

# **Direct evidence of poison-driven widespread population decline in a wild vertebrate**

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**Toxicants like organochlorine insecticides, lead ammunition or veterinary drugs have caused severe wildlife poisoning, pushing the populations of several apex species to the edge of extinction. Yet, poison-driven declines in wild populations remain poorly supported by direct evidence. Using field data at fine spatiotemporal scale, we show a straightforward relationship between poison-induced individual mortality and population changes in the threatened red kite (*Milvus milvus*). Our findings provide compelling evidence of poisoning causing countrywide population declines, including local extinctions, of a once-abundant vertebrate. Because poisoning threatens the global populations of at least 2,475 animal species, a greater understanding of its population-level effects will help to improve regulatory processes of chemical substances contributing to biodiversity conservation.**

Despite populations being a major target for both ecological risk assessments of toxicants (1, 2) and conservation management actions (3), evidence showing straightforward relationships between the effects of toxic compounds at individual and population levels remains scarce, especially in vertebrates (4). The paramount cases of DDT and other organochlorine pesticides (1, 5), lead ammunition (6) and diclofenac (7, 8) exemplify how most evidence of poisoning impacts at population level is retrieved (see details in Appendix 1). Briefly, scattered data of toxic effects from molecular to individual levels are gathered on the one hand, and then their relationships with the observed population declines are directly suggested or inferred through, for example, computational methods (4). For instance, the strong evidence that identified diclofenac as the major cause of massive *Gyps* vultures mortalities across the Indian subcontinent (7, 8), was linked with the observed

widespread population declines directly by deductive reasoning (7, 8) or using demographic simulations (9).

Inferring the effects of toxic compounds at population level from individual responses is not straightforward as individual parameters known to be affected by toxicants (e.g. survival, fecundity) do not always correlate with population changes (2). Indeed, certain number of individuals can be removed from a population (e.g. poisoned) without necessarily leading to its decline due to processes such as density-dependent productivity or immigration that may compensate for toxic effects at individual level (1). As a result, forecasting the fate of populations is a challenging task (10). Alternatively, the use of real-world population changes (e.g. through observed population growth rates), which already incorporate the complexity of population dynamics, emerges as an ecologically sound option to assess how toxic effects at individual-level upgrade into populations (2, 11). Nonetheless, simultaneously collecting field data at individual and population levels is extremely costly and time-consuming especially at large spatiotemporal scales (10, 11). As a result, the available evidence linking individual responses to toxics and observed population changes is limited to a few studies at local scales (frequently just one concrete location) and/or over short-term periods (e.g. 12, 13). These spatiotemporal limitations, which restrict in turn sampling replication, weaken the strength of the evidence available, precluding the consecution of sound conclusions on the impact of toxics on species populations (11, 14). Counteracting these limitations, we link here field data of toxic-induced individual mortality and population changes in the threatened red kite across hundreds of localities in a 20-year timeframe. To do so, we take advantage of two long-term and large-scale monitoring schemes: i) Spanish national surveys of the presence and

abundance of the species (~3,500 10x10-km squares) in 1994 (15) and 2014 (16), and ii) data on wildlife poisoning events across Spain compiled by the “ANTÍDOTO” program (17) over the same period ( $n = 18,500$  dead animals and 4,175 baits).

A total of 1,075 red kites were registered as poisoned in mainland Spain, i.e. 657 confirmed through toxicological analyses and 418 suspected (but without toxicological confirmation), in the 1995-2013 between-census period (see Methods and Appendix 2). Aldicarb and carbofuran were detected in >82% of the poisoned red kites analyzed (i.e. 316 and 228 individuals respectively). Despite being banned in Europe since 2003 and 2007, respectively, these insecticides are still used illegally to kill wildlife (17). To assess the role of these and other poisons on the observed changes in the distribution and abundance of breeding red kites in 274 10x10-km squares between 1994 and 2014 (Fig. 1), we included the observed data on individual poisoning of red kites and other animal species (e.g. dogs, raptors; see Methods and Appendix 3) in each square as explanatory variables in generalized linear models (GLMs). We accounted also for land use changes known to determine the species' habitat suitability (18).

Poison-driven red kite mortality (both suspected and confirmed) showed a negative impact on red kite abundance (Fig. 2a,b). The decrease in the number of breeding pairs as poisoned red kites increased in a 10x10-km square highlights a pernicious effect of poisoning on the species population. This result was further supported by the negative relationship between the number of poisoned dogs (considered as a good indicator of poison use) (19) (see below and Methods), and changes in the breeding population of red kite (see Appendix 3).

Contrastingly, while the presence of poisoned dogs negatively influenced also red kite

occupancy (Fig. 2c), the opposite effect was observed for the poisoned red kites, that is, higher probability of occupancy in sites with higher number of poisoned kites (Fig. 2d). This apparently counterintuitive result might be explained because dead red kites, and thus the probability of locating them, is higher at locations with larger breeding populations. Also, as shown by the variable “breeding red kite abundance in 1994” included in the model explaining changes in occupancy (Fig. 2e), locations with larger breeding populations seem to be more resistant to local extinction despite suffering high kite mortality, including poisoning, perhaps due to density-dependent or immigration processes. Indeed, red kite populations can expand to some extent even when exposed to toxic substances as observed for some reintroduced or isolated populations (20, 21).

Our results are in accordance with existing demographic models on the impact of poison on the species (13, 22) and support previous works suggesting a marked impact of poisoning on small and isolated populations (21, 23), while agreeing with poisoning being considered as a major global threat for the species (24). Furthermore, we detect local extinctions at 107 10x10-km squares, underlining the important impact of poisoning on the occurrence of red kite. It is therefore expected that, in the absence of effective measures to eradicate or minimize poisoning, further local extinctions may occur. Besides poison, our models showed a positive association of irrigated crops on red kite presence and abundance (Appendix 3) that could be due to irrigated farmlands harboring a more heterogeneous landscape with trees for breeding and open areas for foraging. This is particularly so in the main species strongholds in northwestern Spain (25).

