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Unmasking the impact of second-generation anticoagulant rodenticides on Masked owls (*Tyto novaehollandiae castanops*) in Tasmania

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ABSTRACT

Second-generation anticoagulant rodenticides (SGARs) are widely available in Australia for control of mammalian pests, without species distinction but particularly intended for rats and mice. Increasing rates of secondary exposure of non-target wildlife to these pesticides are well documented in avian and mammalian predators worldwide, including threatened species. We measured exposure levels of the threatened Tasmanian Masked Owl (*Tyto novaehollandiae castanops*) to a suite of anticoagulant rodenticides (ARs) available in Australia. We detected SGAR residues in 16 (94%) livers from 17 Masked Owls dying of varied causes. Residues of more than one AR were present in three birds. Likely toxic liver SGAR concentrations (>0.1 mg/kg wet weight) were present in nine (53%) of the owls. SGAR toxicosis was confirmed at necropsy for two birds. This study adds to the growing evidence for widespread exposure of non-target wildlife to SGARs and further emphasises the need for stricter regulatory control of the sale and use of these pesticide products in Australia.

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Introduction

Anticoagulant rodenticides (ARs) are used throughout the world to control rodent pest populations in urban and agricultural settings. ARs act by disrupting normal blood clotting processes, leading to uncontrolled bleeding (Rattner and Mastrota 2018). The discovery of resistance to first-generation ARs (FGARs) in the 1970s led to the development of second-generation ARs (SGARs). SGARs are far more dangerous to species that prey on rodents (such as owls) than FGARs because they are toxic in smaller amounts and take months, rather than days, to break down in body tissues (Erickson and Urban 2004; van den Brink *et al.* 2018). This prolonged presence increases the risk of bioaccumulation in predatory and scavenging species through multiple ingestions of poisoned rodents, leading to an increased risk of secondary poisoning for non-target species (Eason *et al.* 2002; Nakayama *et al.* 2019; Elliott *et al.* 2022).

There is a growing body of research evidence demonstrating non-target exposure and toxicity of SGARs in various species worldwide, particularly raptors (e.g. Stone *et al.* 1999, 2003; Albert *et al.* 2010; Okoniewski *et al.* 2021; Niedringhaus *et al.* 2021; Thornton *et al.* 2022). Much of this research has been carried out in Europe and North America where increasing exposure levels over time and increasing rates of exposure to multiple SGARs have been documented in various species, including owls (Walker *et al.* 2014; Huang *et al.* 2016; Murray 2017, 2020).

Concerns over the effects of unregulated use of SGARs on non-target species (Rattner *et al.* 2014) has led to restrictions of the sale and use of these products in many international jurisdictions including the USA (USEPA 2008), Canada (PMRA 2010) and Europe (European Union 2012). Particularly stringent restrictions have been adopted more recently in some regions such as the State of California in the USA (California Ecosystems Protection Act 2020) and British Columbia in Canada (British Columbia 2023).

In Australia, pest control products containing SGARs are still widely available to the general public in supermarkets, hardware stores and other retail outlets (Mooney 2017; Lohr and Davis 2018; Scammell *et al.* 2024). However, the Australian Pesticides and Veterinary Medicines Authority (APVMA) is currently reviewing the regulation of these products (APVMA 2020). Quantification of contemporary exposure rates of non-target Australian wildlife to SGARs under

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Table 1. First and second-generation anticoagulant rodenticides (ARs) tested for in livers of 17 Tasmanian Masked Owls.

Anticoagulant rodenticides (ARs)		
First generation ARs	Second generation ARs (SGARs)	Examples of products sold in Australia containing ARs
Warfarin		Ratsak® Double Strength
Coumatetralyl		Racumin®, Surefire® Couma Blocks, Ratex™
	Brodifacoum	Ratsak® Fast Action, Talon®, Bainbridge bait, Tomcat® II,
	Bromadiolone	Mouseoff® Bromadiolone, Ratsak® Rapid Strike, Bromakil®, Contrac® Blox, Muskil®
	Difenacoum	Ratsak® Rapid Strike, Ratshot® Blocks Blue, Surefire® Difenate, Muskil®
	Difethialone	Generation® First Strike
	Flocoumafen	Storm® Secure, Stratagem®
Pindone		Barmac® Bunny Bait, Rabbait®, Shotgun® rabbit bait

current regulations is important, both as input to the APVMA's review process and for comparison with future data collection if tighter restrictions on the availability of SGARs in Australia become implemented.

