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# Risk assessment of bearded vulture (*Gypaetus barbatus*) exposure to topical antiparasitics used in livestock within an ecotoxicovigilance framework

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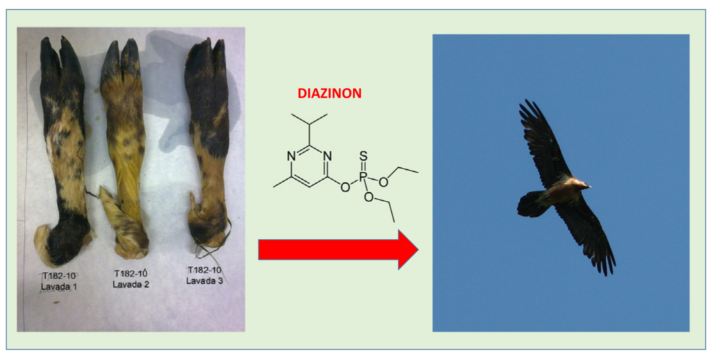
## HIGHLIGHTS

- Antiparasitics used in livestock were found in dead bearded vultures.
- Diazinon was the most prevalent external antiparasitic detected in lamb feet.
- Toxicity–exposure ratio reveals potential risk of acute poisoning in worst-case scenarios.
- Estimated diazinon exposure may impair thermoregulation in bearded vultures.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Between 2004 and 2013, 486 suspected scavenger poisoning cases, including 24 bearded vultures (*Gypaetus barbatus*), were investigated in the Pyrenees and surrounding areas in Spain as part of a monitoring programme regarding accidental and intentional poisoning of wildlife. Poisoning was confirmed in 36% of all analysed cases where scavenger species were found dead within the distribution range of bearded vultures. Organophosphates and carbamates were the most frequently detected poisons. Four of the bearded vulture cases were positive for the presence of topical antiparasitics (3 with diazinon and 1 with permethrin). These likely represented accidental exposure due to the legal use of these veterinary pharmaceuticals. In order to confirm the risk of exposure to topical antiparasitics in bearded vultures, pig feet ($n = 24$) and lamb feet ($n = 24$) were analysed as these are one of the main food resources provided to bearded vultures at supplementary feeding stations. Pig feet had no detectable residues of topical antiparasitics. In contrast, 71.4% of lamb feet showed residues of antiparasitics including diazinon (64.3%), pirimiphos-methyl (25.4%), chlorpyrifos (7.1%), fenthion (1.6%), permethrin (0.8%) and cypermethrin (27.8%). Washing the feet with water significantly reduced levels of these topical antiparasitics, as such, this should be a recommended practice for lamb feet supplied at feeding stations for bearded vultures. Although the detected levels of antiparasitics were relatively low (≤1 μg/g), a risk assessment suggests that observed diazinon levels may affect brain acetylcholinesterase and thermoregulation in bearded vultures subject to chronic exposure.



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## 1. Introduction

The use of chemicals for productive activities can be a risk for the environment in general, and in particular, for wildlife (Guitart et al., 2010). Studies regarding the bioaccumulation and biomagnification of persistent organic pollutants (POPs) have demonstrated the importance of monitoring programmes which utilise top predators such as birds of prey (Gómez-Ramírez et al., 2014). However, the risk of released chemicals to wild animals is not only limited to POPs. Recognised differences in sensitivity to toxicants among animal species highlights the need for greater toxicovigilance of all chemical substances used in the environment (Hutchinson et al. 2014), since these can often be causative agents of wildlife mortality (Pain et al., 2004; Berny, 2007; Martínez-Haro et al., 2008). Recent studies have shown that toxicovigilance should also consider veterinary drugs that can be present in carcasses that are eaten by scavengers and opportunistic predators (Oaks et al., 2004; Taggart et al., 2009). In addition to *accidental poisoning* of wildlife (due to the legal use of pesticides or veterinary drugs), predatory species are also especially at risk of *intentional poisoning* due to human–wildlife conflicts. This occurs especially when predators attack livestock or game species with a high economic value (Whitfield et al., 2003; Wobeser et al., 2004; Venkataramanan et al., 2008; Richards, 2012). Accidental and intentional sources of poisoning can also be present at the same time in a geographic area and together represent a risk for wildlife conservation that has to be unequivocally identified and quantified if necessary corrective measures are to be effectively implemented (Mineau et al., 1999; Elliott et al., 2011).

