both species, however, are protected under the Australian State of Victoria's *Wildlife Act 1975*, where it is illegal to persecute them (Victorian Legislation, 2023).

Considering the accumulation of SGARs in powerful owls (Cooke et al., 2022, 2023) and their primarily non-rodent diet (Bilney, 2013; Cooke et al., 2006), it is plausible that possums are being exposed to SGARs, either unintentionally or through intentional illegal targeting, and subsequently acting as transfer routes for the bioaccumulation of these substances in powerful owls. Studies undertaken in New Zealand, where brushtails are classified as introduced pests, have demonstrated that these possums readily consume rodenticide baits, and these baits effectively eliminate possum populations (Eason et al., 2020). No studies have been conducted in Australia to investigate the potential exposure of either brushtails or ringtails to non-target poisoning with rodenticides. In Europe, AR exposure in small non-target mammals have been suggested as a route for secondary poisoning of predators (e.g., Brakes and Smith, 2005; Tosh et al., 2012; Geduhn et al., 2014); however, these studies often focus on non-target rodents and other small mammals such as shrews. An investigation of medium-sized non-target mammals such as possums as a potential route for secondary poisoning of predators, will help improve our understanding of how ARs are impacting ecosystems.

This study examines the current missing link in the route of AR exposure in an apex predator, the powerful owl, by assessing AR exposure in its main prey species, brushtail and ringtail possums. This

research aims to determine the prevalence of ARs in wild brushtail and ringtail possums, and if ARs are present, the extent of AR exposure in these two possum species, and establish whether this exposure differs across different landscape types.

We hypothesise that there will be some AR exposure in both possum species, as both occur in proximity to human settlements (Hill et al., 2007), and are capable of consuming rodenticide baits. We predict that the degree of AR exposure will be higher in brushtails with their more generalist diet (Hermsen et al., 2015; Marsh et al., 2006), making them more likely to consume baits. Further to this, we envisage the degree of AR exposure will be higher in possums inhabiting human-modified landscapes (i.e., urban, and agricultural) compared to those inhabiting more natural environments (i.e., forest), as the availability of ARs in human-modified areas is likely to be greater than elsewhere.

2. Materials and methods

2.1. Study site and sample collection

Throughout 2022 we opportunistically collected carcasses of deceased brushtails and ringtails from Victoria, Australia. Our primary objective was to maximise the sample size, so we used a diverse range of channels to promote our collection efforts including government and non-government organisations, vets, wildlife shelters, and social media platforms. Our collection strategy aimed to encompass a wide geographic distribution to capture different landscape types and therefore investigate potential variations in rodenticide exposure in these different environments.

2.2. Tissue samples

In Deakin University's laboratory, whole possum specimens were dissected, and their livers extracted. Prior to delivery to the analytical testing facility, the Australian Government's accredited National Measurement Institute (NMI), all liver samples were macerated to a smooth paste and stored at −20 °C. All equipment was thoroughly cleaned between processing each sample to prevent cross contamination. Of the possums collected ($n = 160$), 135 livers were suitable for analysis, 53 brushtails and 82 ringtails. We were unable to analyse samples that were too decomposed, or where significant trauma had destroyed the liver. We also discarded samples where accurate locational data was unavailable.

2.3. Toxicological analysis

All liver samples were analysed at the National Measurement Institute (NMI) with accredited methods for determination of rodenticides in liver samples. Each of the 135 liver samples were screened for residues of eight rodenticides that are registered for the management of rodents in Australia: three FGARs (warfarin, coumatetralyl and pindone) and five SGARs (brodifacoum, bromadiolone, flocoumafen, difenacoum and difethialone). Two grams of liver sample from each possum was weighed, and the sample was homogenised with 5 ml of Milli Q water, followed by vigorous shaking on a horizontal shaker for five minutes. The sample was further extracted with 10 ml of 5 % formic acid in acetonitrile, before being shaken for an additional 30 min. Agilent EN-QuEChERS extraction salts were added to the sample, and it was shaken for two minutes before being centrifuged at 5100 rpm for 10 min at 2 °C. Next, 3 ml of the supernatant was pipetted into a 15 ml analytical tube, 5 ml of hexane was added, and the tube was shaken for two minutes, then centrifuged for 10 min at 5100 rpm. The hexane layer was removed using a vacuum pipette and discarded. A 1 ml aliquot of the supernatant was carefully transferred to a 2 ml QuEChERS dispersive tube. The sample was vortexed for 10 s, then shaken vigorously on a horizontal shaker for two minutes before being centrifuged at 13000 rpm (micro centrifuge) for three minutes. The QuEChERS supernatant was filtered through a 0.45 μm filter. After filtration, 3 μl of coumachlor was added as an internal standard to 497 μl of the filtered extract and vortexed before being transferred to a LCMS-MS vial for analysis.