To the best of our knowledge, our results provide the first straightforward evidence linking the effects of poisoning on individuals (i.e. mortality) with the population trends of a wild species using field data at a fine spatiotemporal scale across hundreds of locations. This response of red kite population to poison suggests a strong potential of this species as a sentinel of toxic environmental risk in natural ecosystems (26). Interestingly, the density of dogs confirmed as poisoned per km<sup>2</sup> and year best predicted the observed changes in occupancy of the red kite breeding population (Fig. 2c; Appendix 3). Dog owners are expected to actively look for their missing pets, thereby increasing the detectability of the poisoning cases affecting them. Considering the low detectability of wildlife poisoning events (17, 27), poisoned domestic animals emerge thus as a reliable index of the real incidence of poison on wildlife (19).

Overall, even considering the low detectability of wildlife poisoning (17, 27) and the difficulty of gathering population data over large areas (10), our results highlight poisoning as a major driver of countrywide population decline in a threatened vertebrate. Changes in distribution and abundance as those we assess here are major criteria used to evaluate the conservation status of a species (28). Thus, our findings further support the listing of the species as “Endangered” in Spain, where the number of red kite breeding pairs has decreased at least 31% in 20 years (16) and poisoning seems to be a major and persistent cause of such reduction (16, 28). Poisoning in all its different forms (i.e. poaching, pest and predator control, anthropogenic pollutants) threatens the global populations of at least 2,475 animal species (i.e. ~3.4% of the 70,999 animal species included in the IUCN Red List; Appendix 4) (29), including ~7.7% of the vertebrates listed under threatened categories. From them, the 28.5% of the species of raptors and carnivores (N = 245) are

threatened by poisoning, including the 50.0% of the species listed as “Critically Endangered”, “Endangered” and “Vulnerable”. Surprisingly, although ample evidence highlight the pernicious effects of poisoning on individuals (1, 4-8), to upgrade the impact of toxics at high levels of biological organization (e.g. populations) remains a challenging task mostly resting on the use of inferential methods (4). As a result, straightforward evidence is lacking that links field data on the impact of toxic compounds at individual and population levels at detailed spatiotemporal extents large enough to further support a widespread (rather than local) consistent effect (11). Direct evidence such as the one we show here (i.e. on the effect of poisoning on the population of a wild species at fine spatiotemporal scales across hundreds of locations) strengthens the still weak bridge between population dynamics and conservation biology (10), e.g. providing further support to take effective actions against the use of toxic compounds pernicious for wildlife (e.g. lead ammunition, rodenticides) (4, 11). Such evidence will help also to improve the regulatory processes of chemical substances, including post-approval adaptation (11, 30). In this context, we illustrate here the strong potential of existing monitoring schemes such as the “ANTÍDOTO” program in Spain (17) or the SAGIR monitoring in France (27) to provide straightforward evidence of the impact of toxic substances across species over large spatiotemporal scales (11).

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## **List of Supplementary materials:**

### **Materials and Methods**

**Supplementary text.** Accumulated evidence on paramount toxicants causing wildlife population declines.

**Table S1.** Red kite mortality by toxic compound in Spain in 1995-2013.

**Tables S2 and S3.** Main results of the models of changes in occupancy and abundance of breeding red kites in 1994-2014.

**Table S4.** IUCN data on poisoning as a major threat for species populations.

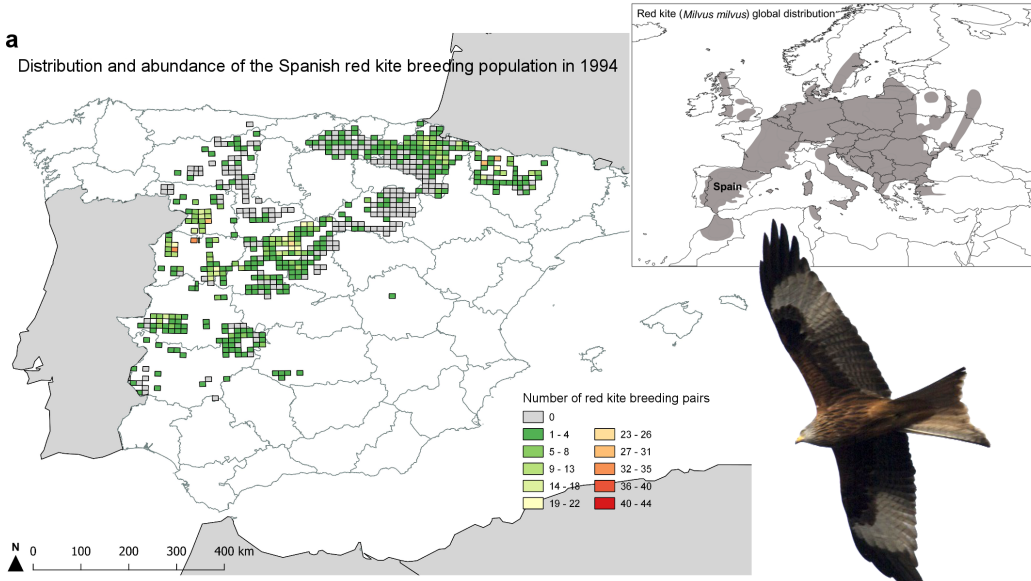
**Author contributions**

J.V. designed and coordinated first national red kite census and conceived the study. RM's lab produced most toxicological analyses in the data base of poisoned animals that was managed by E.M.; J.V., P.M.-T. and P.P.O. designed the study; J.V. and P.M.-T. coordinated the work. P.M.-T. and P.P.O. analyzed the dataset; P. M.-T. and P.P.O. led the writing. All authors contributed to revisions of the manuscript.

