

Baiting location affects anticoagulant rodenticide exposure of non-target small mammals on farms

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Abstract

BACKGROUND: Commensal rodents such as Norway rats (*Rattus norvegicus* Berk.), black rats (*R. rattus* L.) and house mice (*Mus musculus* L.) damage stored produce and infrastructure, cause hygienic problems and transmit zoonotic pathogens to humans. The management of commensal rodents relies mainly on the use of anticoagulant rodenticides (ARs). ARs are persistent and bio-accumulative, which can cause exposure of non-target species. We compared the baiting strategies to use brodifacoum (BR) in bait boxes indoors only versus in and around buildings in replicated field trials at livestock farms to assess resulting BR residues in non-target small mammals.

RESULTS: When bait was used indoors only, the percentage of trapped non-target small mammals with BR residues as well as BR concentration in liver tissue was about 50% lower in comparison to bait application in and around buildings. These effects occurred in murid rodents and shrews but not in voles that were generally only mildly exposed. During the baiting period, BR concentration in murids was stable but decreased by about 50% in shrews.

CONCLUSION: Restricting the application of BR bait to indoors only can reduce exposure of non-target species. The positive effect of this baiting strategy on non-target species needs to be balanced with the need for an effective pest rodent management within a reasonable time. More research is needed to clarify which management approaches strike this balance best.

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Keywords: baiting in and around buildings; brodifacoum; commensal rodents; risk mitigation; rodent management

1 INTRODUCTION

Commensal rodents, such as Norway rats (*Rattus norvegicus* Berk.), black rats (*R. rattus* L.) and house mice (*Mus musculus* L.) can damage stored produce, animal feed and infrastructure by consumption and contamination.^{1,2} In addition, commensal rodents pose a considerable health risk and other hygiene problems. They are hosts of various zoonotic pathogens and parasites that can be transmitted to humans, companion animals and livestock where they can cause serious diseases.^{3,4}

In many countries, populations of commensal rodents are controlled by using chemical rodenticides. Anticoagulant rodenticides (ARs) are used most often because bait products with ARs are easy to use, usually effective and accidental uptake by humans, livestock and pet animals can be treated with the antidote vitamin K1.^{5,6} ARs impede blood clotting by inhibition of the vitamin K epoxide reductase multiprotein complex, which is necessary for the production of several blood coagulation factors.^{7,8} The delayed effect on blood coagulation⁹ results in another significant advantage of the use of ARs in pest rodent management because this prevents learned bait aversion, as rodents cannot relate bait uptake to symptoms.^{1,10} However, the efficacy of ARs can be hampered by genetic resistance^{11,12} leading to insufficient efficacy of all first generation and some second

generation ARs (SGARs) such as bromadiolone^{13,14} and difenacoum,¹⁴ in some populations of some pest rodent species.

SGARs are persistent¹⁵ and bio-accumulative¹⁶ and they are toxic not only for target rodents but also for other homoeothermic species.^{17,18} There are two pathways of exposure of non-target taxa: (i) primary exposure when bait is consumed directly¹⁹ because non-target species are able to enter bait stations as it is the case for small mammals^{20,21} and small birds,²² (ii) secondary

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exposure when target or non-target animals that have fed on AR bait are consumed by predators or scavengers.²³ Numerous examples demonstrate exposure of non-target species including small mammals,^{20,22,24} terrestrial predators and scavengers,^{25–27} predatory birds²⁸ (reviewed in Nakayama et al.²⁹), non-predatory birds (reviewed in Vyas³⁰) and fish (reviewed in Regnery et al.³¹). Modelling exercises suggest that the transfer of ARs to mammalian predators is mainly driven by use patterns.³² For some non-target species adverse population effects have been suggested such as in non-target rodents,³³ American badger (*Taxidea taxus* L.),³⁴ red fox (*Vulpes vulpes* L.)³⁵ and mustelids.³⁶

The ecotoxicological risk associated with widespread application of ARs in crop protection has increasingly led to restrictions imposed by registration authorities, for instance at the European Union (EU) and at the national level. As a result, the number of AR compounds approved at the EU level and AR products registered nationally for agricultural use has decreased in recent years.⁵ Presently, in the EU, two AR compounds are approved in four Member States (bromadiolone in France, The Netherlands, Romania and difenacoum in Portugal).³⁷ In contrast, eight AR compounds are approved in the EU for the biocide sector, and almost 1500 products are registered.³⁸