A Waters TQS Tandem Quadrupole Detector Liquid Chromatograph-Mass Spectrometer (LC-MS/MS) and an ACQUITY UPLC CSH C18 100 × 2.1 mm column were used for detection and quantification of concentrations of each rodenticide. For each analytical batch, a matrix blank, solvent blank, seven points (0.0–0.030 mg/kg) matrix matched calibration and four spike levels (0.001, 0.002, 0.005 and 0.010 mg/kg) were performed to ensure all the required quality assurance and quality control were met for the reportable results. Duplicate results were performed for every 10th sample. Recovery rates for each AR were calculated using chicken liver samples spiked with additional standards. Chicken liver was used as it is the best matrix available commercially to use for the blank and control. The limits of detection (LOD) were 0.0005 mg/kg for warfarin and coumatetralyl and 0.001 mg/kg for all other rodenticides, with limits of reporting (LOR) of 0.001 mg/kg for warfarin and coumatetralyl and 0.005 mg/kg for all other rodenticides. Values below the LOR and above the LOD are reported here as trace detections, indicating presence but at low concentrations.

2.4. Statistical analysis

Rodenticide exposure was determined by calculating the proportion of samples containing detectable levels of each rodenticide (i.e., above the LOD) for both possum species. Where a rodenticide was detected, we calculated the mean, median and standard error of the concentrations for that species.

When analysing the number of SGARs detected in a sample, we used the categories: no SGARs, one SGAR, two SGARs and three or more SGARs. We used a Chi-square test of independence to investigate whether the trend in the number of SGARs detected was different between the two possum species. We then summed SGAR concentrations for each liver sample ('total SGAR concentration'). All SGAR compounds have similar molecular weights and mode of action (Rattner and Harvey, 2021) and therefore, total SGAR concentration can indicate the impact of SGARs on a given individual, although we acknowledge that different SGAR compounds can have different impacts. FGARs were not summed as their molecular weights differ greatly (Rattner and Harvey, 2021). We conducted an independent sample *t*-test to investigate whether the total SGAR concentrations were different between the species of possum.

Littin et al. (2002) found that brushtails that died of brodifacoum exposure had liver concentrations of 0.53 mg/kg. Given this finding, and that the LD_{50} (lethal dose of 50 % of individuals) of brodifacoum in rodents is 0.5 mg/kg (Kaukeinen, 1982), we created potential SGAR impact categories with 0.5 mg/kg as the threshold for potential mortality. Littin et al. (2002) also found that brushtails showed behavioural changes with brodifacoum liver concentrations of 0.17 mg/kg. Further to this, Mosterd and Thijssen (1991) found that Norway rats fed 0.2 mg/kg brodifacoum showed inhibited blood clotting; however, some studies focussing on birds reported 0.1–0.2 mg/kg total SGAR concentration as the low limit for mortality (Christensen et al., 2012; Shore et al., 2019). As such, the potential impact categories we used were:

1. Possibly lethal (>0.5 mg/kg liver weight);
2. Likely toxicological impacts (0.1–0.5 mg/kg);
3. No toxicity (<0.1 mg/kg);
4. Trace detection (LOD – LOR); and
5. No SGARs detected (below LOD).

We used a Chi-square test of independence to investigate whether there was a difference in the distributions of these categories between the two possum species.

All possums collected were also identified as either male or female and adult or juvenile during dissection, based on physical examination of the pouch or testes. We first used a two-way ANOVA to investigate

any potential differences in total SGAR concentration between males and females as well as adults and juveniles for both species. This was followed by chi-square tests of independence to investigate any differences in SGAR impact category between males and females for both species, and between adult and juvenile brushtails. Ringtails could not be analysed in this manner as our sample did not contain enough juveniles.

Spearman's rank correlation coefficient tests were conducted to investigate whether there was a relationship between possum weight and total SGAR concentration in the two species. When running this test for ringtails, samples with no detection of SGARs were removed to better visualise a potential relationship.

All possums were recorded with a location of where the carcass was found. Most samples were collected in urban areas; however, some were from forest and agricultural environments. To categorise the landscape type from which each possum came, we established a 500-m buffer around the point of collection for each possum in QGIS version 3.30.3 (QGIS.org, 2023). We chose 500 m because brushtails have been documented having home ranges up to this size (McKay and Winter, 1989), while ringtails generally have smaller home ranges (Smith et al., 2003). The goal of this was not to determine individual home ranges, but rather to establish the type of landscape in which a possum was living. We used the Victorian government's Land Cover Time series for 2015–2019 (DEECA, 2023) to define the proportion of urban, modified open (i.e., agricultural areas), and natural woodland land cover types in each buffer. A K-means cluster analysis was then undertaken using the R package 'stats' (R Core Team, 2023) to establish landscape clusters for all possums. We conducted one-way ANOVA tests to investigate whether landscape type influenced the total SGAR concentration and the number of SGARs detected in both brushtails and ringtails.

