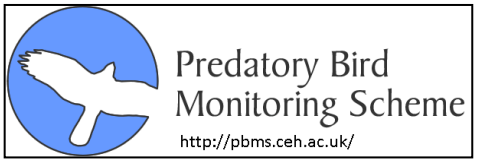

Power analysis of liver second generation anticoagulant rodenticide (SGAR) residue data in barn owls from Britain: a Predatory Bird Monitoring Scheme (PBMS) report

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1 Executive Summary

Five Second Generation Anticoagulant Rodenticides (SGARs) - difenacoum, bromadiolone, brodifacoum, flocoumafen and difethialone - are currently authorised for use in Britain. Only difenacoum and bromadiolone have been authorised for outdoor use. The other more acutely toxic compounds have been restricted to indoor use so as to mitigate against non-target exposure. However, it is not possible to clearly distinguish between the five SGARs in terms of environmental toxicity (risks to non-target species) and the UK regulatory view is that they should be treated identically in terms of authorisations. As there is a need for outdoor use for protection of public health, infrastructure and to prevent economic loss due to rodent infestations, it has been proposed that all SGARs should receive authorisations that include use in open areas. This change would be accompanied by industry-led stewardship designed to deliver responsible outdoor use and thereby minimize exposure and risk to non-target species.

Such proposals represent a major change to current authorisations in the UK and there is a need to monitor outcomes. The Predatory Bird Monitoring Scheme (PBMS - https://pbms.ceh.ac.uk/) has previously monitored SGAR exposure in barn owls (*Tyto alba*), a sentinel for generalist predators of small mammals in rural areas. The PBMS dataset represents a large and unique baseline against which to assess impacts on non-target exposure arising from changes to SGAR authorisation. The current project investigated whether monitoring liver SGARs in barn owls could be used as a decision support tool for assessing impacts on non-targets of changed SGAR authorisations and accompanying stewardship. The key issue was to determine the power of the monitoring to detect change. Our specific objective was to conduct an analysis that elucidated how many barn owls would have to be analysed each year over a 10 year period to detect, with adequate power, a 5%, 10%, 20%, and where appropriate, 50% change in exposure from that recorded in owls that died between 2007 and 2012. Exposure is characterised as the magnitude (or for floumafen and difethiolone the prevalence) of liver SGAR residues in barn owls.

Statistical analysis of liver concentration data for SGARs indicated that values had heavily skewed distributions that could not be overcome by data transformation. Data were therefore split on a statistical basis that also had toxicological relevance. This split was such that residues were classed as low concentrations (< 0.1 µg/g wet wt) or high concentrations (> 0.1 µg/g wet wt). The relationship between the number of birds analysed per year and the time taken to detect changes of specified magnitude are presented in the report. This was done for change scenarios that had adequate (≥ 70%) power using the following metrics:

- Change in low concentrations of brodifacoum, difenacoum, bromadiolone, summed SGARs (∑SGARs)
- Change in high concentrations of brodifacoum, difenacoum, bromadiolone, ∑SGARs
- Changes in the ratio “number of owls with high concentrations: number of owls with low concentrations” for brodifacoum, difenacoum, bromadiolone, ∑SGARs

- Change in the ratio of birds with detectable residues of flocoumafen and difethiolone (too few owls had detected residues of these compounds to permit an analysis using concentration data)

Generally, changes of 10% or more could be detected with adequate power for all metrics for individual SGARS and ∑SGARs. The timeframe over which such changes could be detected varied between compounds and depended on the number of birds that would be monitored in each year.

The outcome of the analysis is that it demonstrates monitoring of barn owls for liver SGAR residues could provide a regulatory tool for assessing the impact of changes in authorised use and stewardship on exposure of non-target species. These data would provide information on: (i) changes in overall exposure and risk, and (ii) variation in prevalence of different individual compounds in barn owls. SMART (specific, measurable, achievable, realistic, timely) targets could be set using the metrics outlined above. It would also be possible to use the data to explore if differences in prevalence or concentrations of individual SGARs were related to presence of resistance in rats, although we did not conduct power analyses for this metric.

Measurement of liver SGARs in barn owls provides information on changes in predator secondary exposure that are mediated through consumption of non-target small mammals in rural areas. It does not provide evidence of changes in exposure primarily mediated through consumption of exposed/poisoned rats or other foodchains, nor on the effects of changed use in non-rural areas. Use of liver SGAR residues in barn owls, as described in this document, for monitoring the outcomes of changed authorisation and stewardship of SGARs should be done in conjunction with other more limited data available for other species.

2 Introduction

2.1 Background to the PBMS

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the Centre for Ecology & Hydrology's long-term contaminant monitoring and surveillance work on avian predators. The PBMS is a component of CEH's National Capability activities.

By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife. Our monitoring provides the scientific evidence needed to determine how chemical risk varies over time and space. This may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change. Our monitoring also allows us to assess whether detected contaminants are likely to be associated with adverse effects on individuals and their populations.

Overall, the PBMS provides a scientific evidence base to inform a range of regulatory policy, including product authorisation under the Biocidal Product Regulation (BPR, Regulation (EU) 528/2012), the effectiveness of the REACH directive (EC) No 1907/2006 and OSPAR convention in controlling or banning emissions to the environment of harmful chemicals and the impact in the UK of the United Nations Environment Programme (UNEP) Minamata Convention on Mercury .

Currently, the PBMS has two key objectives:

(i) to detect temporal and spatial variation in exposure, assimilation and risk for selected pesticides, biocides and pollutants of current concern in sentinel UK predatory bird species and in species of high conservation value

(ii) in conjunction with allied studies, to elucidate the fundamental processes and factors that govern food-chain transfer and assimilation of contaminants by top predators.

Further details about the PBMS, copies of previous reports, and copies of (or links to) published scientific papers based on the work of the PBMS can be found on the PBMS website.

2.2 Exposure of non-target predators and their prey to second generation anticoagulant rodenticides (SGARs) in Britain

A diverse range of avian and mammalian predators and scavengers in rural Britain are known to be widely exposed to Second Generation Anticoagulant Rodenticides (SGARs) – see previous PBMS reports and also Dowding et al., 2010; Hughes et al., 2013; McDonald et al., 1998; Newton et al., 1999; Shore et al., 2003a, 2003b, 2006; Walker et al., 2008a, 2008b). Defra's Wildlife Incident Monitoring Scheme (WIIS)[2] and the PBMS have shown that some mortalities result from this exposure.

Exposure is generally thought to be secondary in most predators and scavengers but many species rarely feed on commensal rodents. It is assumed that exposure arises as a result of preying on non-target small mammal species, especially wood mice *Apodemus sylvaticus* and bank voles *Myodes glareolus,* which will feed on bait they encounter (Brakes and Smith, 2005; Tosh et al., 2012). It has been argued that this exposure scenario is most likely to be significant where SGARs are used outdoors[3]. The prevalence of difenacoum and bromadiolone (the compounds that have been licensed for outdoor use in Britain) residues in barn owl livers is consistent with the assumption of outdoor use as a significant exposure pathway but these are also the compounds that are most widely used in Britain; residues in predators may simply reflect predominant usage.

2.3 Potential changes in SGAR usage and potential implications for wildlife

Five SGARs are currently authorised for use in Britain. These are difenacoum, bromadiolone, brodifacoum, flocoumafen and difethialone. Of these SGARs, only difenacoum and bromadiolone have been authorised for outdoor use. The other three more acutely toxic compounds have been restricted to indoor use as a mitigation measure to prevent unintentional, incidental primary and secondary exposure and poisoning of non-target species. However, a recent Health & Safety Executive (HSE) review of the risk assessments for the five SGARs found that, on the basis of the available ecotoxicological data, it is not possible to distinguish between them in terms of environmental toxicity (risks to non-target species) (Health & Safety Executive, 2012) . This led to the conclusion that they should all be treated in the same way in terms of authorisation.

[2] Annual WIIS reports are available at www.pesticides.gov.uk/environment.asp?id=58

[3] Use can be defined as indoors, in and around buildings and outdoors. *Indoors* is defined by the registration authorities as:- i) situations where the bait is placed within a building or other enclosed structure and where the target is living or feeding predominantly within that building or structure; and ii) behind closed doors. Open barns or buildings and tamper-resistant bait stations in open areas are not classified as indoors. Sewers or closed drains are considered to be 'indoors situations'. *In and around buildings* is understood as the building itself, and the area around the building that needs to be treated to deal with the infestation of the building. This covers uses in sewer system or ships but not in waste dumps or open areas such as farmlands, parks or golf courses (Health & Safety Executive, 2012)

Government departments have also concluded that outdoor use is required for the protection of public health, infrastructure and to prevent economic loss due to rodent infestations. It has therefore been proposed that all SGARs should receive similar authorisations which would include future outdoor use (including in open areas), but that any such changes would be accompanied by an industry-led stewardship scheme. The aim of stewardship would be to coordinate and deliver responsible outdoor use of SGARs and thereby minimise exposure and risk to non-target species.

This proposed change in authorisation may result in changes in usage and in associated secondary exposure to non-target small mammal species and their predators. It might be anticipated that there would be future greater outdoor use of brodifacoum, flocoumafen and difethialone, particularly in areas where there is resistance amongst commensal rodents to bromadiolone and/or difenacoum. This may be accompanied by a concomitant decrease in outdoor use of bromadiolone and difenacoum. Given current exposure patterns in wildlife, it might be expected that changes in usage will be reflected in contamination patterns in non-target species, assuming that outdoor use is indeed the predominant cause of secondary exposure in predators. Brodifacoum, flocoumafen and difethialone are the most acutely toxic of the five SGARs licensed for use in Britain and increased exposure of non-target species to these compounds could significantly increase likelihood of mortality. However, if stewardship is successful, overall exposure (and associated risk) in non-target species may decrease. Any such changes in secondary exposure and risk are likely to be dependent on the degree of conformance with changed label restrictions and adherence by users to the practice and behaviour changes required by stewardship. The potential uncertainties surrounding the impacts of changes in authorisations and of stewardship highlight the need to monitor actual outcomes.

2.4 Use of PBMS monitoring to determine the effects of changes in use

The PBMS has monitored exposure of non-target species to SGARs predominantly using three sentinel species, the barn owl (*Tyto alba*), the red kite (*Milvus milvus*) and kestrel (*Falco tinnunculus*). Of these, data for barn owl are the most extensive with monitoring dating back to the 1980s and, in more recent years, approximately 50 owls per year being analysed for liver SGAR residues (Table 1). This monitoring has demonstrated long-term increases in exposure of barn owls to SGARs, as described in previous PBMS reports (https://pbms.ceh.ac.uk/).

The barn owl is a sentinel for species that are generalist predators of small mammals in rural areas. The PBMS data on SGAR residues in barn owls represents a large and unique base-line dataset against which it is possible to assess the effects of change in SGAR use on non-target exposure. A key issue however is to understand the power of any such monitoring to detect change in exposure. The number of barn owls examined in each year will influence the sensitivity of monitoring in terms of the size of any change that can be detected and how long it takes to detect that change.