Given the enormous difference in availability of AR compounds and products for agricultural and biocidal application,⁵ it seems plausible that in the EU most AR exposure of non-target species is related to the biocide sector. To minimize this exposure, several risk mitigation measures (RMMs) have been introduced in the biocide sector at the EU and national level.^{39–42} Despite diversity in national guidelines and regulations there seems to be consensus about some RMMs.⁴³ For instance, SGAR products are to be used only by qualified users, usually only in and around buildings and in tamper-resistant bait stations or similar structures. Best practice regulations require restrictions of permanent baiting, that dead rodents present during and after operations have to be searched for and safely disposed of and that bait remaining at the end of management operations has to be removed and safely disposed of.^{43,44} Buckle and Prescott⁴⁰ provide a full account of RMMs including roles of regulators, manufacturers and users as well as a notion of the general lack of scientific evidence for benefits and impacts even of intensely applied RMMs. In the United States, the introduction of RMMs led to a decrease in the use of products with SGARs and to an increase in the registration of products containing acute compounds.⁴⁴

A wide range of RMMs is applied with the highly relevant aim to protect human and environmental health. However, benefits and disbenefits of RMMs are usually not known because of missing data.^{40,45} In fact, it is often unclear whether the continued presence of AR residues in non-target wildlife results from inconsequent application of existing RMMs⁴⁶ or existing RMMs being ineffective.⁴⁵ While effects of RMMs are likely, to our knowledge, there is no empiric quantitative information published for any RMM that deals with wildlife protection. This is highlighted in a recent publication that concludes: 'Until these knowledge gaps are better addressed, the discussion over the need for, or required extent of, mitigation or other interventions will continue'.⁴⁷ The dire need for scientific data extends to the restriction to use AR products only indoors and in the close surroundings of buildings usually termed 'in and around buildings'. As outlined in an EU guidance document the latter 'shall be understood as the building itself, and the area around the building that needs to be treated in order to deal with the infestation of the building'.⁴⁸

It has been recently proposed to introduce further RMMs to limit secondary exposure of non-target species.⁴⁵ In this context the restriction of the use of ARs to indoors only could be a potential measure.⁴⁹ This is likely to reduce access of non-target species to bait and the access of predators and scavengers to prey and carcasses of rodents that have been exposed to ARs,³⁹ but consequences regarding AR exposure of non-target species are unclear. Therefore, we conducted a replicated field experiment to compare AR residue patterns in non-target small mammals between the baiting strategies indoor only and in and around buildings. Non-target small mammals can be exposed to bait directly (primary exposure) and a source for secondary exposure of terrestrial wildlife and predatory and scavenging birds because they are a major pathway for the transfer of ARs from bait stations along the food chain.^{20,22,24,50,51}

The assessment of existing and potential future RMMs is a research priority to considerably reduce the AR exposure of non-target animals and to help regulators in decision-making processes.⁴⁷ The combination of the scientific assessment of their effectiveness and of the effectiveness of necessary rodent management action is needed to strike the optimal balance of the action of RMMs and management approaches such as baiting strategy that both affect management outcomes and non-target risk of AR exposure.

In this study, rodenticidal bait with the AR brodifacoum (BR) was placed either in and around buildings or indoors only to manage Norway rats at livestock farms in north-western Germany. BR exposure of non-target small mammals during and after management operation was compared between the bait placement strategies to assess potential benefits to the small mammal community usually present at farms.

2 MATERIALS AND METHODS

We used farms located in the Münsterland region (52°N, 8°E) in western Germany where cattle or pigs were held. The surrounding area consisted of 63% agriculturally used land with a patchy mix of crops (corn, grain, rapeseed, permanent grassland) and 15% broad-leaved forest patches (mainly beech *Fagus* spec. and oak *Quercus* spec.).⁵² The long-term annual mean temperature was 9.9 °C and mean precipitation was 782 mm.⁵³ A typical farm consisted of buildings for holding animals, storing farm equipment and animal feed and housing farmer families. All structures used were brick buildings with timber elements mostly constructed in the middle of the 20th century. None of them was completely rodent-proof due to passageways for rodents under gates, through sewers, etc., which reflects the status of most farm buildings in the area. Prior to trials, several farms were surveyed visually for signs of rat activity (fresh droppings, runways, burrows, etc.) and farms were selected for the trials where Norway rats were present. Farms were at least 3 km apart and all of them showed signs of rat infestation in further visual surveys immediately prior to baiting.

