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Review

The perfect threat: Pesticides and vultures

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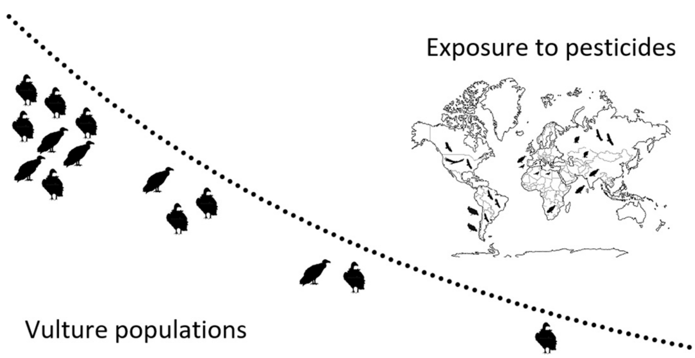
HIGHLIGHTS

- Exposure to pesticides is probably the main threat for vultures.
- Information about this threat is sparse and geographically biased.
- The most used pesticides affecting vultures are carbamates and organophosphorus.
- Massive poisoning events occur, in some cases, within protected areas.
- If this situation is not reversed, some vulture populations could disappear.

GRAPHICAL ABSTRACT



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ABSTRACT

Probably the most important threat currently affecting vultures worldwide is exposure to pesticides, both accidentally and through deliberate abuse. This is of special concern since around 70% of vulture species are threatened by human activities. However, information about this threat is sparse and geographically biased. We compiled existing knowledge about pesticide exposure in vulture species globally, providing unifying criteria to mitigate this problem with a joint global effort. Most information available about accidental exposure to pesticides in vultures is related to organochlorine pesticides. Non-lethal exposure to these compounds occurs on every continent that vultures inhabit. While concentrations of organochlorine pesticides reported in different samples appear to be too low to produce health impacts, some studies show vultures with levels compatible with health impacts. In addition, there are some reports of vultures contaminated accidentally by anticoagulant rodenticides and external antiparasitic drugs used in veterinary practices. Deliberate abuse of pesticides to poison wildlife also occurs on every continent where vultures live, affecting most (78%) vulture species. However, little information is available for some regions of America, Asia and Europe. The exact number of vultures killed due to deliberate poisoning with pesticides is not well known, but the available figures are alarming (e.g. up to 500 individuals in a single event). The most widely used pesticides affecting vulture populations, and associated with deliberate poisoning, are carbamates and organophosphorus compounds. Of particular concern is the fact that massive poisoning events with these compounds occur, in some cases, within protected areas. This suggests that if this situation is not reversed, some vulture populations could disappear. A combination of measures such as banning pesticides, controlling their distribution-acquisition and environmental education could produce better results that banning pesticides alone. If poisoning with pesticides is not stopped, this threatened avian group could inadvertently go extinct very soon.

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Contents

1. Introduction

Vultures and condors (hereafter, vultures) are the avian guild most affected by different human activities (Buechley and Şekercioğlu, 2016; Ogada et al., 2012a). This avian guild is suffering important declines in their populations that may lead to the extinction of several species (Buechley and Şekercioğlu, 2016). In fact, around 70% of vulture species are globally threatened (IUCN 2019). This is particularly concerning given the important ecosystem services they perform by removing organic material from the environment, which in turn likely diminishes the spread of disease (Markandya et al., 2008; Moleon et al., 2014; Ogada et al., 2012b). Different threats, such as lead contamination (Plaza and Lambertucci, 2019), veterinary drug intoxication (Green et al., 2004), human persecution (Ogada et al., 2012a), food shortages (Thiollay, 2006a) and the trade of parts for traditional medicine (Buij et al., 2016), are considered responsible for the decline of vultures around the world. However, exposure to pesticides is probably the main threat for these birds because it is increasing worldwide and producing high mortality rates (Alarcón and Lambertucci, 2018a; Margalida, 2012; Ogada et al., 2016a). Nonetheless, in some regions and for some species, the real magnitude of this problem is not well known, or available information is sparse.

Exposure of vultures to pesticides can be accidental when a compound that is used for approved targets and in correct doses accidentally harms non-target species (Martínez-Haro et al., 2008; Ogada, 2014). On the other hand, exposure is usually a result of deliberate abuse of pesticides in illegal attempts to poison predators or herbivores with toxic compounds. This impacts vultures either as unintentional casualties or in some cases when vultures are specifically targeted (Martínez-Haro et al., 2008; Ogada, 2014; Pauli et al., 2018). While accidental exposure to pesticides can produce different health impacts and even mortality, the deliberate abuse of pesticides to poison wildlife is producing alarming mortality rates in a range of vulture species (Margalida, 2012; Pauli et al., 2018), especially in Africa (Ogada et al., 2016a). Worryingly, the abuse of pesticides to kill animals is a widespread practice around the world. In fact, intentional poisoning is considered one of the major causes of death of different wildlife species in Europe (Berny, 2007; Guitart et al., 2010), with vulture mortality reaching huge numbers (Margalida, 2012). In addition, this practice has become an important conservation problem in South America where it impacts emblematic species such as the Near Threatened Andean condor (*Vultur gryphus*) (Alarcón and Lambertucci, 2018a).

Given the serious health impacts, large mortality rates and associated population declines of vultures due to pesticides, there is a need to evaluate the state of knowledge about this threat worldwide. Therefore, we compiled scientific and popular information about pesticide exposure (accidental and deliberate abuse) on vulture species to place this problem in a global context, and propose ways to mitigate this threat with a joint global effort. To do this, we performed a scientific bibliographic search using different search engines such as Google Scholar (www.scholar.google.com) and Scopus (www.scopus.com). Considering that many poisoning events are not published because they are chance or isolated encounters, we also performed a Google search (in English and Spanish) of newspaper reports mentioning events of vulture poisoning. Finally, given the different and extensive terminology used to study this topic, we examined the references of articles we reviewed for additional reports not found in our searches. We focused particularly on accidental and deliberate abuse of pesticides (Fig. 1) such as carbamates, organophosphorus, organochlorine, pyrethroids and anticoagulant rodenticides because these compounds are the most common pesticides implicated in wildlife poisoning (Hernández and Margalida, 2009, 2008; Richards, 2011). We excluded from this review the use of fungicides, molluscicides and herbicides because they affect vertebrates to a much less degree compared to the compounds mentioned above (Guitart et al., 2010; Martínez-Haro et al., 2008). We noted the location of existing studies, species affected, number of individuals affected, pesticide compounds used and the samples necessary to diagnose exposure. Finally, we analyzed the role of protected areas and different conservation actions needed to mitigate this global problem.

2. Accidental exposure

Most information available about accidental exposure to pesticides in vulture species is related to persistent organic pollutants (e.g.

