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The occurrence of second generation anticoagulant rodenticides in non-target raptor species in Norway

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HIGHLIGHTS

- Brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen were measured in non-target species.
- Total concentrations of 11 to 255 ng/g were detected in golden eagles and eagle owls.
- One or more rodenticides were detected in 53% of samples.
- No rodenticides were detected in peregrine or gyrfalcons, or osprey.

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ABSTRACT

Second generation anticoagulant rodenticides (SGARs) are commonly used for rodent pest control in Norway resulting in the potential exposure of non-target raptor species. In this study the occurrence of flocoumafen, difethialone, difenacoum, bromadiolone and brodifacoum was determined in the livers of five species of raptors found dead in Norway between 2009 and 2011. The SGARs brodifacoum, bromadiolone, difenacoum and flocoumafen were detected in golden eagle (*Aquila chrysaetos*) and eagle owl (*Bubo bubo*) livers at a total SGAR concentration of between 11 and 255 ng/g in approximately 70% of the golden eagles and 50% of the eagle owls examined in this study. In the absence of specific golden eagle and eagle owl toxicity thresholds for SGARs, a level of >100 ng/g was used as a potential lethal range, accepting that poisoning may occur below this level. Thirty percent (7/24) of the golden eagle and eagle owl livers contained total SGAR residue levels above this threshold. Further estimation of the potential mortality impact on the sampled raptor populations was not possible.

1. Introduction

Rodenticides have been commercially available for rodent pest control for over 60 years in one form or another. They are used commercially to protect crops and stored human and animals feed as well as to prevent the spread of disease. They are also easily accessible for private household use although this may change in Norway in the near future. In Europe rodenticides are regulated by the EU Biocides directive 98/8/EC and due to their persistence, bioaccumulative and toxicity potential, they will be re-evaluated every 5 years instead of 10 years as is usually stipulated (EU, 2010).

First generation rodenticides (FGAR), such as warfarin, were introduced in the late 1940s (Laakso et al., 2010) but after some rodents developed resistance, second generation anticoagulant rodenticides (SGAR) were developed and introduced in the 1970s. SGARs are based on derivatives of 4-hydroxycoumarins and include flocoumafen, difethialone, difenacoum, bromadiolone and brodifacoum among others. FGARs and SGARs act as vitamin K antagonists and interfere with normal blood clotting processes and damage capillaries making vertebrates vulnerable to hemorrhage and death (Stone et al., 2003; Vandenbroucke et al., 2008).

Compared to FGARs, SGARs show greater acute toxicity and slower elimination rates (6–12 months; Eason et al., 2002). Initial consumption of SGARs generally provides a lethal dose but death can take up to 10 days, whereas FGARs are rapidly metabolized and excreted (up to 1 month for warfarin; Eason et al., 2002) and often require several feeding incidents before death occurs. The increased acute toxicity and longer liver half-life leads to SGARs being more hazardous to non-target species such as avian and mammalian predators, exposed via secondary poisoning through consumption of poisoned prey. Predator species can accumulate SGARs with multiple feedings on poisoned prey until a cumulative lethal dose is consumed (Godfrey, 1985). Rodent feeding habits and food storage behavior may also have an effect on secondary poisoning routes, as can climatic conditions. Some species, for example the water vole (*Arvicola terrestris*), store collected food which means successive generations can be poisoned, resulting in prolonged exposure potential for predators. The potency of the stored food is dependent on climatic conditions

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(Sage et al., 2007) which also has a bearing on the effect on predator species. Rodents that die above ground following SGAR poisoning rather than return to their burrows will in turn increase the chance of secondary exposure to scavenging species.

Risk assessment and establishing a lethal dose are difficult for SGAR poisoning of non-target species. A non-target species may only need to consume a small number of bait pellets to exceed the lethal dose for brodifacoum, for example, compared to the unlikely event of consuming a larger number of bromadiolone pellets required to reach the lethal dose (Erikson and Urban, 2004). It has been speculated that exposure to rodenticides may alter an individual's behavior causing lethargy or reduced censorial capacity leading to accidents and death (Albert et al., 2010; Lemus et al., 2011) in addition to death caused by hemorrhage after consumption of a lethal dose.

From 1983 to 2010, the detection of SGAR residues in barn owls (*Tyto alba*) has shown a steady increase in the UK with increased usage (Walker et al., 2010). A similar increase in SGAR usage in Norway over recent years (Follestad et al., 2012) is likely to have a similar consequence for secondary consumers in Norway as in the UK.

This study aims to establish the extent of secondary poisoning in raptors found dead in Norway. There were approximately 100 SGAR products on the market in 2010 compared to only 50–60 in 2009 and over 10 new bromadiolone products were registered between 2009 and 2010 (Follestad et al., 2012). There is a paucity of data on their occurrence in Norwegian non-target species and this study aims to address this.

2. Materials and methods

2.1. Sample collection

Archived liver samples were kindly provided by the Norwegian School of Veterinary Science and the Veterinary Institute in Oslo, Norway. The birds were all discovered already dead in the wild from unknown causes at various locations all over Norway (Fig. 2). The raptors were autopsied and the livers stored frozen until analysis. Samples details are presented in Table 1.