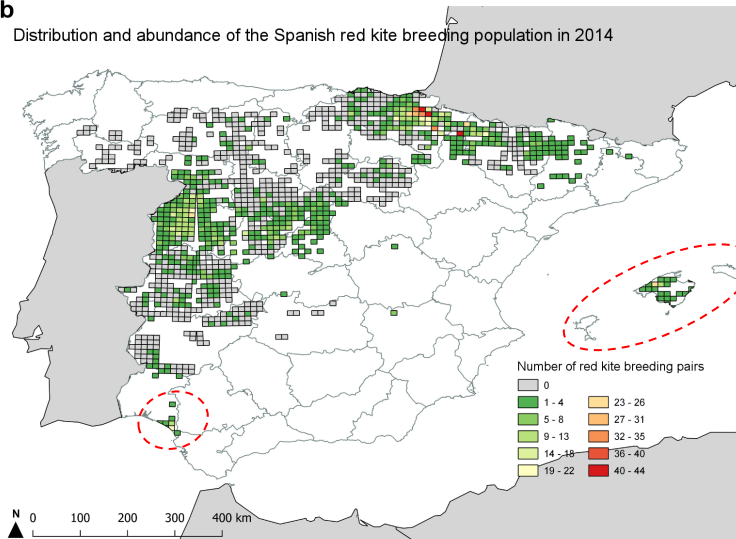
**Competing interests** The authors declare no competing interests.

**Figure 1.** Distribution and abundance of the red kite breeding population in 10x10-km squares in Spain according to the censuses performed in **a)** 1994 (15) and **b)** 2014 (16). From both censuses we obtained data on changes in occupancy and abundance per square **(c)**. Dashed circles indicate red kite populations in the Balearic Islands and Doñana excluded from the analyses (see text). The global distribution of the species according to IUCN (24) is shown in the top-right map. Red kite image from [www.publicdomainpictures.net](http://www.publicdomainpictures.net) under CC0 Creative Commons license.

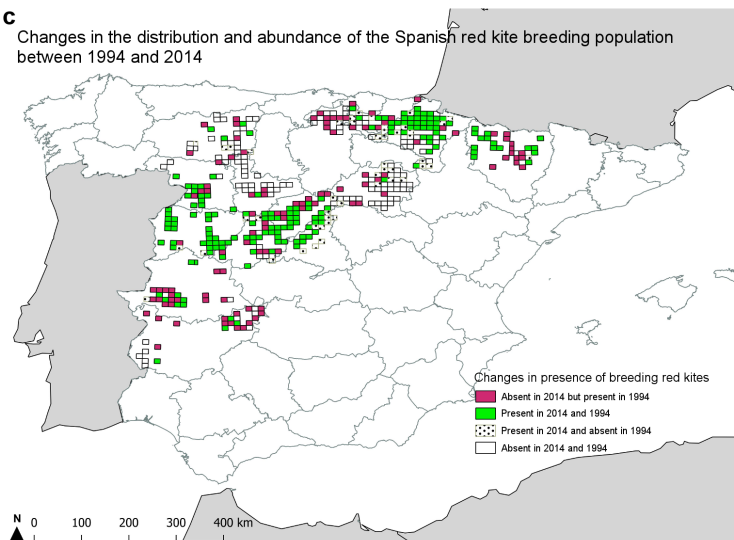
**a** Distribution and abundance of the Spanish red kite breeding population in 1994



**b** Distribution and abundance of the Spanish red kite breeding population in 2014

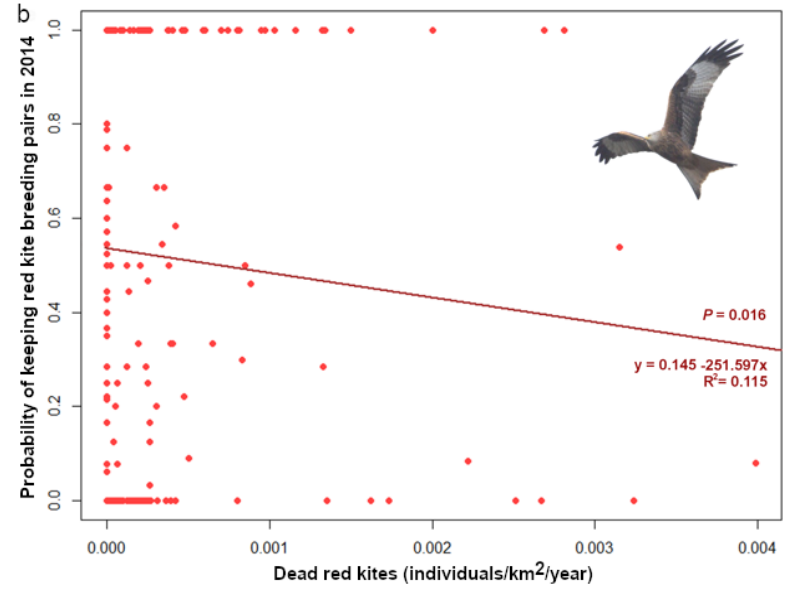
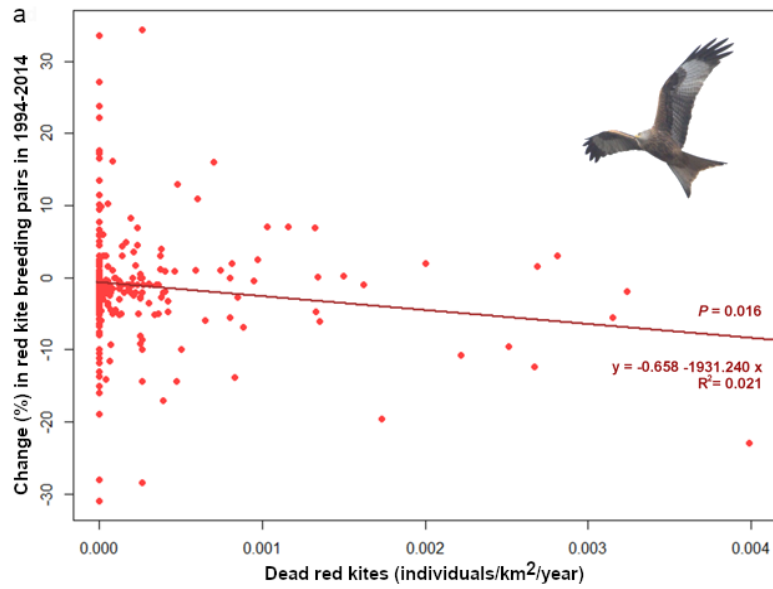