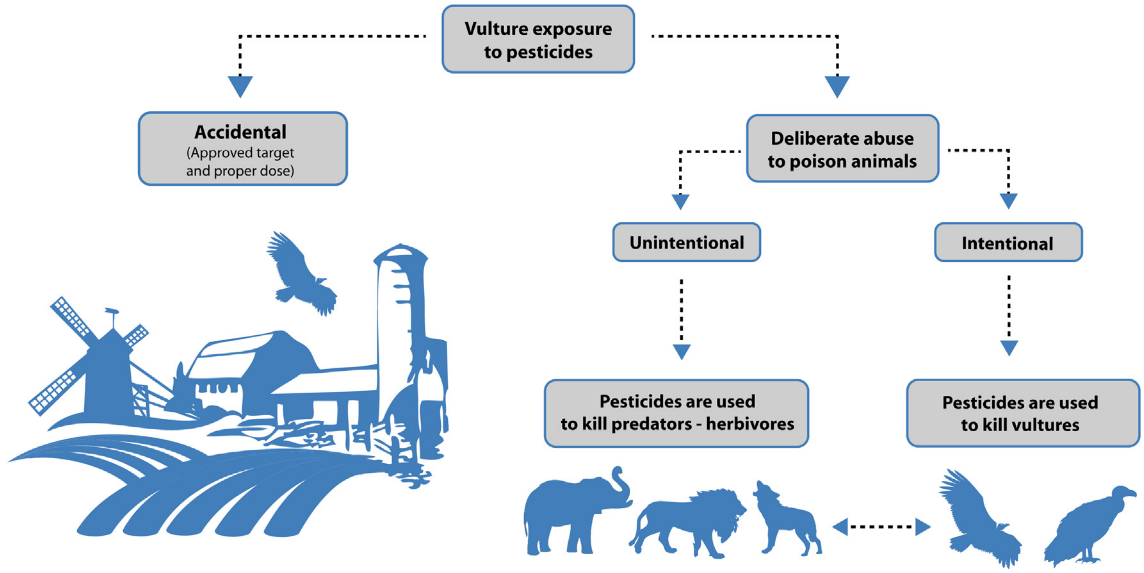

Fig. 1. Conceptual scheme showing the different types of vulture exposure to pesticides.

organochlorine pesticides such as lindane or dichlorodiphenyltrichloroethane). In addition, although there is little information available, some vultures can be affected accidentally by anticoagulant rodenticides (Hosea, 2000; Kelly et al., 2014; McMillin, 2012; Stone et al., 2003) and external antiparasitic drugs used in veterinary practices (Hernández and Margalida, 2008; Mateo et al., 2015). Persistent organic pollutants are used as pesticides-insecticides (e.g. DDT or lindane), but are also generated for industrial uses (e.g. PCBs) (Jones and De Voogt, 1999). In this section, we only focus on accidental vulture exposure to different organochlorine pesticides, anticoagulant rodenticides and external antiparasitic drugs used in veterinary practices. We did not include articles showing vulture exposure to other persistent organic pollutants generated by industrial processes such as polychlorinated biphenyls, which are not used as pesticides.

2.1. Organochlorine pesticides

The following compounds were reported in scientific literature to affect vulture species around the world: α-benzene hexachloride, β-hexachlorocyclohexane (β-BHC), lindane (γ-BHC), α-(cis)-chlordane, β-(trans)-chlordane, dieldrin, endosulfan sulfate (endosulfan S), α-endosulfan, β-endosulfan, endrin, heptachlor epoxide, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE). These lipophilic compounds are considered some of the most important environmental pollutants around the world because they are persistent (lasting in the environment for years or decades) and bio-magnify through food webs (Jones and De Voogt, 1999). Vultures can be exposed to these compounds by direct contact, but also due to the ingestion of contaminated food items such as carcasses of marine mammals or livestock (Kurle et al., 2016; Sallam and Morshedy, 2008; Wiemeyer et al., 1986). These compounds produce harmful impacts such as reproductive alterations (e.g. structure egg alteration; Ratcliffe, 1970), but they are also hormonal disrupters and produce alterations in immune system function (Fry, 1995; Jones and De Voogt, 1999).

Vulture exposure to these types of compounds has been reported in South America, North America, Asia, Africa and Europe, almost all of the regions were vultures live with the exception of Central America (Fig. 2). In South America, feathers sampled from turkey vultures (*Cathartes aura*) and black vultures (*Coragyps atratus*) from Patagonia showed exposure to different organochlorine pesticides in two locations 400 km from each other (Martínez-López et al., 2015). Moreover, while these types of compounds are currently banned in this geographical area, some birds showed ΣDDT at sub-lethal levels, and levels of different organochlorine compounds higher than the levels reported for birds of prey in Asia and Europe (Martínez-López et al., 2015). However, this is the only article evaluating this type of contamination in vultures in Latin America.

In North America, most studies were performed on the Critically Endangered California condor (*Gymnogyps californianus*), and sometimes evaluated turkey and black vultures as they can be potential surrogates for condors. Four decades ago, a strong relationship ($r = -0.93$) between eggshell thickness and DDE levels was reported for California condors (Kiff et al., 1979). Contaminated eggs were thinner than uncontaminated eggs, and this structural alteration was associated with breeding failure due to eggs breaking under the weight of brooding parents (Kiff, 1989). The negative relationship between organochlorine pesticides and eggshell thickness was also studied in turkey and black vultures in Florida and Texas (USA), where eggshell thickness was comparable to levels attributed to reproductive failure (Kiff et al., 1983). In the eighties, DDE levels compatible with reproductive alterations were reported in different tissues of two dead California condors (Wiemeyer et al., 1983), and in their food sources (Wiemeyer et al., 1986). Most of these studies suggest that exposure to these compounds could be an important factor affecting population declines in California condors. Nevertheless, Snyder and Meretsky (2003) re-evaluated this threat and suggested that DDE was an unlikely cause of condor decline. More recently, a study performed between 2006 and 2010 showed egg shell alterations that produced egg failures in California condors, which were probably associated with DDT residues discarded by a factory in California (Burnett et al., 2013). Moreover, California condors foraging on marine mammal carcasses were exposed to DDE, and had higher levels than condors that scavenged on fewer marine carcasses, highlighting the potentially harmful levels of marine contaminants transferred to terrestrial scavengers (Kurle et al., 2016). This fact is relevant not only to California condors, but also potentially to other scavengers and regions (Blázquez et al., 2016; Lambertucci et al., 2018). Nonetheless, there is no consensus as to whether the DDE levels reported can produce reproductive anomalies such as egg failure in California condors (Meretsky and Snyder, 2017). Thus, further research is needed on this topic.