One conservation measure which can be adopted to protect certain avian scavengers regards the use of supplementary feeding stations or so-called *vulture restaurants*; wherein, food security and food safety can potentially be better assured (Margalida et al., 2014). Such feeding stations have also reduced the impact of (for example) EU regulations against the free disposal of carcasses of livestock in the field (Margalida et al., 2010) and have also reduced the risk of poisoning due to illegal baits used to kill predators (Oro et al., 2008), and, of lead poisoning caused by ammunition residues left within shot carcasses of wild game animals (Mateo et al., 1997; Garcia-Fernandez et al., 2005; Hernández and Margalida, 2009a). However, these supplementary feeding stations may also alter the natural foraging behaviour of vultures and may represent a risk due to chronic exposure to veterinary drugs used in livestock (Oro et al., 2008; Taggart et al., 2009). As a result, various scavengers, including vultures, may be exposed to a range of potent/highly bio-active compounds — the implications of which remain very poorly characterized (Shore et al., 2014; Cuthbert et al., 2014). For example, poisoning due to topical antiparasitics has been described in relation to the use of anticholinesterasic compounds (Henny et al., 1987; Mineau et al., 1999); and across Asia, exposure to diclofenac (a non-steroidal anti-inflammatory drug — NSAID) through this pathway has caused the near global extinction of at least three species of Old World *Gyps* vultures (Oaks et al., 2004; Taggart et al., 2009; Cuthbert et al., 2014). Further, Zorrilla et al. (2015) also identified (in southern Spain) a suspected case of a griffon vulture being poisoned by flunixin (another anti-inflammatory drug used in livestock). These cases have highlighted the urgent need for far better pharmacovigilance and the requirement for improved, more comprehensive Life-Cycle Assessments for the myriad of pharmaceutical products currently in use (Shore et al., 2014).

Despite the observed impact of NSAIDs in Asia on scavengers, surprisingly little monitoring data currently exists regarding the fate of pharmaceuticals in the environment and their potential effects on higher wildlife. European vultures provide an obvious starting point for further work in this arena. As such, here, we present data regarding the exposure of bearded vulture (*Gypaetus barbatus*) to topical antiparasitics in north-east Spain. Bearded vulture is a species listed by the IUCN as Near Threatened and the European population is considered Endangered. The European population comprises of only 170 breeding units, 117 of them located in Spain (Margalida et al., 2014); with the core stronghold area being in Aragón in north-eastern Spain (Gómez de Segura et al., 2012). Several studies have compiled details regarding poisoning cases recorded for bearded vultures from the Pyrenees (Margalida et al., 2008; Berny et al., 2015), but antiparasitics have not been thoroughly considered. Between 2004 and 2013, we investigated cases of suspected poisoning in the Pyrenees and surrounding mountains of bearded vultures and other scavenging species. We considered the risk of exposure in bearded vulture to topical antiparasitics used to treat ovine livestock specifically because lamb feet are one of the main food resources consumed by bearded vultures in supplementary feeding stations; in addition, sheep carcasses are also consumed in the wild, within the wider landscape. The antiparasitic compounds and concentrations found in/on lamb feet were then used to assess the risk that these residues posed to bearded vultures. The risk of adverse effects that could compromise adult or nestling survival was evaluated in light of current toxicological knowledge with regard to the detected topical antiparasitics.

## 2. Materials and methods

### 2.1. Species and study area

Bearded vulture is a large raptor (mean weight: 5.79 kg) of the Accipitridae family, which feeds mostly on carcasses of mammals (95%), followed by birds (4%) and reptiles (1%). Medium sized mammals (especially sheep and goat, followed by Southern chamois *Rupicapra pyrenaica*, wild boar *Sus scrofa* and deer species) form the core of their diet (74%) (Margalida et al., 2009). The study area was the Spanish Pyrenees and the surrounding mountains where the bearded vulture is distributed (Fig. 1). Poisonings involving other scavengers in provinces where bearded vulture are present (Alava, Navarra, Zaragoza, Huesca, Lleida, Barcelona and Girona) were also studied to consider the prevalence of poisoning involving various types of toxicant and to compare results obtained with those for bearded vultures. Some of these cases were located outside of the normal distribution range for bearded vultures, but, the data may give an indication of the risk of poisoning if breeding or foraging range expansion occurred. The studied scavengers (facultative and opportunistic) were Egyptian vulture (*Neophron percnopterus*), griffon vulture (*Gyps fulvus*), cinereous vulture (*Aegypius monachus*), black kite (*Milvus migrans*), red kite (*Milvus milvus*), golden eagle (*Aquila chrysaetos*), Eurasian buzzard (*Buteo buteo*), red fox (*Vulpes vulpes*) and domestic dog (*Canis familiaris*).

### 2.2. Poisoning monitoring of bearded vulture and other scavengers

Cases of suspected poisoning of bearded vulture (n = 24) and other facultative or opportunistic scavengers (n = 462; Fig. S1) were submitted to our laboratory by Wildlife Rehabilitation Centres within the study area between 2004 and 2013. Samples of liver, gastric content, brain, pellets or carcass remains were taken by the veterinary staff of these Centres and were submitted with a report containing the more relevant clinical and necropsy findings.

### 2.3. Sampling of food supplied at feeding stations

Feet of lamb (n = 126) were sampled from five slaughterhouses (n = 102) and five supplementary feeding stations (n = 24). In order to study contaminant removal efficiency from the lamb feet, a washing procedure (with water) was utilised. Washed (n = 55) and unwashed (n = 47) lamb feet from the slaughterhouses were analysed. Lamb feet from supplementary feeding stations (n = 24) were all washed. Moreover, pig feet (n = 24) from slaughterhouses were also analysed (Fig. S2).