Anticoagulant rodenticides in red kites (*Milvus milvus)* in Britain 2016

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Contents

1. Executive Summary

Second generation anticoagulant rodenticides (SGARs) can be toxic to all mammals and birds. Various studies have shown that, in Britain, there is widespread exposure to SGARs in a diverse range of predatory mammals and birds, including red kites (*Milvus milvus*) which scavenge dead rats, a target species for rodent control. The Wildlife Incident Monitoring Scheme (WIIS) and the Predatory Bird Monitoring Scheme (PBMS) have shown that some mortalities result from this secondary exposure.

Our aim in the present study was to provide an update on previous reports by analysing liver SGAR residues in 29 red kites that had been found dead in Britain in 2016. The carcasses were submitted to the Disease Risk Analysis and Health Surveillance (DRAHS) programme, the PBMS or the WIIS. The livers of birds submitted to the DRAHS/PBMS, to the WIIS for England & Wales, and to the WIIS for Scotland were analysed for SGAR residues by the Centre for Ecology & Hydrology, Fera Science and SASA (Science & Advice for Scottish Agriculture), respectively. All four organisations are partners in the WILDCOMS network that promotes collaboration among surveillance schemes that monitor disease and contaminants in vertebrate wildlife.

In all, 21 kites (95%) from England & Wales and 5 of the 7 red kites from Scotland had detectable residues of at least one SGAR. When considering the sample of kites as a whole, difenacoum and brodifacoum were each detected in 23 kites and bromadiolone in 18. Difethialone was found in five individuals while flocoumafen was not detected in any birds

Sum liver SGAR concentrations ranged between non-detected and 1800 ng/g wet wt. (arithmetic mean: 339 ng/g, median 112 ng/g). Post-mortems indicated that 7 kites had internal haemorrhaging that was not associated with detectable trauma and SGARs were considered a contributory cause of death; these birds typically had elevated sum SGAR liver concentrations.

Overall, the proportion of red kites with detectable residues, the magnitude of residues and the proportion of birds thought to have been poisoned by SGARs were similar to those reported for red kites that died in 2015.

2. Introduction

2.1 Second generation anticoagulant rodenticides in predatory birds

Second generation anticoagulant rodenticides (SGARs) can be toxic to all mammals and birds. The PBMS (see previous reports, also Newton et al., 1999, Shore et al., 2006, Walker et al., 2008a,b) together with other studies (Dowding et al., 2010, McDonald et al., 1998, Ruiz-Suárez et al., 2016; Sainsbury et a;l., 2018; Shore et al., 2003;2015) have shown that there is widespread exposure to SGARs in a diverse range of predators in Britain. This is also true in many other countries around the world (van den Brink et al., 2018).

In response to conservation concerns over the potential impacts of SGARs on predators, the Centre for Ecology & Hydrology's Predatory Bird Monitoring Scheme (PBMS) has measured liver SGAR residues in a range of predatory birds to determine the scale and severity of secondary exposure. The red kite (*Milvus milvus*) is one of the species that we have monitored. It is a conservation priority species that was reintroduced to England and Scotland in the late 20th Century as part of an official species recovery programme (Carter and Grice 2002). Red kites are scavengers and their diet typically includes dead rats. This propensity to scavenge species that are the target of anticoagulant rodenticide control may mean that red kites are particularly vulnerable to secondary exposure and potential poisoning, and SGAR-induced deaths of kites have been documented.

Up until 2007, only a small number of red kites were received and analysed by the PBMS each year although the analysis undertaken (Walker et al. 2008a) indicated that a large proportion of reintroduced birds may be exposed to SGARs. Subsequent development of a collaboration with the Disease Risk Analysis and Health Surveillance (DRAHS) programme, run by the Institute of Zoology, has meant that the number of red kites available for chemical analysis and reporting of SGAR residues has increased. Red kite necropsies are performed predominantly by the DRAHS, occasionally by the PBMS, and analysis of liver SGARs is undertaken by the PBMS.