Trials were run in October and November 2014 and 2015, because at this time rats tend to migrate from fields to farms where they accumulate.⁵⁴ It was planned to use the same six farms in each session in each year but one farm had to be replaced in 2015 because rats were not present. No AR was used for rat management for 6 months (2014) and 11 months (2015) at any farm prior to trials. Norway rats were managed with BR bait (Ratron Brodifacoum Flocken, rolled oats bait base, 0.05 g/kg BR, frunol delicia GmbH, Germany) in bait stations (Rattenköderbox

'B', Detia Garda GmbH, Germany) according to label instructions. BR was used because genetic resistance to ARs is likely in the region.^{13,55,56} In the context of this study, all small mammals except Norway rats, black rats and house mice were considered non-target animals because bait application was intended to manage Norway rats and house mice and no other rodent species was covered by the product registration.

At three farms, 20 bait stations were placed only in buildings (in strategy). In three other farms, ten bait stations were placed in buildings and ten bait stations were placed around buildings (in and around strategy). The general baiting approach followed best practice guidelines issued by the German national competent authority⁵⁷ and recommendations by Endepols *et al.*⁵⁸ Before the use of BR bait, stations were placed appropriately and left undisturbed for 14 days to accustom rats to the bait stations. Then, they were pre-baited with untreated rolled oats. Bait stations were checked daily for 4 days and consumed rolled oats were replenished. After the 4 days pre-baiting, remaining rolled oats were removed and replaced with BR bait (150 g). During the 21-day baiting period, bait stations were checked at intervals of 3 to 4 days and bait added as or replaced as necessary. After baiting, remaining bait was removed.

Non-target small mammals were trapped at each farm within 10 m around buildings with 20 multiple capture live traps (Ugglan, Grahnab, Sweden). Each trap was baited with a mix of rolled oats, peanut butter, peanut curls, rodent food pellets (Altromin 1324, Altromin Spezialfutter GmbH & Co KG, Lage, Germany) and a piece of apple. Wood wool was provided for bedding. There were two trapping sessions: one starting 3 days after the beginning of the BR baiting period and one starting directly at the end of BR baiting period. Traps were pre-baited for four nights and set for three 24 h periods. Traps were checked about every 12 h in the morning and in the evening resulting in six trap checks per trapping session per farm (20 traps \times 6 farms \times 6 checks \times 2 sessions \times 2 years = 2880 trap checks). Small mammals were sacrificed by cervical dislocation, the sex was determined and whole carcasses were frozen at -20°C .

Later, animals were thawed and body weight was measured with a laboratory scale (U6100, Sartorius GmbH, Göttingen, Germany) to the nearest tenth of a gram. The liver was removed, weighed to the nearest tenth of a gram and stored at -80°C for at least 7 days to inactivate pathogens. Residue analysis was based on the method of Geduhn *et al.*²⁴ Each liver sample was spiked with the surrogates acenocoumarol, coumachlor, diphacinone- d_4 , and phenprocoumon to check the validity of the analyses. The tissue was homogenized in methanol and water (2:1, v/v, Ultra-Turrax T25, IKA, Königswinter, Germany) and purified (ChemElut, Agilent, Santa Clara, CA, USA). Residues of the AR compounds BR, bromadiolone, chlorophacinone, coumatetralyl, difenacoum, difethialone, flocoumafen and warfarin were analysed with liquid chromatography coupled with tandem mass spectrometry in electrospray ionization mode (Ultimate 3000 RS, Dionex, USA + Qtrap 5500, AB Sciex, Darmstadt, Germany). The analytes were identified with one precursor-product-ion transition by multiple reaction monitoring and with spectra comparison between sample and reference based enhanced product ion-spectra (> 1000 cps). The quantification of 1,3-indandiones was conducted using the internal standard chlorophacinone- d_4 and for all other analytes warfarin- d_5 . The eight point calibration curves from 0.1 to 100 ng/mL in methanol/water (1:1) resulted in $R^2 > 0.99$. The signal-to-noise ratios of the lowest concentration level were always $> 6:1$. The limits of quantifications were 1 ng/g