Recent studies have detected SGARs in various Australian avian predators, including Powerful Owls (*Ninox strenua*), Tawny Frogmouths (*Podargus strigoides*), Eastern Barn Owls (*Tyto javanica*) and Australian Boobooks[1] (*Ninox boobook*) in Victoria (Cooke *et al.* 2022, 2023), Australian Boobooks in Western Australia (Lohr 2018), and Tasmanian Wedge-tailed Eagles (*Aquila audax fleayi*), White-bellied Sea Eagles (*Icthyophaga*[2] *leucogaster*), and Grey Goshawks (*Accipiter novaehollandiae*) in Tasmania (Pay *et al.* 2021; Tasmanian Museum and Art Gallery unpublished data). Information from additional species, particularly predators that consume rodents, will add to this important body of knowledge and provide further evidence to support the need for stricter regulation of SGARs in Australia.

The Tasmanian Masked Owl (*Tyto novaehollandiae castanops*) is a subspecies of Masked Owl, occurring only in Tasmania, whose population is estimated at approximately 500 breeding pairs (Bell *et al.* 1997). The species is listed as Endangered under the Tasmanian Threatened Species Protection Act 1995 and as Vulnerable under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 (Threatened Species Section 2025). The focus of this short note is the quantification of exposure rates of Tasmanian Masked Owls to the suite of FGARs and SGARs available in Tasmania.

Methods

Necropsy and sample collection

The zoology department of the Tasmanian Museum and Art Gallery (TMAG) performs necropsies and sample collection on threatened Tasmanian raptor species, including Wedge-tailed Eagles, White-bellied Sea Eagles, Grey Goshawks and Tasmanian Masked Owls. Necropsies are carried out to confirm or determine cause of death and tissue samples are collected for genetics, toxin testing and diet. Museum specimens, including study skins, skeletal material and spirit specimens are prepared from carcass remains.

Deceased Tasmanian Masked Owls found opportunistically at localities throughout Tasmania were submitted to TMAG where they were stored frozen at −20°C prior to preparation as museum specimens. Recorded locations of death varied in precision from 1–25 km. The small sample size of Owls with precise location data precluded analysis of spatial correlations between AR exposure and habitat composition. Seventeen carcasses submitted to TMAG between 2015 and 2024 were thawed, necropsied and sampled between 2 June 2023 and 20 February 2024.

Ultimate causes of death were allocated to one of five categories based on necropsy findings and bird history: i) trauma (cause unknown), ii) vehicle collision, iii) disease, iv) AR toxicity and v) unknown. AR toxicity was only allocated as the ultimate cause of death if the following three conditions were met: i) total AR concentration was >0.1 mg/kg ww, ii) there was evidence of internal or external haemorrhage and iii) no evidence of another cause was found.

Liver samples (2–5 g per bird) were stored at −20°C for up to 10 months before being sent to Analytical Services Tasmania (AST) for quantification of concentrations of a suite of first- and second-generation ARs (Table 1).

Toxicological analysis

Approximately 1 g of liver sample was weighed into a 50 ml centrifuge tube along with two ceramic homogenisers. To each tube 5.0 ml ACN (Acetonitrile, LC-MS grade or equivalent) and 25 µL of Surrogate Spike (10 µg/mL Warfarin-d5 and Brodifacoum-d4) were added. Tubes were vortexed for 60 seconds, then ultrasonicated for 10

[1]Previously Southern Boobook.
[2]Previously *Haliaeetus*.

minutes. Samples were then centrifuged for 10 minutes at 4200 rpm, or until the solids separated from the solvent. A 100 µL aliquot of each upper acetonitrile layer was transferred to individual 2 mL autosampler vials with 900 µL UHQ water added. Samples were centrifuged again to remove particulates if required.

