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Investigation of the distribution of anticoagulant rodenticide residues in red fox (*Vulpes vulpes*) livers to ensure optimum sampling protocol

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ABSTRACT

The lobular distribution of 9 different anticoagulant rodenticide (AR) residues in the liver of a sentinel predator/scavenger i.e. the red fox (*Vulpes vulpes*) was determined following multi-residue analysis using Ultra (High) Performance Liquid Chromatography tandem Mass Spectrometry (UHPLC-MS/MS). The aim of the study was to address concerns that if distribution of AR residues in the liver was significantly heterogeneous, analysis of random sub-samples or finite remnants of liver could result in false negatives or lead to incorrect toxicological diagnoses. Intact livers excised from animals shot as part of routine legal pest control activities in Scotland during 2018 and 2019 were sub-sectioned and the lobular concentration of AR residues was investigated. Analysis of individual lobes from 10 different fox livers revealed that AR residues initially detected in a randomly selected small portion of liver tissue were present throughout the liver. Also, in cases where AR residues were not found in the initial randomly selected portion, they were not found in the subsequent more detailed examination. The limit of quantitation was 3 μg kg^{-1} and AR residue concentrations ranged from 3 to 885 μg kg^{-1}. Statistical analysis of variance (ANOVA) was performed on the eight and six out of ten livers that tested positive for bromadiolone and brodifacoum, respectively. No statistical evidence was found of differences in mean residue levels of bromadiolone throughout the liver i.e. within and between liver lobes. However, brodifacoum showed a statistically significant difference ($p < .001$) in mean residue concentration between the lobes but there was no statistical evidence of mean differences within the lobes.

1. Introduction

Anticoagulant Rodenticides (ARs) are used globally to control commensal rodent populations in urban and agricultural environments. The efficacy of the associated products relies on the capability of the product's active ingredient(s) to prevent synthesis of vitamin K-dependent blood-clotting factors in the target pest. Complete suppression of the coagulation process ultimately results in the death of the target animal via fatal hemorrhaging within days of the initial exposures [1,2]. Several active ingredients are currently authorised for use in the United Kingdom i.e. brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difenacoum, difethialone, flocoumafen and warfarin [3]. Diphacinone is no longer authorised for use in the UK, but it is currently included in our multi-residue AR target list as a legacy chemical.

Exposure of non-target animals to anticoagulant rodenticides (ARs), particularly predators and scavengers that feed regularly on rodents and other small mammals, is widespread and of international concern given the global use of AR products.

Routes of exposure can be:

- Primary exposure via direct ingestion of bait formulations
- Environmental: via secondary exposure following ingestion of dead or dying prey contaminated with ARs
- Accidental i.e. careless use or misuse of products and baits
- Illegal i.e. deliberate attempt to poison animals

Consequently, the impact of AR use on non-target animals is monitored in various countries to carry out environmental impact assessments, investigate suspected poisoning incidents or enforce and amend related legislation and regulatory policies [4-6].

It is widely recognised that the liver is the main organ for accumulation and storage of the ARs [7], and as such, is the primary tissue used in laboratory tests to identify AR exposure. Consequently, testing laboratories engaged in the determination of multiple AR residues use liver as the biological specimen of choice [8–10]. When testing small mammals, the whole liver is usually analysed [11,12] and on occasion the whole animal

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[13]. However, for larger species e.g. herbivores, omnivores, carnivores [14], the red fox [15] or red deer, pigs, cattle [16], sub-samples of liver tissue are used i.e. from a single liver lobe, from within a lobe or via biopsy. However, there is an inherent concern that sample heterogeneity could significantly contribute to the uncertainty of measurements [17]. Sample heterogeneity could be compounded by the practice of random sub-sampling or analysis of a limited amount of liver tissue. Therefore, there is a tangible risk that such practices could result in false negatives or incorrect toxicological diagnosis if AR residue concentrations are under- or over-estimated. Although there are no direct publications about the validity of residue measurements due to AR residue heterogeneity in liver, researchers have considered the consequences of heterogeneity of various metals in the liver. For example, it was noted that care should be taken in relying on the copper content of a small sample of liver as an indication of total liver content [18]. Alternatively, studies into the distribution of copper in the liver of pigs and sheep revealed that there was no significant variation in mean copper concentration according to the site from which the sample was taken. It was therefore concluded that the (aspiration) biopsy technique gave copper values reasonably representative of those for whole livers [19]. In more recent times, studies into the lobular distribution of various elements in cattle liver indicated that the distribution of all elements except cobalt and zinc varied significantly across the liver. The left lobe showed higher trace element concentrations and the caudate lobe showed the lowest [20].

