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Non-invasive monitoring of red fox exposure to rodenticides from scats

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ABSTRACT

Exposure of wildlife to anticoagulant rodenticides is mainly assessed by analysing residues in the tissues, notably liver, of dead animals. Recent finding suggested that the analysis of active ingredients in mammal scats sampled in the field could be used as a non-invasive method to monitor non-lethal exposure in populations. Here, we measure experimentally the persistence of 6 anticoagulant rodenticides in fox scats when placed under natural conditions. Six foxes were fed with voles dosed with brodifacoum, bromadiolone, chlorophacinone, warfarin, difenacoum and difethialone in controlled conditions and their faeces were collected. Then, the scats were placed outside, thus exposed to weathering, and sampled up to four months later to measure the concentrations of the 6 rodenticides. We showed that both the concentrations and the occurrence of residues in the scats decreased rapidly for all these pesticides. Based on concentrations, the degradation half-lives ranged from 5.26 days for chlorophacinone to 7.98 days for bromadiolone. Furthermore, the probability of sampling a scat containing detectable residues decreased by 10% after 7d, 2d, 10d, 5d, 3d and 10d for warfarin, chlorophacinone, bromadiolone, brodifacoum, difenacoum and difethialone respectively. Thus, in terms of using residues in scats to monitor fox exposure to rodenticides, we recommend first, to clear the studied areas of old faeces and then, sample scats after a short period, ideally <5 days.

1. Introduction

Wildlife can be exposed to anticoagulant rodenticides (ARs) when they are used as biocides or for plant protection to limit damage caused by rodents. Lethal poisoning due to primary or secondary exposure is widely reported (Berny et al., 1997; Coeurdassier et al., 2014) and is commonly diagnosed by checking both haemorrhages and significant levels of residues in the liver (Berny, 2007). Furthermore, high sub-lethal exposure rates have also been found in carnivores such as raptors (Albert et al., 2009; Christensen et al., 2012; Walker et al., 2008) and mammals (Elmeros et al., 2011; Shore et al., 2003; Tosh et al., 2011) by analysing residues in animals killed by road traffic, hunting, trapping or with unknown cause of death. In France, the AR bromadiolone has been applied in field to control Water vole *Arvicola scherman* outbreaks since the 1980's. This has led to massive poisoning of wildlife, notably between 1998 and 2002. Common buzzards *Buteo buteo* and Red foxes *Vulpes vulpes* were the species the most frequently found poisoned with several hundreds of cases reported (Coeurdassier et al., 2014). Since then, treatment practices have evolved successfully to mitigate wildlife poisoning and the number of poisoned animals has subsequently decreased with 5 cases reported in 2012 and 2013 (Coeurdassier et al., 2014). However, Sage (2008) showed that sub-lethal exposure of foxes was high, 85% of the individuals (41/48) shot for an epidemiological survey program had detectable bromadiolone residues in liver.

To assess consistently sub-lethal exposure rates using animal corpses collected by wildlife surveillance networks, large sample sizes are needed and thus, it is less applicable at small spatial scales, i.e. <10 km^2, notably for small, non-abundant and/or cryptic

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species. Moreover, an increased susceptibility of animals poisoned by ARs to other threats, due for instance to lethargic or aberrant behaviour, remains currently under question (Rattner et al., 2014). This could lead to an overestimation of sub-lethal exposure rates of ARs when using animal carcasses found randomly in nature. An alternative and non-invasive way of monitoring sub-lethal exposure of wildlife to ARs is to look for residues in raptor pellets or mammal scats. This has been developed successfully for the barn owl in both laboratory and field experiments (Eadsforth et al., 1996; Newton et al., 1994). More recently, as most of the ARs are excreted in faeces by mammals (World Health Organization, 2016), Sage et al. (2010) have investigated whether fox exposure can be assessed by analysing ARs in scats. First, a sensitive LC–ESI–MS method to quantify bromadiolone in fox scats was developed and the toxicokinetic of bromadiolone in foxes was studied. Red foxes were fed with bromadiolone-poisoned voles for 2–5 days and then with uncontaminated food under controlled conditions. Bromadiolone residues were detected in fox faeces 15 h after the first exposure and increased dramatically during the exposure period. Then, when the exposure stopped, residues decreased rapidly but they remained detectable in faeces for at least 26 days (end of the experiment) (Sage et al., 2010). Moreover, when bromadiolone was analysed in fox scats sampled in an area treated with this AR, 48% of the faeces collected between 15 and 45 days after the treatment had detectable residues (Sage, 2008). The authors concluded that scat sampling and analysis had promise as a mean of monitoring the exposure of foxes in the natural environment. However, they underlined that key points remained to be addressed before using it as a routine standard method, notably the persistence of ARs in scats following excretion in natural environment. Most of the ARs are persistent in animal tissues (e.g., degradation half-life DT50 of bromadiolone ranging from 28 to 170 days in rodent liver, Crowell et al., 2013) but they disappear faster in baits (e.g., DT50 bromadiolone: 5 days; Sage et al., 2007) or standardized soils (e.g., DT50 bromadiolone 4.6–53 days; Lewis et al., 2016). No data on the fate of ARs in mammal droppings is available while such information is needed to check the usefulness and to calibrate the sampling of scats as indicator of fox and more generally wildlife exposure.