(coumatetralyl), 2 ng/g (warfarin, difenacoum), 3 ng/g (bromadiolone, bromadiolone) and 5 ng/g (difethialone, flocoumafen, chlorophacinone). Mean recovery rates [determined with turkey (*Meleagris gallopavo* L.) liver samples spiked with analytes and surrogates] were $58 \pm 6\%$ (BR), $77 \pm 4\%$ (bromadiolone), $83 \pm 14\%$ (chlorophacinone), $100 \pm 6\%$ (coumatetralyl), $78 \pm 7\%$ (difenacoum), $41 \pm 7\%$ (difethialone), $65\% \pm 4\%$ (flocoumafen), $118 \pm 4\%$ (warfarin) and $112 \pm 5\%$ (acenocoumarol), $91\% \pm 2\%$ (coumachlor), $106 \pm 9\%$ (diphacinone- d_4) and $101 \pm 1\%$ (phenprocoumon). Sample cleaning did not cause interferences in blank liver and matrix effects were not observed. AR concentrations stated refer to the fresh weight of liver tissue samples and were not corrected for recovery rates, which is in line with the reporting format of previous studies and allows comparison.^{24,59,60}

Statistical analyses were conducted with the program R.^{61,62} R-packages lme4⁶³ and MASS⁶⁴ were used for fitting models by maximum-likelihood (Laplace approximation). The occurrence of BR residues and BR concentrations were modelled in generalized linear mixed models (GLMMs) following a binomial distribution respectively a negative binomial distribution. Both models included the explanatory variables years (2014, 2015), baiting strategy (in, in and around), trapping period (start, end of bait application), sex and taxon and in the BR concentration model also their interactions. Farm was selected as random factor to account for repeated measures. Model simplification and selection was based on the Akaike information criterion⁶⁵ and validity was checked by graphical evaluation of residuals.

During model simplification, the random factor farm was eliminated because it explained little variance and analyses resulted in overdispersion or singular fits. In the final GLMM, the variance of BR residues occurrence was explained by years, baiting strategy and taxon. The final GLMM of BR concentrations included years and baiting strategy as well as the interaction of trapping period and taxon. For *post hoc* analyses, factors were compared pair-wise using the R-package emmeans⁶⁶ (Tukey contrasts).

3 RESULTS

We captured and removed 315 small mammals of three (sub)families and eight taxa and tested them for AR residues (Table 1). Small mammals were grouped into Soricidae, Arvicolinae and Murinae for further analyses because some species were caught in low numbers that did not allow analysing data at the species level.

3.1 ARs in small mammal community

ARs were present in liver tissue of 134 of 315 (42.5%) individuals tested with BR present in 40.3% of all animals screened. BR (the AR used in bait) was detected in 94.8% of individuals ($n = 127$) with AR residues. The presence of other ARs was rare and not considered in further analyses: chlorophacinone (4.1% of all animals screened, mean residue concentration of these 13 animals was 181 ± 31 ng/g), difenacoum (2.2%, mean residue concentration of these seven animals was 176 ± 57 ng/g), flocoumafen (1.3%, mean residue concentration of these four animals was 88 ± 33 ng/g) and bromadiolone (0.6%, mean residue concentration of these two animals was 48 ± 1 ng/g) occurred in harvest mice (*Micromys minutus*), *Sorex* spp. and white-toothed shrews (*Crocidura russula*) but not in *Apodemus* or *Arvicolinae* species. Difethialone, coumatetralyl and warfarin were not found. There was a single AR compound present in 116 samples (36.8% of all animals

Table 1 Sample size (*n*) and occurrence of brodifacoum (BR) residues in liver tissue of non-target small mammals removed from farms where BR bait was used to manage Norway rats (*Rattus norvegicus*) either in and around buildings or indoors only

Taxon	Bait stations in and around buildings		Bait stations indoors only	
	<i>n</i>	With BR residues	<i>n</i>	With BR residues
Total	159	86 (54.1%)	156	41 (26.3%)
Arvicolinae	18	2 (11.1%)	58	6 (10.3%)
Bank vole (<i>Clethrionomys glareolus</i>)	13	2 (15.4%)	29	6 (20.7%)
Common vole (<i>Microtus arvalis</i>)	5	0 (0.0%)	26	0 (0.0%)
Field vole (<i>Microtus agrestis</i>)	0	0 (0.0%)	3	0 (0.0%)
Murinae	42	17 (40.5%)	32	6 (18.8%)
Harvest mouse (<i>Micromys minutus</i>)	33	13 (39.4%)	16	3 (18.8%)
Wood mouse (<i>Apodemus sylvaticus</i>)	8	4 (50.0%)	12	2 (16.7%)
Yellow-necked mouse (<i>Apodemus flavicollis</i>)	1	0 (0.0%)	4	1 (25.0%)
Soricidae	99	67 (67.7%)	66	29 (43.9%)
White-toothed shrew (<i>Crociodura russula</i>)	98	67 (68.4%)	60	26 (43.3%)
Eurasian/Crowned shrew (<i>Sorex araneus/S. coronatus</i>)	1	0 (0.0%)	6	3 (50.0%)