Unpublished studies using radio-labelled ARs in rats seemed to suggest that ARs were relatively evenly distributed throughout rat livers (A. Buckle, pers. comm). The rat is a relatively small mammal and this radio-isotope work was designed to test whole body distribution and may not have been sensitive enough to detect within organ distribution. However, it is recognised that ARs bind to the vitamin K epoxide reductase (VKOR), a membrane enzyme located within the endoplasmic reticulum of the liver cells and other tissues. Furthermore, these enzymes may be relatively evenly distributed throughout the liver tissue [21].

When we had the opportunity to obtain livers from foxes shot during routine pest control activities throughout Scotland, we realised that this presented a unique opportunity to investigate the distribution of AR residues in livers from a top predator/scavenger. Determination of the lobular distribution and concentration of AR residues in the liver of a sentinel non-target predator/scavenger, will provide evidence to confirm or refute that prevailing (sub) sampling protocols of laboratories tasked with associated monitoring activities are reliable. Furthermore, results obtained can be extrapolated to other species, especially the Canidae family.

2. Material and methods

2.1. Analytical method

Each lobe sub-section was prepared separately for subsequent analysis in accordance with the published method developed and utilised routinely by us [22] and accredited to requirements of ISO/IEC 17025:2017 Standard [23]. The only difference in the approach for this study, was that the whole (lobe) sub-section was processed and 1.0 g of the resultant homogenised tissue was then taken for further examination. The extraction was achieved by adding 5.0 mL of acetonitrile and vortexed for 1 min. The QuEChERS extraction salt was then added and after vigorous shaking, the samples were centrifuged for 5 min at 2500 $\times g$ at room temperature. 3 mL of supernatant was subsequently transferred to dispersive SPE tube, vortexed and centrifuged for 5 min at 3000 $\times g$. The supernatant was filtered by syringe filter to vials for chromatographic analysis.

2.2. Sample provenance, selection and processing

Livers were taken from randomly selected foxes (3 females and 7 males, all aged between 1 and 2 years) that had been shot during legal pest and disease control activities conducted throughout Scotland between 2018 and 2019. Livers were obtained either from carcases submitted directly to our laboratory from a range of sources or via another laboratory undertaking disease monitoring that removed the livers and passed them on to us. The intact livers were weighed and frozen (−20 °C) on receipt. Livers were thawed before being prepared, extracted and analysed. After initial testing of a randomly selected sub-sample (approximately 1 g of tissue) from the ten different and intact fox livers, eight livers tested positive for residues of brodifacoum, bromadiolone, coumatetralyl, difenacoum and/or flocoumafen and 2 livers tested negative for all 9 target ARs. All ten livers were then subject to more detailed consideration and investigation.

The liver of the fox is comprised of 6 lobes i.e. right and left medial lobes; right and left lateral lobes; quadrate lobe, caudate lobe and the papillary process of the caudate lobe [24]. The ten individual livers (including the 2 livers that did not contain ARs) were subdivided as follows: outer/inner right and left medial lobe, outer/inner left and right lateral lobe, quadrate lobe, caudate lobe and papillary process of the caudate lobe (Fig. 1). Therefore, each individual liver (average weight = 172 g ± 29.2) yielded 11 separate samples.