In Asia, Indian vultures were reported to be exposed to organochlorine pesticides such as DDT and lindane (Kaphalia et al., 1981; Malik et al., 2018). Different studies demonstrated exposure in white-

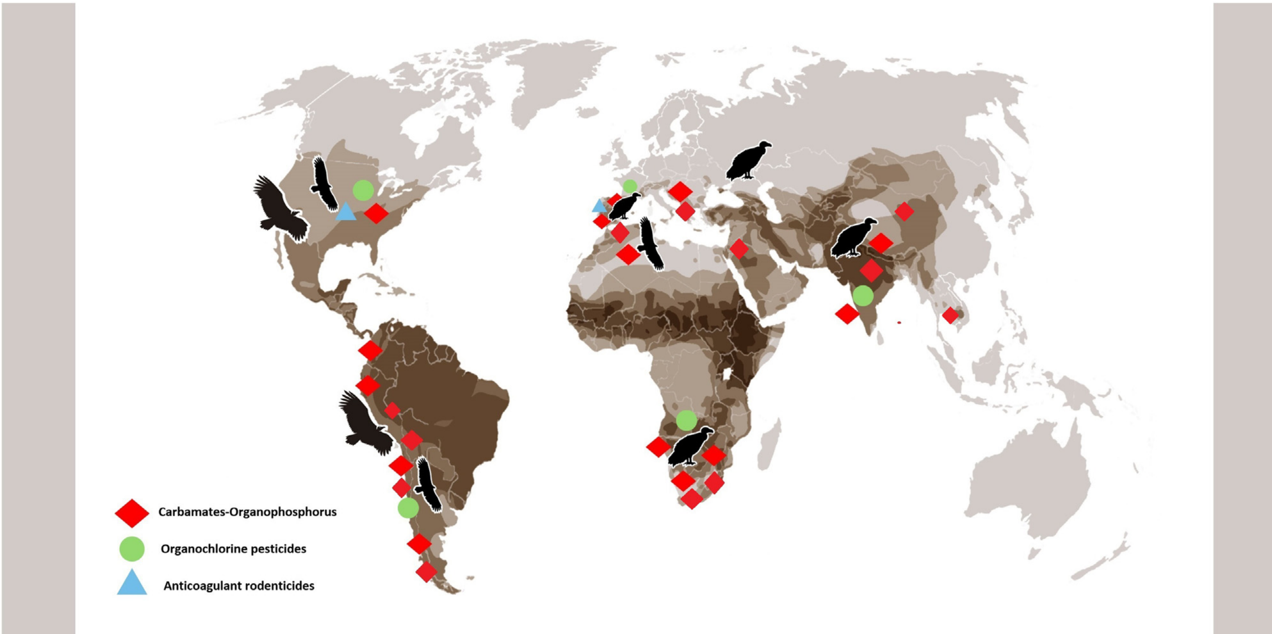

Fig. 2. World map showing the locations of exposure to pesticides in diverse vulture species. The world distribution of vultures is shown in brown (the darker the color the higher species richness). The map with the world distribution of vultures was obtained from www.nationalgeographic.com.

rumped vultures (*Gyps bengalensis*) to these toxins, especially DDE, from different tissues such as blood, brain, liver, muscle and even eggs (Dhananjayan et al., 2011; Dhananjayan and Muralidharan, 2013; Kaphalia et al., 1981; Muralidharan et al., 2008). Similarly, residues of these compounds were present in blood samples of white-rumped vultures and in Egyptian (*Neophron percnopterus*) and griffon vultures (*Gyps fulvus*) (Dhananjayan et al., 2011). However, all of these studies concluded that the levels found in the different species and sample matrixes are below the thresholds that can produce adverse effects.

A similar situation has been reported in Africa. In South Africa and Kenya, species such as white-backed vulture (*Gyps africanus*), lappet-faced vulture (*Torgos tracheliotos*) and Cape vulture (*Gyps coprotheres*) were reported to be affected by organochlorine pesticides through analysis of these toxins in blood, muscle, brain, liver, kidney and eggs (Frank et al., 1977; Mundy et al., 1982; Van Wyk et al., 2001; Van Wyk et al., 1993). While the concentrations found were generally low in the different studies, some individuals showed exposure levels that can produce negative impacts. Thus, exposure to these compounds should not be underestimated (Van Wyk et al., 1993).

In Europe, low values of organochlorine pesticides were found in various samples from Egyptian, griffon, bearded (*Gypaetus barbatus*) and cinereous vultures (*Aegypius monachus*), in Spain and France (Berny et al., 2015; García-Fernández et al., 2008; Gómara et al., 2004; Monclús et al., 2019). In addition, a study on bearded vultures from the Pyrenean region showed that these compounds are present in the eggs of this species, but there was no relationship between contaminant levels and egg shell thickness (Hernández et al., 2018). Finally, organochlorine pesticides have been detected in the blood, liver and fat of griffon vultures, and in the blood of cinereous vultures from Greece with levels compatible with sub-lethal effects (Goutner et al., 2011; Hela et al., 2006).

In summary, while almost all studies about organochlorine pesticides in vultures concluded that the levels found are too low to produce important health impacts, some studies showed individuals with concentrations compatible with potential negative impacts, especially on reproductive fitness (Malik et al., 2018; Martínez-López et al., 2015; Van Wyk et al., 1993; Wiemeyer et al., 1983). Therefore, these compounds continue impacting vultures around the world due to their persistence in the environment from current and historical use (Kurle et al., 2016; Malik et al., 2018; Martínez-López et al., 2015; Monclús et al., 2019). Moreover, they may be producing sub-lethal effects that are not being considered by scientists, authorities and wildlife managers compared to other threats to which vultures are exposed. This lack of consideration may produce important biases in conservation policies that could result in deceptive protection and recovery of vulture populations.

2.2. Anticoagulant rodenticides

Anticoagulant rodenticides are also implicated in accidental exposure in vulture species. These compounds are used worldwide to control rodents by inhibiting vitamin K recycling, which interferes in the synthesis of some clotting factors (II, VII, IX, X) (Rattner et al., 2014; Thijssen, 1995). This produces hemostatic disorders that result in the death of both target and non-target animals. There are two generations of anticoagulant rodenticides-first generation (e.g. warfarin) and second generation (e.g. brodifacoum, bromadiolone, diphacinone), which differ in their persistence and toxicity (Garcıa-Fernández, 2014). Therefore, accidental exposure to these compounds is often related to exposure to second-generation compounds due to their longer half-life and accumulation in tissues such as the liver (Garcıa-Fernández, 2014).

Vultures are exposed to anticoagulant rodenticides when they ingest carcasses with residues of these compounds, but there is very little information about vulture exposure to these types of pesticides. Some cases are from the USA, were anticoagulant rodenticides such as diphacinone and brodifacoum were found in different tissues of turkey vultures (Hosea, 2000; Kelly et al., 2014; McMillin, 2012; Stone et al., 2003). Additionally, in Europe, residues of anticoagulant rodenticides were reported in griffon vultures from Spain (Sánchez-Barbudo et al., 2012). Future research should seek to determinate if vultures are being killed by rodenticides but not reported, are not overlapping with areas where rodenticides are being used, are less sensitive to these compounds because they are not prone to ingesting contaminated rodents, or have an inherent resistance to these compounds.