Sample analysis was carried out using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) methods incorporating multiple reaction monitoring (Zweigenbaum *et al.* 2009; Mastovska *et al.* 2017), using an Agilent 1290 Infinity II UHPLC coupled to an Agilent 6495 iFunnel triple quadrupole LCMS (Agilent Technologies). Prior to LC-MS/MS quantitation, gradient separation of target analytes was achieved using an ammonium acetate/methanol mobile phase gradient on a Waters X Select HSS T3 analytical column. Quality control samples were prepared and run every 20 samples, including blank samples, duplicate extractions, blank recoveries, sample recoveries and calibration verification standards. See Supplementary Appendix 1 for manufacturer details of analytical standards and surrogates.

Data analysis

AR concentrations were reported in mg/kg wet weight (ww) for each AR within each liver sample. Limits of quantification (LOQ) were 0.01 mg/kg for all ARs except pindone which was 0.02 mg/kg. Results were collated along with any other available information for each bird, including sex, weight, location, circumstances of death and post-mortem findings.

Combined total SGAR concentrations were calculated for each Masked Owl to estimate the overall levels of SGAR contamination and their possible effects on the birds due to their similar modes of action (Rattner and Harvey 2021). Estimated toxicological effects were based on published contamination thresholds (Lohr 2018; Pay *et al.* 2021) as follows: <0.01 mg/kg ww, probably no toxicity; 0.01–0.1 mg/kg ww, possible toxicity; 0.1–0.5 mg/kg ww, likely toxicity/possibly lethal; 0.5–0.7 mg/kg ww, probably lethal; >0.7 mg/kg ww, lethal.

Results

AR residue and total SGAR concentrations

SGARs were detected at concentrations above LOQ in 16 (94%) of the livers from 17 Masked Owls. Pindone, a FGAR, was detected in a single individual. Residues of more than one AR were present in three birds. Brodifacoum was the most commonly detected SGAR, present in 15 (88%) of the 17 birds. Bromadiolone was detected in two birds (along with brodifacoum) and difenacoum and pindone were detected in one individual. Combined total AR residue concentrations for each bird are shown in Table 2. Individual AR data are provided in Supplementary Appendix 2.

On the basis of published contamination thresholds (Lohr 2018; Pay *et al.* 2021), lethal levels of SGARs were recorded for one (6%) bird, likely toxic/possibly lethal levels in eight (47%) birds, possibly toxic levels in seven (41%) birds and no toxicity in one (6%) bird (Figure 1).

Spatial distribution and ultimate causes of death

The Masked Owls tested came from a range of localities around Tasmania (Figure 2) and died from a variety of causes. The most common ultimate cause of death was trauma ($n = 12$), definitively attributable to vehicle collision in four cases. Cause of death and pertinent necropsy findings for each individual are provided in Table 2. AR toxicity was only allocated as the ultimate cause of death for two birds; however, likely toxic or lethal concentrations of ARs were found in nine (53%) of the 17 birds and may have contributed to many of these deaths, either directly (haemorrhage) or indirectly (anaemia and reduced fitness).

Discussion

The frequency and extent of SGAR exposure in Tasmanian Masked Owls suggest that exposure to these chemicals may be both common and widespread across the population, with 94% of birds tested showing detectable levels in their livers. More than half of the birds tested had SGAR concentrations >0.1 mg/kg ww, indicative of likely toxicity with possibly lethal outcomes (Lohr 2018; Pay *et al.* 2021. These results are similar to those of Cisterne *et al.* (2023) who found detectable SGAR residues in 85% of 20 Tasmanian Masked Owls tested, with 65% of the birds having concentrations >0.1 mg/kg ww.

