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Spatial and temporal exposure patterns in non-target small mammals during brodifacoum rat control

Anke Geduhn [a,b,*], Alexandra Esther [a], Detlef Schenke [c], Hermann Mattes [b], Jens Jacob [a]

[a] Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forests, Vertebrate Research, Toppheideweg 88, 48161 Münster, Germany
[b] University of Münster, Institute of Landscape Ecology, Heisenbergstrasse 2, 48149 Münster, Germany
[c] Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Strasse 19, 14195 Berlin, Germany

HIGHLIGHTS

- Brodifacoum residues: detected in all non-target small mammal taxa during rat control
- There was a negative correlation of distance to bait stations on residue occurrence.
- High residue concentrations were largely restricted to 15 m around bait stations.
- Higher concentrations but less residues occurred during baiting than after baiting.
- The highest maximal residue concentrations occurred in *Apodemus* species.

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ABSTRACT

Worldwide pest rodents on livestock farms are often regulated using anticoagulant rodenticides (ARs). Second generation ARs in particular can cause poisoning in non-target species due to their high toxicity and persistence. However, research on exposure of small mammals is rare. We systematically investigated spatial and temporal exposure patterns of non-target small mammals in a large-scale replicated study. Small mammals were trapped at different distances to bait stations on ten farms before, during and after brodifacoum (BR) bait application, and liver samples of 1178 non-target small mammals were analyzed for residues of eight ARs using liquid chromatography coupled with tandem mass spectrometry. BR residues were present in 23% out of 742 samples collected during and after baiting. We found clear spatial and temporal exposure patterns. High BR residue concentrations mainly occurred within 15 m from bait stations. Occurrence and concentrations of residues significantly decreased with increasing distance. This pattern was found in almost all investigated taxa. After baiting, significantly more individuals contained residues than during baiting but concentrations were considerably lower. Residue occurrence and concentrations differed significantly among taxa, with the highest maximal residue concentrations in *Apodemus* species, which are protected in Germany. Although *Sorex* species are known to be insectivorous we regularly found residues in this genus. Residues of active agents other than brodifacoum were rare in all samples. The confirmation of substantial primary exposure in non-target small mammals close to the baiting area indicates considerable risk of secondary poisoning of predators, a pathway that was possibly underestimated until now. Our results will help to develop risk mitigation strategies to reduce risk for non-target small mammals, as well as their predators, in relation to biocidal AR usage.

1. Introduction

Anticoagulant rodenticides (ARs) are commonly used in many parts of the world for the control of pest rodents for plant protection and hygiene purposes in both rural and urban environments (Buckle and Smith, 1994). ARs are usually coumarin or indandione derivates that inhibit blood clotting in vertebrates and result in a delayed death of poisoned individuals (Maroni et al., 2000; Thijssen and Janssen, 1994; Valchev et al., 2008). This mode of action avoids bait shyness and vitamin k_1 is available as an antidote against warfarin associated ARs (Bjornsson, 1984; Bull, 1976; Lowenthal and Taylor, 1959; Markussen et al., 2003). Exposure of non-target animals to AR, however, can occur by direct bait intake (primary exposure) or when residues of ARs are passed through the food web via prey and carrion (secondary exposure). Second generation ARs (SGARs; e.g. brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen) were introduced to the

* Corresponding author at: Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forests, Vertebrate Research, Toppheideweg 88, 48161 Münster, Germany. Tel.: +49 251 8710642; fax: +49 251 8710633.
E-mail address: anke.geduhn@jki.bund.de (A. Geduhn).

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market in the 1970s (Lund, 1984; Thijssen et al., 1989) because of resistance of Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus* spp.) to first generation ARs (FGARs; e.g. chlorophacinone, coumatetralyl and warfarin). SGARs have a higher toxicity to vertebrates and persist longer in animal tissue than FGARs (Eason et al., 2002; Fisher et al., 2003).

Predators are at great risk of secondary poisoning because the persistent ARs accumulate in the liver (Eason et al., 2002). There is worldwide evidence of secondary exposure to ARs in aerial and terrestrial predators (e.g. France: Berny et al., 1997; Raoul et al., 2003; New Zealand: Eason et al., 2002; Spurr et al., 2005; Denmark: Christensen et al., 2012; USA: Riley et al., 2007; UK: McDonald et al., 1998; Walker et al., 2010). AR-residues in population studies of mammalian predators have been reported for example in 84% of red foxes (*Vulpes vulpes*; Tosh et al., 2011a), 23% of stoats (*Mustela erminea*; McDonald et al., 1998), 30% of weasels (*Mustela nivalis*; McDonald et al., 1998) and 36% of polecats (*Mustela putorius*; Shore et al., 2003). AR-residues are highly variable in birds of prey, ranging from 10 to 100%, but most studies found 65 to 85% of bird affected (e.g. Christensen et al., 2012; Hughes et al., 2013; Murray, 2011; Newton et al., 1990; Walker et al., 2010). Large variance of AR-occurrence in these studies may reflect different application practices such as aerial applications in New Zealand (Dowding et al., 1999), field application of bromadiolone in France (Sage et al., 2008) and the restriction of BR to bait station usage in the UK (Tosh et al., 2011b).

The source of secondary exposure of predators can be target, as well as non-target individuals that consumed ARs. Barn owls (*Tyto alba*) often nest in farm buildings (De Bruin, 1994) and may be at a particularly high risk of exposure to AR poisoned animals, whether they are target, or non-target species (Newton et al., 1990; Walker et al., 2010). In Greece, the most common prey species of barn owls is the house mouse (*Mus musculus domesticus*, 26%; Bontzorlos et al., 2005), whereas in other regions of the world, including Germany, the diet of barn owls mainly consists of non-target small mammals like voles and wood mice (Görner, 1979; Langenbach, 1982; Smith et al., 1972). In these regions, bait uptake through non-target animals could play an important role for predators.