2.3. External antiparasitic drugs used in veterinary practices

Vultures may accidentally be exposed to multiple veterinary drugs such as antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), and antiparasitics (Blanco et al., 2017; Mateo et al., 2015; Oaks et al., 2004). These compounds can produce important health alterations leading in some cases to severe decline in populations as was reported with NSAIDs in Asia (Oaks et al., 2004). Moreover, some veterinary drugs used as external antiparasitics contain pesticides as an active ingredient, which may produce health alterations on vultures if they ingest carcasses contaminated with these drugs (Mateo et al., 2015). There is not much information about this potential threat for vultures in many regions of the world. However, at least four bearded vultures were reported dead in Spain as a consequence of external antiparasitics associated probably with the consumption of livestock carcasses (Mateo et al., 2015). In fact, residues of different external antiparasitics such as diazinon, pirimiphos-methyl, chlorpyrifos, fenthion, permethrin and cypermethrin were found in lamb feet (Mateo et al., 2015), showing a potential threat for vultures. Similarly, in the same country, two cinereous vultures were reported dead as a consequence of an authorized veterinary topical insecticide (Hernández and Margalida, 2008). Therefore, given that these drugs are commonly used in veterinary practices globally, they should be considered potential threats and included in future research to put this problem in context for different vulture species around the world.

3. Deliberate abuse of pesticides

Vultures can be unintentional victims of deliberate abuse of pesticides when they ingest a poisoned carcass that was targeted for predators such as feral dogs (*Canis lupus familiaris*), wolves (*Canis lupus*), hyenas (*Crocuta crocuta*), or pumas (*Puma concolor*) (Ogada, 2014; Ogada et al., 2016a; Pauli et al., 2018; Richards, 2011). Moreover, vultures can be intentionally poisoned because some people consider them nuisance species (Ogada et al., 2016a) (Fig. 1). Currently, the deliberate abuse of pesticides is producing massive mortalities of vulture species globally, which result in population declines (Alarcón and Lambertucci, 2018a; Margalida, 2012; Ogada, 2014; Table 1). However, the actual magnitude of this threat for vultures remains unknown for several geographic regions and species.

3.1. Geographic patterns

3.1.1. Africa

Africa has the highest number of mortalities of vultures associated with deliberate abuse of pesticides known, although this may be influenced by the greater amount of research on this topic for this region compared to others (e.g., Asia or Latin America). Common reasons for poisoning wildlife on this continent include the control of damage-causing animals, harvesting animals for traditional medicine, poaching for wildlife products, and killing wildlife sentinels (Botha et al., 2015; Ogada, 2014). In fact, the practice to deliberately kill wildlife in Africa has a long history, beginning in South Africa at the end of the 17th century as a form of mitigating human-animal conflicts with species such as lions (*Panthera leo*), hyenas and antelopes (Ogada, 2014). In fact, until the last century, governments from this region encouraged and implemented poisoning programs, enhancing hostile attitudes especially toward predator species (Ogada, 2014). The form of poisoning most frequently reported is the baiting carcasses; others include soaking grains in pesticide solutions, mixing pesticides to form salt licks, and even tainting waterholes (Craig et al., 2018; Odino and Ogada, 2008; Ogada, 2014; Santangeli et al., 2016). This continent is inhabited by almost half of the extant vulture species (11) including the Critically Endangered white-backed vulture, Ruppell's griffon (*Gyps rueppellii*), hooded vulture (*Necrosyrtes monachus*) and white-headed vulture (*Trigonoceps occipitalis*), the Endangered Egyptian vulture, Cape vulture and lappet-faced vulture, the Near Threatened bearded vulture and cinereous vulture and the griffon and the palm-nut vulture (*Gypohierax angolensis*) that are considered as of Least Concern (IUCN 2019). Most of these species are subject to important conservation problems across the entire continent leading to declines in their populations (Ogada et al., 2016b) (Table 1). In this sense, the deliberate abuse of pesticides is one of the most important threats impacting these populations (Di Vittorio et al., 2018; Ogada et al., 2016b).

Several articles reported large numbers of unintentional and intentional vulture deaths due to the deliberate abuse of pesticides. For instance, Basson (1987) reported that at least 593 vultures died as a consequence of poisoning with organophosphorus compounds, carbamates and strychnine in South Africa between the years of 1985–1987. Similarly, 166 poisoning events caused by pesticides such as monocrotophos, diazinon, parathion, carbofuran and strychnine involving different animal species, with vultures among the most affected, were reported in Africa over a period of 7 years (1988–1995) (Fourie, 1996). Diverse poisoning events involving white-backed vultures, white-headed vultures and lappet-faced vultures killed at least 175 individuals in Namibia between 1995 and 2000 (Bridgeford, 2001). Dozens of individuals of the white-backed vulture and the white-headed vulture were reported dead in Zambia and Malawi due to pesticides such as aldicarb (Roxburgh and McDougall, 2012). Accordingly, several studies on different vulture species including the hooded vulture (Mullié et al., 2017; Odino et al., 2014; Ogada and Buij, 2011) and the Egyptian vulture (Hille and Collar, 2011), suggest that deliberate poisoning is the main threat producing declines in these populations (Di Vittorio et al., 2018; Garbett et al., 2018; Ogada et al., 2016b; Ogada and Keesing, 2010; Thiollay, 2006a; Thiollay, 2006b; Virani et al., 2011). This decline averages 70–90% (Mullié et al., 2017; Ogada et al., 2016b; Ogada and Keesing, 2010).

Worryingly, since 2012 the practice of deliberate poisoning of vultures showed an important increment associated with ivory poachers (Ogada et al., 2016a). Previously, vulture mortality had been associated mainly with unintentional poisoning targeted at carnivores (Ogada et al., 2016a). However, since 2012 ivory poachers have used pesticides to kill elephants (*Loxodonta africana*) and even to kill vultures because of their habit of circling above carcasses acting as wildlife sentinels and calling attention to poaching activity (Ogada et al., 2016a). For instance, between 2012 and 2015 at least 165 elephants and 2044 vultures were killed by poison (Ogada et al., 2016a). This was registered in Zimbabwe (Groom et al., 2013), Botswana (Bradley, 2014), Mozambique, South Africa and Zambia (Ogada et al., 2016a). Therefore, the deliberate abuse of pesticides is currently the main threat in Africa, which could lead some vultures to the brink of extinction if this activity is not reverted (Murn and Botha, 2018).

3.1.2. Europe

In Europe, the deliberate use of pesticides to kill wildlife appears to be a common practice in Spain and France, and is mostly associated to human-wildlife conflicts with predators (Berny, 2007; Guitart et al., 2010; Margalida, 2012; Margalida et al., 2008; Mateo-Tomás et al., 2012; Soler Rodríguez et al., 2006). For instance, in France, the abuse of carbamates and organophosphorus compounds impact species such as bearded vultures, Egyptian vultures, and griffon vultures (Berny et al., 2015). In Spain, several mortality events due to illegal poisoning with organophosphorus and carbamate pesticides were reported in cinereous vultures (Hernández and Margalida, 2008), bearded vultures, griffon vultures and Egyptian vultures (Cortés-Avizanda et al., 2009; Hernández and Margalida, 2009; Margalida et al., 2014; Mateo et al., 2015; Mateo-Tomás et al., 2012). The practice of poisoning wildlife with organophosphorus compounds, carbamates and, in some cases, with strychnine, is also common in Greece, Croatia, Bulgaria and Macedonia (Antoniou et al., 1996; Grubač et al., 2014; Parvanov et al., 2018; Pavoković and Sušić, 2005; Xirouchakis et al., 2000). Consequently, this practice has led to declines in populations of vultures

Table 1
List of vulture species impacted by deliberate abuse of pesticides and their conservation status, global population, populations trend, maximum of individuals killed in a single poisoning event and % of population killed in a single event.