SGAR residues in red kites from England & Wales that are suspected of being poisoned are analysed and reported by Fera Science as part of the Wildlife Incident Investigation Scheme (WIIS) for England &Wales. The WIIS is a post-registration monitoring scheme designed to inform the pesticide approval process, and investigates the death or illness of wildlife, pets and beneficial invertebrates that may have resulted from pesticide poisoning. Monitoring through the WIIS for England & Wales and PBMS/DRAHS is complimentary in that carcasses/tissues of kites that died in England & Wales are exchanged so that birds suspected of being poisoned are analysed by WIIS while birds that would not qualify for analysis under the WIIS (typically because poisoning is not suspected) are analysed by the PBMS. The WIIS for Scotland is run by SASA (Science & Advice for Scottish Agriculture) and examines SGAR residues in any raptors found dead in Scotland. Kite carcasses from Scotland that are offered to the PBMS are redirected so that they are submitted to the Raptor Health Scotland study for post-mortem investigation and then onto SASA for chemical analysis. WIIS data (for England & Wales and for Scotland) are collated and published quarterly on line[2]. Data for birds that died in 2016 and analysed by the WIIS have been made available for the current report so that as full a picture as possible can be presented for SGAR exposure in red kites in Britain that died in

[2] http://www.hse.gov.uk/pesticides/topics/reducing-environmental-impact/wildlife/wiis-quarterly-reports.htm

2016. This complex collaboration between five separate organizations/schemes (PBMS, DRAHS, WIIS for England & Wales, Raptor Health Scotland and the WIIS for Scotland) has been facilitated by the WILDCOMS network, in which all are partners.

2.2 Aims of the current study

Our aims were to provide an update on previous studies by reporting the liver SGAR residues in 29 red kites found dead in 2016 and submitted to the DRAHS/PBMS, WIIS for England & Wales, or the WIIS for Scotland for analysis. We describe the current incidence, magnitude and likely toxicological significance of the liver SGAR residues detected in these birds.

3. Methods

The carcasses of 29 red kites that died in 2016 were collected as part of either the PBMS or the DRAHS programmes, WIIS for England & Wales, or WIIS for Scotland (Table 1 and Figure 1). Both PBMS and DRAHS projects rely on citizen science in that members of the public send in dead birds that they find. All carcasses were subject to a post-mortem examination and various tissue samples, including the liver, were excised and stored at -20ºC.

Liver SGAR residues in kites submitted to the PBMS were quantified by Liquid Chromatography Mass Spectrometry; analytical methods are outlined in the report by Shore et al (2018). The methods used by Fera Science and SASA as part of the WIIS are similar in principle to those used by the PBMS but the precise methodology, limits of detection and recoveries will differ to some extent –limits of detection and recoveries for the different laboratories are given in Appendix 1). Anticoagulant rodenticide residues are reported for compounds individually and as the sum of all compounds (ΣSGARs) and concentrations are expressed as ng/g wet weight (wet wt.). Data were statistically analysed using Minitab 16.1 (Minitab Ltd., Coventry, U.K.) and illustrated using Graphpad Prism version 5.04 for Windows (GraphPad Software, San Diego, USA).

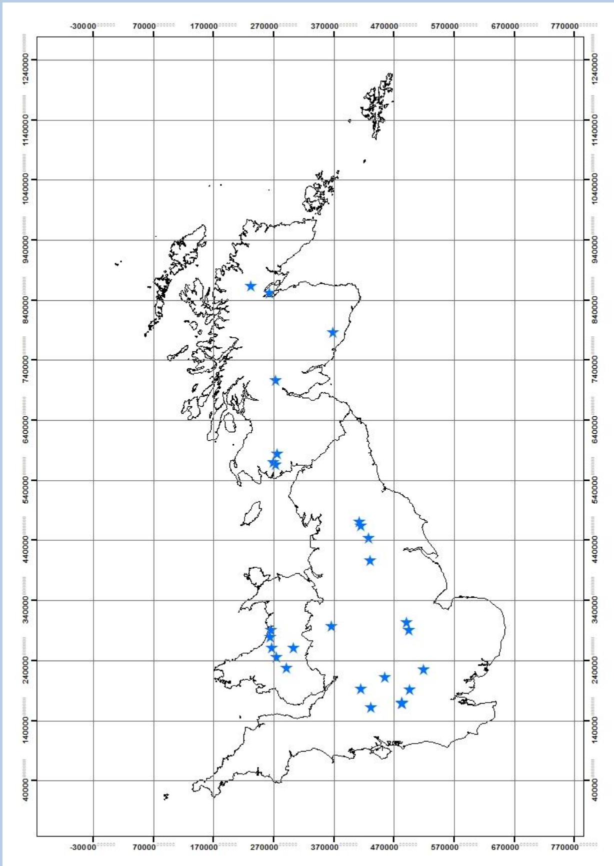

Figure 1. Location of red kites, *Milvus milvus*, found dead in 2016 for which livers were analysed for second generation anticoagulant rodenticides (SGARs) by the PBMS, SASA and Fera Science.