To provide a holistic assessment of influential variables, we attempted Generalised Linear Model (GLM) analyses to assess the differences in SGAR exposure based on species, sex, age and landscape types. The overall sample size limitations and zero-inflated data, especially in ringtails, prevented us from being able to model the total SGAR concentration data using this approach. Based on this, we converted SGAR exposure data to presence/absence data and ran a GLM with all variables using a binomial distribution. A GLM with a quasi-Poisson distribution was also run to assess the influence of these variables on the number of SGAR compounds detected in samples. R packages 'stats' (R Core Team, 2023) and 'tidyverse' (Wickham et al., 2019) were used for these analyses.

We also investigated the proportion of different SGAR compounds detected in brushtails and ringtails and compared this to that of powerful owls. As powerful owls predate on both possum species (Cooke et al., 2006), we investigated whether the proportion of SGARs was similar between powerful owls and possums. Data for powerful owls (n = 24) was provided by (Cooke et al., 2023). All statistical tests were undertaken across R version 4.2.1 (R Core Team, 2022), SPSS version 29 (IBM Corporation, 2022) and Microsoft Excel version 16 (Microsoft, 2023).

3. Results

3.1. Detection of rodenticides

At least one AR was detected in 48/53 (91 %) brushtails and 33/82 (40 %) ringtails. FGARs were rarely detected, comprising of the detection of warfarin in three brushtails and no ringtails (Table 1). All samples that contained FGARs also contained at least one type of SGAR, and SGARs were detected far more often than FGARs. Brodifacoum was the most detected SGAR, present in 89 % of brushtails and 35 % of ringtails, and bromadiolone was also frequently detected, being present in 17 % of brushtails and 6 % of ringtails. The other SGARs were less frequently detected in both species (Table 1). One ringtail sample had a detection of 186 mg/kg ww bromadiolone; even though this result was re-tested and verified by the NMI laboratory, it was a clear outlier and was therefore excluded from any further analysis where total SGAR concentration was used.

Table 1
Summary statistics of rodenticide detection and concentrations (mg/kg ww) in liver samples of common brushtail possums (*Trichosurus vulpecula*) and common ringtail possums (*Pseudocheirus peregrinus*) collected in Victoria, Australia in 2022. The minimum value for each rodenticide represents the lowest detected concentration. SE = standard error of the mean; ND = not detected; ww = wet weight. Total SGAR are the summed weights of all SGARs in an individual

		First-generation anticoagulant rodenticide			Second-generation anticoagulant rodenticide					
		Pindone	Coumatetralyl	Warfarin	Difenacoum	Brodifacoum	Bromadiolone	Difethialone	Flocoumafen	Total SGAR
Common brushtail possum (n = 53)	Number exposed	0/53 (0 %)	0/53 (0 %)	3/53 (6 %)	2/53 (4 %)	47/53 (89 %)	9/53 (17 %)	3/53 (6 %)	2/53 (4 %)	48/53 (91 %)
	Maximum (mg/kg ww)	ND	ND	0.057	0.007	1.708	9.610	0.010	1.400	10.428
	Minimum (mg/kg ww)	ND	ND	0.001	0.001	0.001	0.065	0.002	0.035	0.001
	Mean (mg/kg ww)	ND	ND	0.028	0.004	0.223	1.748	0.007	0.711	0.577
	Median (mg/kg ww)	ND	ND	0.025	0.004	0.021	0.420	0.009	0.711	0.065
	SE (mg/kg ww)	ND	ND	0.016	0.003	0.056	1.028	0.002	0.676	0.227
Common ringtail possum (n = 82)	Number exposed	0/82 (0 %)	0/82 (0 %)	0/82 (0 %)	4/82 (5 %)	29/82 (35 %)	5/82 (6 %)	1/82 (1 %)	5/82 (6 %)	33/82 (40 %)
	Maximum (mg/kg ww)	ND	ND	ND	0.003	0.045	186.038	0.066	1.497	186.902
	Minimum (mg/kg ww)	ND	ND	ND	0.001	0.001	0.025	0.066	0.001	0.001
	Mean (mg/kg ww)	ND	ND	ND	0.002	0.006	37.294	0.066	0.305	5.728
	Median (mg/kg ww)	ND	ND	ND	0.002	0.003	0.061	0.066	0.001	0.004
	SE (mg/kg ww)	ND	ND	ND	0.0003	0.002	37.186	0.000	0.298	5.662

3.2. Interspecific differences

As there were only three detections of FGARs, we focussed all analysis on SGARs. We found that the number of SGAR compounds detected within a sample was different between brushtails and ringtails (Chi-squared: $\chi^2 = 35.5$, df = 3, p < 0.001). Detection of one SGAR was more frequent than expected in brushtails, whereas no detection of SGARs was more common than expected in ringtails (adjusted standardised residuals 4.7 and 5.8, respectively) (Fig. 1).