Scientific name	Common name	IUCN Red list status	Global population[a] (mature population)	Trend	Poisoning reported in scientific articles	Poisoning reported in newspaper	Poisoning reported as a threat in the Red List	Maximum individuals killed in a single event (reference)[b]	% of total population killed in a single event (mature population)
Gyps bengalensis	White-rumped vulture	Critically endangered	3500–15,000 (2500–9999)	Decreasing	Yes	No	Yes	23 (Clements et al., 2013)	0.15–0.65 (0.23–0.92)
Gyps africanus	White-backed vulture	Critically endangered	270,000 (NA)	Decreasing	Yes	Yes	Yes	324 (Bradley, 2014)	0.12
Gyps tenuirostris	Slender-billed vulture	Critically endangered	1500–3750 (1000–2499)	Decreasing	Yes	Yes	Yes	18 (Newspaper report 6, Table S1)	0.48–1.2 (0.72–1.8)
Gyps indicus	Indian vulture	Critically endangered	45,000 (30,000)	Decreasing	No	No	Yes	NA	NA
Gyps rueppellii	Ruppell's vulture	Critically endangered	30,000 (22,000)	Decreasing	Yes	No	Yes	NA	NA
Gyps coprotheres	Cape vulture	Endangered	8000–10,000 (9400)	Decreasing	Yes	Yes	Yes	49 (Botha et al., 2015)	0.49–0.61 (0.52)
Gyps fulvus	Griffon vulture	Least concern	500,000–999,999 (648,000–688,000)	Increasing	Yes	Yes	Yes	30 (Newspaper report 1, Table S1)	0.003–0.006 (0.0043–0.0046)
Gyps himalayensis	Himalayan griffon	Near threatened	100,000–499,999 (66,000–334,000)	Stable	No	Yes	Yes	36 (Newspaper report 54, Table S1)	0.007–0.036 (0.01–0.05)
Neophron percnopterus	Egyptian vulture	Endangered	18,000–57,000 (12,000–38,000)	Decreasing	Yes	Yes	Yes	70 (Grubač et al., 2014)	0.12–0.38 (0.18–0.58)
Necrosyrtes monachus	Hooded vulture	Critically endangered	197,000 (NA)	Decreasing	Yes	Yes	Yes	14 (Newspaper report 26, Table S1)	0.007 (NA)
Trigonoceps occipitalis	White-headed vulture	Critically endangered	5500 (3685)	Decreasing	Yes	Yes	Yes	6 (Bridgeford, 2001)	0.11 (0.16)
Sarcogyps calvus	Red-headed vulture	Critically endangered	3500–15,000 (2500–9999)	Decreasing	Yes	No	Yes	7 (Clements et al., 2013)	0.04–0.2 (0.07–0.28)
Torgos tracheliotos	Lappet-faced vulture	Endangered	8500 (5700)	Decreasing	Yes	No	Yes	30 (Groom et al., 2013)	0.35 (0.52)
Gypaetus barbatus	Bearded vulture	Near threatened	2000–10,000 (1300–6700)	Decreasing	Yes	Yes	Yes	3 (Newspaper report 34, Table S1)	0.03–0.15 (0.04–0.23)
Aegypius monachus	Cinereous vulture	Near threatened	23,400–31,500 (15,600–21,000)	Decreasing	Yes	Yes	Yes	10 (Ntemiri et al., 2018)	0.03–0.04 (0.04–0.06)
Coragyps atratus	Black vulture	Least concern	NA	Increasing	Yes	Yes	No	61 (Newspaper report, 20, Table S1)	NA
Cathartes aura	Turkey vulture	Least concern	NA	Stable	Yes	No	No	NA	NA
Vultur gryphus	Andean condor	Near threatened	10,000 (6700)	Decreasing	Yes	Yes	Yes	34 (Alarcón and Lambertucci, 2018a)	0.34 (0.50)
Gymnogyps californianus	California condor	Critically endangered	231 (150)	Increasing	No	No	No	NA	NA
Gypohierax angolensis	Palm-nut vulture	Least concern	NA	Stable	No	No	No	NA	NA
Cathartes burrovianus	Yellow-headed vulture	Least concern	NA	Stable	No	No	No	NA	NA
Cathartes melambrotus	Greater yellow-headed vulture	Least concern	NA	Decreasing	No	No	No	NA	NA
Sarcoramphus papa	King vulture	Least concern	1000–10,000 (670–6700)	Decreasing	No	No	No	NA	NA

NA = not available.
[a] Total population estimates (mature and immature) obtained from the IUCN Red List.
[b] This may be a conservative estimate since higher numbers have been reported for some areas but there is no a detailed description of numbers for species.

(Demerdzhiev et al., 2014; Grubač et al., 2014; Xirouchakis et al., 2001). For instance, between 2000 and 2016, Ntemiri et al. (2018) described 1015 poisoning incidents in Greece associated with carbamates such as methomyl and carbofuran, but also with organophosphorus compounds. The authors concluded that vultures were the most impacted species. However, the lack of information available about this problem in other countries from this continent is concerning.

3.1.3. Other regions

In Asia, there is less information regarding deliberate poisoning with pesticides compared to Africa and Europe. However, MaMing and Xu (2015) called the attention to the decline of different vulture species in China and suggested that poisoning could be the main cause, but more research is needed in this case. In Cambodia, the decline of species such as the Critically Endangered white-rumped vulture, slender-billed vulture (*Gyps tenuirostris*), and the red-headed vulture (*Sarcogyps calvus*), has also been attributed to poison with organophosphorus and carbamate pesticides (Clements et al., 2013; Loveridge et al., 2019). In Saudi Arabia, one lappet-faced vulture was poisoned probably with carbamates or organophosphorus compounds, as it was found with low cholinesterase activity in the blood (Ostrowski and Shobrak, 2001). In India, newspapers reported different poisoning incidents killing species such as slender-billed vultures and Himalayan griffon vultures (*Gyps himalayensis*), mostly related to poisoning aimed at killing feral dogs (Table S1, reports 23, 54 and 55). In Korea, at least five cinereous vultures were reported dead by different pesticides from 2010 to 2013 (Kim et al., 2016).