The high frequency and widespread detection of SGARs in Masked Owls is similar to findings in other owl species in Mainland Australia and other countries. For example, Lohr (2018) detected AR residues in 72% of Southern Boobook Owls found dead or moribund in WA, half of which showed concentrations >0.1 mg/kg in liver tissue. Cooke *et al.* (2022) detected ARs in 83% of livers from dead Powerful Owls in Victoria, 28% of which showed SGAR concentrations >0.1 mg/kg.

Table 2. Total AR levels (mg/kg ww) and necropsy data for the 17 Tasmanian Masked Owls tested.

TMAG ID	Total AR (mg/kg ww)	Ultimate Cause of Death	Necropsy findings	Date died or found	Sex	Body weight (g)	Stomach contents	Locality
B9826	**0.00**	Trauma	Internal bleeding.	28-Jun-23	M	500	Fur and mouse bones	Mayfield
B9832	**0.02**	Trauma	Fractured humerus. Lethargic.	06-May-23	M	500	Not recorded	Brighton
B9841	**0.02**	Trauma	Spinal trauma.	22-May-23	F	850	Not recorded	Newtown
B9883	**0.04**	Trauma	Fractured leg.	10-Nov-21	F	546	Empty	Black River
B9988	**0.05**	Vehicle collision	Vehicle collision. Haemorrhagic intestines.	05-May-18	M	608	At least 4 baby rodents	Cranbrook
N/A	**0.07**	Unknown	Damaged wing. Haemorrhagic gastritis. Thin.	17-Feb-24	F	340	Empty	Low Head
B9861	**0.09**	Trauma	Fractured leg. Abscess on foot.	07-Sep-22	F	1000	Not recorded	Police Point
B9986	**0.09**	Trauma	Fractured wing.	28-Oct-23	M	528	Fur and rodent bones	Cambridge
B9827	**0.12**	Unknown	Found incapacitated and emaciated.	2022	M	300	Empty	Flowerdale
B9862	**0.13**	Disease	Euthanised after developing aspergillosis.	14-May-22	M	428	Not recorded	Lillydale
B9987	**0.20**	Vehicle collision	Vehicle collision. Fractured wing. Emaciated.	31-Oct-22	F	790	Empty	Mt Nelson
B9825	**0.23**	Trauma	Large wound. Poor body condition.	29-Oct-22	F	700	Empty	Cygnet
B10022	**0.23**	Vehicle collision	Vehicle collision. Fractures and internal injuries.	01-Mar-23	F	1013	Not recorded	Moogara
B9155	**0.24**	Toxin - Rodenticide	Blood-tinged fluid in coelom and mouth.	01-Oct-15	M	593	Not recorded	Granton
B9828	**0.29**	Vehicle collision	Hit by truck. Broken wing.	30-Nov-20	M	900	Empty	Cressy
B9985	**0.40**	Trauma	Fractured leg.	16-Nov-21	F	524	Empty	Montague
B9844	**0.80**	Toxin - Rodenticide	Internal haemorrhage.	04-Dec-22	F	926	Empty	Leslie Vale

Shading from light to dark indicates four levels of estimated toxicity: None (<0.01 mg/kg), possible (0.01–0.1 mg/kg), Likely (0.1–0.5 mg/kg) and Lethal (>0.7 mg/kg).

Similar frequencies of exposure have been found in owls in Canada and the USA (Albert *et al.* 2010; Stansley *et al.* 2014; Gomez *et al.* 2023). Moreover, there is evidence that exposure levels are increasing over time as SGAR usage becomes more widespread (Huang *et al.* 2016).

Although most of the birds in our study died from traumatic causes, particularly vehicle collision, their likelihood of death may have been exacerbated by behavioural changes, such as lethargy, induced by SGAR toxicity (Stone *et al.* 2003; Rattner *et al.* 2014; Alexandrov *et al.* 2024). There is also evidence that tytonid owls (which includes Masked Owls) are more sensitive to the toxic effects of SGARs than other raptors (Thomas *et al.* 2011; Elliott *et al.* 2024).