Once SGAR concentrations were pooled for each sample, the mean total SGAR concentrations was greater for brushtails (0.52 ± 0.21 mg/kg ww, s.e.) than for ringtails (0.03 ± 0.02 mg/kg ww, s.e.) ($t = 2.4$, df = 53, $p = 0.020$).

Using 0.5 mg/kg ww of SGARs as the threshold for potential mortality, we found that 13/53 (25 %) brushtails and 2/82 (2 %) ringtails had likely lethal liver SGAR concentrations. Further to this, 9/53 (17 %) brushtails and 1/82 (1 %) ringtail were likely suffering from toxicological impacts of SGARs (Fig. 2). The proportions of possums within toxicity categories were different between the two species ($\chi^2 = 50.2$, df = 4, $p < 0.001$). Higher SGAR concentrations (>0.1 mg/kg ww, i.e., 'likely toxicological impacts' and 'possibly lethal' categories) occurred more frequently than expected in brushtails (adjusted standardised residuals 6.4 and 4.0, respectively) and less frequently than expected in ringtails (adjusted standardised residuals −3.4 and − 4.0, respectively). Conversely, possums with no detection of SGARs were more frequent than expected in ringtails and less frequent than expected in brushtails (adjusted standardised residuals 5.8 and − 5.8, respectively) (Fig. 2). Overall, these results indicate that brushtails are more frequently exposed to higher levels of SGARs than ringtails.

3.3. Intraspecific differences

There was no difference in the total SGAR concentration between both males and females as well as adults and juveniles, and nor was there any interaction between sex and age for either brushtails (sex F = 0.31, df = 1, $p = 0.578$; age F = 0.22, df = 1, $p = 0.640$; interaction F = 1.7, df = 1, $p = 0.197$) or ringtails (sex F = 0.16, df = 1, $p = 0.693$; age F = 0.05, df = 1, $p = 0.831$; interaction F = 0.460, df = 1, $p = 0.503$).

When total SGAR concentrations were analysed using the potential SGAR toxicity impact factors, we found a difference between SGAR exposure in adult and juvenile brushtails within the lower end of the impact categories ($\chi 2 = 10.7$, df = 4, $p = 0.030$). Trace detections (< 0.005 mg/kg) of SGARs was more likely than expected in juveniles (adjusted standardised residual 2.8), while 'no toxicity' levels (0.005–0.099 mg/kg) of SGARs was more likely than expected in adults (adjusted standardised residual 2.0). There was no difference in the likelihood of detecting higher levels of SGARs (i.e., 'likely toxicological impacts' and 'potentially lethal' impact categories) between adults and juveniles (Fig. 3).

We found no relationship between weight and total SGAR concentration in both brushtails ($r = 0.16$, n = 53, $p = 0.265$) and ringtails ($r = -0.26$, $n = 31$, $p = 0.164$).

3.4. Rodenticides across landscape types

The K-means cluster analysis revealed four clusters that best described the landscape type where individual possums were found. The landscape clusters were: very high urban (means of land-use categories: 75.3 % urban, 11.3 % modified open and 6.1 % natural woodland), high urban (means of land-use categories: 51.8 % urban, 22.1 % modified open and 22.2 % natural woodland), agricultural (means of land-use categories: 54.5 % modified open, 23.5 % natural woodland and 18.1 % urban), and forest (means of land-use categories: 73.1 % natural woodland, 8.6 % urban and 11.0 % modified open). For brushtails, 32 were from very high urban, eight from high urban, five from agricultural, and eight from forest; for ringtails, 55 were from very high urban, 14 from high urban, four from agricultural, and eight from forest. This highlights that our samples were more concentrated in urban environments.

Landscape type did not influence total SGAR concentrations ($F_{(3, 49)} = 0.34$, $p = 0.795$ and $F_{(3, 77)} = 2.17$, $p = 0.098$ for brushtails and ringtails, respectively) or the number of SGARs detected ($\chi^2 = 6.9$, df = 9, $p = 0.648$ and $\chi^2 = 3.8$, df = 9, $p = 0.924$ for brushtails and ringtails, respectively), suggesting that SGAR exposure is consistent across different landscapes from which our samples were collected.