2.3. Multi-residue analysis

Quantitation of AR residues was achieved following external multi-point (n = 6) calibration using matrix-matched standards. The linear calibration range was 0.0005 to 0.05 μg ml^{-1}. Analytical quality control (AQC) was assured following analysis of organic liver (i.e. chicken liver purchased from local retail outlets) fortified at two different fortification levels 0.01 and 0.05 mg kg^{-1}. A typical analytical batch comprised AQC, matrix-blank and chemical blank controls and 22 liver sub samples (i.e. from 2 foxes). The limit of quantitation (LOQ) was determined to be 3 μg kg^{-1} for all 9 ARs following attainment of signal:noise (S:N) values ≥3:1 for the lowest calibration level. All method performance criteria were met for these studies i.e. recoveries determined at each fortification level ranged from 73 to 107%: target mean recovery values were set at 60–140% with corresponding coefficient of variation (%CV) set at ≤20%. Results were not corrected for recovery in accordance with guidelines specified in the EU guidance document for method validation and quality control procedures for pesticide residue analysis in food and feed [25]. This practice ensures consistency of measurements and method performance with our laboratory's other main operational function i.e. the determination of

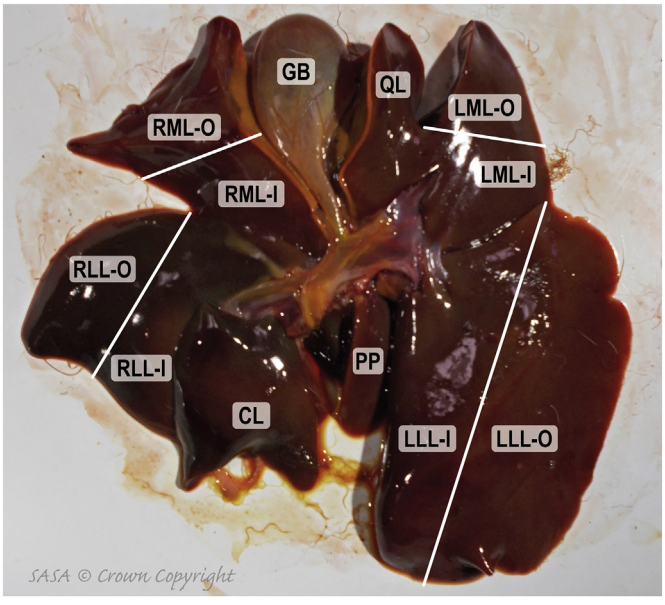

Fig. 1. Liver lobe identification and sub-section protocol. RML-O (right medial lobe-outer), RML-I (right medial lobe-inner), GB (gall bladder), QL (quadrate lobe), LML-O (left medial lobe-outer), LML-I (left medial lobe-inner), LLL-O (left lateral lobe-outer), LLL-I (left lateral lobe-inner), PP (papillary process), CL (caudate lobe), RLL-O (right lateral lobe-outer) and RLL-I (right lateral lobe-inner).

pesticide residues in food and feed. We are however, aware that other practitioners opt for internal calibration and/or adjust results for recovery.

Fig. 2 shows the reconstructed total ion chromatogram containing multiple reaction monitoring (MRM) ion chromatograms characteristic of the target ARs from the 0.001 μg mL^{-1} matrix-matched calibration standard.

3. Statistical analysis

Analysis of variance (ANOVA) is widely used to compare the means of different experimental treatments [26] or in this case locations within the liver. A general statistical package, Genstat [27] was used to test whether the dependent variable (logarithm base 10) of anticoagulant rodenticide concentrations differ between livers' lobes (e.g. right medial lobe vs left medial lobe) and/or between inner and outer portion of the lobe (e.g. right medial-inner vs right medial-outer). The probability value (p) is a value i.e. a number between 0 and 1, used in statistical hypothesis testing that is intended to determine whether the obtained results are significant. Differences are considered statistically significant when $p < .05$.

The measured concentrations were logarithm (base 10) transformed prior to statistical analysis. Mean transformed concentrations between locations were compared separately for each AR by an analysis of variance incorporating blocking for foxes. A constant difference between two log-transformed concentrations reflects a proportional change on the original scale between the two concentrations irrespective of their absolute values. Hence blocking for foxes in the analysis accounts for proportionally higher ARs across all locations in some foxes than in others. The transformation not only helps with the assumption of homogeneity of variances underpinning ANOVA but also converts the data onto a scale which facilitates evaluation in terms of proportional change.