screened). Seventeen animals (5.4%) contained two AR compounds (eight contained brodifacoum + chlorphacinone, six brodifacoum + difenacoum, two brodifacoum + flocoumafen, one bromadiolone + flocoumafen) and one (0.3%) contained three AR compounds (brodifacoum, bromadiolone, flocoumafen).

There was a higher mean percentage of small mammals with BR present in liver tissue when bait stations were placed in and around buildings (54.1%, 86 of 159 with BR residues) compared to farms where bait stations were placed indoors only (26.3%, 41 of 156 with BR residues, $P < 0.001$). Shrews carried BR residues more often than mice and voles ($P < 0.001$) but there was no difference between the latter two taxa ($P = 0.08$). The occurrence of BR in shrews (67.7% versus 43.9%) and mice (40.5% versus 18.8%) was higher when bait was used in and around buildings versus indoors only ($P = 0.02$). Among voles, BR residues were only present in bank voles (*Clethrionomys glareolus* Tilesius). The occurrence of BR residues in voles was similar for the two baiting strategies (11.1% versus 10.3%). However, this was based on only eight individuals (one at the beginning/seven at the end of the baiting period) trapped on only one farm per baiting strategy (Table 1).

3.2 BR concentration in small mammal taxa

The mean BR concentration in liver tissue from non-target small mammals carrying BR residues ($n = 120$) was more than twice as high when bait stations were placed in and around buildings (1409 ng/g, $n = 79$, excluding seven statistical outliers, see later) compared to farms where bait stations were placed indoors only (617 ng/g, $n = 41$, $P = 0.006$) (Table 2, Fig. 1). Including non-target small mammals without BR residues, the mean BR concentration in individuals from farms with baiting in and around buildings was more than four times higher (732 ng/g, $n = 152$, excluding seven statistical outliers, see later) than from farms with indoor only baiting (162 ng/g, $n = 156$).

The correlation of BR liver residues with physiological effects varies considerably among species.^{17,18,67} For the non-target species considered here, there is no information about median lethal dose (LD₅₀) values for BR or other toxicity thresholds available. To compare BR residue levels among taxa, BR residues were grouped in four concentration classes: (i) individuals without detectable AR residues, (ii) lower tertile of individuals with BR residues

(9–395 ng/g), (iii) middle tertile of individuals with BR residues (396–1384 ng/g) and (iv) upper tertile of individuals with BR residues (1385–4283 ng/g). Seven individuals (5.5%) with highest BR residues (5397–19 068 ng/g) were statistical outliers and had to be excluded from analyses. These were captures from farms with baiting in and around buildings: five white-toothed shrews with BR residues of 5397 to 9731 ng/g and two wood mice with BR concentrations of 15 056 and 19 068 ng/g. However, 44% ($n = 35$) of individuals carried BR residues of the upper concentration class when bait was used in and around buildings while only 15% ($n = 6$) did when bait was applied indoor only.

In both baiting strategies voles showed the lowest BR concentrations (0–442 ng/g, $n = 8$) (Table 2). BR residue concentrations of all but one vole were in the lower concentration class (Fig. 2). Mean BR concentrations in mice with residues caught on farms with indoor application (699 ng/g, $n = 6$) were less than twice as low as in mice from farms with application in and around buildings (1802 ng/g, $n = 15$, $P < 0.006$) (Table 2). There was no difference in BR concentrations between samples taken at the beginning and at the end of the baiting campaign ($P = 0.794$) (Fig. 1). On farms with indoor application, one of six mice (17%) had BR residue at the upper BR concentration class and on farms with bait stations in and around buildings, nine of 15 mice (60%) had residues of the upper BR concentration class (Fig. 2). In shrews, the mean BR concentration in animals with residues was about twice as high when baiting was used in and around buildings (1357 ng/g, $n = 62$) versus indoors only (684 ng/g, $n = 29$, $P = 0.006$) (Table 2). Residues were generally higher at the beginning than at the end of the baiting period ($P < 0.001$) (Fig. 1). BR residues of the upper concentration class occurred in five of 29 (17%) shrews when bait was used indoor only and in 26 of 62 (42%) shrews for bait application in and around buildings (Fig. 2). BR residue concentrations in Murinae and Soricidae were similar at the beginning of the baiting period ($P = 0.899$) but lower in Soricidae versus Murinae at the end ($P = 0.002$) (Fig. 1).