2.5 Aim and generic approach

The current overall aim was to determine if PBMS monitoring could be used as a decision support tool in assessing the impacts of any changes in SGAR authorisations and accompanying stewardship schemes.

This involved investigating the relationships between:

(i) the number of birds monitored per year,
(ii) the size of any change in exposure that could be detected in terms of change in mean liver concentrations and/or the presence or absence of detectable residues of individual SGARs and summed SGARs (ΣSGARs),
(iii) the number of years to detect a change of specific magnitude and
(iv) the power that any such monitoring would have

Our specific objectives were to conduct a power analysis that would provide information on the number of barn owls that need to be sampled each year over a period of up to 10 years to detect (with adequate power) a 5%, 10%, 20%, and where appropriate, 50% change in exposure. In this context, exposure was classified as (i) the % of owls with detectable liver residues of each SGAR or any SGAR, and (ii) the magnitude of residues of each SGAR or all SGARs combined additively (summed SGARs – ΣSGARs).

3 Data description, initial analyses and selection of exposure scenarios

3.1 Collection of owls and chemical analyses

We used data on liver SGAR residues in barn owls that had died between 2006 and 2012. Data were limited in this way so that all chemical analysis were by a single analytical method (see below), and this would be the method employed in any future analysis of barn owl livers. Barn owl carcasses were submitted to the PBMS by members of the public. The birds died from various causes, but mainly from road traffic collisions and from starvation. All barn owls received by the PBMS were autopsied and liver SGAR residues were analysed for difenacoum, bromadiolone, brodifacoum, flocoumafen and, in the last two years, for difethialone (Walker et al., 2012).

Chemical determination of residues was by Liquid Chromatography Mass Spectrometry and a summary of the analytical methods can be downloaded at http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Rodenticides_Methods.pdf. Anticoagulant rodenticide concentrations are reported as µg/g wet weight (ww) throughout this report. Limits of detection for each compound were between 0.001 and 0.002 µg/g ww.

3.2 Description of the dataset

Liver SGAR data were available for 395 barn owls (Table 1). This was a mix of adult and juvenile birds, where juveniles were defined as individuals that hatched in the current or previous year to that in which they were found dead. The mean (lower-upper 95% confidence limits) proportion of adults, expressed as a percentage of birds of known age, was 29.5% (20.4 – 38.7%).

Table 1. Number of adult and juvenile barn owls analysed for liver SGAR residues each year

	Number of barn owls analysed				
Year	**Adult**	**Juvenile**	**Unknown [1]**	**Total**	**% adults[2]**
2006	16	53	2	71	23.2
2007	14	33	2	49	29.8
2008	20	23	6	49	46.5
2009	17	32	4	53	34.7
2010	13	34	6	53	27.7
2011	8	48	2	58	14.3
2012	19	43	0	62	30.6
Total	107	266	22	395	28.7

[1]age of birds was not determined. [2]calculated as the % of owls of known age class (excludes owls of unknown age class)

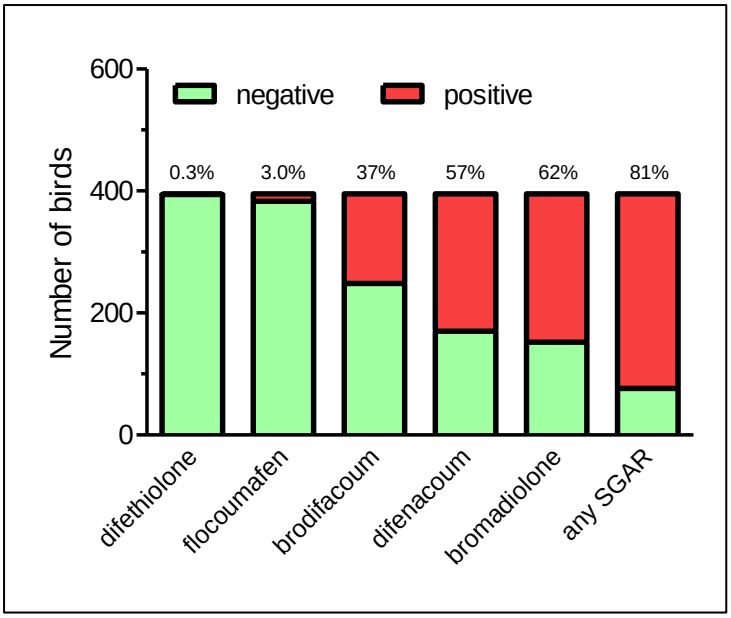

Figure 1. Number of barn owls with detected (green bars) and non-detected (red bars) residues for individual and any SGAR. % of all birds with detected residues indicated at top of column

The proportion of barn owls with detectable residues of individual or any SGAR varied from 0.3% to 81% (Figure 1). Mean wet weight concentrations (for which non-detected values were treated as zeros when calculating the mean) for the whole data set for each SGAR were:

- 0.000 µg/g (difethialone)
- 0.002 µg/g (flocoumafen)
- 0.023 µg/g(brodifacoum)
- 0.017 µg/g (difenacoum)
- 0.023 µg/g (bromadiolone)
- 0.063 µg/g (ΣSGARs)

3.3 Initial approach and analyses

The original intention had been to conduct power analyses on adult and juvenile birds separately as recent preliminary analysis of the available data for SGAR analyses in barn owls indicates that residue magnitude is significantly greater in adults than juveniles (Shore – *unpub data*). We had also intended to explore the potential for using a combined analysis of adults and juveniles to determine whether such an approach yielded significantly greater power than using data for adults or juveniles alone. However initial analyses carried out as part of this project indicated that splitting data by age class would reduce power to unacceptable levels, and so subsequent exploration of the data focused on using the dataset for all birds.

It was also initially envisaged that exploration of the power to detect change would use data both on the presence/absence of detectable residues and on data on changes in mean concentrations of individual or summed SGARs. However, initial data screening suggested that, for compounds for which there were a large number of detected values (brodifacoum, bromadiolone, difenacoum and ΣSGARs, use of concentration values would be more sensitive and provide greater power than simple changes based on the binary function of presence or absence. It would also provide a more sensitive tool for decision making as residue concentrations can to some extent be related to potential toxic risk, whereas changes in presence/absence may be due to changes in prevalence of very small residues (may represent little risk), high residues (may present significant risk) or a mixture of high and low residues. Furthermore, the already high prevalence of ΣSGAR residues in barn owls (Figure 1) meant that presence/absence data would have little sensitivity for detecting any increase in overall exposure to SGARs.

It was decided therefore that subsequent effort in the study should centre on using concentration data only for individual compounds and ΣSGARs. The only exception was for assessing exposure to flocoumafen and difethialone. This was because there have been few detected residues to date for these compounds in barn owls analysed by the PBMS. Examination of ability to detect change in exposure to these compounds had to focus solely on presence/absence data for liver residues.

4 Selection of exposure scenario and associated power

Statistical analysis of concentration data indicated that values show heavily skewed distribution (D'Agostino & Pearson normality test; K2 = 263 – 943, P<0.0001 for all compounds and ΣSGARs), resulting in high variance and extremely low power to detect any effect. Data transformation could not overcome this problem, with even the logged data showing heavy skew, and it was necessary to split the data into two sets. These sets needed to be defined on the basis that: (i) data sets were normally, log-normally or gamma distributed and could be used for power calculations; (ii) the threshold for the split was toxicologically relevant.

On a statistical basis, a logical split point for the data was 0.1 µg/g ww. Both data sets either side of this split showed data that conformed to either a log-normal distribution (>0.1 µg/g wet wt) or gamma distribution (<0.1 µg/g wet wt); the gamma distribution was used due to the high proportion of zeros in the (<0.1 µg/g wet wt) concentration data. This concentration also has some toxicological relevance. Liver ΣSGAR residues of > 0.1 µg/g ww (Newton et al., 1998) and > 0.2 µg/g ww (Newton et al., 1999) in barn owls have been previously described as in the "potentially lethal range". These concentration thresholds have been so classed on the basis of two sets of observations. The first was that owls diagnosed at post-mortem of having died from rodenticide poisoning (because they had characteristic signs of haemorrhaging from such organs as the heart, lungs, liver, brain and/or subcutaneous areas) almost all had liver residues > 0.1 µg/g ww. The second was that owls that had been experimentally poisoned had residues of the range 0.2 – 1.72 µg/g ww (Newton et al., 1999). The concentration threshold of 0.1 – 0.2 µg/g ww has therefore been used to indicate the potential of adverse effects in individuals. A more recent probabilistic analysis of residues (Thomas et al., 2011) has suggested that ΣSGAR concentrations in barn owls of 0.1 µg/g ww may be associated with a 10% probability of mortality.

For the purposes of this power analysis, we therefore split the dataset for each SGAR into two the data sets and defined them as:

- *Low concentrations*: liver SGAR residues < 0.1 µg/g ww. The bulk (>80%) of birds in each dataset fall into this category and change in these concentrations represent changes in average low level contamination
- *High concentrations*: liver SGAR residues > 0.1 µg/g ww. Changes in mean concentrations in this group are likely to be of toxicological significance

We then determined for low and high residue datasets of brodifacoum, difenacoum, bromadiolone and ∑SGARs [the datasets for which there was sufficient concentration data], the power with which 5%, 10%, 20% and 50% (high residues only) changes from mean concentrations could be detected over 1, 2, 3, 4, 5 and 10 years with the hypothetical number of birds analysed each year ranging from 10 to 200. We likewise determined the power to detect similar changes in the mean proportion of residues that were in the low and high categories.

The power to detect changes under the scenarios listed above was calculated by using simulated data based on the observed distribution of concentrations in the PBMS base datasets. The base data was used to estimate parameters of the distributions assumed for each SGAR in either of the low residue or high residue cases. Once the parameters were evaluated, the power analysis proceeded by drawing values from these distributions under the scenarios in a parametric bootstrap type approach. This approach followed the algorithm as listed below:

1. Determine the parameters of the assumed distribution from the PBMS base data
2. Randomly sample $N \cdot p$ observations from this distribution, where N represents the number of birds analysed each year and p represents the observed proportion of birds in the low/high residue case in question.
3. Copy these simulated values NYr times where NYr is the number of years we are investigating change over.
4. Multiply the $Np \text{ x } NYr$ matrix of simulations by the vector $\{0, \dots, NYr\} \cdot (Ch/NYr)$, where Ch is the proportion of change (eg 10% = 0.1). This provides a matrix of simulations that possess the inherited variability of the base data and are subject to change according to the scenario under investigation.

Following this algorithm ensured that we had a full dataset as defined by the scenario that we could analyse to test for a change over time.

The hypothesis of no change over time against the alternative hypothesis that there were changes in the concentrations over the period was analysed using Generalised Linear Models (GLMs). GLMs were used to account for the use of the gamma distribution in the low residue data and this parametric approach was favored as previous studies have shown it to be more powerful than non-parametric alternatives when there are sufficient data.

Power was estimated as the proportion of times a significant p-value corresponding to time covariate was returned from the GLMs across 100 different simulated datasets as derived according to the algorithm above. The same approach was taken across all the change, number of years and number of birds scenarios under investigation. An initial assessment of the potential power across the number of birds and number of years scenarios was made for each hypothetical change and each SGAR. This provided us with an indication of the level of power that could be achieved and whether it was sufficient.