The treatment structure adopted in the ANOVA reflected the biological distinction between lobes (where the papillary process was regarded as a seventh lobe) and within the same lobe (inner and outer). This enabled separate testing of whether there were mean differences between lobes (e.g. right medial lobe vs left medial lobe) and also between portions of the same lobe (e.g. right medial-inner vs right medial-outer). Unless a significance level is stated, the term 'significance' throughout the paper refers to statistical significance at the 5% level. Confidence intervals for mean differences between example pairs of liver locations were computed and then back-transformed. The back-transformed 95% confidence intervals represent approximate 95% confidence limits for the ratios of (geometric) mean concentrations expressed on the original scale at the two locations. They enable assessments of 1) whether statistically significant mean differences are of a sufficient magnitude to also be of biological importance or not and 2) whether in the absence of formal statistical significance the observed mean difference is nevertheless still consistent with a range of outcomes which include underlying differences of biological significance. Additionally, as decisions are made on an individual liver basis, corresponding 95% prediction intervals were computed for a single future sample.

The above ANOVA approach comparing means investigated whether any differences between locations in AR concentrations were consistent across foxes and so whether sampling routinely from any one of these specific locations would lead to a biased estimate of the true concentration of an individual AR. Where the observed differences on the transformed scale between locations are not consistent across foxes, it is relevant to consider the extent of the variability of measurements within livers. To address this question the ANOVA model was refitted excluding location and the resulting residual variance used in confidence interval estimation. The confidence interval for a liver sample based on a single randomly selected location was calculated using the residual variation in this dataset and therefore reflects both inconsistent variation between locations and any measurement error inherent in the analytical process adopted.

4. Results and discussion

Residues of brodifacoum, bromadiolone and difenacoum were detected in 7, 8 and 5 foxes, respectively, whereas residues of coumatetralyl and flocoumafen were detected in single foxes. Six fox livers contained residues of 3 different ARs and 3 fox livers were found to contain residues of 2 different ARs. Seven out of the eight livers that tested positive contained residues of both bromadiolone and brodifacoum. Ion chromatograms (i.e. quantifier MRMs) shown in Fig. 3, correspond to residues of brodifacoum, bromadiolone and difenacoum detected in the right medial lobe inner and outer sub-sections of fox #3 to further illustrate the data acquisition and evaluation process. Whenever initial results were outside the highest calibration level the liver extracts were diluted accordingly (x10) and re-tested. The identity and levels of residues detected were consistent with the range of residues detected in red fox liver from our own and other general surveillance studies [28,29]. Furthermore, the frequency and abundance of residues detected reflected rural and urban rodenticide usage in Scotland [30,31].

Table 1 contains the results summary for each fox liver and all liver sub-sections i.e. 8× positives and 2× negatives. Ultra-low levels of AR residues i.e. below the LOQ, indicate exposure and are generally considered semi-quantitative by us in routine surveillance activities. Results for one fox's right lobe (inner/outer) and its papillary process were not available due to irretrievable error in sample collection. Formal statistical analysis was restricted to brodifacoum and bromadiolone as there were insufficient livers with concentrations at or above the LOQ from the other ARs for this to be meaningful. Additionally, a liver was excluded from the statistical analysis if the respective AR was not present in any of the samples from it since complete absence is uninformative with respect to location differences.

Mean AR concentrations by location within the liver are presented in Table 2. There was very strong evidence ($p < .001$) for differences in mean brodifacoum concentration between lobes. In particular, there was very strong evidence ($p < .001$) that the concentration in the papillary process was lower than in any of the other lobes and some evidence ($p < .05$) that the mean concentration was higher in the quadrate lobe than in the lateral and medial lobes.