Despite ample data for secondary exposure in non-target predators that was stated above, information about non-target small mammals is lacking. Primary exposure caused by AR application for crop protection was shown in target *Arvicola* and *Microtus* species (Giraudoux et al., 2006; Hernandez et al., 2013; Sage et al., 2008). Exposure through plant protection products in the field is unlikely in Germany, because most ARs that are authorized at the EU level for plant protection are prohibited in Germany for field application. There is evidence for primary poisoning in commensal target rodents during biocidal bait usage (e.g. Cowan et al., 1995; Rowe et al., 1978). Beside commensal target rodents, many non-target small mammal species, like *Apodemus* species, bank voles (*Myodes glareolus*) and greater white toothed shrews (*Crocidura russula*) are regularly present in rural and peri-urban environments (Braun and Dieterlen, 2005). Therefore, they can be exposed to ARs (Brakes and Smith, 2005; Tosh et al., 2012; Townsend et al., 1995). Other species such as common voles (*Microtus arvalis*) and field voles (*Microtus agrestis*) mainly live in (scrub-) grassland (Braun and Dieterlen, 2005) and exposure to ARs is rare (Brakes and Smith, 2005; Cox and Smith, 1990; Elliott et al., 2014). Some of the non-target small mammal species are legally protected in Germany (e.g. *Apodemus* species as well as *Sorex* and *Crocidura* shrews; BMJV, 2005) and their exposure to ARs would be of concern.

Biocidal AR bait requires covered application (e.g. bait stations) in Germany. However, non-target small mammal species may be prone to primary exposure to biocidal ARs because their similar body size to target species allows them access to bait stations. There is anecdotal (Cox and Smith, 1990) and qualitative evidence (Brakes and Smith, 2005; Tosh et al., 2012) from the UK that wood mice (*Apodemus sylvaticus*) consume AR bait at bait stations placed on farms. The presence of individuals with bait-residues was restricted to a maximum distance of about 80–110 m from the baited area (Tosh et al., 2012; Townsend et al., 1995). However, there are no detailed data from systematic approaches, on spatial and temporal patterns of AR residues in non-target small mammals.

The identification of species-specific associations of residue concentration and distance from baited areas in natural settings for the application of an AR product could help to evaluate differences among locations and species regarding the risk of primary exposure. Such knowledge may also be used to estimate the risk of secondary exposure for predators at and around farms where ARs are applied and to derive spatially targeted risk mitigation approaches.

The aim of our study was to identify temporal and spatial patterns of residue distribution caused by an AR application. We used a systematic, replicated, quantitative approach to determine whether primary exposure occurs in non-target small mammals before, during and after a coordinated baiting campaign on farms in NW Germany using BR for the control of Norway rat populations. BR is regularly used in rodent control operations in the area (Buckle et al., 2012) because resistance to FGARs and to some SGARs has developed (Pelz, 2007). We were particularly interested in small mammal non-target inter-species differences in residue occurrence and concentrations, seasonal effects, and the temporal and spatial patterns of residue distribution. These aspects are highly relevant for assessing the risk for small mammals as well as predators when ARs are applied in and around buildings for biocidal or plant protection use.

2. Material and methods

2.1. Study area

In four trapping events (autumn (October/November) 2011 and 2012 as well as in late winter (February/March) 2012 and 2013) small mammals were screened for rodenticide residues during baiting campaigns with brodifacoum (BR) to control Norway rat populations. The work was conducted on farms in the Münsterland region (Fig. 1, 51.960665N, 7.626135E, North Rhine-Westphalia, Germany). The area is a mosaic of farmland (about 60%) and small forest sections (about 15%) used for timber production (GENESIS-online-database, 2013). Main crops are corn (*Zea mays*), wheat (*Triticum* spp.) and barley (*Hordeum vulgare*; GENESIS-online-database, 2013). In the region, the mean temperature is 9.2 °C and annual precipitation is 782 mm (DWD, 2014). On all farms included in this study, livestock (cattle, poultry and/or pigs) was held in stables and/or on surrounding meadows. Small mammal populations were investigated on six farms at all four trapping events (autumn 2011 and 2012 and winter 2012 and 2013; gray dots in Fig. 1), whereas populations at four farms were investigated less often (empty dots in Fig. 1) due to insufficient rat numbers based on visual surveys before bait application. At which trapping events these farms were investigated are indicated in Fig. 1. The minimum linear distance between farms where we applied BR bait was 2.9 km. Neighboring farms not included in the study were on average 310 m ± 120 m standard deviation (sd) away (minimum 160 m). Prior to the study, farmers were interviewed about AR usage behavior and rat occurrence. All farmers stated regular occurrence of rodents on their farms and used ARs to control them. Brodifacoum was used most often (5 of 10 farmers). All farmers used covered bait stations for baiting around buildings. On average farmers used ARs twice a year for three to four weeks for each baiting event. The last time farmers applied bait was several weeks to two years prior to the start of the study.

2.2. Norway rat control

Baiting was conducted according to label instructions and following the standard practice of farmers in the region as indicated in the pre-study questionnaire (see above) and lasted for three weeks. Twenty bait stations (Rattenköderbox “B”, Detia Garda GmbH) were placed at each farm where rat feces and footprints were observed. Where signs