The Americas have the least information available about this threat. However, some articles reported its occurrence in both North America (Fleischli et al., 2004; Mineau et al., 1999) and South America (Pavez and Estades, 2016). In fact, the increase in poisoning events with pesticides such as carbofuran affecting large numbers of the Near Threatened Andean condor are currently of high concern (Alarcón and Lambertucci, 2018a). Throughout the Andes range, new world vultures many times die in remote areas, so to find a poisoned carcass is a fortuitous event, making this a hidden threat.

In brief, deliberate poisoning of vulture species with different pesticides occurs around the world (Fig. 2), especially in Africa and Europe where the figures are alarming. However, there is a lack of information for some vast regions such as Eastern Europe, Latin America and Asia. The little information available for some geographical areas is concerning since this problem may be larger than previously thought. Future research and efforts are needed in the areas where little information is available to evaluate the existence and intensity of this threat for each vulture species. For this purpose, economic resources for research intending to study this topic in those regions are needed, as well as collaborations with research groups and labs working on poisoning in other regions. Nonetheless, it will be necessary to develop specialized laboratories for toxicology diagnosis in each region, which will help with the rapid determination of the problem, and efficient responses.

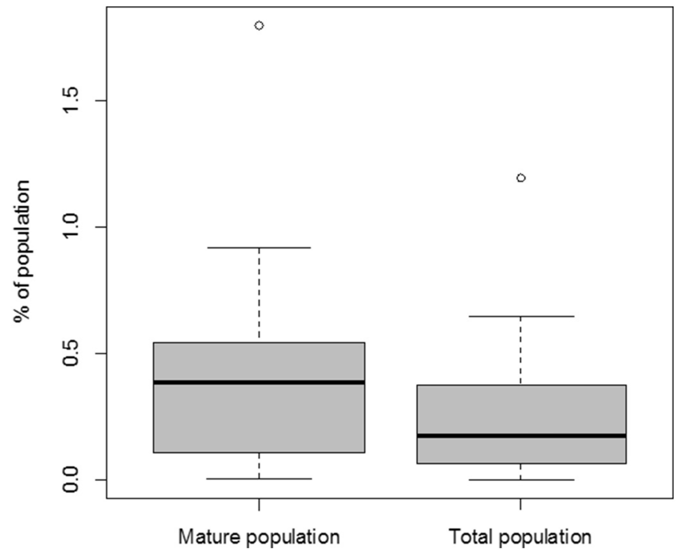

Fig. 3. Percentage of the total and mature population for different vulture species killed by single poisoning events (Boxplots show minimun, first quartile, median, third quartile, maximum and outliers).

3.2. Species affected

The scientific literature, newspaper reports and the IUCN Red List show that the deliberate abuse of pesticides affects at least 18 of the 23 extant vulture species (Table 1). Eight of these species are cataloged by the IUCN as Critically Endangered, three are cataloged as Endangered, four as Near Threatened and three as Least Concern (IUCN, 2019) (Table 1). Therefore, this problem affects most vulture species of the world (78%, 18/23), and particularly species of important conservation concern (83%, 15/18, of the species affected are Critically Endangered, Endangered or Near Threatened). It is difficult to determine those species most affected by this problem because there is a lack of information from many regions, and thus the figures could be biased toward the most studied species or areas. Moreover, it is often difficult to determine the species involved in massive poisoning events because their carcasses are found long after death (Bradley, 2014), and some parts such as the beak may be missing (Groom et al., 2013). However, based on the available information, it seems that African vultures are the most affected by this threat (Botha et al., 2015; Ogada et al., 2016a, 2016b), especially the Critically Endangered white-backed vulture given the high mortality rates associated with poisoning by ivory poachers (Ogada et al., 2016a, 2016b). It should be noted that this threat may be important to all vulture species because a single poisoning event could kill an important percentage of their populations. Indeed, a sole poisoning event could cause mortality values of 1.2% of the total population and 1.8% of the mature population (Table 1, Fig. 3).

There are no reports of the Critically Endangered California condors dying as a consequence of deliberate abuse of pesticides (Rideout et al., 2012) (Table 1). This is probably associated with the strict vigilance established by the reintroduction program developed for this species. In addition, there are few reports of poisoning associated with the deliberate abuse of pesticides for turkey vultures and there are no reports for yellow-headed vultures (*Cathartes burrovianus*), greater yellow-headed vultures (*Cathartes melambrotus*) or king vultures (*Sarcoramphus papa*) (Table 1). Some of the New World vultures (*Cathartes* spp.) possess a well-developed sense of smell (Ferguson-Lees and Christie, 2001), and thus it would be interesting to evaluate if this characteristic allows them to detect poisoned carcasses. Finally, as expected, there is no information on poisoning for the palm-nut vulture. This species has a particular diet, composed mostly of fruits and grains (Ferguson-Lees and Christie, 2001), which probably reduces the risk of direct poisoning.

Future research is needed to improve the detection of poisoning events discriminating the vulture species and numbers affected, particularly at sites where there is no available information. Regional registers of mortality due to deliberate poisoning could help in our understanding of the true demographic magnitude of this threat, not only for the scientific community but also for wildlife managers and policy makers.

3.3. The tip of the iceberg

Considering the lack of information available regarding this concerning threat, the number of vultures killed reported here is an underestimation and represents only a tiny fraction of reality, and thus should be considered as 'the tip of the iceberg' (Ogada, 2014). Even so, the number of individuals killed due to deliberate poisoning with pesticides is impactful. For instance, in Africa between 1970 and 2012 at least 3967 vultures were killed in association with poisoning events with different pesticides and 2044 vultures were killed between 2012 and 2014, reaching a total of 6011 (Ogada et al., 2016a). In Spain, between 1990 and 2010 at least 4000 vultures were killed by pesticides (Margalida, 2012). In fact, in this country during this time period 53 bearded vultures, 366 Egyptian vultures, 759 cinereous vultures and 2877 griffon vultures died as a consequence of illegal poisoning (Margalida, 2012). Similarly, 224 vultures died between 1987 and 2017 in Greece, Bulgaria and Macedonia (Parvanov et al., 2018), and in Croatia, a single poisoning event with carbofuran killed 17 griffon vultures (Muzinic, 2007). Finally, in South America at least 66 Andean condors died due to poisoning with pesticides such as carbofuran in a period of 13 months between 2017 and 2018 (Alarcón and Lambertucci, 2018a). All of these figures demonstrate that the tip of the iceberg is sufficiently large to put several vulture species at risk of extinction.