In order to assess whether statistically significant differences between locations were of a sufficient magnitude to be of biological significance, confidence intervals were computed for example comparisons. For the

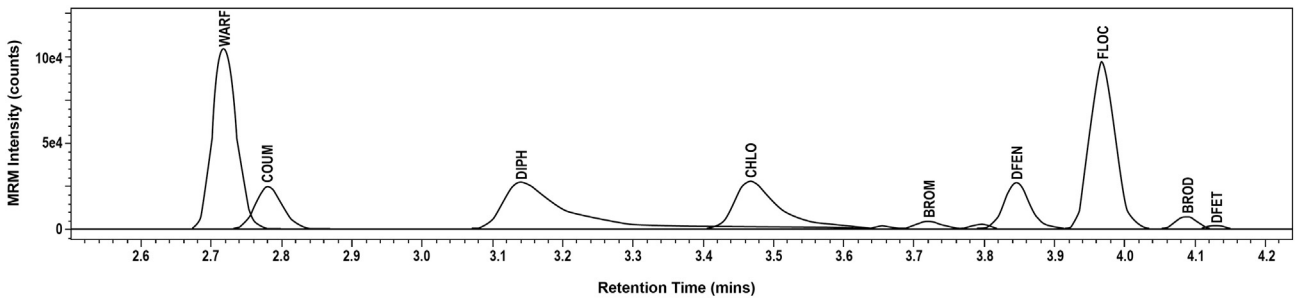

Fig. 2. Reconstructed multi-residue MRM ion chromatogram yielded by a 0.001 μg mL^{-1} matrix-matched AR standard. Warfarin (WARF), coumatetralyl (COUM), diphacinone (DIPH), chlorophacinone (CHLOR), bromadiolone (BROM), difenacoum (DFEN), flocoumafen (FLOC), brodifacoum (BROD) and difethialone (DFET).