4. Results and Discussion

Of the 29 kites analysed, eight were adult[3] females, eight adult males, and there was no age class data available for 13 birds. Summary data for the magnitude of the liver SGAR concentrations detected are given in Table 1. Twenty-six individuals (90%) had detectable liver residues of one or more SGAR. This was similar to the prevalence rate in previous years; 95% of kites analysed since 2010 have contained ≥ 1 liver SGAR residues (Walker *et al.*, 2010, 2012, 2013, 2016, 2017).

In all, 21 (95%) of the 22 kites from England & Wales had detectable residues of at least one SGAR. The proportion for birds from Scotland with detectable residues (5/7 kites; 71%) was a little (but not statistically) smaller. This difference was not unduly influenced by the slightly higher average analytical detection limits applied in the analysis of Scottish birds (Appendix 1). When the higher limit of detection of 5 ng/g wet wt. was applied across all birds, 20 (91%) of the 22 kites from England & Wales still had detectable residues. This pattern of greater contamination in red kites from England & Wales compared with Scotland is consistent with that found in 2015 (Walker et al., 2017).

In terms of the prevalence of individual compounds, difenacoum and brodifacoum each occurred in 23 kites (79% of the birds analysed) and bromadiolone in 18 (62%). Difethialone was detected in five individuals (17%) but flocoumafen was not detected in any birds. This exposure pattern was again broadly similar to that reported for red kites that died in 2015 (Walker et al., 2017).

Sum liver SGAR (ΣSGAR) concentrations ranged between non-detected and 1800 ng/g wet wt.[4] and the arithmetic mean concentration was 339 ng/g wet wt. As in previous reports (Walker *et al.*, 2016; 2017) the majority of birds (55%) had ΣSGAR liver concentrations that exceeded 100 ng/g wet wt., the median concentration was 112 ng/g wet wt.

Post mortem examinations by the Institute of Zoology, SAC Consulting: Veterinary Services on behalf of SASA, Fera Science and CEH indicated that seven of the 29 kites (24%) had internal haemorrhaging that was not associated with detectable trauma. These birds on average had higher ΣSGAR liver concentrations than those with no haemorrhaging or with haemorrhaging associated with physical trauma (Figure 2). Given the lack of evidence for trauma and relatively high liver residues, it is probable that SGARs were a contributory factor in the deaths of these birds.

We compared whether there had been any change in average liver ΣSGAR residue magnitude between 2015 (prior to implementation of rodenticide stewardship) and 2016, the year that stewardship was implemented. In both years, birds for which SGARs were thought to be a contributory factor in their deaths had significantly higher residues than kites that had died from other causes. However, there was no significant difference between years in ΣSGAR concentrations for groups with the same cause of death (Figure 2). There was likewise no difference between years in the proportion of birds diagnosed as poisoned by SGARs (10/32 in 2015, 7/29 in 2016, Fisher's Exact test, P=0.58).

We pooled data from both 2015 and 2016 to improve our characterisation of liver residues in birds diagnosed as poisoned by SGARs (Figure 3). Overall, the median ΣSGAR concentration

[3] For the purposes of this study, adults are classed as individuals that hatched before 2015.
[4] Liver SGAR residues are sometimes given in units of µg/g wet wt. Concentrations of 1800 ng/g wet wt. are equivalent to 1.8 µg/g wet wt.

in SGAR-poisoned kites was 10 fold higher than that of birds that had died from other causes. Kites with residues >700 ng/g wet wt. all had haemorrhaging unassociated with trauma (SGARs were thought to be a contributory cause of death) whereas none of the kites with liver residues <240 ng/g wet wt. had non-trauma related haemorrhaging. However, there was considerable overlap between the "poisoned" and "other" group (Figure 3) for birds with ΣSGAR liver concentrations in the 240-700 ng/g range, presumably at least in part reflecting inter-individual susceptibility to SGARs. Thus, there is no clear diagnostic threshold for residues indicative of SGAR poisoning. The probabilistic approaches to interpreting the significance of liver residues, as proposed by Thomas *et al.* (2011), may be a better means of understanding the likely impact of ΣSGAR residues in this range at least. The current dataset may also be useful in testing the validity of such probabilistic approaches.