4 DISCUSSION

This study demonstrated for the first time the efficacy of a (potential) RMM to reduce the exposure of non-target animals to ARs. In our replicated field experiments on livestock farms the mean

Table 2 Concentrations of brodifacoum (BR) residues in liver tissue of non-target small mammals from farms where BR bait was used to manage Norway rats (*Rattus norvegicus*) either indoors only or in and around buildings. Five white-toothed shrews (*Crocidura russula*) (5397–9731 ng/g) and two wood mice (*Apodemus sylvaticus*) (15 056 and 19 068 ng/g) were excluded from dataset (see text for details)

Taxon	Bait stations in and around buildings (ng/g liver tissue)				bait stations indoors only [ng/g liver tissue]			
	n	Mean ± standard error	Median	Minimum–maximum	n	Mean ± standard error	Median	Minimum–maximum
Total	79	1409 ± 128	1312	18–4283	41	617 ± 103	360	9–2571
Arvicolinae	2	98 ± 56	98	18–177	6	206 ± 52	184	55–442
Bank vole (<i>Myodes glareolus</i>)	2	98 ± 56	98	18–177	6	206 ± 52	184	55–442
Common vole (<i>Microtus arvalis</i>)	0	0	0	0	0	0	0	0
Field vole (<i>Microtus agrestis</i>)	0	0	0	0	0	0	0	0
Murinae	15	1802 ± 346	2144	24–3836	6	699 ± 319	466	9–2192
Harvest mouse (<i>Micromys minutus</i>)	13	2074 ± 341	2176	104–3836	3	1041 ± 507	869	62–2192
Wood mouse (<i>Apodemus sylvaticus</i>)	2	38 ± 10	38	24–52	2	512 ± 365	512	9–1015
Yellow-necked mouse (<i>Apodemus flavicollis</i>)	0	0	0	0	1	48	48	48
Soricidae	62	1357 ± 134	1303	38–4283	29	684 ± 123	396	17–2571
White-toothed shrew (<i>Crocidura russula</i>)	62	1357 ± 134	1303	38–4283	26	708 ± 134	462	17–2571
Eurasian/crowned shrew (<i>Sorex araneus/S. coronatus</i>)	0	0	0	0	3	484 ± 234	360	61–1032

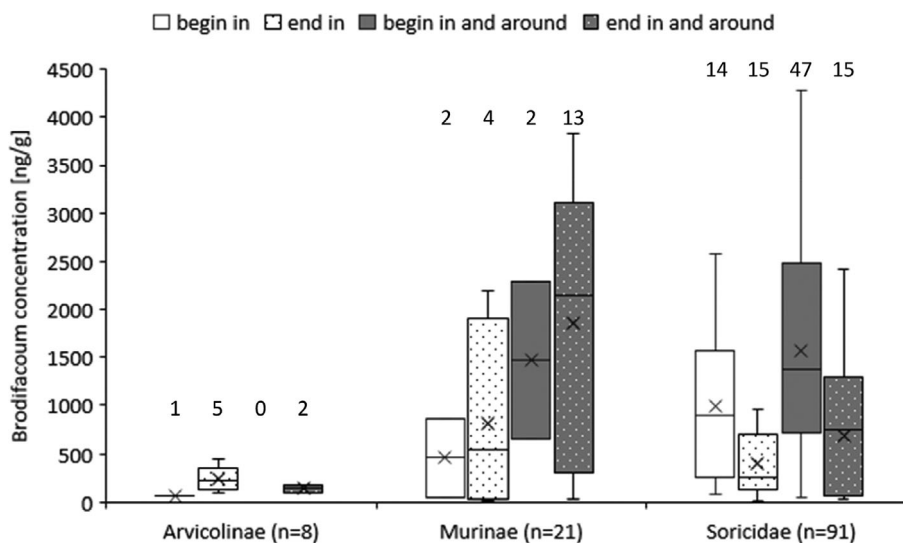


Figure 1 Brodifacoum (BR) concentration in liver tissue of animals of Arvicolinae, Murinae and Soricidae that carried BR residues detected at the beginning (begin) and at the end (end) of Norway rat (*Rattus norvegicus*) control with BR bait application either indoors only (in) or in and around buildings (in and around). Numbers above boxplots indicate sample size for each category (n). Stations = 25–75% quartile; X = mean; horizontal line = median; whiskers = minimum and maximum values.