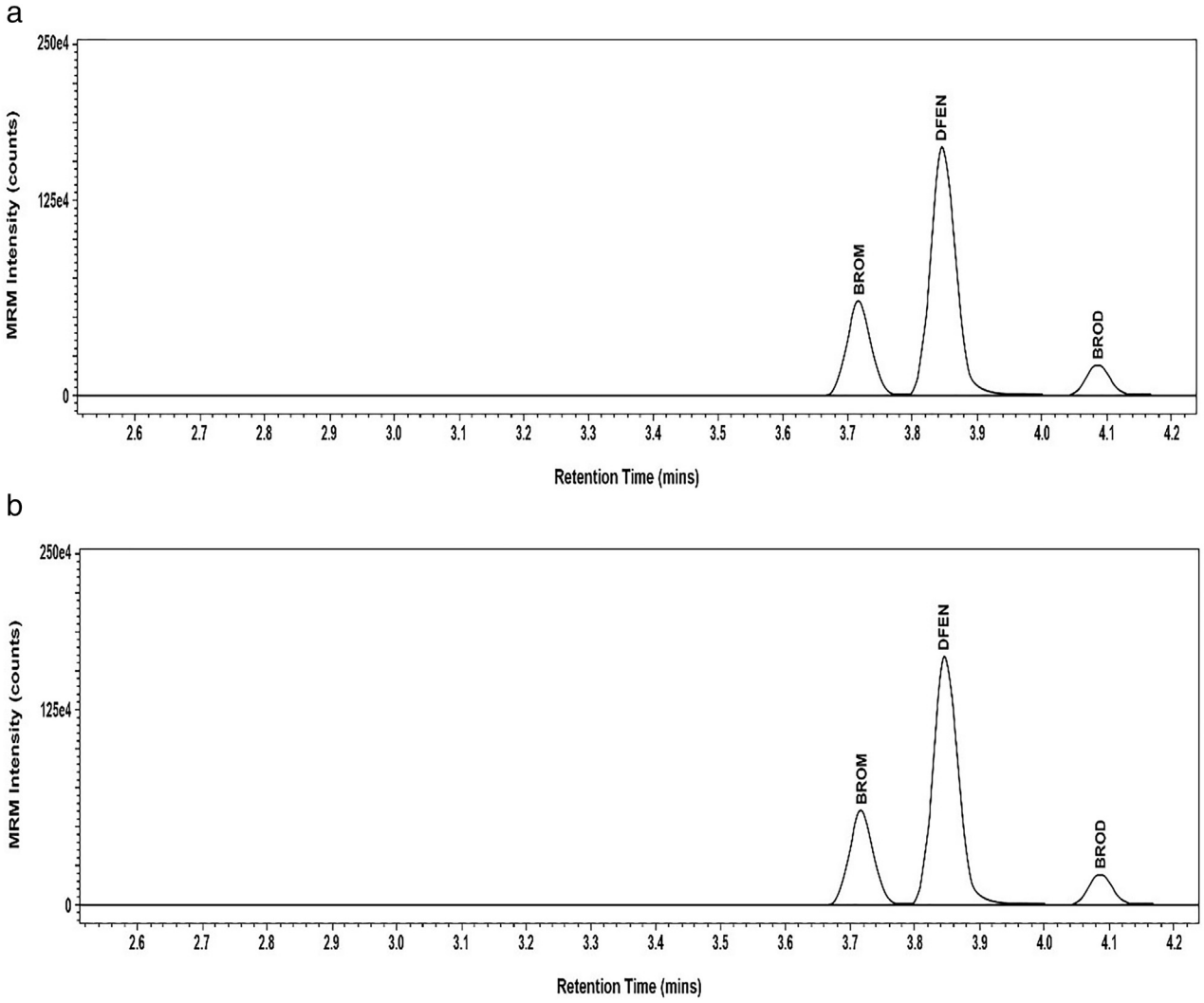

Fig. 3a. a. Re-constructed MRM ion chromatogram of AR residues detected in fox # 3 - right medial lobe-inner. Residues detected were bromadiolone (BROD), difenacoum (DFEN) and brodifacoum (BROD). b. Re-constructed ion chromatogram of AR residues detected in fox # 3 - right medial lobe-outer. Residues detected were bromadiolone (BROD), difenacoum (DFEN) and brodifacoum (BROD).

largest observed, statistically significant difference in mean brodifacoum concentrations, which was between the quadrate lobe and the papillary process, the 95% confidence interval for the ratio of the (geometric) mean quadrate: mean papillary concentrations was (1.39, 1.97). That is to say, the confidence interval for the mean quadrate concentration spanned from 39% more to 97% more than the mean papillary process concentration. The corresponding 95% prediction interval for an individual liver's quadrate concentration spanned from 8% to 154% more than the papillary process concentration.

The largest observed mean difference in brodifacoum concentrations within lobe was in the left medial lobe. Although this was not statistically significant, the 95% confidence interval for the ratio of the (geometric) mean of the outer: mean inner left medial lobe was (0.89, 1.26). That is to say, the confidence interval for the mean outer concentration spanned

Table 1
ARs detected and quantified in fox liver lobe sub-sections.

Liver ID	AR concentration range in liver lobe sub-sections (μg kg^{-1}) $n = 11$								
	BROD	BROM	CHLO	COUM	DFEN	DFET	DIPH	FLOC	WARF
Fox 1	47.8–101.0	133.8–340.0	ND	2.3–6.0	ND	ND	ND	ND	ND
Fox 2*	98.0–154.0	303.0–365.3	ND	ND	ND	ND	ND	4.1–5.8	ND
Fox 3	4.3–6.0	84.0–107.5	ND	ND	113.5–148.5	ND	ND	ND	ND
Fox 4	2.0–2.8**	105.8–176.0	ND	ND	6.5–10.0	ND	ND	ND	ND
Fox 5	24.3–52.8	129.0–248.3	ND	ND	1.0–2.3**	ND	ND	ND	ND
Fox 6	8.5–21.0	131.0–311.0	ND	ND	ND	ND	ND	ND	ND
Fox 7	38.9–67.3	30.0–48.0	ND	ND	2.00–4.3**	ND	ND	ND	ND
Fox 8	ND	597.5–885.0	ND	ND	72.5–120.0	ND	ND	ND	ND
Fox 9	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fox 10	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not Detected; *$n = 8$; **results < 3 μg kg^{-1} (LOQ) were considered as semi-quantitative and not included in the statistical analysis.