**c** Changes in the distribution and abundance of the Spanish red kite breeding population between 1994 and 2014

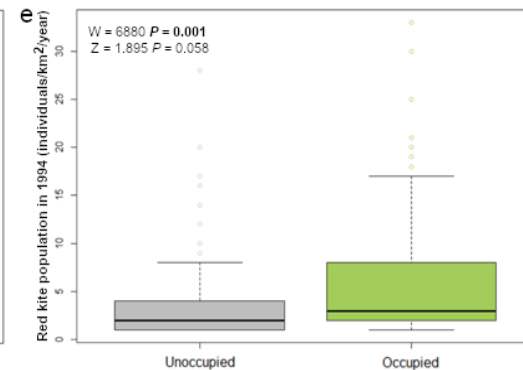
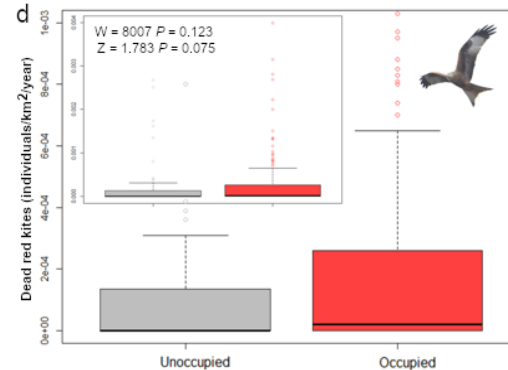
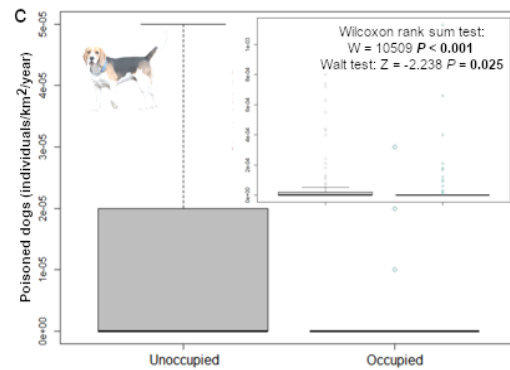


**Figure 2.** Density of dead red kites (both confirmed and suspected as poisoned) explained the observed changes in the abundance of breeding pairs both **(a)** when raw data were considered, and **(b)** when included in the best model, while keeping the remaining variables constant at their mean values. Agreeing with the occupancy models for breeding kites, raw data showed that locations where red kite breeding pairs disappeared between 1994 and 2014 had **(c)** more dogs confirmed as poisoned, **(d)** less dead red kites (both confirmed and suspected as poisoned), and **(e)** less breeding population in 1994. Wilcoxon tests show differences for the raw data. Wald tests for each variable once included in the best model is provided for comparisons. Significant *P* values (<0.05) in bold. See additional model details in Methods and Appendix 3. Red kite image from [www.publicdomainpictures.net](http://www.publicdomainpictures.net) and dog image from pixabay.com, under CC0 Creative Commons license.

Changes in abundance



Changes in distribution





## **MATERIALS AND METHODS**

### **Study species and study area**

The red kite (*Milvus milvus*) is an endemic raptor of the western Palearctic (Fig. 1), globally listed as “Near Threatened” (24). The European population, estimated in 25,200-33,400 breeding pairs, that currently account for >95 % of the global species population, has decreased ~30 % in the last three generations (34.5 years), mainly due to declines within its core breeding areas, e.g. Germany, France and Spain (26). Spain (our study area) holds ~2,000 breeding pairs and ~50,000 wintering individuals (16), becoming one of the main breeding strongholds and wintering areas for red kites in the world (31, 32). Nonetheless, both the Spanish red kite wintering and breeding populations have experienced sharp decreases of 7-19 and 31-43 %, respectively, in the last two decades (15, 16, 33). Although the species trend in the last decade could be considered globally stable (16, 33), in Spain the red kite is legally considered as “Endangered”, meaning that the species probability of survival is low if the main threats are still in place (34).

Red kites are opportunistic raptors that feed on a wide range of food resources, from small prey such as rodents, passerines or young rabbits to all type of meat remains, including organic waste at dumps and carcasses of any size (e.g. from large ungulates to reptiles, birds or rodents) (24). This makes the species highly susceptible to different toxic compounds, from those legally administered for pest control to deliberately poisoned baits for illegal killing of predators (26, 35-37). Illegal poison use is currently considered among the main threats for the species (24) and it has been suggested to be a major factor behind red kite population declines in SW Europe (35). Mortality caused by various toxic compounds is considered to delay the expansion of the Scottish reintroduced population of

red kites (22), contrasting with reintroduced populations in England (20). However, albeit the reported high mortality caused by toxicants, these two populations are expanding (20, 22). In contrast, extensive anticoagulant rodenticide use in agrarian landscapes in NW Spain has been highlighted as a major driver of short-term population declines at regional level (38). For instance, high mortality rates and demographic modelling support that illegal poison use is a major cause of red kite population declines in small isolated populations in Doñana National Park and the Balearic Islands (13, 21, 23). However, the suggested main role of poisoning as a driver of large-scale and long-term declines of this species remains unproven.

### **Red kite breeding population**

Data on the distribution and abundance of breeding red kites in mainland Spain were obtained from two national censuses performed in 1994 and 2014 (i.e. the longest monitoring period available up to date) (15, 16). Census methodology is described in detail in [15] and [39]. Briefly, breeding kites were located within 10x10-km UTM squares in March-July of each monitoring year by trained volunteers through both: i) car surveys at low speed (~40 km/h) to obtain the individuals observed per km, and ii) active search of breeding territories from vantage observation points or intensive nest searching. Data obtained by both methods were converted to population estimates by using the equations described in [15] and [39].

