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Assessment of anticoagulant rodenticide exposure in six raptor species from the Canary Islands (Spain)

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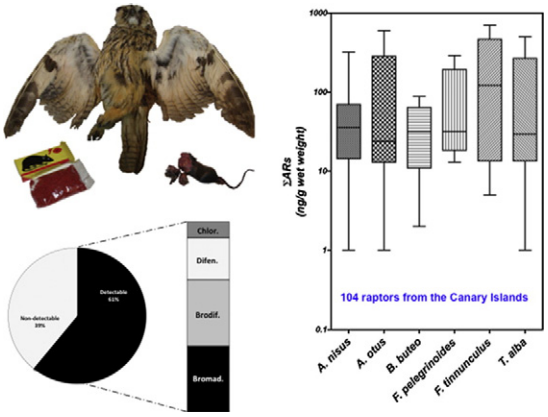
HIGHLIGHTS

- Monitoring of seven anticoagulant rodenticides in six species of birds of prey
- 35% of raptors exceeded the toxicity threshold.
- Higher levels in nocturnal and mammal-eater birds of prey
- High levels in birds of prey that feed on other birds

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides are highly toxic compounds that are widely used for pest control of rodents, but that also may threaten the wildlife's health. This work aimed to assess the exposure to first- and second-generation anticoagulant rodenticides (ARs) in six birds of prey species from the Canary Islands (Spain). The concentrations of seven widely used ARs were determined by LC–MS/MS in 104 liver samples of six species of birds of prey (*Buteo buteo*, *Accipiter nisus*, *Falco pelegrinoides*, *Falco tinnunculus*, *Asio otus*, and *Tyto alba*). We determined that 61% of the livers had detectable residues of at least one AR. The most frequently detected AR was bromadiolone, which was detected in 60.3% of the positive cases. The detection frequencies of these compounds varied widely, depending on the species. More than 75% of the *A. nisus*, *T. alba*, and *A. otus* individuals had detectable rodenticide residues in the liver. However, *F. tinnunculus* exhibited the highest concentrations of AR, with median values above 100 ng/g w.w. We did not detect first-generation ARs in any of the samples. When grouped, nocturnal species exhibited higher AR concentrations than diurnal species ($P < 0.001$). The residue levels were higher among small mammal-eaters than bird-eaters ($P < 0.01$). While most animals exhibited no macroscopic signs of coagulation disorders, approximately 35% exceeded the threshold levels of toxicity, which suggests that these compounds could weaken these animals in their natural environment. In conclusion, the control of rodent populations by ARs suggests that these compounds will enter the food chain and thus threaten the vulnerable

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populations of raptors on the Canary Islands. Our findings require authorities to ban or strictly control the use of these rodenticides in the natural environment for the conservation of raptors and other predatory species.

1. Introduction

In the agricultural sector, rodent populations remain one of the primary causes of economic losses in crops not only prior to harvesting but also during storage (Colazo, 1997). Public health authorities also target rodent populations because these animals can transmit zoonotic diseases, such as leptospirosis and plague (Bharti et al., 2003; Collins et al., 1996; Schelotto et al., 2012).

The most preferred and widely used method for rodent population control is the use of anticoagulant rodenticides (ARs), which are chemical products that interfere with normal blood clotting and cause death by inducing diffuse hemorrhages. The first rodenticide anticoagulants started being used in the 1940s, and these chemicals are currently referred to as the first-generation anticoagulants. Because of widespread use and the continuous exposure to these products, resistance to first-generation ARs developed in rodents. This motivated the development of new chemical formulas, and second generation rodenticides (SGARs) appeared in the market in the 1970s. These new chemicals include bromadiolone, brodifacoum, difenacoum, flocoumafen, chlorophacinone, and diphacinone (Murphy, 2007; Pelfrène, 2010). The SGARs are much more powerful and persistent than the first-generation ARs and are considered toxic after a single dose (Pelfrène, 2010). The primary mechanism of toxicity for these substances is the inhibition of vitamin K epoxide reductase. This enzymatic inhibition blocks vitamin K regeneration, and as a result, the vitamin K-dependent coagulation factors II, VII, IX and X are incorrectly synthesized and do not bear the post-translational carboxylation required for activation. This impairs normal blood coagulation and predisposes the animal to death due to bleeding (Murphy, 2007; Pelfrène, 2010).