3.5. Generalised linear models

When influences of species, sex, age and landscape type were assessed together on the detection of any SGAR, the model indicated an influence of the species only ($P < 0.001$) with SGARs being detected less often in ringtails ($\beta = -2.608$), and there was no influence of sex, age or landscape type. A model on the number of SGAR compounds detected indicated a similar trend, with the ringtails having fewer numbers of SGARs detected in their livers compared with brushtails ($P < 0.001$, $\beta = -0.89052$ for ringtails) and none of sex, age or landscape type having an influence.

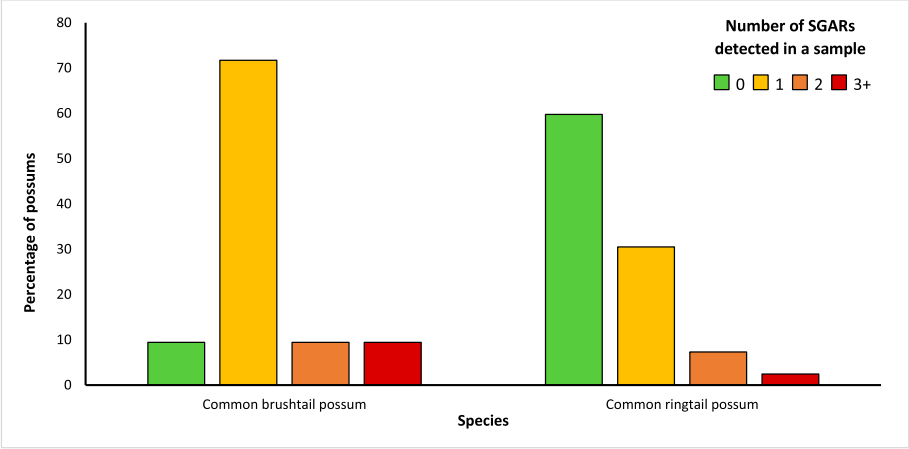

Fig. 1. Percentages of the samples with numbers of second-generation anticoagulant rodenticide (SGAR) compounds detected in a liver sample of common brushtails possums (*Trichosurus vulpecula*) ($n = 53$) and common ringtail possums (*Pseudocheirus peregrinus*) ($n = 82$). There was one instance of four SGARs being detected and so this result was assigned to the group of three or more SGARs detected.

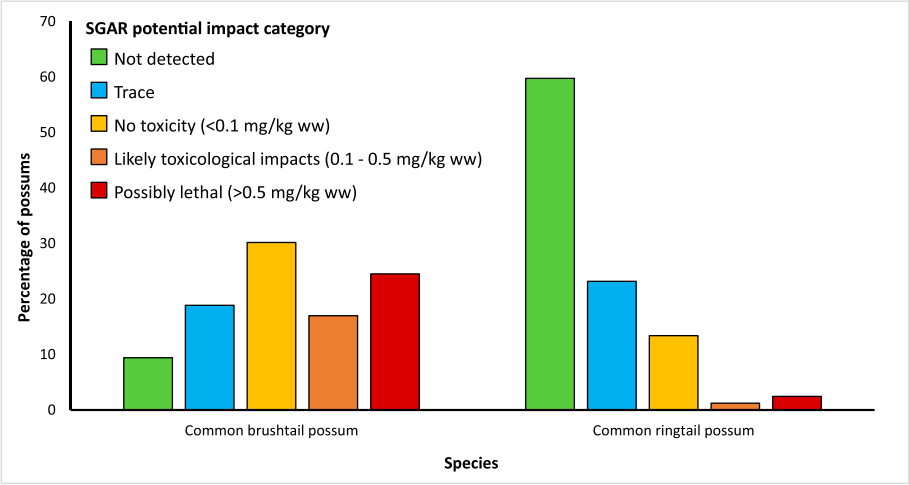

Fig. 2. Percentages of the samples at five levels of total second-generation anticoagulant rodenticide (SGAR) concentrations in liver samples of common brushtail possums (*Trichosurus vulpecula*) ($n = 53$) and common ringtail possums (*Pseudocheirus peregrinus*) (n = 82). 'Trace' category indicates that the SGAR was detected but at a concentration below the limit of reporting range (i.e., < 0.005 mg/kg ww of liver).