Data on the presence and abundance of breeding red kites in 1994 were obtained for a total of 2,990 10x10-km squares (i.e. ~300,000 km<sup>2</sup>, 60 % of the country); the species presence

was confirmed in 386 squares (i.e.  $\sim 38,600 \text{ km}^2$ ), with a total breeding population estimated at 3,333-4,044 pairs (Fig. 1a) (15). In 2014, the species census covered 1,400 10x10-km squares (i.e.  $\sim 140,000 \text{ km}^2$ ) and presence of red kites was reported in 554 squares with an estimated population of 2,312-2,440 breeding pairs (Fig. 1b) (16). Nonetheless, after corrections for potential overestimations of breeding pairs detected in the last census, the Spanish breeding population was estimated at  $\sim 2,000$  breeding pairs and its distribution was considered to decrease  $\sim 40\%$  in the last two decades<sup>10</sup>. Data on red kite presence from both censuses were combined to obtain a response variable of the change in occupancy of breeding red kites per 10x10-km square in the 20-years period considered (Fig. 1c; Appendix 3). Those squares with breeding pairs in both censuses (i.e. 1994 and 2014) were codified as presences (value: 1). Squares with breeding kites in 1994 but not in 2014 were considered as absences (or losses, codified as 0). Changes in the abundance of breeding pairs among censuses were codified in a variable with two vectors: i) “number of succeeds”, considered as the number of breeding pairs estimated per 10x10-km square in 2014, and ii) “number of failures”, as the number of breeding pairs that disappeared between 1994 and 2014 (40). Those squares with no change or increase in the abundance of breeding pairs in the considered period were assigned a 0 number of failures.

We excluded from the analyses data from Balears islands and Doñana, because these populations are geographically isolated from the remaining Spanish red kite population and seem to present different habitat preferences (20). Nonetheless, both populations were known to be heavily impacted by poisoning (13, 21, 23, 41).

### **Wildlife poisoning data and other environmental variables**

To assess the potential influence of wildlife poisoning on the observed changes in distribution and abundance of the breeding population of red kite, we used a large and exhaustive database of confirmed or suspected cases of wildlife poisoning compiled over more than three decades in Spain by WWF Spain and SEO/BirdLife (17). Most data were gathered by survey programs coordinated by governments of autonomous regions in Spain and toxicological analyses were performed at ecotoxicology labs. We considered twelve variables related to the incidence of poisoning on the fauna in general, and on raptors and kites in particular (Appendix 3). According to the low detectability of wildlife poisoning (estimated in 5-15 % of the total cases) (17, 27), we considered also the number of poisoned dogs per km<sup>2</sup> and year as a potentially more accurate index of the real incidence of wildlife poisoning (19). Considering the red kite trophic ecology (i.e. feeding on small carcasses and meat remains) (24), we also calculated the density of poisoned baits as a proxy for the incidence of poisoning in natural ecosystems. Poison-related variables were calculated in two ways: i) considering all the episodes registered in the database in the period between censuses (i.e. 1995-2013, N = 18,500 animals and 4,175 baits), and ii) considering only those episodes in which the presence of a toxic compound susceptible of poisoning wildlife was confirmed (N = 9,562 animals and 3,257 baits). All these variables were initially considered in the models.

To account for other factors able to influence the observed changes in the red kite breeding population, we considered several environmental factors previously identified as important to determine habitat suitability for the species in Spain. Seoane *et al.* [18] highlighted the importance of different land uses such as cropland, forests and pastures as well as of topographic variables such as elevation and slope, and climatic variables such as

temperature on the distribution and abundance of red kites. From these variables considered as important for the red kite in 1994, we incorporated into our analyses those susceptible of meaningful and noticeable changes in the considered period (i.e. 1994-2014). Thus, we used the CORINE Land Cover information from 1990 and 2012 (42) to calculate the percentage of change in crop, forest and pasture surface per 10x10-km square among the considered red kite censuses (Appendix 3). We assumed that the remaining variables considered important for explaining habitat suitability for the red kite (e.g. climate, topography) had not changed noticeably in the period analyzed.

We finally considered the number of breeding pairs of red kite estimated per 10x10-km square in 1994 (Table 1). Squares with a higher breeding population in 1994 could be more resilient to species threats such as poisoning and land use change, thereby having a higher probability of being occupied in 2014.

## **Analysis**

We used generalized linear models (GLMs), with binomial error distributions and “logit” link, to explain the observed changes in distribution and abundance of breeding red kites between 1994 and 2014. From the explanatory variables significantly correlated (i.e. Spearman correlations  $P < 0.05$ ), we included in the models the one with more ecological significance or, if not easily discriminated, the variable with best results in a univariate GLM (e.g. lower corrected Akaike Information Criteria, AICc; Appendix 3) (43).

We used multimodel selection to choose the best models (43). We randomly selected the 70% of the data to train the models and the remaining 30% to test the results. We used the

training dataset to generate a set of models with all possible combinations of the considered explanatory variables. We tested both linear and quadratic responses as well as interactions between pairs of variables. The resulting models were ranked by lower AICc and higher relative weight ( $\omega_m$ ), which indicates the probability that a model was the best one among all the candidate models evaluated. From these ranked models, we selected a set of best models comprising those whose weights added to 0.95 ( $\Sigma\omega_m = 0.95$ ). From the set of best models we depleted the redundant ones, i.e. those including the same variables of other more explicative model (i.e. with lower AICc and higher  $\omega_m$ ) but with some additional variable that complicates the model without improving its predictive ability. Once filtered, model weights were recalculated to sum 1. The models resulting from this selection process were combined into a single one considered as the best minimum model (43, 44). The coefficients of the explanatory variables included in the best minimum model were calculated by weighting each variable coefficient by the weight of the model where the variable was included.

The relative contribution of each variable to the best minimum model ( $\omega_i$ ) was calculated by summing the weights of the best models ( $\omega_m$ ) which included the considered variable. The most important variables were those with the highest relative weight ( $\omega_i = \Sigma\omega_m$ ). We expected that our modelling approach selecting non-correlated variables to include in the models minimizes the multicollineality, thus minimizing in turn the issues associated with pondered models (44). Nonetheless, to get additional information on the performance of the explanatory variables, we calculated also  $Z$  and  $P$  values of the Wald test (Appendix 3) for each variable included in the best minimum model (see above). All the statistical analysis were performed in R (45).