The aim of this study was to quantify the persistence of six ARs frequently used in Europe, brodifacoum, bromadiolone, chlorophacinone, difenacoum, difethialone and warfarin in fox scats placed in natural conditions and thus, exposed to weathering.

2. Materials and methods

2.1. Exposure of foxes in controlled conditions and faeces collection

The experiment was conducted at the experimental farm of the Agence Nationale de Sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES, Nancy – France; agreement A54747). The animal used was the silver fox, which belongs to the same species as the 'wild' red fox, (*Vulpes vulpes*). Six adult foxes (mixed sex group) and that had never been exposed to ARs were individually caged, 5 were fed with Water voles spiked with ARs and 1 control was fed with uncontaminated voles. Both duration and dose of exposure of foxes to ARs were determined according to Sage et al. (2008) who showed that voles trapped in a plot treated with bromadiolone may contain over 300 μg of bromadiolone per individual a few days after bait application. According to Artois (1989), adult red foxes eat 0.3–0.6 kg of food per day, which represents 4–8 water voles. The water voles used to feed the foxes were trapped in an organic farm (village of By, 5.89°E, 47.01°N France) where bromadiolone has not been applied in fields for several years. Trapped voles were stored at −20 °C. The day the voles were given to the foxes, they were defrosted and spiked at five different intraperitoneal points with 1 ml of ethanol (Carlo Erba analytical grade) solution containing 50 μg each of the 6 following active ingredients (a.i.): brodifacoum, bromadiolone, chlorophacinone, difenacoum difethialone and warfarin. The final amount of ARs was 300 μg per vole in total. During 5 days four water voles were given to each fox. At the end of exposure, foxes received uncontaminated water voles for the next 3 days. The faeces of the 6 foxes were collected from the first to the sixth day after the beginning of exposure. Due to the photosensitivity of ARs, the faeces were stored separately at −20 °C, in the dark.

Although the administrated doses of ARs were realistic, the daily dose and the duration of fox exposure were potentially sufficient to cause lethal haemorrhages. Indeed, the lowest lethal dose reported for bromadiolone is 150 $\mu g\,kg^{-1}\,d^{-1}$ during 5 days (Kolf-Clauw et al., 1995). There is no such data available for the other a.i tested here. However, the acute toxicity of bromadiolone for mammals is in the same range as the other second generation ARs and higher than that of first generation ARs (i.e., warfarin and chlorophacinone) (Erickson and Urban, 2004). In our experiment, each fox weighed ∼7 kg, thus, the daily dose of ARs given to a fox, i.e., 1200 $\mu g\,fox^{-1}\,d^{-1}$, was higher than the lowest lethal dose reported above for bromadiolone, 1050 $\mu g\,fox^{-1}\,d^{-1}$. Therefore, the antidote, vitamin K1 was administrated to each fox from the first day of experiment until one month after exposure. The use of vitamin K1 does not modify the toxicokinetic of ARs (Gamelin and Harry, 2005) and prevents bleeding. This protocol was approved by an ethic committee (Opinion No. 13/09/11-6).

2.2. Experimental design to assess ARs persistence in faeces under natural conditions

Each collected scat was separated into three parts: the central part was placed outside to assess the variation of ARs concentrations over time. The two tops were pooled and immediately refrozen and stored at −20 °C for later determination of the initial concentration of ARs. For all the faeces, the central part was placed in an enclosure strictly prohibited to public at the Botanical Gardens of the City of Besançon (France). The experimental enclosure was a drained area delimited by walls (L: 3.5 m, l: 1.5 m, h: 0.5 m) with a 10 cm layer of soil and opened to weathering. In order to limit the impact of an unexpected gradient of environmental conditions (humidity, sunlight...), the enclosure was split into six blocks. In each block, 15 faeces were deposited on the soil the 25th October 2011. They were distributed in order to have at least one scat from each fox and each exposure day per block. Then, 6 faeces (one by block) were sampled at each sampling time, i.e., 0, 1, 2, 4, 7, 10, 15 days, 1 month, 2 months, 3 months and 4 months. The strategy was to collect at least one faeces of each fox and each exposure time at each sampling time. Then, the sampled scats were stored at −20 °C in the dark until analysis. Faeces collected the first day of fox exposure were not used because of their low number. Sixteen faeces were selected at day 0 and a total of 76 scats was used in the experiment. In order to determine if ARs were homogeneously distributed in faeces, 16 faeces from each fox and sampled at days 4, 5 and 6 of exposure were split into three parts as described above and then immediately stored at − 20 °C until analysis. Daily meteorological conditions including minimum and maximum temperatures, rainfall and sunshine duration measured at the weather station of Besançon were obtained from MeteoFrance.

2.3. Dosage of anticoagulants rodenticides in faeces

Rodenticide concentrations in fox faeces were measured as described by Sage et al. (2010). Briefly, scats were first crushed and then, dried in an oven for 24 h at 60 °C. An aliquot of 0.5 g