Table 2. Power analysis for low and high concentrations and for the ratio of high to low for liver SGAR residues in barn owls. Analysis is the power to detect a 5%, 10%, 20% or 50% change from mean values; 50% change scenarios were only run for high residues. Percent power values <70% are highlighted in red.

Compound	Change	% Power to detect change		
		Low concentrations (< 0.1 ug/g ww)	High concentrations (> 0.1 ug/g ww)	High/low ratio
Brodifacoum	5%	71	23	74
	10%	80	63	71
	20%	74	71	71
	50%	-	74	-
Difenacoum	5%	71	71	71
	10%	72	73	72
	20%	80	71	73
	50%		100	
Bromadiolone	5%	71	63	71
	10%	72	72	72
	20%	72	73	75
	50%	-	72	-
∑SGARs	5%	72	45	59
	10%	71	71	71
	20%	76	71	72
	50%	-	72	-

We found that it was possible to detect all the % change scenarios with acceptable (>70%) power with the low residue dataset and with almost all the low/high ratio datasets (Table 2). Power was unacceptable for some of the 5% change scenarios for the high residue datasets but was >70% for almost all change scenarios of 10% and greater (Table 2).

For flocoumafen and difethialone, the power to detect changes of 5%, 10% and 20% in the proportion of birds with detectable residues of either compound was between 71% and 76%.

From all the estimates of power obtained across the scenarios, we used simple logistic regression models to fit the power relationship between the number of birds analysed per year and number of years taken to detect the specified change with sufficient power. This enabled us to investigate and easily read off the number of birds needed over a set number of years to be able to detect a hypothetical change with sufficient power.

Changes in the proportion of birds with low and high residues was assessed in a similar way using data simulated in an analogous way to the algorithm presented earlier with the obvious adaption that the total number of birds was simulated and changes were imposed on the relative proportion between the two classes. These data were also analysed using a GLM with a binomial distribution due to the proportional nature of the response.

The presence/absence data were subjected to the same change scenarios and analysed using non-parametric Chi-squared tests, examining the difference in frequency counts.

Wherever insufficient data was available to complete a parametric based test, non-parametric Chi-squared tests on the frequency counts were used to investigate the significant impact of the imposed change

In summary, we examined the relationship between number of bird livers analysed per year and duration of monitoring for:

- Change in mean concentration of low residues for brodifacoum, difenacoum, bromadiolone and ∑SGARs or change in proportion of owls with detected concentrations of flocoumafen and difethialone (Section 5)
- Change in mean concentrations of high residues (Section 6)
- Change in proportion of birds with low and high residues (Section 7).

In the following sections, the diagrams depicting this relationship show values for mean time in years (solid black line) and the upper and lower confidence limits (grey lines). Furthermore, the mean time taken to detect changes when monitoring 50 or 100 barn owls per year has been highlighted (red dotted lines). Fifty birds per year is the typical sample size analysed per year to date, and 100 birds is approximately the total number of barn owls that might be gathered by the PBMS each year (see section 10.2).

5 Low residues: relationship between number of birds analysed per year, duration of monitoring and size of change that can be detected

5.1 Mean low concentrations of SGARs

The % change scenarios used in these analyses below represent changes from existing mean values (Table 3).

Table 3. Mean liver SGAR low concentrations (µg/g ww) in barn owls

	Brodifacoum	Difenacoum	Bromadiolone	∑SGARs
Mean	0.005	0.011	0.014	0.024
Standard Error	0.001	0.001	0.001	0.001
Lower 95% CL	0.004	0.009	0.012	0.021
Upper 95% CL	0.007	0.013	0.016	0.027
No/ barn owls	381	375	376	329

5.2 Low concentration of brodifacoum

Changes from the mean liver low brodifacoum concentration (0.005 µg/g ww) of 5% or more would be detectable with adequate power. These are shown in Figure 2 and can be summarised as a:

- 5% change (i.e. a change from a change from 0.005 to 0.00475 or 0.00525 µg/g ww) should be detected within 8 years when analysing 100 owls/year but it would take more than 10 years when analysing 50 owls/year
- 10% change should be detected within one year (monitoring 100 owls/year) or approximately three years (monitoring 50 owls/year)
- 20% change should be detected within one year when analysing either 50 or 100 owls per year

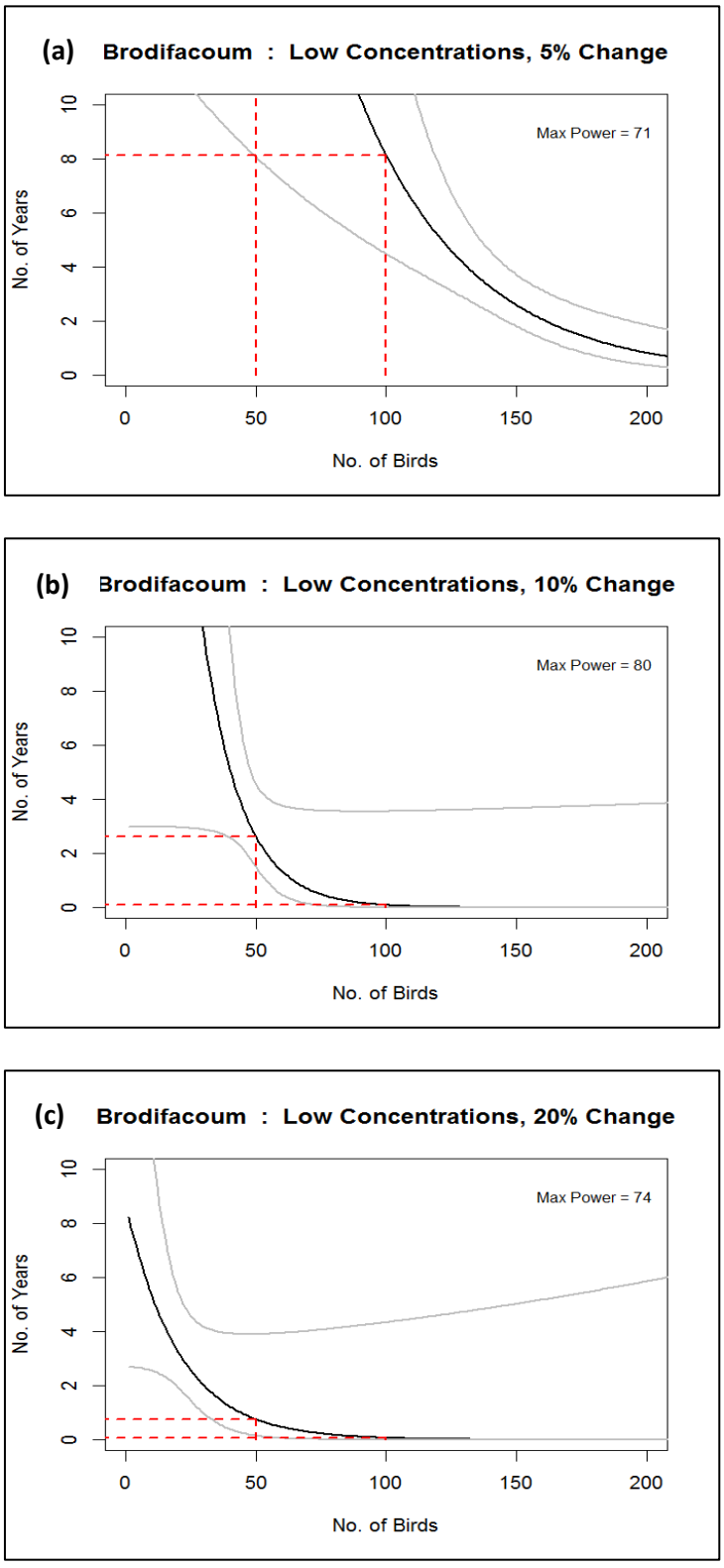

Figure 2. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 5%, (b) 10% and (c) 20% from mean low concentration (Table 3) of brodifacoum. Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

5.3 Low concentration of difenacoum

Changes from the mean liver low difenacoum concentration (0.011 µg/g ww) of 10% and 20%, but not 5%, could be detected with adequate power. These are shown in Figure 3 and can be summarised as a:

- 10% change should be detected within one year (monitoring 100 owls/year) or approximately 10 years (monitoring 50 owls/year)
- 20% change should be detected within one year when analysing either 50 or 100 owls per year

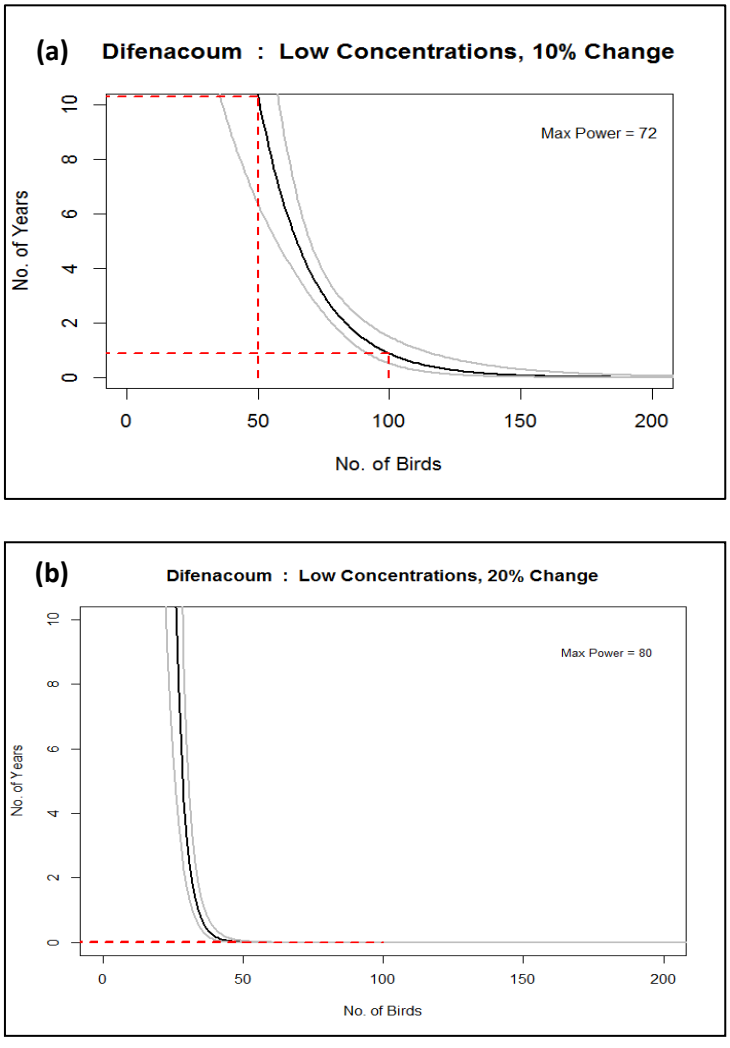

Figure 3. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 10% and (b) 20% from mean low concentration (Table 3) of difenacoum. Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