3.4. Pesticides used

Different pesticides can be used to deliberately kill wildlife, mostly correspond to carbamates and organophosphorus compounds such as aldicarb, carbofuran, methomyl, monocrotophos, diazinon, parathion and fenthion-ethyl (Basson, 1987; Fourie, 1996; Hernández and Margalida, 2009; Parvanov et al., 2018). However, strychnine, although forbidden for many years throughout the world, was still used until a decade ago in some regions of Europe (Hernández and Margalida, 2009, 2008; Parvanov et al., 2018). In recent years, the most common pesticide used to deliberately kill wildlife has been carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl-Nmethylcarbamate) (Alarcón and Lambertucci, 2018a; Ogada, 2014; Richards, 2011). Moreover, carbofuran has been the most widely used pesticide to kill vulture species around the world. For instance, it is the pesticide responsible for the death of many vultures in South Africa (Botha et al., 2015), Namibia (Bridgeford, 2001), Kenya (Odino and Ogada, 2008), and other areas of the African continent (Ogada et al., 2016a; Ogada, 2014). In addition, carbofuran was implicated in vulture mortalities in Europe (Hernández and Margalida, 2009, 2008; Muzinic, 2007), but was also was responsible for the death of numerous Andean condors in South America (Alarcón and Lambertucci, 2018a). Therefore, this section focuses on this compound, although it should be taken into account that several other pesticides can be used to kill wildlife as well (Botha et al., 2015; Giorgi and Mengozzi, 2011).

Carbofuran is a pesticide (insecticide, nematicide and acaricide) with a broad spectrum of activity (Gupta, 1994). It has been used around the world to control different crop pests, and is especially effective in to controlling pests resistant to organophosphorus (Richards, 2011). The mode of action of carbofuran is the same as other carbamates and organophosphorus pesticides, producing the reversible inhibition of the enzyme acetylcholinesterase (AChE) (Gupta, 1994; Richards, 2011). This results in the accumulation of the neurotransmitter acetylcholine at the junction of the nerve cell and the receptor sites, producing alterations such as salivation, tremors, convulsions and ultimately death (Gupta, 1994). Carbofuran formulations are typically sold in granular form in different concentrations, but also in liquid form in some regions of the world (Richards, 2011).

Several studies have addressed the use patterns of pesticides, especially carbofuran (Craig et al., 2018; Odino et al., 2014; Sakellari et al., 2016; Santangeli et al., 2016). In these studies, a common finding is that farmers admit using this poison to kill wildlife because it is easy to acquire and it is especially used in dealing with conflicts with predators. Moreover, the available alternatives are less widely known by farmers and more expensive, making carbofuran the preferred and more accessible pesticide (Odino and Ogada, 2008). For instance, in a study in Kenya carbofuran was available at 100% of agro-vet stores and was sold daily (Odino and Ogada, 2008). In some countries, such as Argentina until 2018, it was possible to buy this pesticide online. Given that carbofuran has been continuously unregulated in some regions of the world (Richards, 2011), it will be key to consider regulations and control on its use in these areas.

3.5. Samples useful to diagnose poisoning in vultures

One of the main obstacles to addressing pesticide poisoning events in vulture species is the correct diagnosis and identification of the compounds involved. Unfortunately, it is very difficult to establish a poisoning diagnosis because samples are generally not extracted adequately for analysis (Berny, 2007; Vyas, 1999). Large numbers of vultures in otherwise good body condition found dead lying face-down, with the wings spread near a suspected carcass, can be considered clinical evidence of poisoning (Groom et al., 2013; Richards, 2011). However, this is not pathognomonic of poisoning because infectious diseases in some species can also produce large numbers of dead individuals at a single site (Kock et al., 2018). Traditional samples to identify pesticides such as carbamates and organophosphorus compounds include the gastrointestinal track (e.g. crop and stomach) to evaluate the presence of the compound implicated, and the brain to evaluate cholinesterase inhibition (Richards, 2011). In addition, samples of muscle, liver and brain, where traces of pesticides can be found, can be useful to evaluate the compound involved (Berny, 2007; Espín et al., 2016). To avoid the deterioration of samples, they should be put in plastics bags (Espín et al., 2016), and the samples can be frozen (-20ºC) if needed (Richards, 2011). Regrettably, it is often not possible to obtain these types of samples, especially when a poisoning event occurs in remote areas or the carcasses of vultures are degraded by the time they are found well after an event occurs.

Recent studies suggest that other types of samples could help in assessing if a mortality event was produced by a pesticide, even identifying which toxin is implicated (e.g., talon and beak samples; Richards, 2011). For instance, aldicarb was detected in talons from different poisoning events (Otieno et al., 2011, 2010; Richards et al., 2017). Moreover, the palate and tongue of poisoned vultures, but also of other animals that died from poisoning events, can be useful samples (Richards et al., 2015). Interestingly, the analysis of soil samples associated with mortality events when there is a suspected poisoning can show poison residues and provide forensic evidence (Otieno et al., 2010). Finally, when some vultures are found alive at the site of the poisoning event, cholinesterase levels can be measured in plasma as a non-destructive biomarker to diagnose the exposure of birds to carbamates and organophosphorus compounds (Naidoo and Wolter, 2016; Oropesa et al., 2017; Roy et al., 2005). In conclusion, when traditional samples are not available, other sample types may be useful as alternatives to evaluate exposure to poison and should be considered.

3.6. The role of protected areas

There is little information on the role of protected areas in prevent the deliberate abuse of pesticides to kill vultures. In fact, the available information shows contradictory results. On the one hand, some studies show that protected areas are not sufficient to avoid the impacts associated with this threat. For instance, monitoring efforts to measure abundance of birds of prey in a protected area in Kenya concluded that vultures are the avian guild most impacted probably by poisoning (Ogada and Keesing, 2010). Similar conclusions were obtained in Botswana, where there were important declines of birds of prey in protected areas, with poisoning being a potential threat (Garbett et al., 2018). However, these studies assumed limitations because the decline in abundances can also be subject to changes in space use associated with different variables such as weather condition or food shortages (Garbett et al., 2018; Ogada and Keesing, 2010). Moreover, several vulture species exhibit large home ranges and travel long daily distances, and thus they may be impacted by pesticides outside protected areas because these areas are not sufficiently large (Alarcón and Lambertucci, 2018b; Lambertucci et al., 2014). In fact, the most high priority areas for vulture conservation in southern and eastern Africa, South Asia and the Iberian Peninsula remain largely unprotected (Santangeli et al., 2019).

Some studies show that protected areas help to diminish the impacts of deliberate abuse of pesticides. For instance, a study in South Africa showed that the survival rates of the white-backed vulture are higher in a protected area compared to non-protected areas, perhaps because of different poisoning rates between sites (Monadjem et al., 2018). Similarly, a study in Sudan described important declines in vulture species that were more pronounced outside than inside protected areas, and concluded that these areas are very important to maintaining vulture populations (Thiollay, 2006b).

While the information about the role of protected areas to mitigate deliberate abuse of poisoning is contradictory, it suggests that in some cases, poisoning events could occur within these areas. Diverse massive poisoning events that killed several individuals of different vulture species have been reported inside protected areas in Zimbabwe, Mozambique, Namibia, Zambia, South Africa, Malawi, Botswana and Democratic Republic of Congo (Bradley, 2014; Groom et al., 2013; Ogada et al., 2016a; Roxburgh and McDougall, 2012). Similarly, in Spain, protected areas were identified as an important factor that can enhance the likelihood of illegal use of poison to kill wildlife, possibly as a consequence of large numbers of wolves that predate on livestock (Mateo-Tomás et al., 2012). This has also been reported in some protected areas in Asia (Gupta, 2018). Therefore, it is clear that vultures are impacted by the deliberate abuse of poisoning with pesticides, even inside protected areas, which is of great concern. Authorities and policy makers of these areas must improve the control of this threat, and these areas should play an important role in educational programs.