Masked Owls feed mainly on small mammals, including introduced rodents and rabbits, as well as native marsupials such as bandicoots, possums and small macropods (Mooney 1993; Todd 2012; Young

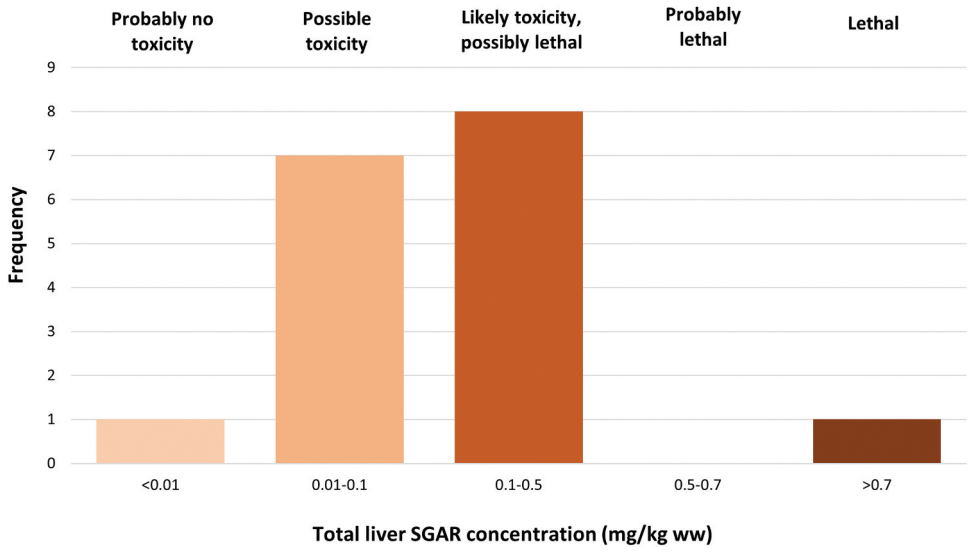

Figure 1. Frequencies of total liver SGAR concentrations (mg/kg ww) for 17 Tasmanian Masked Owls in each toxicity threshold (as per Lohr 2018; Pay *et al*. 2021).