## **Global assessment of poisoning as a species threat**

We searched the IUCN Red List (29) for animal species threatened by poisoning at global scale according to the IUCN criteria for the inclusion of threats. We considered a species threatened by poisoning when threat categories “5.1.2. Unintentional effects (species is not the target)”, “5.1.3. Persecution/control” or “9.3.3. Herbicides and pesticides” were recorded in the IUCN assessment of the species. We restricted our search to only global species assessments. Additionally, we also did a search for only threatened species in the wild (i.e. those listed as “Vulnerable”, “Endangered”, and “Critically Endangered”). We also considered these figures for raptors and carnivores, as predators either frequently prosecuted through illegal poisoning or affected by secondary poisoning (e.g. by consumed poisoned preys such as rodents) (1, 4-8, 27, 35-38, 46-47). The main results of this search are detailed in Appendix 4.

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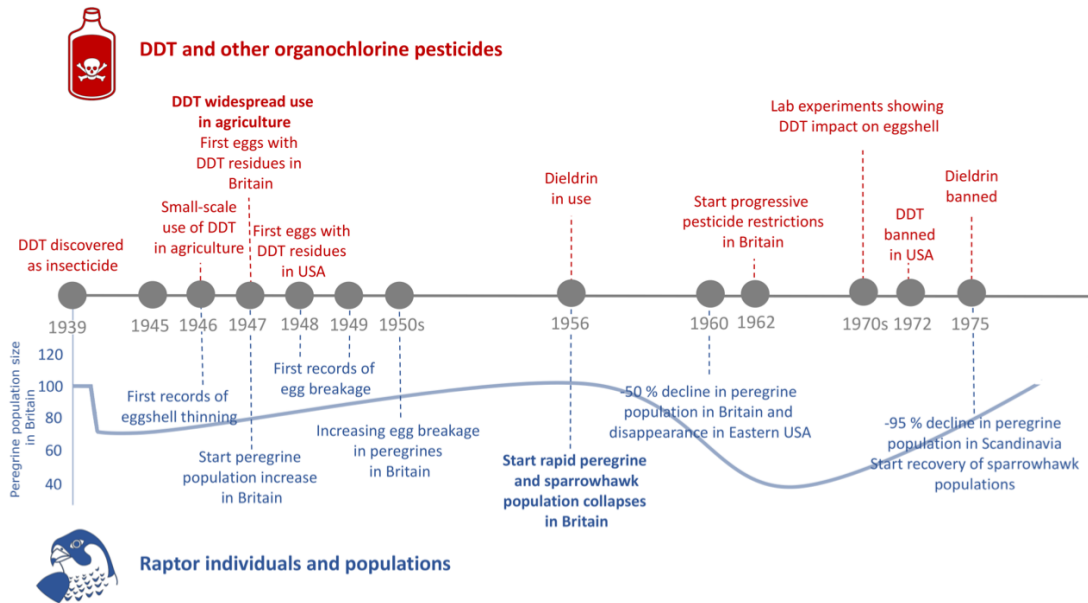
**Supplementary text.** Summary of the accumulated evidence on toxicants, like the organochlorine insecticide DDT, lead ammunition and the anti-inflammatory drug diclofenac, known to be the cause of large population declines in falcons, condors and vultures, respectively.

### **DDT and the decline of the peregrine falcon populations in Europe and North America**

Organochlorine pesticides, including the DDT (i.e. dichlorodiphenyltrichloroethane), are thought to be the most harmful pesticides for wildlife, especially for birds of prey (1,2). Although synthesized in the XIX century, the DDT was not discovered as insecticide until 1939 (Figure S1). It was used first to control mosquito outbreaks during the Second World War, and, from 1946 onwards, DDT was widely used against agricultural pests as well as to kill mosquitoes to eradicate malaria (2).

DDT is a fat-soluble, persistent compound that biomagnifies up in the food chain, passing from preys to predators, and thus tending to bioaccumulate, reaching higher concentrations in predators; it is also toxic to fishes (1,2). Inside the animal's body, DDT biodegrades to DDE, which is thought to be responsible for eggshell thinning, and their consequently breakage, in birds. Because of their position at the top of food webs, raptors were the species most affected by DDT (1). Indeed, the populations of bird-eating species such as the peregrine falcon *Falco peregrinus* and the sparrowhawk *Accipiter nisus* showed the strongest declines attributed to DDT (1). In the 1950s and 1960s, peregrine falcon populations plummeted in many parts of Western Europe and North America. In the 1960s, the species had already disappeared in the half eastern of the United States; in Great Britain the breeding population in the early 1960s (estimated in 241 occupied territories in 1962)

had declined 50 % relative to the population of 1930-1939; in Southern England the decline was of 92 % (3). The Sweden peregrine population crashed from 350 pairs in the 1950s to 15 pairs in 1975 (i.e. >95 % decline) (4), and in West Germany, from about 400 pairs in 1950, to 40-50 pairs in 1973 (5) .



**Figure S1.** Timeline showing the use of DDT and other pesticides (in red at the top) and their potential impacts on raptor populations (in blue at the bottom). The population trend of the peregrine falcon in Britain is shown according to Ratcliffe [5]. Illustrations of Chris Homan and Martina Krasnayová from The Noun Project.

Between 1951 and 1956, Ratcliffe [6] reported for the first time an unusual number of cases (13 out of 59 eyries) of broken eggs in peregrine nests in Britain. Later studies indicated that this phenomenon had increased in Britain from the 1950s, although the first records of egg breakage date back to 1948-1949 (6, 7). The incidence of broken eggs in peregrine nests increased over an order of magnitude, from <2.7 % in the period 1904-1950 to 28 %

in 1950-1966 (8). A similar incidence of egg breakage had also been found in the sparrowhawk in Britain (8). The increase in the egg breakage led researchers to examine any possible change in the thickness of eggshells, which, in the peregrine and the sparrowhawk, decreased significantly since 1946 in both Britain (8) and the United States (9). This synchronic change of the eggshell thickness in both regions just after the widespread usage of DDT directly pointed to pesticides in general and DDT in particular as major causes. Hickey & Anderson [9] studying herring gulls and Cade *et al.* [10] in Alaskan peregrine falcons found a negative relationship between the shell thickness and DDE residues in eggs. Studies in captive birds demonstrated that a diet containing DDE in environmentally realistic doses caused the thinning of the eggshells in American kestrels *Falco sparverius* (11). Accumulated evidence pointed to the DDT as responsible of the eggshell thinning and egg breakage in different species, but some authors still raised doubts, arguing that eggshell thinning started too soon, i.e. in 1946 and 1947 in Britain and USA, respectively, just when DDT came into general use (1946-1947) (12). However, these concerns were dismissed when Peakall [13] was able to find DDE residues in the dried membranes of peregrine eggs collected in California between 1948 and 1950, but not in eggs collected before that date; two years later similar studies were conducted in Britain by Peakall *et al.* [14] who found DDE residues in peregrine eggs from 1946 and 1947.