Table 2
Mean log (base 10) transformed, p values, standard errors of difference (SED) and least significant differences (LSD) of brodifacoum and bromadiolone AR concentrations in liver lobe sub-sections (μg kg^{-1}).

Lobe sub-section	Brodifacoum	Bromadiolone
Left lateral lobe - Inner position	1.513	2.275
Left lateral lobe – Outer position	1.532	2.293
Left lateral lobe – Average	1.522	2.284
Left medial lobe – Inner position	1.524	2.284
Left medial lobe – Outer position	1.550	2.295
Left medial lobe – Average	1.537	2.289
Right lateral lobe – Inner position	1.502	2.280
Right lateral lobe – Outer position	1.520	2.293
Right lateral lobe - Average	1.511	2.287
Right medial lobe – Inner position	1.541	2.303
Right medial lobe – Outer position	1.529	2.272
Right medial lobe – Average	1.535	2.288
Quadrate lobe	1.606	2.288
Caudate lobe	1.561	2.288
Papillary process of the caudate lobe	1.388	2.201
Overall mean	1.524	2.279
Number of foxes testing positive (above LOQ)	6	8
	SED[a] LSD[b] (5%)	SED LSD (5%)
Between lobes with both inner & outer	0.0267	0.0248
	0.0538	0.0494
Between two lobes with one and two locations, respectively	0.0328	0.0303
	0.0659	0.0606
Between lobes each with a single location	0.0378	0.0350
	0.0761	0.0699
Between locations within lobes	0.0378	0.0350
	0.0761	0.0699
P value (Between lobes including papillary process)	$p < .001$	$p = .104$
P value (Within lobes)	$P = .904$	$p = .863$

[a] SED = Standard Error of the Difference.
[b] LSD = Least Significant Difference.

from 11% less to 26% more than the mean inner concentration. The corresponding 95% prediction interval for an individual liver's outer concentration spanned from 31% less to 63% more than the inner concentration.

There was no statistical evidence of differences in mean bromadiolone concentration between lobes ($p = .104$). As for brodifacoum, the lowest mean concentration was from the papillary process, but this did not attain statistical significance with the protected least significant difference – LSD [32]. Nor was there statistical evidence ($p = .863$) of mean differences within lobes. Nevertheless, the ratio of mean inner: mean outer right medial lobe concentrations (for example) was (0.91, 1.26). The corresponding 95% prediction interval for an individual liver's inner concentration span from 32% less to 69% more than the outer concentration.

Given no statistical evidence of bias from choice of sampling, the 95% confidence interval for an individual liver's bromadiolone concentration based on a single randomly selected location were computed to indicate precision of the estimate. The lower and upper limits were 72% and 138% respectively of the observed concentration on the original scale (i.e. from 28% lower to 38% higher than the estimated concentration).

The differences in brodifacoum distribution between liver lobes are difficult to interpret given the absence of absorption, distribution, metabolism, and excretion (ADME) studies for this specific species/chemical combination. Although there is a general dearth of studies into the distribution of chemicals in liver, it is possible to hypothesise what factors may exert an influence on the distribution of brodifacoum in the liver. Studies into the toxicokinetic properties of brodifacoum and other ARs that exist as diastereoisomers in commercial products have indicated that *trans-isomers* are eliminated more rapidly than *cis-isomers* [33]. This behaviour could affect brodifacoum residue (re)distribution and therefore levels between lobes. Alternatively, or co-incidentally, the presence of bromadiolone in the liver could also affect brodifacoum (re)distribution, concentration and elimination by suppression of the VKOR binding activity of brodifacoum. Brodifacoum has been shown to interfere with the microsomal warfarin binding via occupation of warfarin binding sites in rat liver microsomes [34].