5.4 Low concentration of bromadiolone

Changes from the mean liver low bromadiolone concentration (0.014 µg/g ww) of 10% and 20%, but not 5%, could be detected with adequate power. These are shown in Figure 4 and can be summarised as a:

- 10% change should be detected within approximately three (monitoring 100 owls/year) or 9 years (monitoring 50 owls/year)
- 20% change should be detected within one year when analysing either 50 or 100 owls per year

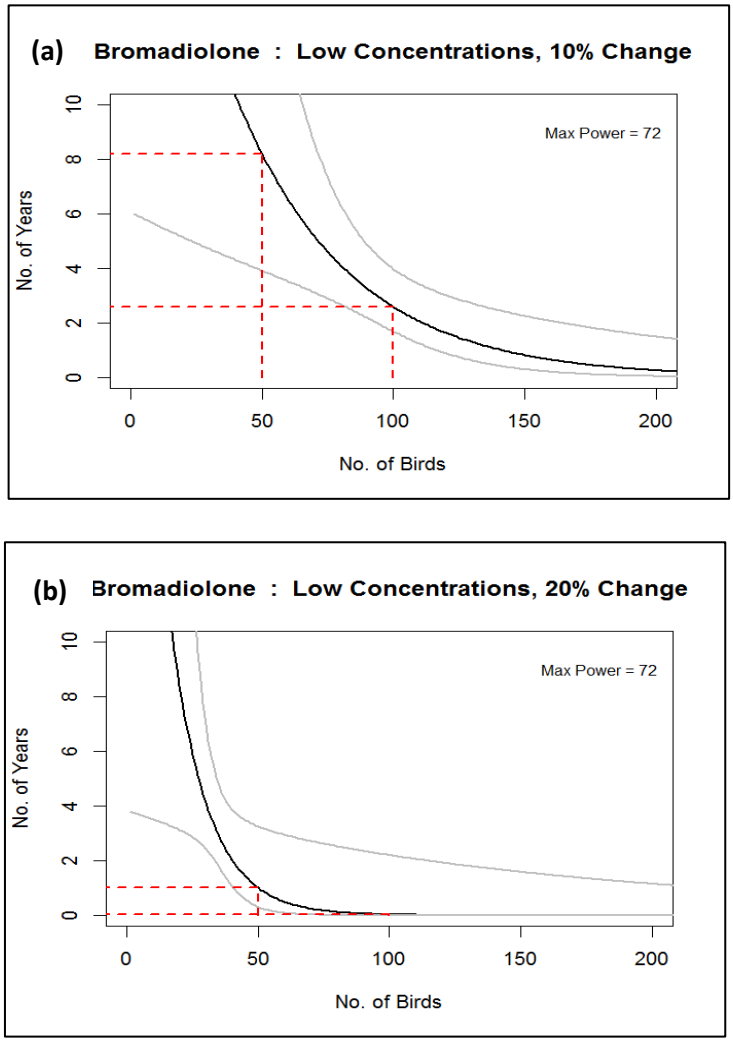

Figure 4. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 10% and (b) 20% from mean low concentration (Table 3) of bromadiolone. Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

5.5 Low concentration of ΣSGARs

Changes from the mean liver low ΣSGAR concentration (0.024 µg/g ww) of 10% and 20%, but not 5%, could be detected with adequate power. These are shown in Figure 5 and can be summarised as a:

- 10% change should be detected within 5 years (monitoring 100 owls/year) but it would take more than 10 years when analysing 50 owls/year
- 20% change should be detected within one year when analysing 100 owsl/year or two years when analysing 50 owls/year

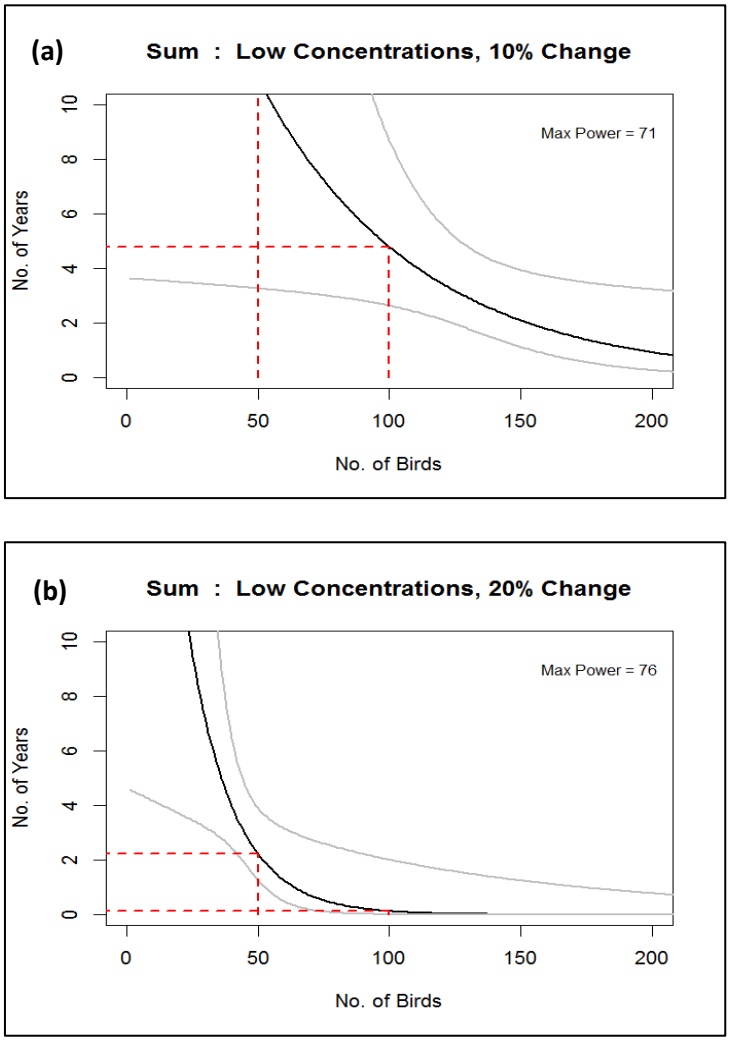

Figure 5. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 10% and (b) 20% from mean low ΣSGAR concentration (Table 3). Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

6 High residues: relationship between number of birds analysed per year, duration of monitoring and size of change that can be detected

6.1 Mean high concentrations of SGARs

The % change scenarios used in these analyses below represent changes from existing mean values (Table 4).

Table 4. Mean high liver SGAR concentrations (µg/g ww) in barn owls

	Brodifacoum	Difenacoum	Bromadiolone	∑SGARs
Mean	0.510	0.140	0.194	0.260
Standard Error	0.116	0.006	0.022	0.031
Lower 95% CL	0.259	0.127	0.148	0.198
Upper 95% CL	0.761	0.153	0.241	0.323
No/ barn owls	14	20	19	66

6.2 High brodifacoum concentration

Changes from the mean high liver brodifacoum concentration (0.510 µg/g ww) of 20% would not be detected within 10 years when monitoring 100 owls/year or less (Figure 6); smaller % changes would not be detected at all with adequate power (Table 2). However, a 50% change should be detectable within one year (monitoring 100 owls/year) or approximately 5 years if monitoring 50 owls/year (Figure 6).

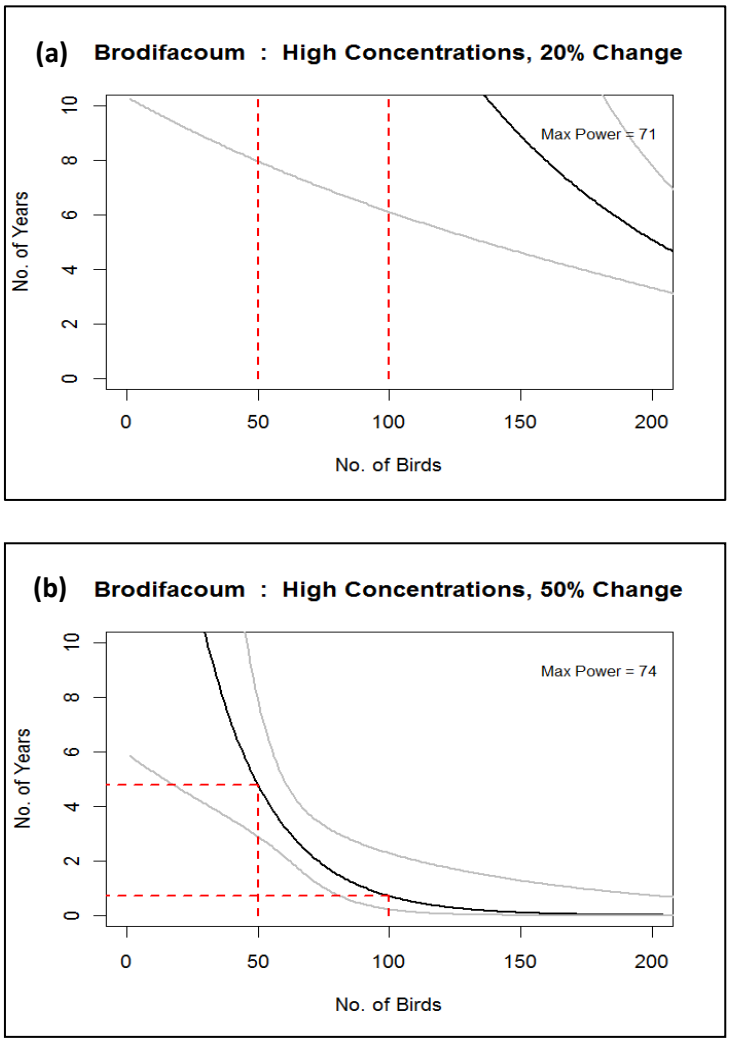

Figure 6. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 20% and (b) 50% from mean high concentration (Table 4) of brodifacoum. Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

6.3 High difenacoum concentration

Changes from the mean high liver difenacoum concentration (0.140 µg/g ww) of 5% or greater could be detected with adequate power. These are shown in Figure 7 and can be summarised as a:

- 5% change should be detected within 7 years when monitoring 100 owls/year but only after > 10 years when monitoring 50 owls/year
- 10% change should be detected within one and three years when analysing 100 and 50 owls per year, respectively
- 20% change or greater should be detected within one year when analysing 50 owls or more per year