The mechanism by which DDT affected eggshell was elucidated in the late 1960s and early 1970s using captive non-raptorial birds under laboratory conditions. DDE produces a decrease in calcium content in eggshells thorough inhibiting the carbonic anhydrase and Ca-ATPase activity, enzymes involved in the transport of calcium in the avian shell-forming gland (15-17). This in turn produces eggshell thinning which occurs only 4 days

after the female ingests DDE (40 ppm); the decrease in eggshell thickness reduces both breaking strength and pore density in the shells (18), which leads to egg breakage and breeding failure (19).

Therefore the evidence obtained during decades allowed to elucidate how DDT could be affecting reproduction in birds in the field: i) organochlorine residues are incorporated by parent birds when feeding upon contaminated preys with these pesticides; ii) once in the bird's body, DDT biodegrades to DDE, which alters the calcium transport in the shell-forming gland, iii) this produces a decrease in calcium content in the eggshell, iv) eggshell thinning, v) egg breakage and, finally, vi) breeding failure.

*Have DDT-induced effects on eggshell and breeding success caused the collapse of peregrine falcon populations in late 1950s and 1960s?*

In Britain, DDT residues in peregrine eggs were present as soon as 1946 and 1947, and the decrease in the thickness of eggshell was detected from 1946 onwards (Fig. S1). However, despite the almost immediate effect of DDT on egg breakage (18), the sharp collapse in the breeding population of peregrine in Britain began long after that, i.e. in 1956 (2, 7). Even more, from the late 1940s to 1955 the British population of peregrine falcon was increasing after the population control exerted during the Second World War (7). The decrease of the peregrine population after 1955 was so rapid (i.e. 50 % in less than 10 years) that it was suggested that adult mortality had to be involved (7). This temporal pattern of population decline matched the introduction of other organochlorine pesticides: the seed dressing cyclodienes (7). The cyclodienes aldrin, dieldrin and heptachlor cause (sub)lethal toxicity increasing mortality rates<sup>2</sup>. Similar to the peregrine, the sparrowhawk population in Britain

suffered a post-1955 collapse too, supporting the hypothesis that DDT did not was the ultimate responsible of such a rapid population crash. Indeed, it was rather related to the use of dieldrin (2, 19, 20), as this pesticide was in use from 1956 and once dieldrin was definitively banned in 1975, the population of sparrowhawk rapidly recovered (19). Although some evidence suggested a positive relationship between DDT-induced eggshell thinning and decreasing populations of peregrine (21), the population changes in the peregrine and sparrowhawk observed over time do not seem to support it (at least in Britain, see above). DDT was the first pesticide subject to environmental regulations; it was banned in the United States in 1972 (21). In Great Britain restrictions for use of DDT started in 1962 and it was completely banned in 1986.

This shows that although the toxicological effects of DDT on eggshell thinning and reproduction have been proven, these effects have not been well reflected at the population level, likely because other density-dependent factors have compensated the reduction in breeding success driven by DDT (20). Therefore evidence linking DDT effects at the individual level (i.e. shell thinning and breeding failure) with effects at the population level (i.e. population collapse and declines) is weak or not-well supported (9, 21). Indeed, Ratcliffe [7] stated that “the respective contributions of adult mortality and breeding failure to the peregrine population decline will perhaps never be known”. Several decades later, Walker *et al.* [2] highlight the same idea “although it is well established that lethal toxicity [of DDT] and eggshell thinning have occurred in the field in the UK, difficulties arise in quantifying them and relating them to population change”.

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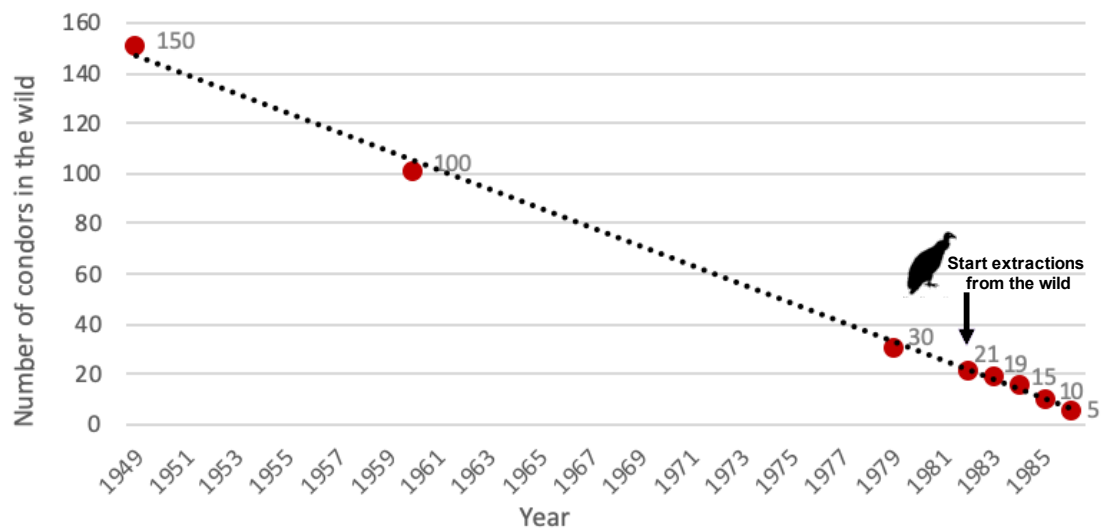
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## **Lead ammunition and the extinction of the California condor in the wild**

The California condor *Gymnogyps californianus* case study represents one of the few ones in the world in which a toxicant has been recognized to be the most important factor driving the global extinction of a species in the wild.