It is important to note that a lack of statistical significance does not mean that rodenticide concentrations are necessarily uniform across the liver. A lack of statistical significance can arise where there are differences between locations but these differences are not consistent across foxes. Any lack of consistency is reflected in the underlying variation and hence in LSDs and confidence intervals for the differences between locations. While there were no statistically significant differences between locations in bromadiolone concentration, nevertheless the observed mean bromadiolone concentration in the papillary process was lower than in any of the other locations, and this was the same trend as was statistically significant for brodifacoum. Taking the results for bromadiolone and brodifacoum together and considering the underlying biological processes it is reasonable to suspect that mean bromadiolone concentration also differs between locations.

The false negative rate is the proportion of livers with an AR present that is not detected. However, the proportion of all livers tested that are incorrectly declared negative depends not only on the test's ability to detect ARs when present but also the prevalence of AR presence in the foxes tested. The following points are clear from the concentration ranges in each fox for both brodifacoum and bromadiolone, (Table 1). Firstly, variation between locations is greater in livers where the concentrations detected are higher, thus supporting the log transformation and considering proportional change. Secondly, the ratio of maximum:minimum within a liver never exceeded three. Thirdly, all the observed bromadiolone concentrations where this AR was detected were more than ten times the LOQ. Fourthly, only two foxes had samples with any quantifiable brodifacoum concentrations less than three times the LOQ.

Given the paucity of concentrations very close to the LOQ it is not surprising that there were no cases for these two rodenticides where an AR was detected in some locations and not in others. Nevertheless, the results do indicate that the magnitude of mean differences between locations are such that the risk of failing to detect a serious level of AR in a sample would be very low. Across foxes the median concentration from the single randomly located sample was very close to the median concentration over the 11 locations for both brodifacoum and bromadiolone.

5. Conclusion

There was clear evidence of mean differences between location in brodifacoum concentration. There are grounds to believe that the lack of statistical significance for location differences in bromadiolone could be due to the small number of livers with the AR present. Hence consistent location differences may be present in the range of ARs. Always sampling from the same location within the liver will give AR concentrations which are either consistently over- or consistently under-estimates of individual livers' concentrations depending on the location chosen. While randomly selecting the location in each liver should give an unbiased estimate of the average AR concentration over the population of livers, it clearly will lead to over- or under-estimates for individual livers.

If poisoning (especially by brodifacoum) is suspected it is recommended that, where possible, the whole liver should be processed to ensure an accurate determination of AR residue levels. If this is not possible then there is a case for at least avoiding the papillary process. Our results indicate that where a liver is truly positive, the risk of a false negative will be very low unless the underlying AR concentration in the liver is approaching the LOQ. Hence the risk of failing to detect serious levels of ARs in any given sample is likely to be negligible. Over the entire testing programme, however, the risk of failing to detect the presence of ARs will depend on both how close any concentrations in individual samples are to the LOQ and the distribution of AR concentrations in the population of foxes.

Due to the close phylogenetic relationship, we expect these results to be equally applicable to other susceptible terrestrial mammals especially the Canidae family e.g. domestic dog (*C. lupus familiaris*), coyote (*C. latrans*), wolf (*C. lupus*), jackal (e.g. *C. aureus*) and in the liver of other terrestrial

mammals with 6 liver lobes e.g. domestic or wild cats (*Felis catus* and *Felis silvestris*).

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CRediT authorship contribution statement

Michael J. Taylor: Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Project administration, Validation.**Ian Nevison:**Methodology, Formal statistical analysis, Writing - original draft, Writing - review & editing.**Fabio Casali:**Formal analysis, Investigation, Writing - original draft.**Marta Giergiel:**Investigation, Writing - review & editing, Funding acquisition, Validation.**Anna Giela:**Investigation, Methodology, Writing - original draft, Validation.**Steve Campbell:**Investigation, Writing - review & editing.**Elizabeth A. Sharp:**Investigation, Methodology, Writing - review & editing, Validation, Project administration.**Gill Hartley:**Conceptualization.**Andrzej Posyniak:**Supervision, Project administration, Writing - review & editing, Funding acquisition.

Declaration of competing interest

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

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