percentage of non-target small mammals with BR residues was more than twice as high when bait was applied in and around buildings (54.1%) compared to bait application indoors only (26.3%). Accordingly, mean BR concentration in liver tissue samples of non-target small mammals with residues was about twice as high for baiting in and around buildings (1409 ng/g) versus baiting in buildings only (617 ng/g). However, results also indicate that baiting indoors only does not completely prevent non-target exposure of species that are active around buildings. These effects were present in murid rodents and shrews but not in voles. This reflects earlier findings that voles on farms rarely

consume bait from bait stations³³ while murid rodents and shrew species access AR bait, which results in high levels of residue concentrations.^{20,21,24,33,68}

The duration of AR baiting is usually limited. Permanent baiting without proof of continuing rodent problems is not permitted or highly restricted to prevent long-term exposure of non-target species.⁶⁹ Our findings support such a regulation because about more than half of non-target small mammals were exposed to BR within a 3 week application of BR bait in and around buildings. About 42% of shrews and 60% of murine rodents carried BR residues of the upper concentration class (1385–4283 ng/g) that may

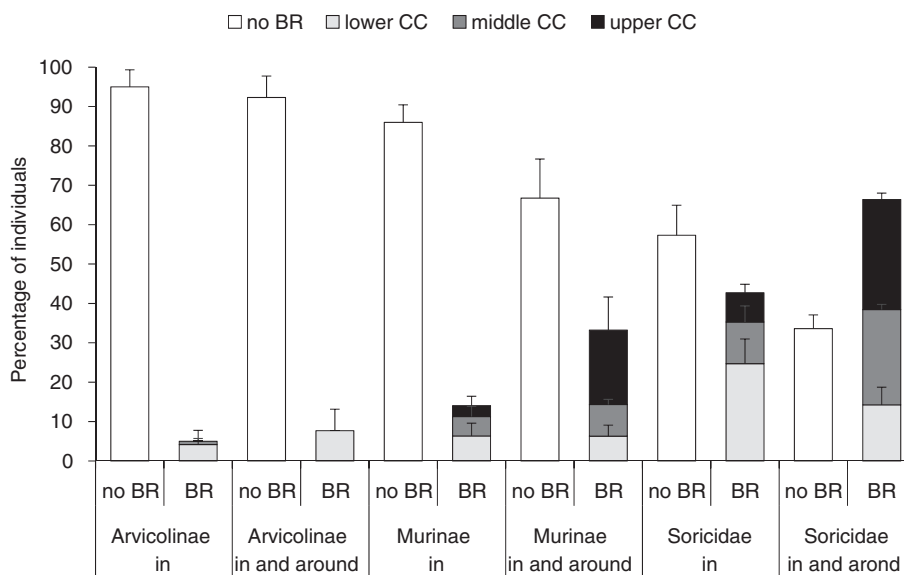


Figure 2 Percentage of brodifacoum (BR) residues per concentration class (CC) of non-target small mammal taxa from farms where bait stations with BR-bait were placed indoors only (in) or in and around buildings (in and around) to manage Norway rats (*Rattus norvegicus*). Concentration classes represent individuals without detectable BR residues (no BR), the lower tertile individuals with BR residues (lower CC; 9–395 ng/g), the middle tertile individuals with BR residues (middle CC; 396–1384 ng/g) and the upper tertile individuals with BR residues (upper CC; 1385–4283 ng/g). Five white-toothed shrews (*Crocidura russula*) (5397–9731 ng/g) and two wood mice (*Apodemus sylvaticus*) (15 056 and 19 068 ng/g) were excluded from the dataset (see text for details). Values are means of farms, error bars are standard errors.

indicate lethal effects.^{70,71} However, the correlation of BR residues and physiological effects is rarely known and large differences exist among species and individuals.^{17,18}

It is likely that AR exposure will be more pronounced in these taxa when AR bait is available outdoors for longer periods. The reason could be further bait uptake by resident small mammals or by immigrants recolonizing territories from former owners that died of AR poisoning. This illustrates not only the disadvantage of extended baiting periods regarding non-target exposure but also the importance of complete bait removal at the end of management operations mandated in best practice guidelines.^{72,73}