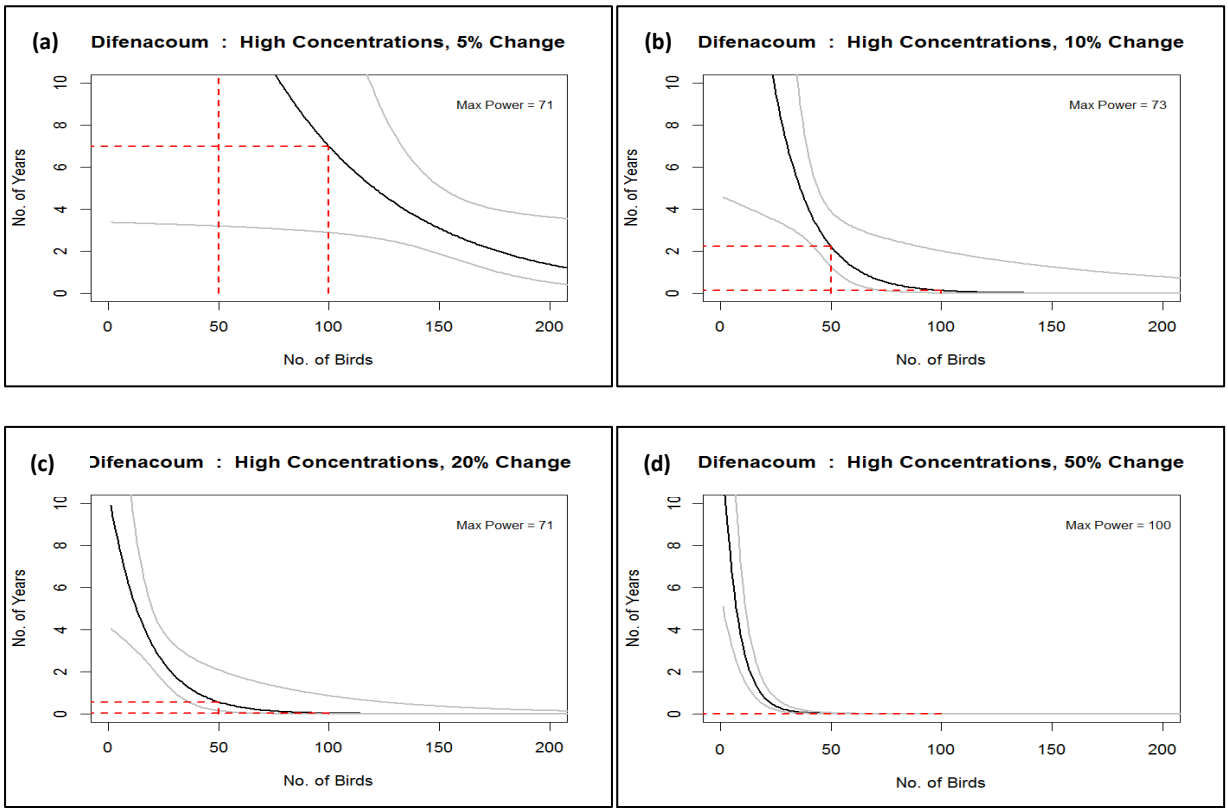

Figure 7. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 5%, (b) 10%, (c) 20% and (d) 50% from mean high concentration (Table 4) of difenacoum. Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

6.4 High bromadiolone concentration

Changes from the mean high liver bromadiolone concentration (0.194 ug/g ww) of 10%, 20% and 50%, but not 5%, could be detected with adequate power. These are shown in Figure 8 and can be summarised as a:

- 10% change should be detected within 9 years (monitoring 100 owls/year) but it would take more than 10 years when analysing 50 owls/year
- 20% change should be detected within one year when analysing 100 owls/year or approximately 5 years when analysing 50 owls/year
- 50% change should be detected within one year when analysing 50 or 100 owls/year

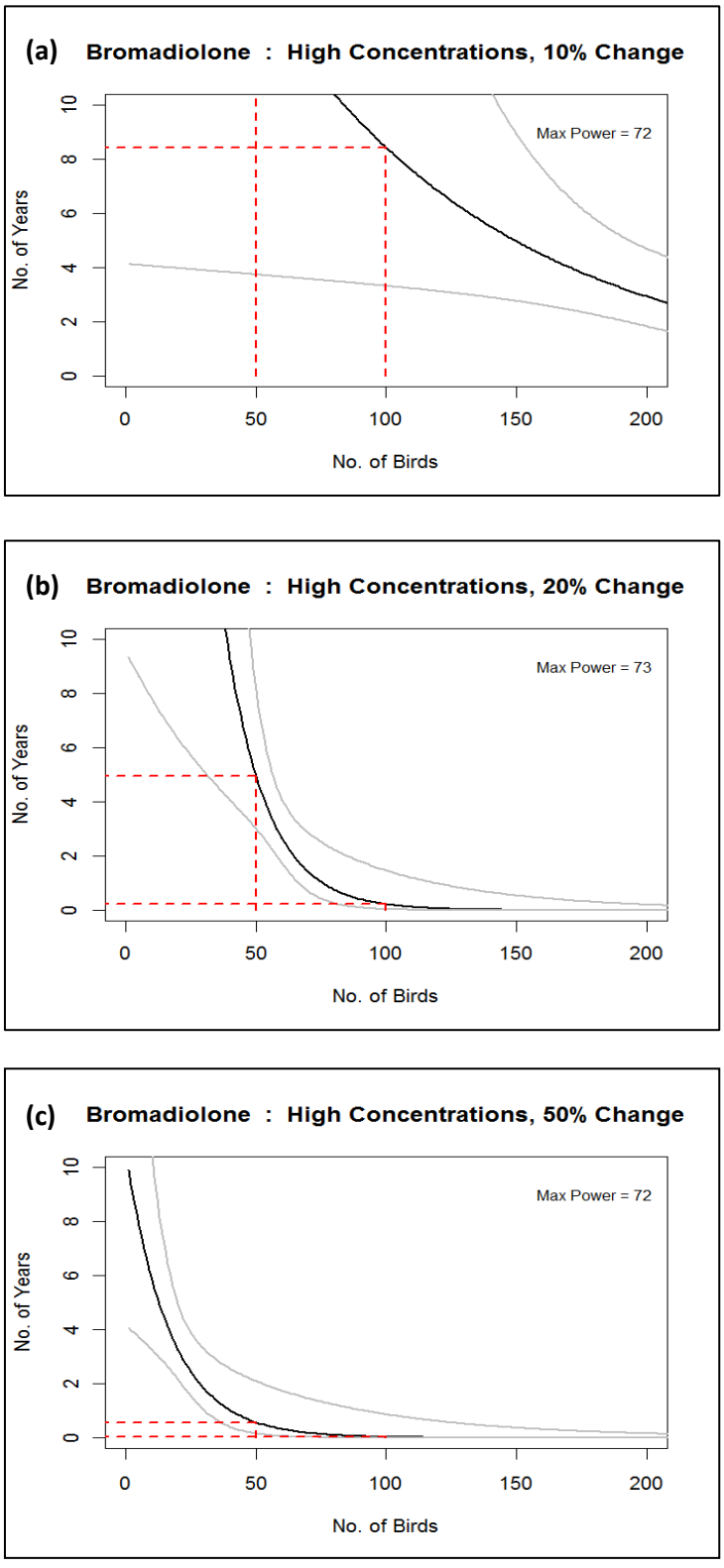

Figure 8. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 10%, (b) 20% and (c) 50% from mean high concentration (Table 4) of bromadiolone. Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

6.5 High ΣSGAR concentration

Changes from the mean high liver ∑SGARs concentration (0.260 µg/g ww) of 10%, 20% and 50%, but not 5%, could be detected with adequate power. Changes of 10% could not be detected within 10 years even if 150 owls were monitored per year (data not shown) but changes of 20% and 50% are shown in Figure 9 and can be summarised as a:

- 20% change should be detected within 4 years when analysing 100 owls/year but it would take > 10 years to detect when analysing 50 owls/year
- 50% change should be detected within one year when analysing 50 or 100 owls/year

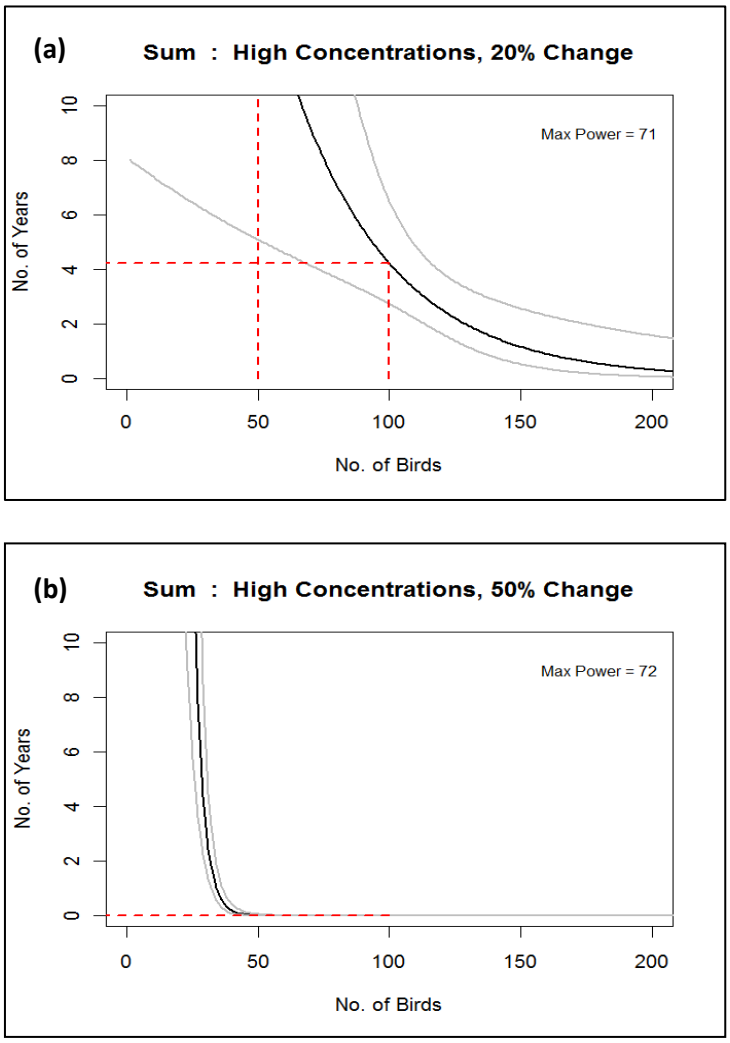

Figure 9. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 20 and (b) 50% from mean high ∑SGAR concentration (Table 4). Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

7 Ratio of high to low residues: relationship between number of birds analysed per year, duration of monitoring and size of change that can be detected

7.1 Ratio of high:low residues

The overall ratio of barn owls with high residues:barn owls with low residues (henceforth termed high:low ratio) over the whole time period is shown in Table 5.

In sections 7.2 to 7.5, we examine the power to detect changes from these high:low ratios for brodifacoum, difenacoum, bromadiolone and ∑SGARs, respectively.