2.2. Analysis

Residues were measured in liver as previous analysis has identified that the liver usually accumulates the majority of the SGAR residue (Gray et al., 1994; Erikson and Urban, 2004). Coumachlor was added to each liver sample (0.5 g) as an internal standard (Langford et al., 2012). 200 μl of zinc chloride solution (10%) was added and mixed thoroughly before the addition of 1.5 ml acetonitrile and further mixing and precipitation. Samples were centrifuged for 5 min at 13,000 rpm and the top layer was removed. Heptane (1 ml) was added to the acetonitrile extract and shaken before centrifugation again. The acetonitrile layer was then removed in preparation for analysis by LC/MS/MS (Acquity UPLC–Quattro Premier XE (Micromass, Sweden)). Spiked control and blank samples were extracted alongside each batch of samples to ensure the robustness of the method.

Analytes were separated on an Acquity BEH C18 column (50 mm × 2 mm × 1.7 μm) with water (10 mM ammonium acetate) and methanol (10 mM ammonium acetate) mobile phases using a gradient elution program from 50% to 1% water (10 mM ammonium acetate) over a period of 2 min with a flow rate of 0.6 ml/min. The optimized MS parameters and method recoveries are shown in Table 1 in the Supplementary material. For both positive and negative modes the source temperature was 100 °C, the desolvation temperature 450 °C, the cone gas (N_2) flow 55 L/h and the desolvation gas (He) flow 880 L/h. The capillary voltage was 3.2 kV.

3. Results and discussion

None of the selected SGARs were detected above detection limits (<2–5 ng/g w/w) in osprey, peregrine falcon or gyrfalcon livers and

Table 1
Concentration of SGAR compounds detected in raptor species found in Norway where – denotes no data available and <LoD denotes less than detection limit.

Species	Location	Year	Age/sex/weight (kg)	SGAR (ng/g w/w)					
				Flocoumafen	Difethialone	Difenacoum	Bromadiolone	Brodifacoum	Sum
Golden eagle (*Aquila chrysaetos*)	Løten	2010	Adult/M/3.745	<2	<5	<2	<5	11	11
	Flekkefjord	2009	Adult/F/–	<2	<5	<2	<5	<5	<LoD
	Vikeså	2009	Adult/F7/4.8	<2	<5	<2	50	<5	50
	Vinje	2010	Juvenile/F/5.0	117	<5	<2	13	<5	130
	Vik i Sogn	2010	Juvenile/M/–	<2	<5	<2	<5	<5	<LoD
	Hol i Tjeldsund	2010	Juvenile/M/3.4	<2	<5	<2	<5	<5	<LoD
	Hansnes	2011	Juvenile/—/—	<2	<5	<2	<5	<5	<LoD
	Åndalsnes	2009	Adult/F/5.4	15	<5	<2	31	<5	46
	Vikeså	2010	Adult/F/–	<2	<5	<2	154	57	211
	Lyngdal	2011	Juvenile/M/3.76	<2	<5	<2	20	<5	20
	Oppdal	2011	Juvenile/M/4.5	<2	<5	<2	<5	110	110
	Engerdal	2009	Juvenile/F/3.8	<2	<5	<2	<5	<5	<LoD
	Balestrand	2009	Juvenile/M/2.78	<2	<5	<2	<5	29	29
	Rena	2009	Juvenile/F/4.05	<2	<5	<2	22	99	121
	Tolga	2011	Adult/F/5.02	<2	<5	<2	43	21	64
	Kyrksæterøra	2009	Juvenile/M/2.8	<2	<5	<2	<5	16	16
Eagle owl (*Bubo bubo*)	Hitra	2011	Adult/F/3.0	<2	<5	<2	<5	<5	<LoD
	Hitra	2011	Adult/F/–	<2	<5	39	<5	<5	39
	Kopervik	2011	Adult/F/1.6	13	<5	<6	<5	133	146
	Hitra	2010	Adult/F/2.345	<2	<5	<2	<5	<5	<LoD
	Mandal	2011	Adult/F/–	<2	<5	<2	<5	158	158
	Halden	2010	Adult/F/1.7	<2	<5	<7	<5	95	95
	Hardbakke	2011	Adult/F/2.522	<2	<5	<2	<5	<5	<LoD
	Fitjar	2009	—/—/1.78	<2	<5	181	<5	74	255
Osprey (*Pandion haliaetus*)	Øvre Rendal	2010	Adult/M/–	<2	<5	<2	<5	<5	<LoD
	Sør Trøndelag	2009	Juvenile/–/1.135	<2	<5	<2	<5	<5	<LoD
	Valldal	2010	Adult/F/–	<2	<5	<2	<5	<5	<LoD
Peregrine falcon (*Falco peregrinus*)	Berlevåg	2011	Juvenile/F/1.48	<2	<5	<2	<5	<5	<LoD
	Roa	2010	–/M/0.77	<2	<5	<2	<5	<5	<LoD
Gryfalcon (*Falco rusticolus*)	Gaupne	2009	Adult/F/–	<2	<5	<2	<5	<5	<LoD