3.7. Mitigation actions

Different actions have been proposed to mitigate the deliberate abuse of pesticides to kill wildlife. However, there is no clear information on the effectiveness of many of these actions. In this section, we analyze the most common actions proposed and implemented.

3.7.1. Banning some pesticides

Currently, there are a number of regulations banning pesticides like carbofuran, aldicarb or endrin in many parts of the world including the USA, Canada and the Europe Union, due to their negative impacts in non-target species (Martínez-Haro et al., 2008; Richards, 2011). Worryingly, these compounds remain legal in some regions of the word including Africa (Ogada, 2014), India and some parts of South America (Richards, 2011), where they are widely used. While it is a priority to ban some of the pesticides used to deliberately kill wildlife in these regions, banning pesticides does not completely guarantee that poisoning will stop because people illegally obtain these compounds or continue with this practice using alternatives (Chiari et al., 2017; Martínez-Haro et al., 2008; Ruiz-Suárez et al., 2015). For instance, although endrin and strychnine have been banned in Spain since 1991 and 1994, respectively, they were still used to kill wildlife many years after the ban (Martínez-Haro et al., 2008; Martínez-López et al., 2006). Therefore, bans should be accompanied by other actions, such as control of manufacturing, distribution and acquisition of the compounds most used to intentionally kill wildlife (Martínez-Haro et al., 2008).

3.7.2. Control of distribution

The control of the acquisition and distribution of pesticides is a key measure to mitigate the deliberate abuse of pesticides. Regulations to ensure the monitoring of a pesticide's manufacturing process and its use by consumers are needed. Interestingly, the use of pesticides to kill animals seems to be strongly related to the lethality and availability of the formulations suggesting that the most toxic and available compounds are the most used (Craig et al., 2018; Martínez-Haro et al., 2008; Odino et al., 2014; Sakellari et al., 2016; Santangeli et al., 2016). Therefore, actions should focus on these highly toxic and available compounds and that there be a move toward regulations that prevent their use. Finally, as mentioned above, it is important to highlight that the ban of pesticides most widely used for poisoning along with the control of the distribution of alternatives produces better results that banning pesticides alone (Martínez-Haro et al., 2008).

3.7.3. Changes in formulations

An alternative to the ban of some pesticides may be the addition of repellent substances. Some manufacturers add to the pesticide formulation compounds that deter ingestion of granular pesticides, causing animals to avoid ingestion of the active component (Martínez-Haro et al., 2008; Mastrota and Mench, 1995). The most effective repellents have been *d* pulegone and quinine hydrochloride methyl anthranilate, 2-heptanone, and lithium chloride (Mastrota and Mench, 1995). However, research is needed to evaluate if vultures are sensitive to these types of repellents or if these compounds can produce adverse effects on them.

3.7.4. Educational programs

Influence human behavior though education is a fundamental strategy to mitigate this problem. However, an understanding of the complexity of human-wildlife conflicts and the reasons for poisoning is needed to implement adequate educational initiatives (Santangeli et al., 2016). The applications of 'canned' educational programs that do not take into account regional and social characteristics are not necessarily effective. Therefore, given the complexity of this environmental problem, it is important to plan educational programs at a regional scale taking into account the particularities of this problem in each geographical area.

3.7.5. Economic resources

It is important to identify funding to mitigate this threat. Resources should be used to develop regional pesticide centers in different geographic areas where they are most needed. The centers would provide chemical testing, public education, training, monitoring and diagnosis (Ogada, 2014). Funds are also needed to increase research, control and mitigation of human-carnivore-scavenger conflicts (Van Eeden et al., 2018). In addition, the use of electronic devices (e.g., GPS) on many individuals can be effective to detect poisoning events (O'Donoghue and Rutz, 2016), and would contribute to educational efforts and poisoning prevention.

3.7.6. Use of animals to detect poison

Another interesting alternative that may be implemented in different geographical areas is the use of dogs to detect poisons. For instance, in Spain there are specialized dogs trained to detect poisons in the field. These dogs detect poisons at different hot spot areas, where there were poisoning events in the past or at sites inhabited by species of conservation concern (Richards, 2011). However, this strategy should only be one aspect of mitigating and controlling the deliberate abuse of poisons.

3.7.7. Laws to penalize deliberate poisoning activity

Laws that penalize those responsible for deliberate poisoning can be controversial and are not always effective or easy to implement. For instance, in Africa 38 countries have laws regarding wildlife poisoning, but there are very few offender prosecutions (Ogada, 2014). In Spain and other European countries, there are strong economic penalties for people that poison wildlife and they could spend time in prison (Richards, 2011). However, the practice of wildlife poisoning persists. Although there is sometimes no other way than judicially prosecuting people who commit these types of offenses, this should be the last resort in a more comprehensive conservation approach. Rather, the priority should be to strengthen research, environmental education, and collaboration among researchers, managers, and farmers to reduce human-wildlife conflicts and promote the coexistence of productive human activities with wildlife conservation. It is relevant to highlight that in some areas, many people have agreed to implementing anti-poisoning actions (Sakellari et al., 2016), but there also needs to be a solution to the conflicts they face with wildlife. If the conflicts between humans and carnivores and scavengers are not mitigated, poisoning will most likely continue.

4. Conclusions

Vultures are suffering important impacts on their populations due to deliberate abuse of pesticides, especially in Africa (Ogada, 2014), but also in Europe and South America (Alarcón and Lambertucci, 2018a; Margalida, 2012). Most impacted species are of conservation concern, and thus if this situation continues, some species could be extinct in a short time (Murn and Botha, 2018). In fact, a single poisoning event can impact important proportions of an entire population. In addition, although at lower levels, some vulture species are contaminated with organochlorine pesticides, which may affect their reproductive performance. They may also be contaminated with anticoagulant rodenticides and external antiparasitic drugs used in veterinary practices. In the case of deliberate poisoning, future efforts should be directed to generate effective measures to mitigate the impact that pesticides have on different vulture species. It is important to develop multiple actions such as banning pesticides, and controlling distribution and acquisition, alongside educational programs, to achieve better results compared to isolated specific actions (Ogada, 2014). In addition, it is necessary to invest enforcement and economic resources in strategies that permit better diagnosis of poisoning events. The establishment of anti-poisoning laboratories that could provide earlier diagnoses and prevention measures is needed, especially in the geographic regions most impacted by this threat. However, seeking solutions for human-carnivore-scavenger conflicts is key to addressing this problem. Collaborative projects between researchers, managers, conservation biologists, governments, stakeholders, sociologists and environmental educators are necessary to end the practice to killing wildlife, and save vultures from extinction.

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