In the European Union (EU), these products are freely sold and distributed. Even more, governmental organizations encourage their use and finance the purchase of these products to farmers and ranchers. This situation leads to an extensive use of these products by unqualified personnel that may apply the rodenticides directly to open spaces. This has been reported as a usual practice and facilitates free access to these chemicals for many animals (SEO/Birdlife, 2012). It should also be noted that after rodents have consumed a lethal dose of ARs, they do not get sick or die instantly but do so over the course of several days (generally 2 to 4 days), experiencing a gradual change in their habits that can include erratic behavior or spending more time in open spaces; thus, they become easy prey for raptors (Cox and Smith, 1992; Stansley et al., 2013). During the period when rodents feed on the baits, they can consume approximately 8–10 times the LD50 of the products most commonly used in rodent control campaigns (Stansley et al., 2013). All of these factors lead to AR exposure in many non-target species, and this has been documented for various raptor species worldwide (Albert et al., 2010; Dowding et al., 2010; Elmeros et al., 2011; Guitart et al., 2010; Hughes et al., 2013; Lambert et al., 2007; Stansley et al., 2013; Stone et al., 2003). In some cases, raptors feed on the rodents against which these substances are used, but some species also feed on granivorous birds that sometimes have accidentally ingested cereal baits (Sanchez-Barbudo et al., 2012). As a result, several studies confirm the presence of AR residues in the tissues of raptors (Albert et al., 2010; Hughes et al., 2013; Rattner et al., 2011, 2012; Sanchez-Barbudo et al., 2012; Thomas et al., 2011; Walker et al., 2008), and it appears that in many cases, this exposure leads the birds to a secondary poisoning that can cause them to weaken or die (Hughes et al., 2013; Sanchez-Barbudo et al., 2012; Stone et al., 1999; Thomas et al., 2011).

Due to the relative isolation and climate of the Canary Islands, the flora and fauna of the islands are completely unique from those of the European and African continents. On this archipelago, many endemic species and subspecies are found in areas of high ecological value. There are 7 species of diurnal birds of prey and 2 nocturnal nesting birds of prey on the Canary Islands. Four of these are subspecies that are endemic to the Canary Islands, and two other species are endemic to the Macaronesian region (which includes the Azores, Madeira, Canaries and Cape Verde regions) (Lorenzo et al., 2012). Several anthropogenic circumstances have provoked a population decline of some of these species in recent decades which, along with their characteristic slow reproductive rates, are threatening their survival: power lines, malicious or accidental poisonings, and high tourist pressure on the territory (the archipelago has four national parks that receive 5.5 million visitors a year (MAGRAMA, 2013)), as well as the extensive past and present uses of pesticides in agriculture, among others. In particular, the rodenticides have been widely used in these islands in recent years because the local public administration has provided these products to the farmers for free (BOP, 2011). Although it has been demonstrated that the exposure of raptors to these chemicals is related to potential risks to their health and that this exposure could be threatening the raptor populations of these islands, there are no data documenting the rodenticide exposure for the populations of birds of prey from this region. To address this gap, we have designed this study with the aim of assessing first- and second-generation AR exposures in six species of raptors on the Canary Islands to determine if raptor species of the archipelago are exposed to toxic quantities of these substances, which could potentially represent a threat to their conservation.

2. Material and methods

2.1. Sample collection and ethics statement

Liver samples were obtained from necropsies of 104 birds of prey from 6 species that were admitted to the Wildlife Recovery Centers (WRCs) of Tafira (Gran Canaria, Spain) and La Tahonilla (Tenerife, Spain) between October 2009 and December 2012. The series included 9 common buzzards (*Buteo buteo*), 14 European sparrowhawks (*Accipiter nisus*), 16 Barbary falcons (*Falco pelegrinoides*), 21 common kestrels (*Falco tinnunculus*), 23 long-eared owls (*Asio otus*), and 21 barn owls (*Tyto alba*). The birds died naturally or were euthanized within one week of admission. Dead animals were kept frozen until the moment of the necropsy. No animal was killed for the purposes of this study. The main cause of death was determined by examining the birds macroscopically at the WRCs, and, when necessary, radiological or toxicological analyses were performed. The causes of death for all of the animals that were included in this study consisted of different types of trauma. The whole livers, the primary organ for the accumulation of rodenticides (Dowding et al., 2010), were excised and stored at −20 °C until sample preparation. Part of the liver samples used in this study was retained from a previous study of anthropogenic persistent pollutant exposure in raptors (Luzardo et al., 2014a).

2.2. Chemicals and reagents

Dichloromethane, hexane, ethyl acetate and cyclohexane were of the highest purity available (>99.9%) and were purchased from Fisher Scientific (Leicestershire, United Kingdom). Ultrapure (UP) water was produced from a Milli-Q Gradient A10 (Millipore, Molsheim, France). Diatomaceous earth was purchased from Sigma-Aldrich (St. Louis, USA). Bio-Beads SX-3 was purchased