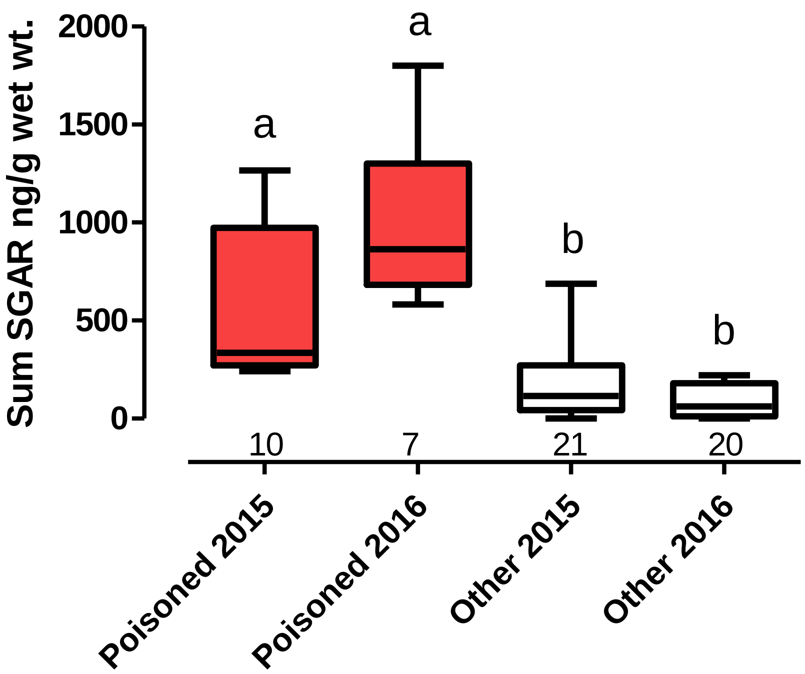

Figure 2. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of sum (Σ)SGAR concentrations in red kites that died in either 2015 or 2016 with haemorrhaging not associated with physical trauma (Poisoned) and those died from other causes (other). A Kruskal-Wallis test with Dunn's Multiple post-hoc analysis was used to analyse the data and significant ($P<0.05$) differences between groups are indicated by different letters. Sample numbers are shown near the x-axis for each group and median values for Poisoned 2015, Poisoned 2016, Others 2015 and Others 2016 were 335, 863, 116 and 62.5 ng/g wet weight, respectively. Two of the 29 birds, namely bird no. 18965 and 33a, in the 2016 cohort (Table 1) were excluded from analysis as it was unclear whether observed haemorrhaging was associated with trauma or not.