Shrews seemed to be especially prone to bait consumption from bait stations to a degree that results in residues level in the upper concentration class. These results confirm an earlier study with white-toothed shrews exposed to AR baiting in a similar environment where 10–20% of BR concentrations found in white-toothed shrews were > 1000 ng/g.²⁴ In contrast to murid rodents, shrews carried higher BR residue levels a few days after the commencement of baiting than at the end of the baiting period. This indicates that shrews succumb quicker to BR than mice^{74,75} resulting in apparently low levels of exposure at the late phase of rodent baiting.

It seems unlikely that secondary exposure via invertebrates can deliver doses within a few days that result in the observed high BR concentrations in shrews.^{22,24,76,77} Therefore, direct consumption of rolled oat-based bait^{78,74} or intake of bait dust⁷⁴ seems the cause of shrew exposure in our study. Shrews are insectivorous but seeds and other plant material are reported from dietary analyses in several species,⁷⁵ in particular in *Crocidura* species.^{79,80} In either case, shrews can be highly exposed to BR bait. This is of concern because shrews are legally protected in Germany.⁸¹

AR compounds that have not been used in the study were very rarely detected. The presence of chlorphacinone, difenacoum, flocoumafen and bromadiolone may have been due to several sources: residues in residents from previous bait application,

residues in immigrants from nearby farms where these compounds have been consumed, or contamination of the brodifacoum product.²⁴ The former seems unlikely because there was no AR bait application at the farms for 6 to 11 months. However, we cannot exclude any of these sources of residues but given the small percentages of rodents with such residues (0.6–4.1%) and the low mean concentrations, the occurrence of other ARs should not have affected the results of the study.

The desirable benefits of RMMs and rodent control outcomes need to be balanced. If baiting strategies are limited to indoor application, there may be adverse effects on the eradication of Norway rat populations. Norway rats are not restricted to indoors but often the majority of the population lives in outdoor areas, rats migrate between indoors and outdoors^{40,82,83} and rats from outdoors can replace individuals eradicated with ARs indoors.^{39,40} In this common scenario, and given the fact that Norway rats tend to consume more AR bait from bait stations around buildings than in buildings,⁸⁴ bait application in and around buildings seems essential to eradicate Norway rat infestations indoors and to prevent an influx of nearby 'outdoor' rats. Non-target small mammals also travel between indoors and outdoors because there were BR residues in individuals trapped outdoors when bait was applied indoors only. In contrast to Norway rats, infestation of house mice tend to be restricted to indoors at least in Europe.⁸⁵ Therefore, for this species, a restriction of AR bait application to indoors may not negatively affect the management outcome, but this requires further studies. More work needs to be done to define the optimal baiting approach for pest rodents regarding pros and cons (positive versus negative effects on management efficacy, duration of baiting, non-target exposure) of bait placement strategies. Further studies should assess the benefits and disbenefits of RMMs to ensure the application of effective measures.

Further aspects of baiting are relevant for non-target protection such as choosing the optimal AR compound for baiting. Compounds of the first generation or less toxic options from the SGARs

should be applied in regions where commensal rodents are fully susceptible to ARs. Only in regions where genetic resistance of rodents to ARs occurs,⁵⁵ highly potent SGARs such as brodifacoum should be used.^{13,56} In addition, bait station design could be modified to limit the access of non-target species^{86,87} but little is known about their effect on the exposure of non-target species. In any case, care has to be taken that bait station design is suitable for quick uptake of an effective dose⁸⁸ to ensure that losses in stored produce or the risk of human infection with rodent-borne disease are minimized.⁴⁴

There is no doubt that adverse effects of ARs in non-target wildlife need to be prevented. Empirical evidence should determine what level of protection can be provided by RMMs and how the efficacy of RMMs can be optimized without putting the rodent management success at risk.

5 CONCLUSIONS

The results demonstrate that restricting the application of BR bait to indoors only reduces the fraction of non-target animals carrying residues and BR concentrations in liver tissue compared to bait application in and around buildings. However, even when bait application is conducted indoors only, non-target species trapped outdoors have consumed bait as indicated by BR residues. Further work is needed to find the optimal balance between the positive effect of baiting indoors only on AR exposure of non-target murid rodents and shrews and the need to achieve an appropriate outcome of commensal rodent management.

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