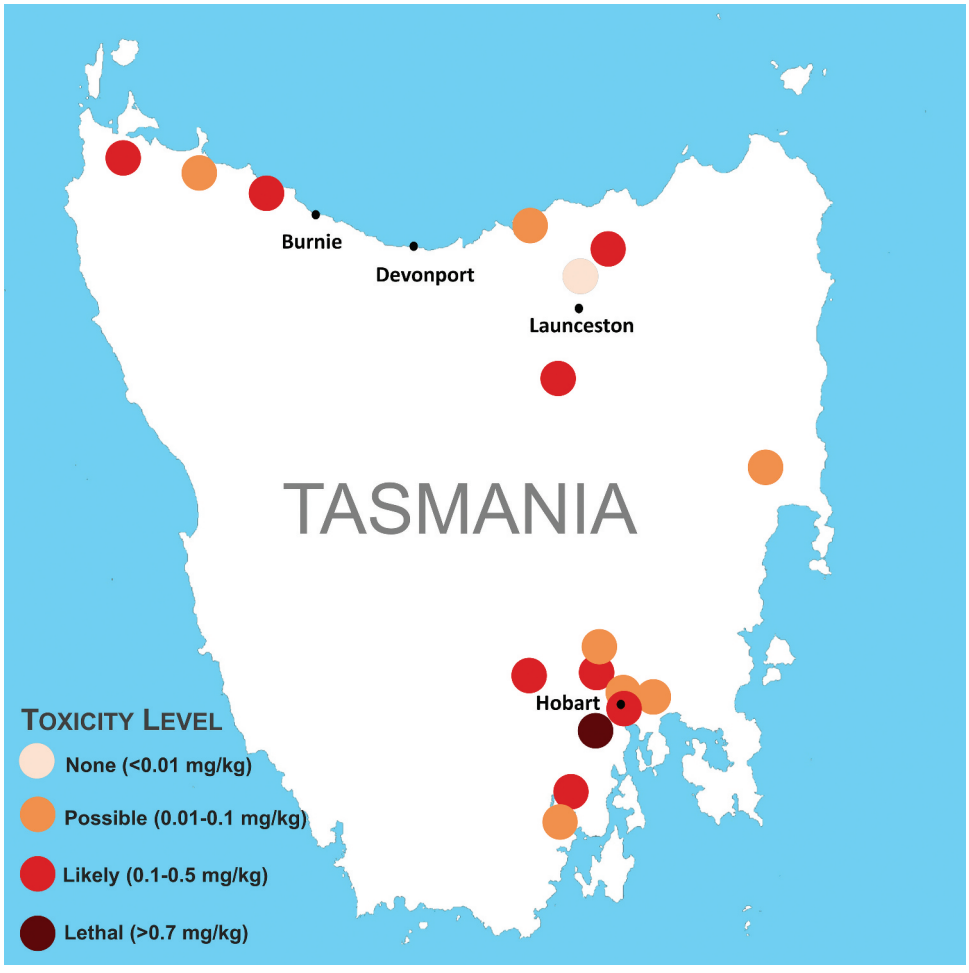

Figure 2. Spatial distribution of the Tasmanian Masked Owls in this study. Toxicity levels of SGARs in each bird are indicated by symbol colour.

et al. 2020). Rodents comprise between 10% and 60% of Masked Owl diet, depending on location and prey availability (Todd 2012). Although Masked Owls have not been recorded scavenging in the wild, small mammals that are debilitated due to SGAR poisoning often have poor situation awareness and are likely to be attractive and easy to catch (Mooney 2017), with potentially devastating consequences for predators such as owls.

SGARs are widely available in Australia, being the active components of many brands of rodent baits that are sold on the shelves of retail outlets, as well as in products used by pesticide companies in rural and urban settings (Table 1). Regulations restricting the availability of SGARs to the general public and regulating their use by pesticide companies are in force in some international jurisdictions (e.g. PMRA 2010, 2012; California Ecosystems Protection Act 2020; British Columbia 2023), in an attempt to reduce harmful effects on wildlife. The APVMA is currently reviewing the regulation of SGARs in Australia and is including data from research on the effects of SGARs on Australian wildlife in its deliberations (APVMA 2020).

The data from this study will be submitted to the APVMA via the national electronic Wildlife Health Information System (eWHIS) database (https://wildlifehealthaustralia.com.au/Our-Work/Surveillance/eWHIS-Wildlife-Health-Information-System) and will add to the growing data set obtained for raptor species in the country. A number of Australian studies have demonstrated high levels of non-target exposure to SGARs in various species, including Wedge-tailed Eagles, Powerful Owls, Australian Boobooks, Barn Owls, Tawny Frogmouths, Common Brushtail Possums (*Trichosurus vulpecula*), Common Ringtail Possums (*Pseudocheirus peregrinus*), Tasmanian Devils (*Sarcophilus harrisii*), four Quoll species (*Dasyurus geoffroii*, *D. viverrinus*, *D. maculatus*, *D. hallucatus*) and even wild reptiles and frogs (Lohr 2018; Lettoof *et al.* 2020; Pay *et al.* 2021; Cooke *et al.* 2022, 2023; Rowley *et al.* 2024; Scammell *et al.* 2024; Lohr *et al.* 2025). We hope that this expanding collection of evidence showing the widespread deleterious effect of SGARs on wildlife will lead to much stricter regulation on the sale and use of these products.

Conclusion

Our results provide evidence of widespread secondary exposure of Tasmanian Masked Owls to persistent SGARs at levels likely to cause toxicity in some individuals (Elliott *et al.* 2024). These findings are similar to those for other owl species in Australia (Lohr 2018; Cooke *et al.* 2022, 2023). Although direct correlations between liver residue concentrations and life-threatening toxic effects on individual birds are unclear (Erickson and Urban 2004; Rattner and Harvey 2021), the growing body of evidence for bioaccumulation of SGARs in a wide range of non-target species is cause for concern, especially for threatened populations. Australia is overdue for stronger legislated restrictions on the availability and use of SGARs in the landscape.

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Disclosure statement

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