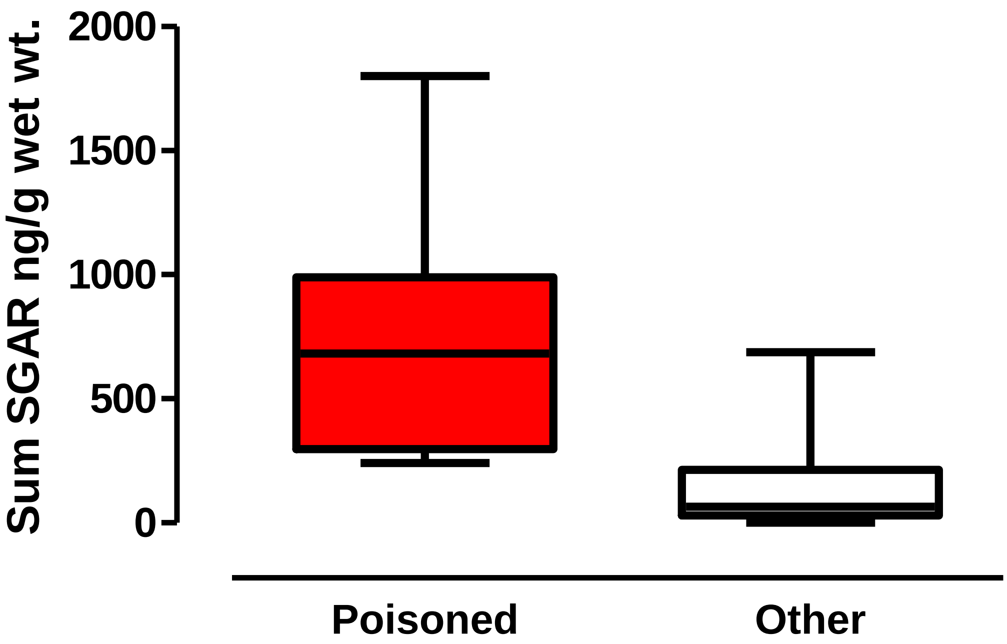

Figure 3. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of sum (Σ)SGAR concentrations in red kites that died in 2015 and 2016 combined, with haemorrhaging not associated with physical trauma (poisoned; n=17) and those that died from other causes (other; n=41). The difference in median concentrations between the "Poisoned" and the "Other" group was statistically significant (Mann-Whitney U test, U=34, $P<0.0001$)

Table 1. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2016.

Lab	Incident/ Bird code	SGAR Poisoning	Month of death	Sex	Age	Location	Concentration of SGAR (ng/g wet wt.)					
							Brom	Difen	Floc	Brod	Difeth	Σ SGARs
Fera Science	98	No	Feb	F	Adult	Cardiganshire	ND	6.8	ND	ND	ND	6.8
Fera Science	102	No	Mar	M	Adult	North Yorkshire	0.9	50.0	ND	8.0	ND	58.9
Fera Science	13	No	May	F	Adult	Radnorshire	18.0	13.0	ND	28.0	ND	59.0
Fera Science	17	No	May	F	Adult	West Yorkshire	180.0	23.0	ND	4.0	ND	207.0
Fera Science	23	No	May	U	U	North Yorkshire	12.0	40.0	ND	60.0	ND	112.0
Fera Science	30	No	May	M	Adult	Cardiganshire	140.0	30.0	ND	30.0	ND	200.0
Fera Science	33 A	Yes	Jul	U	U	Berkshire	ND	2.0	ND	18.0	ND	20.0
Fera Science	33 B	Yes	Jul	U	U	Berkshire	0.2	ND	ND	1800	ND	1800.2
Fera Science	33 C	Yes	Jul	U	U	Berkshire	ND	1.0	ND	1300	ND	1301.0
Fera Science	33 E	Yes	Jul	U	U	Berkshire	ND	10.0	ND	980.0	ND	990.0
Fera Science	37	No	Aug	M	Adult	Cardiganshire	1.0	ND	ND	4.0	ND	5.0
Fera Science	35	No	Aug	U	U	Herefordshire	ND	ND	ND	ND	ND	0.0
SASA	16057/1	No	U	U	U	Highland	30.0	184.0	ND	7.0	ND	221.0
SASA	16074/1	No	U	U	U	Central	35.0	23.0	ND	28.0	ND	86.0
SASA	16082/1	No	U	U	U	D&G	ND	ND	ND	ND	ND	0.0
SASA	16098/1	No	May	U	U	Grampian	29.0	30.0	ND	ND	ND	59.0
SASA	16141/1	No	Jul	M	U	D&G	ND	ND	ND	ND	ND	0.0
SASA	16153/1	No	Aug	U	U	Highland	ND	26.0	ND	ND	ND	26.0
SASA	16202/1	No	Dec	U	U	D&G	2.0	49.0	ND	15.0	ND	66.0
PBMS/IoZ	18965	No	Apr	F	Adult	Breconshire	ND	29.1	ND	978.4	57.0	1064.5
PBMS/IoZ	19116	Yes	Apr	F	Adult	Warwickshire	35.9	535.9	ND	3.2	8.3	583.3
PBMS/IoZ	19119	Yes	Mar	F	Adult	Buckinghamshire	ND	53.5	ND	419.8	209.6	682.9
PBMS/IoZ	19120	No	Apr	F	Adult	Leicestershire	81.2	89.1	ND	20.9	5.4	196.7
PBMS/IoZ	19125	No	Jun	M	Adult	Oxfordshire	127.6	27.3	ND	27.6	ND	182.5
PBMS/IoZ	19238	Yes	Nov	M	Adult	Berkshire	ND	8.1	ND	855.3	ND	863.3
PBMS/IoZ	19239	No	Sep	M	Adult	Wiltshire	69.1	6.3	ND	33.6	ND	109.0
PBMS/IoZ	19240	Yes	Jun	F	Adult	S.-W. Yorkshire	2.1	ND	ND	70.5	638.6	711.2
PBMS/IoZ	19242	No	Apr	M	Adult	Carmarthenshire	13.8	20.4	ND	2.7	ND	36.9
PBMS/IoZ	19243	No	Oct	M	Adult	Shropshire (Salop)	54.4	115.4	ND	3.4	ND	173.2