Table 5. Ratio of high to low liver SGAR concentrations in barn owls

	Brodifacoum	Difenacoum	Bromadiolone	∑SGARs
No/ owls with high residues	14	20	19	66
No/ owls with low residues	381	375	376	329
% owls with high residues	3.5	5.1	4.8	16.7
% owls low residues	96.5	94.9	95.2	83.3

7.2 Change in proportion of owls with high and low residues of brodifacoum

Changes of 10% and 20%, but not 5%, in the overall ratio of barn owls with high or low liver residues of brodifacoum could be detected with adequate power. These are shown in Figure 10 and can be summarised as a:

- 10% change in the ratio of barn owls with high or low liver residues should be detected within 8 years when monitoring 100 owls/year but it would take more than 10 years when analysing 50 owls/year
- 20% change in the ratio of barn owls with high or low liver residues should be detected within one year when analysing 100 owls/year or four years when analysing 50 owls/year

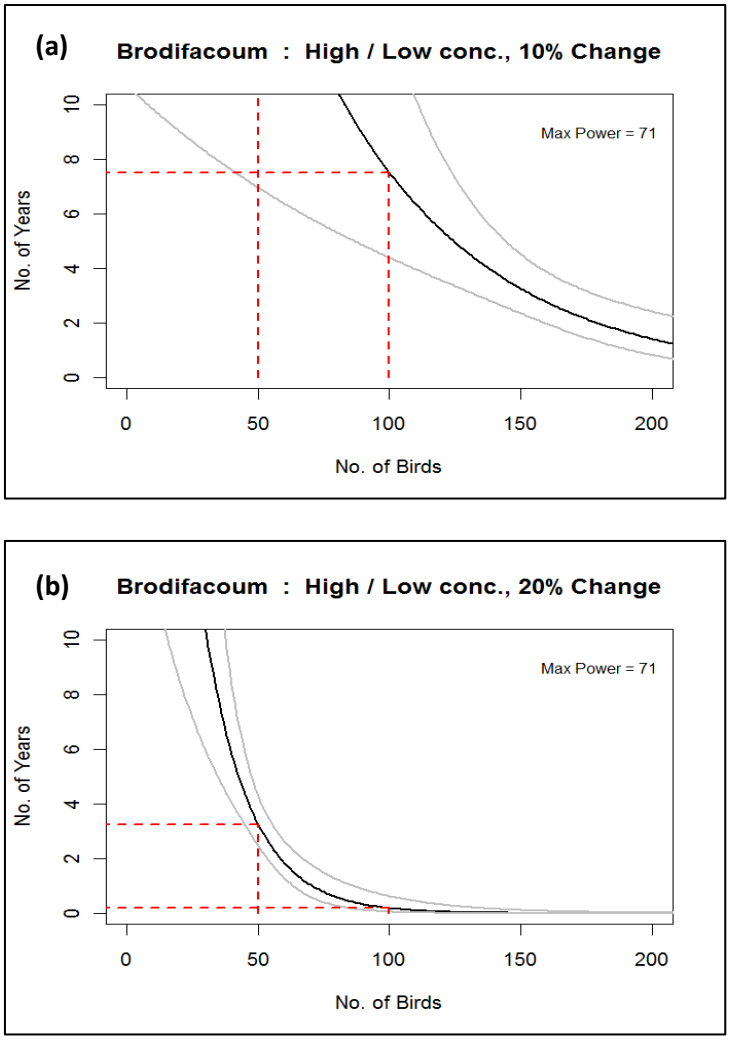

Figure 10. Relationship between number of owls analysed per year and number of years monitoring needed to detect a change of (a) 10% and (b) 20% in the high: low ratio for liver residues of brodifacoum (Table 5). Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

7.3 Change in proportion of owls with high or low residues of difenacoum

Changes of 10% and 20%, but not 5%, in the overall ratio of barn owls with high or low liver residues of difenacoum could be detected with adequate power. These are shown in Figure 11 and can be summarised as a:

- 10% change in the ratio of barn owls with high or low liver residues could not be detected within 10 years when monitoring 100 owls/year or less
- 20% change in the ratio of barn owls with high or low liver residues should be detected within one year when analysing 100 owls/year or approximately 8 years when analysing 50 owls/year

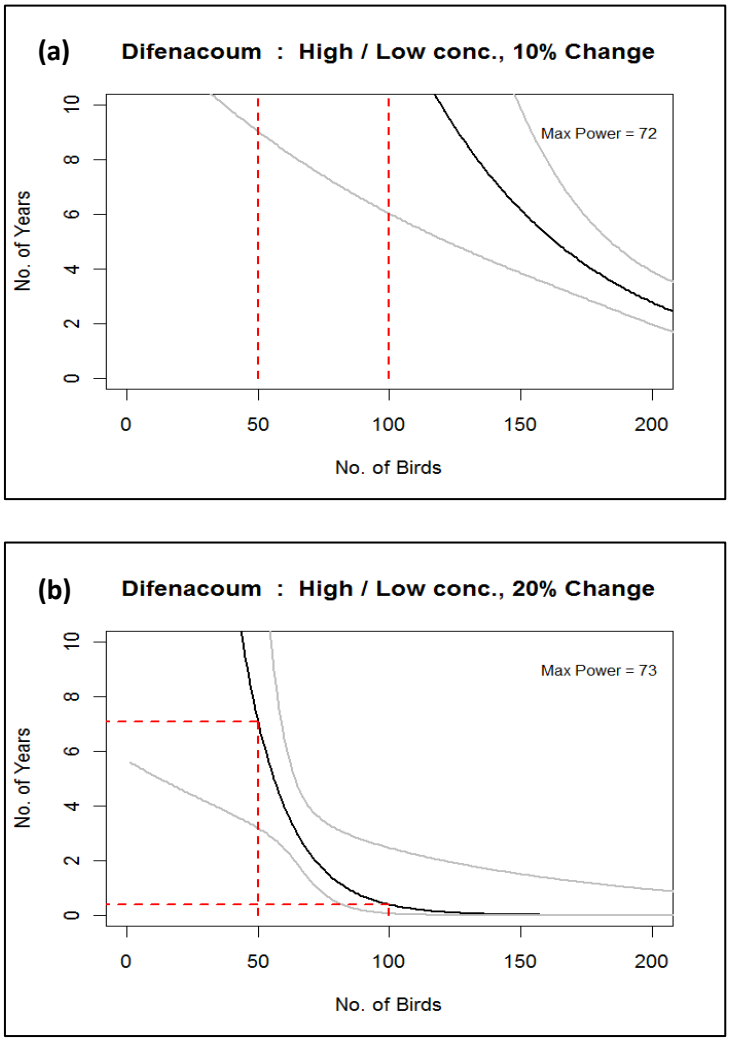

Figure 11. Relationship between number of owls analysed per year and number of years monitoring needed to detect a change of (a) 10% and (b) 20% in the high: low ratio for liver residues of difenacoum (Table 5). Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

7.4 Change in proportion of owls with high or low residues of bromadiolone

Changes of 10% and 20%, but not 5%, in the overall ratio of barn owls with high or low liver residues of difenacoum could be detected with adequate power. These are shown in Figure 12 and can be summarised as a:

- 10% change in the ratio of barn owls with high or low liver residues could not be detected in less than 10 years when monitoring 100 owls/year or less
- 20% change in the ratio of barn owls with high or low liver residues should be detected within one year (analysing 100 owls/year) or approximately 5 years when analysing 50 owls/year

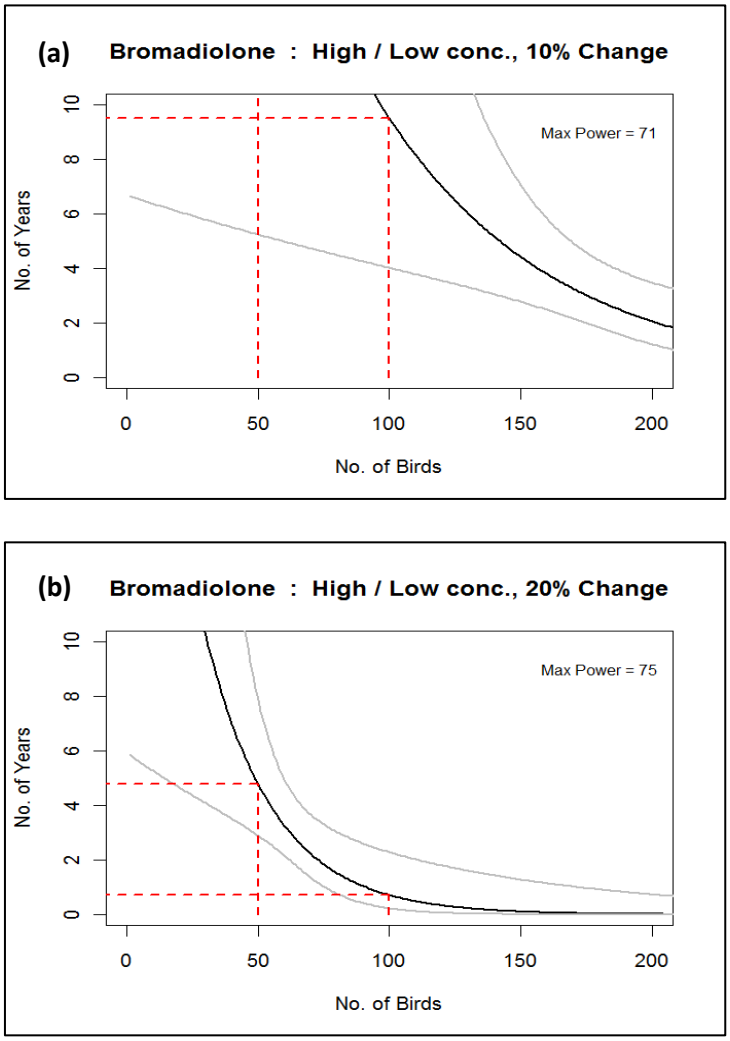

Figure 12. Relationship between number of owls analysed per year and number of years monitoring needed to detect a change of (a) 10% and (b) 20% in the high: low ratio for liver residues of bromadiolone (Table 5). Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

7.5 Change in proportion of owls with high or low ∑SGAR residues

Only a change of 20% in the overall ratio of barn owls with high or low ∑SGAR liver residues should be detected with adequate power and would take approximately three years when analysing 100 owls/year but approximately 10 years when analysing 50 owls/year (Figure 13).

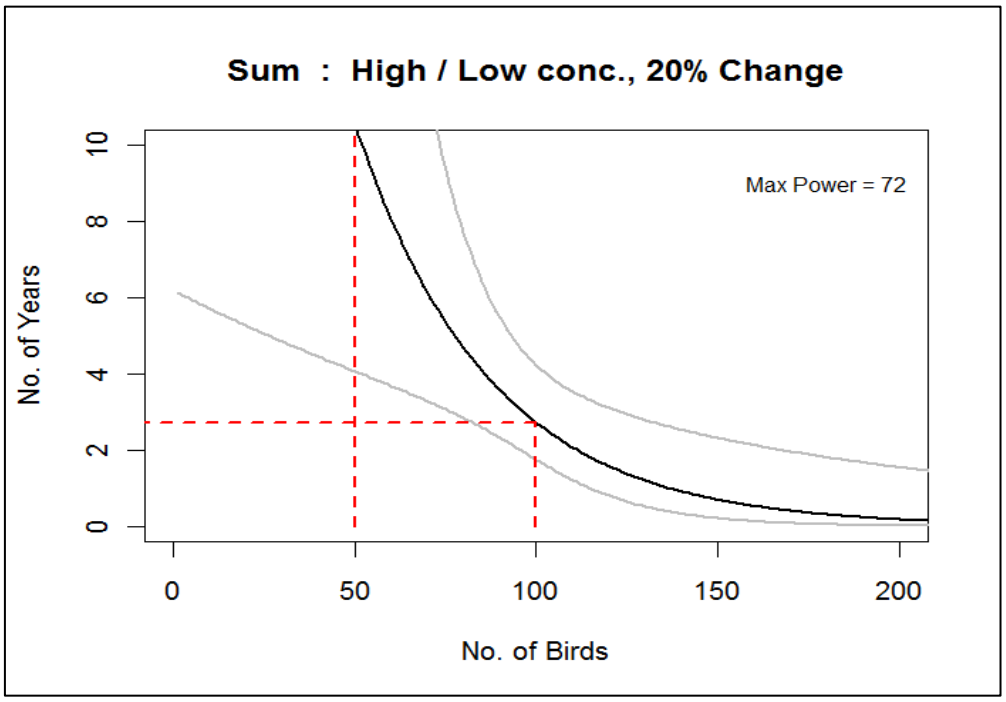

Figure 13. Relationship between number of owls analysed per year and number of years monitoring needed to detect a change of 20% in the high: low ratio for ∑SGAR liver residues (Table 5). Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

8 Change in the proportion of owls with detectable concentrations of flocoumafen and difethiolone

The numbers of barn owls over the period 2006-2012 with concentrations of flocoumafen and difethialone above the detection limit (0.001 - 0.002 µg/g ww) were 12 and 1, representing 3.04% and 0.25%, respectively, of the total number of owls analysed (Figure 1). The low detection rates reflect likely limited use of flocoumafen in the UK and the fact that difethiolone was only recently authorised for use in the UK.