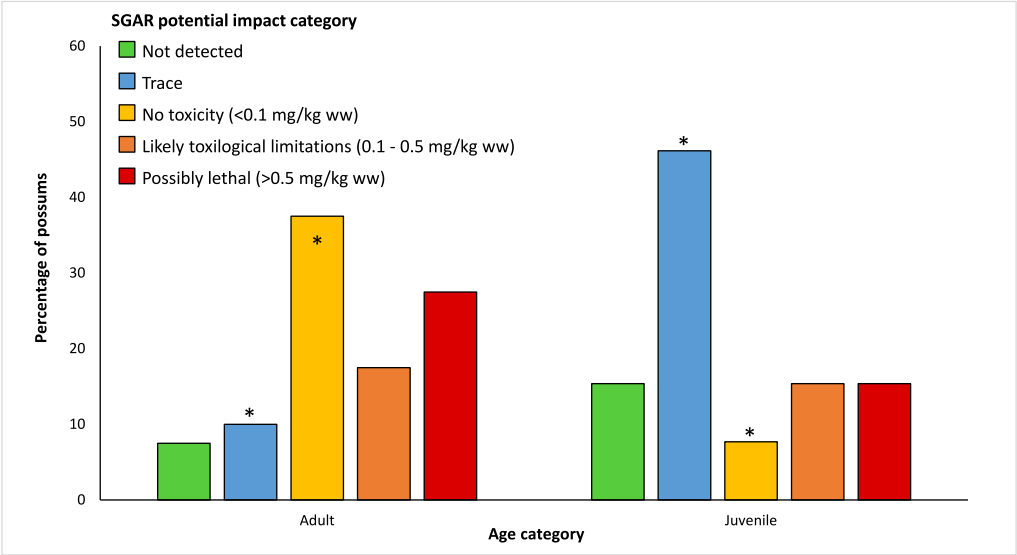

Fig. 3. Percentages of the samples at the five levels of total second-generation anticoagulant rodenticide (SGAR) concentrations of common brushtail possums (*Trichosurus vulpecula*) separated into adults ($n = 40$) and juveniles ($n = 13$). 'Trace' category indicates that the SGAR was detected but at a concentration below the limit of reporting range (i.e., < 0.005 mg/kg ww of liver). Differences between categories are indicated with asterisks (*).

3.6. Proportion of SGARs detected in possums and powerful owls

The proportions of different SGAR compounds detected in samples were similar in brushtails, ringtails, and powerful owls ($\chi^2 = 9.1$, df = 8, $p = 0.334$), indicating similar exposure patterns in possums and a predator that preys on them (Fig. 4).

4. Discussion

SGARs were developed to efficiently kill rodent pests; however, due to their non-specific nature, they are also effective at killing non-target species (Olea et al., 2009) and globally, many studies have detected SGARs in a range of predators (Christensen et al., 2012; Dowding et al., 2010; Murray, 2020; Rodríguez-Estival and Mateo, 2019). Traditionally, this exposure was thought to be due to secondary poisoning via rodents, the primary targets of SGARs (Nakayama et al., 2019); however, predators that do not typically consume rodents have also been found to be exposed to SGARs (Broughton et al., 2022; Elliott et al., 2022; Thomas et al., 2011). We investigated this missing link and confirmed the presence of SGARs in two native Australian marsupials, the common brushtail possum and the common ringtail possum. The poisoning of non-target consumers, including brushtails and ringtails, likely indicates broad contamination of Australian ecosystems, including the flow-on secondary poisoning of one of their key predators, the powerful owl (Cooke et al., 2022, 2023).

4.1. Second-generation anticoagulant rodenticides

Widespread exposure to SGARs was detected in both the brushtails and ringtails we sampled, the majority of which were from urbanised areas. Both species have adapted well to urbanisation, and are common in urban environments (Adams et al., 2013; Kerle, 2001). In Australia, the use of SGARs is not heavily restricted in urban, commercial or industrial areas, or around agricultural buildings (APVMA, 2023); therefore, it is likely that these possums are being exposed to SGARs regularly, either unintentionally or through intentional targeting via the use of

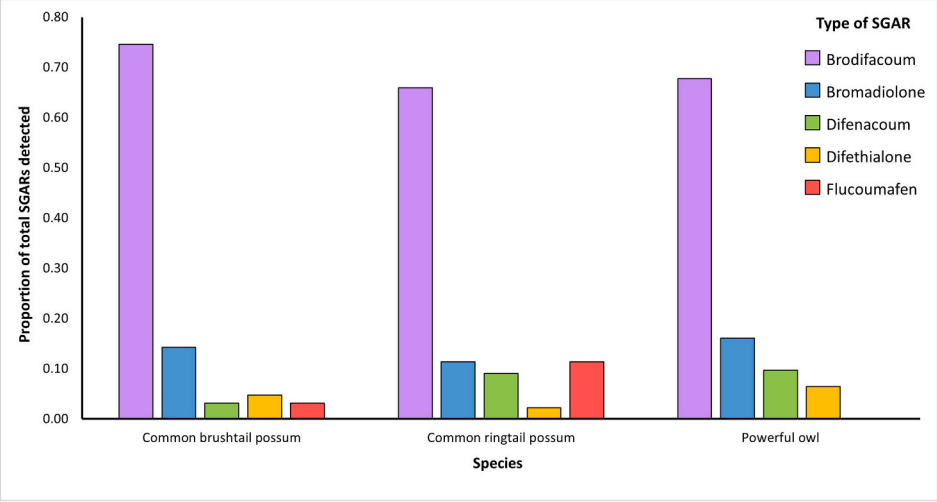

Fig. 4. The proportion of different second-generation anticoagulant rodenticide (SGAR) compounds detected in liver samples of common brushtail possums (*Trichosurus vulpecula*) ($n = 53$), common ringtail possums (*Pseudocheirus peregrinus*) ($n = 82$) and powerful owls (*Ninox strenua*) ($n = 24$) in Victoria, Australia. Data for powerful owls provided by Cooke et al. (2023).