M – male; F- female; U – sex not determined; ND = non-detected; Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth - difethiolone; Those birds that showed signs of haemorrhaging that was not associated with physical trauma are highlighted in yellow. These 7 kites make up the 2016 “poisoned” group in Figure 2.

5. Conclusions

The monitoring of SGAR residues in red kites remains an important contribution to our understanding of SGAR exposure in wildlife, particularly in relation to predators and scavengers that feed directly on target prey, such as the brown rat.

Of the 29 red kites from England, Wales and Scotland analysed overall most had been exposed to SGARs and 7 (24%) were considered likely to have been poisoned by SGARs. This mortality prevalence does not include any other type of poisoning that may occur because of illegal use of other pesticides and through exposure to lead (Pain et al., 2007; Molenaar et al., 2017). Three of the kites that were diagnosed to have been poisoned (Fera Science incident codes 33B, 33C, 33E – Table 1) were from one incident investigated by the WIIS (England & Wales) and were likely a consequence of abuse or misuse of SGARs. It is unknown whether exposure of the four individuals (out of 10 examined) diagnosed as poisoned by PBMS/IoZ were the result of incidental secondary exposure or due to misuse or abuse.

Overall, our results to date suggest that poisoning is a significant mortality factor in red kite populations, especially in England and Wales. Our findings do not indicate any impact of rodenticide stewardship to date on exposure of red kites to SGARs, either in terms of overall exposure or the relative prevalence of different compounds. This is not surprising given that stewardship was only fully initiated part way through 2016.

Data available for kites from Scotland in 2015 and 2016 were consistent in that they indicated lower exposure in birds from Scotland than in those from England & Wales; this has also been noted previously for barn owls *Tyto alba* (Shore et al., 2015).

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We thank all the members of the public who have submitted carcasses to the Predatory Bird Monitoring Scheme (PBMS). Their efforts are key to the success of the scheme. The PBMS was supported by the Natural Environment Research Council award number NE/R016429/1 as part of the UK-SCaPE programme delivering National Capability in 2017-18 with additional funding from Natural England (NE) and the Campaign for Responsible Rodenticide Use (CRRU).

The Wildlife Incident Investigation Scheme in England is under the policy responsibility of the Chemicals Regulation Division of the Health and Safety Executive (HSE) and the WIIS is run on HSE's behalf by Natural England. In Wales, Scotland and Northern Ireland, the WIIS is run by the Welsh Government, the Science and Advice for Scottish Agriculture (SASA) on behalf of the Scottish Government and the Department of Agriculture and Rural Development, respectively.

This report was peer-reviewed by Drs Susan Zappala and Alastair Burn of Natural England and Dr Alan Buckle (Campaign for Responsible Rodenticide Use).

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8. Appendix 1 – Summary of limits of detection and spiked standard recoveries for anticoagulant rodenticides by LC-MSMS analysis across schemes

Table 2 Limits of detection (LoD; ng/g wet wt.) and percentage recovery for spikes used in analysis by PBMS (CEH), WIIS England & Wales (Fera Science) and WIIS Scotland (SASA) laboratories.

	CEH		Fera Science		SASA	
	LoD	% Spike recovery#	LoD	% Spike recovery$	LoD	% Spike recovery*
Brodifacoum	1.3	66	0.6	63.5	5	85
Bromadiolone	1.3	49	0.6	94	5	86
Difenacoum	1.3	-	0.6	94	5	90
Flocoumafen	1.3	-	0.6	104.5	5	79
Difethialone	2.6	-	0.6	82.8	5	84

* Spiked at 20 ng/g wet wt., # spiked with deuterated spiking solution.