It was not possible to conduct a power analysis on the basis of a change from mean concentrations because of the small number of detected liver residues of each compound. We therefore examined the power to detect changes in the proportion of barn owls with detected concentrations of these compounds.

Changes of 10% and 20%, but not 5% in the proportion of barn owls with measurable liver concentrations of flocoumafen or difethialone could be detected with adequate power. These are shown in Figures 14 and 15 respectively and can be summarised as a:

- 10% change in the proportion of barn owls with detectable liver residues of flocoumafen should be detected within 8 years when monitoring 100 owls/year but it would take more than 10 years when analysing 50 owls/year
- 20% change in the proportion of barn owls with detectable liver residues of flocoumafen should be detected within one year when analysing 100 owls/year or four years when analysing 50 owls/year
- 10% change in the proportion of barn owls with detectable liver residues of difethialone should be detected within three (monitoring 100 owls/year) or 8 years (monitoring 50 owls/year)
- 20% change in the proportion of barn owls with detectable liver residues of flocoumafen should be detected within one (monitoring 100 owls/year) or approximately two years (monitoring 50 owls/year)

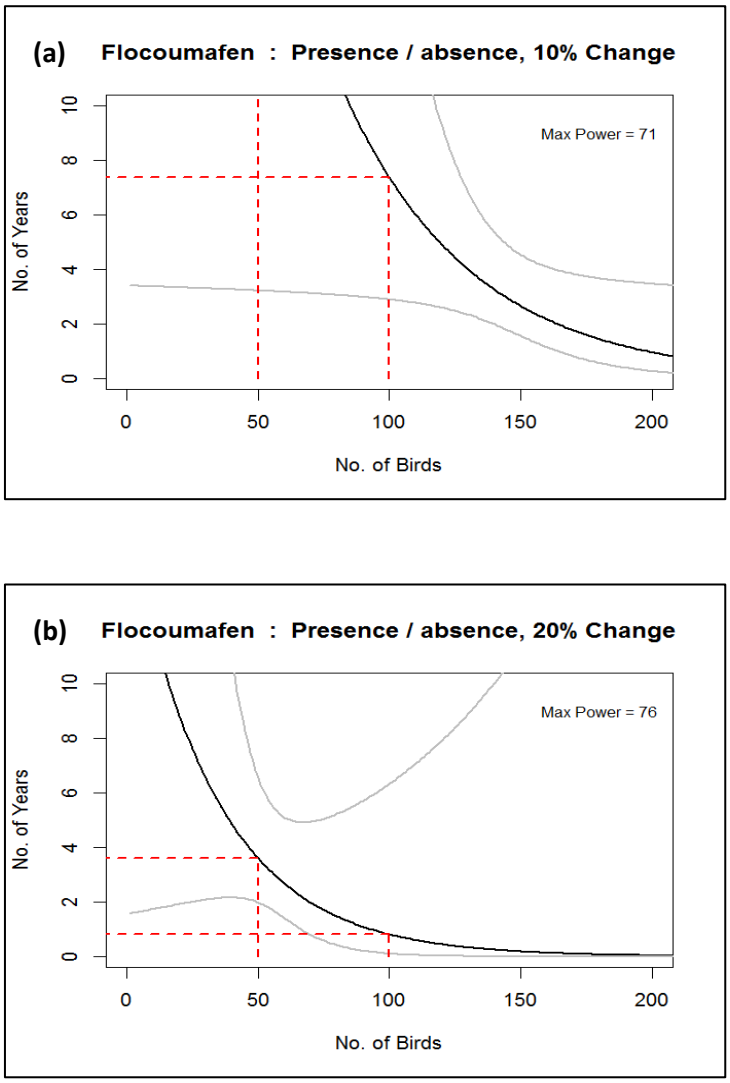

Figure 14. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 10% and (b) 20% in the proportion of barn owls with detectable liver residues of flocoumafen. Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

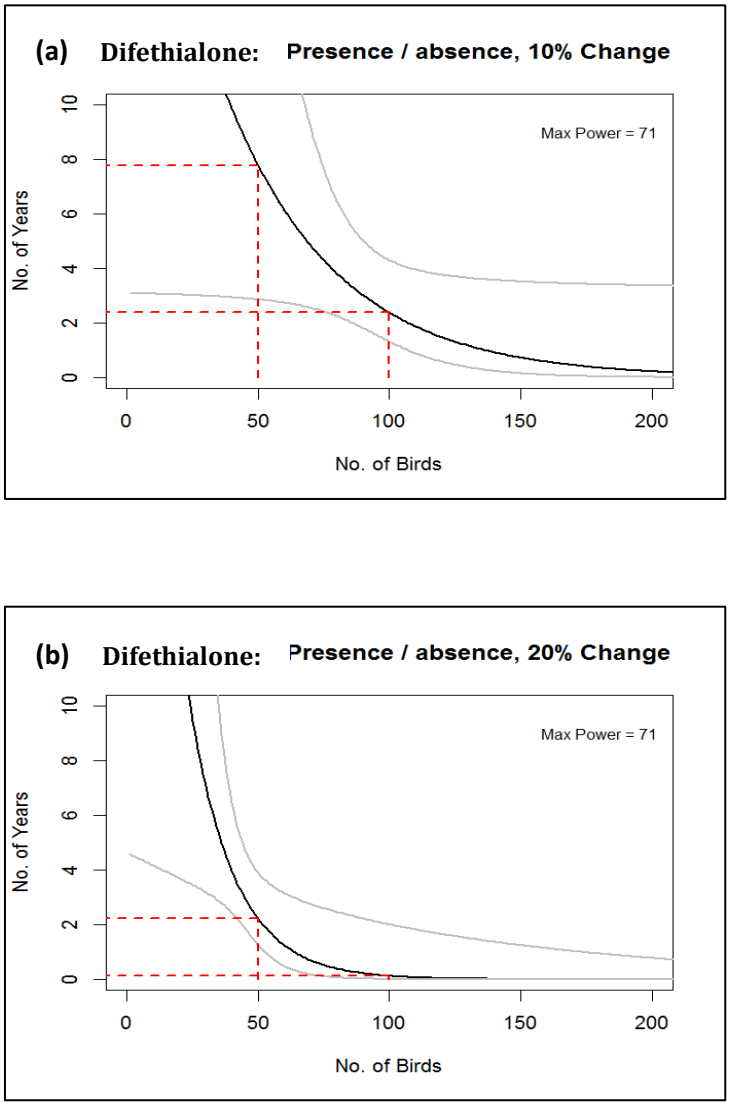

Figure 15. Relationship between number of birds analysed per year and number of years of monitoring needed to detect a change of (a) 10% and (b) 20% in the proportion of barn owls with detectable liver residues of difethialone. Mean and 95% confidence limits are shown. Red dotted lines indicate number of years needed to detect designated % change by analysing 50 or 100 birds per year.

9 Summary of timeframes for detecting change

A summary of the number of years taken to detect changes in low concentrations (as described in Section 5), high concentrations (Section 6) and the ratio of high to low concentrations (Section 7) is given in Table 6. The mean time taken to detect such changes when monitoring 50 or 100 barn owls per year has been highlighted in the previous sections. In Table 6, the lower and upper confidence limits for this time period are given rather than the mean value. Values are colour coded to indicate the potential for monitoring to detect change within a maximum timeframe of 3 years (green highlight), 10 years (orange highlight) or longer (red highlight).

Table 6. Summary of lower (L) and upper (U) 95% confidence limit for the number of years to detect a 10%, 20% (and 50% - high concentrations only) change in low liver concentrations, high liver concentrations and the ratio of owls with high and low liver residues when monitoring either 50 birds/year or 100 birds/year. Data are for brodifacoum (Brod), difenacoum (Difen), bromadiolone (Brom) and ∑SGARs . Mean values (ug/g ww) for the low and high concentrations are indicated in the left hand column by compound. See text for detail on rationale for red, amber and green colour coding on years

	No/ Birds per year	Low (<0.1 µg/g ww) Number of years				High Concentrations (>0.1 µg/g ww) Number of years						Ratio High:Low residues Number of years			
		95% CL for 10% change		95% CL for 20% change		95% CL for 10% change		95% CL for 20% change		95% CL for 50% change		95% CL for 10% change		95% CL for 20% change	
		L	U	L	U	L	U	L	U	L	U	L	U	L	U
Brod *0.005* *0.510*	50	2	5	1	4	NA	NA	8	93	3	9	7	44	3	5
	100	1	4	1	5	NA	NA	7	40	1	3	5	13	1	1
Difen *0.011* *0.140*	50	7	17	1	1	2	4	1	3	1	1	9	102	4	16
	100	1	2	1	1	1	3	1	1	1	1	7	31	1	3
Brom *0.014* *0.194*	50	4	18	1	4	4	55	3	9	1	3	6	80	3	8
	100	2	4	1	3	4	22	1	2	1	1	5	23	1	3
∑SGARs 0.024 0.260	50	4	37	2	4	9	35	6	47	1	1	8	187	5	28
	100	3	9	1	3	9	25	3	7	1	1	7	88	2	5

10 Discussion

Sections 5-7 in this report have described the magnitude of changes in three main sets of metrics that can be detected by PBMS monitoring of barn owls for SGARs. These are:

- Changes in low concentrations for brodifacoum, difenacoum, bromadiolone and ∑SGARs
- Changes in high concentrations for brodifacoum, difenacoum, bromadiolone and ∑SGARs
- Changes in high:low ratios for brodifacoum, difenacoum, bromadiolone and ∑SGARs

There is also a subset metric of a change in the ratio of birds with detectable residues of flocoumafen and difethialone (Section 8)

Generally, changes of 10% or more could be detected with adequate (≥ 70%) power for all three metrics, although the timeframe over which such changes could be detected varied between compounds and depended on the number of birds that would be monitored in each year.