publicly available SGARs in domestic settings. It is also important to note that flocoumafen and difethialone were detected in eight possums. These SGARs are not readily available to the public, but are used by professional pest controllers (Lohr, 2018), suggesting that at least some possums are exposed to SGARs via professional use. Unfortunately, there is a lack of data in Australia regarding the quantity of ARs sold or the specific regions where these sales take place. This absence of information hinders the ability to draw comparisons with wildlife accumulations, as highlighted by Lohr and Davis (2018). It is crucial for Australia and other nations without such data collection to initiate efforts in this regard.

We expected some level of SGAR exposure in brushtails, due to their broad generalist diet (Marsh et al., 2006); however, an exposure rate of 91 % is extremely high given that they are a non-target species of rodent control. Furthermore, these exposures resulted in high concentrations that were likely lethal or would have led to toxicological impacts on a large proportion of the possums. The 40 % exposure rate of ringtails to SGARs is also surprising, especially given their mostly folivorous diet (Hermsen et al., 2015). Whilst the concentration of SGARs was considerably lower in ringtails, their presence nonetheless highlights the non-discriminatory nature of SGARs. SGAR exposure in both species was consistent across age, sex, and weight, with the exception that trace levels were more common in juvenile brushtails and less common in adult brushtails. Exposure to SGARs was also ubiquitous across landscape types, suggesting that possum exposure to SGARs is widespread. Studies conducted in other countries have also found SGAR exposure in non-target herbivores, including generalists such as white-tailed deer (*Odocoileus virginianus*) and more specialist species, such as grey squirrels (*Sciurus carolinensis*) (Hughes et al., 2013; Stone et al., 1999).

Poisoning of non-target primary consumers is a major global concern as it increases the risk of toxins bioaccumulating more broadly in ecosystems through the secondary poisoning of predators (López-Perea and Mateo, 2018). Both brushtails and ringtails are prey species for predators such as powerful owls (Bilney, 2013; Cooke et al., 2006), and the similarity in proportions of SGAR compounds detected in the possums and the powerful owls supports the notion that exposure of these possum species to SGARs contributes to the secondary poisoning of powerful owls (Cooke et al., 2022, 2023). Globally, many studies have found that predators that do not typically consume rodents are exposed to SGARs (Broughton et al., 2022; Dowding et al., 2010; Shore et al., 2018). Our findings add to the growing evidence that the exposure of non-target native species to SGARs is prevalent in ecosystems worldwide (Nakayama et al., 2019; Rodríguez-Estival and Mateo, 2019; Stone et al., 1999), and there is an urgent need to reduce the footprint of SGARs in our ecosystems.

There is a clear, urgent need to better manage the use of SGARs, especially in countries like Australia, where their use is not tightly restricted in urban settings. The sale of SGARs to the public should be restricted so that only trained personnel who are aware of the risks of non-target and secondary poisoning, can deploy them. Furthermore, we need regulations that effectively reduce non-target species' access to SGARs. Restricting the use of SGARs to indoor settings may reduce their access to non-target species that do not use built structures for living. While brushtails do use roof cavities (Matthews et al., 2004), this may reduce some of their exposure. A further key element is regulating that SGARs only be delivered in tamper-proof, rodent-specific bait stations which prevent access by non-rodent species to rodenticides. These restrictions are already present in some parts of the U.S. and several European countries (Rattner et al., 2014). Such changes would limit the exposure of non-target consumers to SGARs, overall limiting some of their accumulation in the ecosystem.

4.2. First-generation anticoagulant rodenticides

In contrast to SGARs, FGARs were rarely detected in either possum species. FGARs are also readily available in Australia, commonly being sold in supermarkets and hardware stores. As such, it is plausible that both species encounter and consume FGARs, and our lack of detections may simply reflect the short latency period of FGARs in the body (Rattner and Harvey, 2021), rather than a lower usage rate or exposure. This finding supports other studies which have detected SGARs far more frequently than FGARs in non-target primary consumers (Stone et al., 1999). Although exposure to FGARs in possums may be higher than what we have detected, their shorter latency period (Rattner et al., 2014) likely means that FGARs contribute less to the secondary poisoning of predators.