Although a single metric may have been simpler from a regulatory perspective for assessing the impact of stewardship on exposure, it was not possible to calculate power from the whole dataset in this way. The different metrics outlined above however, may be beneficial as they provide a means of assessing and contextualising different types of impact and associated risk.

The key question then is how each of these metrics may be used as a monitoring tool and the relative weight that may be attached to each. This is outlined below.

10.1 Use of residues as tools to monitor the outcomes of changes in use and stewardship

The mean ΣSGAR low liver concentration in barn owls as measured by the PBMS between 2006 and 2012 was 0.024 µg/g ww. There is no clearly defined relationship between liver SGAR residues and occurrence of adverse acute toxicity, and a general lack of knowledge of whether low-level exposure results in sub-lethal effects (Shore et al., in press). However, liver SGAR residues below 0.1 µg/g ww have not generally been associated with SGAR-related mortality in barn owls and the study by Thomas et al. (2011) suggests that the maximum probability of mortality associated with accumulation of a ΣSGAR liver residue of 0.024 µg/g ww is less than 5% in barn owls.

Using low liver SGAR concentrations may be a relatively sensitive means of assessing the outcomes of changes in SGAR authorised use and associated stewardship. Increases or decreases of 20% in ΣSGAR liver concentrations are likely to be detectable within 1 – 4 years if 100 owls were monitored per year or 2-4 years if 50 owls are analysed for SGARs per year;

bigger changes would be detected more rapidly. An increase of 20% in ΣSGAR liver concentrations would represent a change from 0.024 µg/g ww to 0.029 µg/g ww, a change unlikely to be of much toxicological significance. Thus, if such an increase was used to trigger a review of stewardship practices or authorisations, this could be implemented when average residues would still be relatively low and there would be little increased risk to barn owl populations. In contrast, if changes in authorisations and stewardship reduced low exposure in barn owls, monitoring should be capable of detecting reductions of 20% or more within 1 – 4 years.

Similar scale changes could be detected over the same timescales in low concentrations of brodifacoum, bromadiolone and difenacoum and, in fact, in the prevalence of flocoumafen and difethialone. This means that it should be possible to determine whether there is a change in the compounds to which birds are being predominantly exposed, even though this may not result in any change in overall ΣSGAR low concentrations. For example, authorisation of outdoor use for brodifacoum, flocoumafen and difethialone may lead to a large-scale increase in usage with a concomitant decrease in difenacoum and bromadiolone use. Such a change would be expected to be reflected by changes in the composition of liver residues accumulated by barn owls. This information is likely to be of significant value as a proxy for interpreting how usage patterns for different compounds may change. Such information can be related to different spatial areas, such as those with and without SGAR resistance in rats, and would provide an independent means of testing changes that may be detected through surveys of use.

High (> 0.1 µg/g ww) liver residues in barn owls are of potential toxic concern, as previously highlighted. Of the barn owls monitored between 2006 and 2012, 16.7% had high liver ΣSGAR concentrations and the mean ΣSGAR concentration in these birds was 0.26 µg/g ww (Table 4). Using the approach of Thomas et al. (2011), a ΣSGAR concentration of 0.26 µg/g ww would be associated with a probability of mortality of approximately 25%. This effectively translates into an estimate that a quarter of barn owls that accumulate high liver ΣSGAR concentrations might be expected to die as a result of their exposure, although there are uncertainties associated with such estimates (Thomas et al., 2011). The timescales over which changes in mean high concentrations could be detected are variable, particularly for changes of 20% or less. However, changes of 50% in high concentrations of individual compounds and of ΣSGARs should generally be detectable within 1-3 years (Table 6). Again, using the approach outlined by Thomas et al. (2011), a 50% rise in current mean ΣSGAR concentrations would equate to an estimated rise in the probability of mortality of 10 – 15% for individual birds that accumulated high residues. This assumes that the probability of mortality is unaffected by the extent to which different compounds contribute to the sum concentration.

A relevant question is whether low concentrations or high concentrations alone could be used as a monitoring tool to assess the outcomes of changed authorisations and stewardship? Use of low residues as a sole tool would have the benefit of using the bulk of the data collected (as most owls fall into this category; Table 5), but it does not necessarily relate to birds that accumulate the highest concentrations and are potentially most at risk. Use of high concentration data alone would provide information that most closely relates to acute toxic risk but may reflect particular practice patterns or hotspots of exposure and would discard

the bulk of the data obtained through monitoring. Furthermore, information on changes in mean low and high concentrations need to be used in conjunction both with each other and with the high:low ratio. This is necessary to gather a picture of how exposure may be changing overall. For example, if a change in exposure altered the high:low ratio, the change in mean values for low and high residues could conceivably be in opposite directions, particularly if there was a relatively large number of birds with residues close to the threshold value of 0.1 µg/g ww.

10.2 Potential use of PBMS collection of barn owls for monitoring stewardship?

The power analysis presented in this report has shown the relationships that describe how many birds (between 0 and 200) need to be monitored each year to detect changes of 5%, 10%, 20% or 50% over a given time period. We have highlighted the length of time needed to detect changes when 50 owls/year have been analysed, which is the number that have been analysed in the past, and double that number.

Because barn owl carcasses are submitted to the PBMS by members of the public, we have little control over how many carcasses are submitted each year and are suitable for rodenticide analysis. The best guide to likely numbers is probably the numbers that have been submitted in previous years and this is shown in Figure 16.

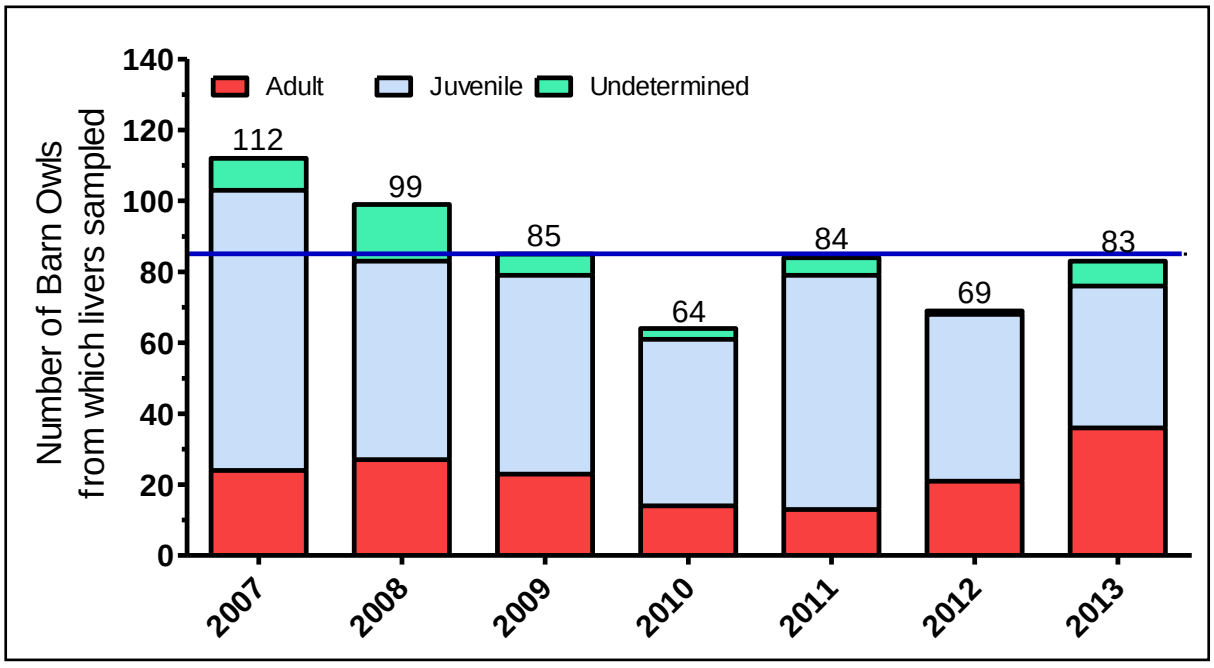

Figure 16. Number of barn owls that were submitted to the PBMS each year and were in a condition suitable for SGAR analysis.

On average, 85 barn owls suitable for SGAR analysis have been collected through the PBMS each year between 2007 and 2013. This suggests that PBMS would be expected to obtain sufficient birds to monitor 50 birds/year and would usually be able to monitor more, although there would have to be an increase in submissions to be able to analyse 100 barn owls regularly each year. Analysis of an intermediate number of birds between 50 and 100 would improve detection sensitivity from 50 birds but would not achieve that outlined in the figures for 100 birds.

A key regulatory issue in term of monitoring is the speed of reporting the results of monitoring. Typically the PBMS monitoring involves stratifying the sample of barn owls collected by within-year date received such that a temporally stratified sample from across the year of approximately 50 birds are analysed. For example, if approximately 100 birds are received each year, every other barn owl as received is selected for analysis. Such temporal stratification requires selection to be made once all birds have been received for any one year. The consequence is that there is a delay in reporting the results of analysis. For example, selection for analysis of barn owls that died in 2012 did not take place until early 2013 and the results were then reported later (Walker et al., 2014).

A similar timeline would be necessary if a temporarily stratified sample is again required. However, if all barn owls submitted to the PBMS were analysed for rodenticides, there would be no need to delay selection of samples for analysis and thus it may be possible to report data on a rolling shorter time period, although this would be less efficient because of duplication of analytical set up and reporting costs. More rapid rolling reporting of small datasets would only be of value in terms of possible early indications of trends and would not have the power of the larger annual datasets that have been outlined in the current report.

11 Conclusions

This report has shown that monitoring of barn owls for liver SGAR residues could provide a means of assessing the impact of changes in authorised use and stewardship on the exposure of non-target species. These data could provide information on changes associated with implementation of changed use and stewardship, and would also provide information on variation in prevalence of different individual compounds. Provenance data for all birds are available and so it would also be possible to determine whether differences in prevalence or concentrations of individual compounds were related to presence of resistance in rats, at least at the vice-county level. Similar spatial analyses were conducted to investigate the impact of SGAR use during Foot and Mouth disease on exposure of barn owls and buzzards (Shore et al., 2006).

Barn owls predominantly eat small mammals but few commensal species such as house mice and rats (Love et al., 2000). Monitoring secondary exposure in barn owls is therefore likely to be of most use for interpreting the effects of changes in authorised use and stewardship on SGAR exposure mediated through non-target small mammals. If changes in usage and stewardship primarily affect the potential for non-target exposure through consumption of poisoned rats, then monitoring barn owls will not detect such an effect. The distribution of barn owls is also such that they occupy rural habitats and so this species will not be a useful sentinel for changes that relate specifically to urban use.

Overall, any use of monitoring liver SGAR residues in barn owls, as described in this document, for monitoring the outcomes of changed authorisation and stewardship should be done in conjunction with other monitoring data that is also already collected. This could include information from the Wildlife Incident Investigation Scheme on the numbers of SGAR-related mortalities of predatory birds and mammals and also information on liver SGAR residues that have been measured, as described by Hughes et al. (2013). Information on residues to date in such species needs to be collated to be able to put any future changes in residue accumulation into context.

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