4.3. Wider implications

The exposure to SGARs in non-target consumers is likely leading to a broader movement of these compounds through the ecosystem, and as such a wider range of predators can be secondarily exposed. This is a concern as predators fulfill vital roles in maintaining ecosystem stability through top-down regulation of primary consumers (Ritchie and

Johnson, 2009; Wallach et al., 2015). Disruption of predator-prey balances because of rodenticide exposure could also lead to significant alterations to natural abundances of primary consumer populations in the absence of a key predator.

We found greater SGAR exposure in brushtails than ringtails, and this disproportional exposure, coupled with the secondary poisoning of their predators, may lead to an overpopulation of ringtails, which can impact the wider ecosystem. As brushtails are exposed to SGARs more often, and at higher concentrations, it is possible that their densities are reduced, which could allow the density of sympatric ringtails to increase. This scenario could potentially create a vicious cycle whereby ringtail populations increase substantially, which can lead to intense defoliation of canopy trees, further exposing ringtails as a native pest species to some people and encouraging further use of rodenticide baits in urban areas. A future study to investigate this possibility is warranted.

A further concern is that the density of predators that prey on possums, such as powerful owls, is reduced through secondary poisoning from SGARs (Cooke et al., 2023). Breeding pairs of powerful owls are estimated to consume approximately 250 prey items per year (Seebeck, 1976; Tilley, 1982), with brushtails constituting 31 % of their diet, and ringtails contributing 64 %, in urban-fringe environments (Cooke and Wallis, 2004). This would equate to the potential consumption of 69 poisoned brushtails and 64 poisoned ringtails per year, resulting in a consistent intake by the owls of SGARs (potential exposure to SGARs every three days). As SGARs are known to remain in liver tissue for 100–300 days (Horak et al., 2018), this consistent intake of poisoned possums will compound in their predators. This clearly demonstrates that the non-target exposure of these possums can lead to significant secondary poisoning of powerful owls. It is possible that the prevalence of SGARs in possums is acting as one of the limiting factors that prevent their predator, powerful owls, from occupying and maintaining long-term territories in more urbanised landscapes, leading to further disruption of the predator-prey balance.

4.4. Limitations

Most possum samples used in our research were collected from highly urbanised areas, and all possums were from areas containing at least some degree of urbanisation. This bias in sampling was due to the opportunistic nature of our collection, i.e., most possums were collected from areas where people are more likely to find possum carcasses. We found no influence of landscape type on SGAR exposure for either possum species; however, it is possible that this is due to the bias in our sample, as it is likely that rodenticide use is higher in human-influenced landscapes, such as agricultural and urban land areas (Gabriel et al., 2012; Hofstader et al., 2021). To achieve more even sampling across landscape types, researchers could focus more on engaging stakeholders in non-urban landscapes (e.g., farmers) in future studies. Given that killing individuals and sampling along a gradient is not feasible, as both brushtails and ringtails are protected in Victoria under the *Wildlife Act 1975* (Victorian Legislation, 2023) and that it raises serious ethical concerns, the current sampling method was considered appropriate.

5. Conclusion

We found extensive exposure of non-target possums to SGARs, with a concerning extent of exposure in common brushtail possums. This study provides evidence that SGARs are accumulating at high levels in non-target species, likely leading to widespread secondary poisoning of an apex predator that generally does not consume rodents, the powerful owl. Globally, other studies (e.g., Broughton et al., 2022; Dowding et al., 2010) have found similar secondary poisoning in predators that do not typically consume rodents. This poisoning of predators may be impacting entire ecosystems due to the important functional roles of predators in food-webs. Further studies of rodenticide consumption by non-target consumers are needed globally to strengthen our understanding of the extent of contamination of these chemicals. Given the current limited regulations of rodenticide use in Australia, structured routine testing for rodenticides in a wide range of species would improve our understanding of their impacts in a range of ecosystems. To mitigate the exposure of non-target native herbivores, and consequent secondary poisoning of native predators in Australia, urgent changes in legislations are needed to restrict the availability and use of rodenticides, and routine testing could be used to determine the effectiveness of such legislations. Critically, further global research is needed to investigate the biological impacts of rodenticide residues in wildlife, and the population level consequences of them.

CRediT authorship contribution statement

Kieran Scammell: Writing – review & editing, Writing – original draft, Investigation. **Raylene Cooke:** Writing – review & editing, Investigation, Conceptualization. **Kaori Yokochi:** Writing – review & editing, Visualization, Investigation. **Nicholas Carter:** Writing – review & editing. **Hao Nguyen:** Writing – review & editing, Methodology. **John G. White:** Writing – review & editing, Visualization, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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