In the nineteenth century, the California condor was widely distributed along the west coast of North America, from British Columbia in Canada to the mountains of northern Baja California in México (22, 23). During the 19<sup>th</sup> and 20<sup>th</sup> centuries, however, the species suffered a drastic and rapid reduction in both range and population until its total extinction in the wild in the 1980s (Figure S2). Rough estimates suggested a condor population of about 150 birds in the 1940s, which was further reduced to about 100 birds in the early 1960s (23). In the late 1970s, the species only consisted of about 30 birds occupying a small area in the San Joaquin Valley of California. In the following years the population continued declining, from 21 birds in 1982, to 5 in 1986 (22, 24). Owing to this rapid and continuing population decline, captive breeding programs began in 1982 with the capture of two condors, a nestling and an immature bird. The last wild condor was captured in 1987, and thus all the condor population was brought into captivity. In 1987 the captive population consisted of 27 individuals (22) taken of the wild: 7 adult birds, 7 birds that were taken as nestlings or immatures and 13 full-grown birds that had been taken as eggs (23).



**Figure S2.** The collapse of the California condor population was so fast that wild birds were captured from 1982 onwards to save the species from extinction. Between 1982 and 1986, 15 free-flying condors died, 6 of them in the winter of 1984-85; the causes of death were only determined for 4 of these birds (i.e. 3 were poisoning by lead and 1 by cyanide) (23).

The remnant condor population in the early 1980s exhibited high mortality rates (26 %) (22) that, according to demographic models, exceeded the levels for self-sustaining populations (i.e. <10 %) (22). Three out of 4 free-living condors found dead in the early 1980s died by lead poisoning, and the other one by cyanide poisoning (22). While habitat loss and direct persecution (shooting, poisoning) seemed to be the main causes of the decline of the California condor during the 19<sup>th</sup> and early 20<sup>th</sup> centuries (25), lead poisoning was attributed as the main cause of decline of the remnant population (22, 25). This evidence was later confirmed when the captive birds were released since 1992. In the 1997-2010 period, 150 condors were released in California. Between 50 % and 88 % of these condors yearly released showed blood lead levels >100 ng/mL (about 3 times higher than blood levels of pre-release condors, 30.3 ng/mL). In addition, a mean of 20 % of the birds per year (range = 0-44 %)

reached the level of clinical lead poisoning (i.e. 450 ng/mL), thus needing chelation treatment for recovery (26).

Evidence shows that condors were poisoned by ingestion of lead ammunition fragments in carcasses or gut piles of shot animals (e.g. big game, small mammals, feral hogs, coyotes, livestock) (25, 26). Isotopic composition of blood lead for many of the free-flying condors (63 %, n=110) was consistent with lead-based ammunition; other sources of lead were background environmental and lead-based paint.

The free-flying condor population currently undergoes intensive management to limit exposure to lead. Blood lead levels are frequently measured for most birds; if lead levels are high (i.e. >450 ng/mL), the birds receives chelation therapy in captivity and are released once the lead levels turn to be low. Demographic models showed that, without this intensive management to limit the high lead exposure of the free-flying condor population, the population in California would decline from 150 individuals to 22 individuals in 11 years if mortality is assumed at blood lead levels of 1000 ng/mL, or in 61 years, assuming mortality at 3000ng/mL (26).

Overall, the evidence of the negative individual-level effects of lead on condors seems to be clear (21). However, how these individual-level effects translate to population-level effects is based on a deterministic, age-based demographic model (26), that indirectly shows the pervasive impact of lead on the California condor population level, but there is not direct evidence linking effects of the toxicant between individual and population levels.

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### **Toxic impact of the anti-inflammatory drug diclofenac on vulture populations in Asia**

The rapid collapse of the Asian vulture populations (>90 % of decline in less than 10 years), which led toward the brink of extinction to three formerly common *Gyps* species (namely, the oriental white-rumped vulture *Gyps bengalensis*, and the long-billed vulture *G. indicus*) (27-29), is frequently highlighted as one of the most dramatic episodes of wildlife poisoning in the last decades. Investigations revealed the anti-inflammatory diclofenac as the cause of these population declines, whereby vultures were contaminated by consuming livestock carcasses treated with this drug (30, 31).

First vulture population declines were reported in the Keoladeo National Park in Rajasthan, northern India. Between 1986 and 1999, the resident populations of the oriental white-rumped and the long-billed vultures plummeted by 95 % (from 1,800 to 86 individuals) and by 96 % (from 816 to 25 individuals), respectively (27). The white-rumped vulture disappeared as breeder in the park (from 204 nests in 1985/86 to none in 1999 and 2001) (28).

Sharp vulture population declines were also reported in other regions of India (28). Between 1991/93 and 2000, the number of white-rumped vultures counted in road transects across the country declined by 95.7 %, from 20,974 (3.3 birds/km) to 883 (0.14 birds/km). In the same period, the number of long-billed vultures censused by road transects declined by 92.2 % (28).

In 2001, high annual mortality rates of 11-18 % were reported for oriental white-backed vultures in the Punjab province, Pakistan (29). Here, a total of 668 sick or dead vultures were

collected between December 2000 and June 2001 (29). Post-mortem analyses were conducted in 259 of these individuals, 219 (85%) of which had urate deposits compatible with visceral gout disease causing kidney failure, hyperuricaemia and uric acid deposits (30). Detailed necropsies and analyses (i.e. liquid chromatography and mass spectroscopy) performed in a subsample of these vultures detected diclofenac residues in 25 out of 25 (100 %) vultures that died of kidney failure, and in 0 out of 13 (0 %) vultures that died from other causes. Experiments with captive vultures were carried out to further demonstrate that diclofenac caused death and renal failure in *Gyps* vultures. Firstly, oral doses of diclofenac were administered to four captive juvenile vultures; three of them died within three days after the diclofenac administration, showing hyperuricaemia. Secondly, 20 captive vultures were experimentally fed with meat of livestock treated with diclofenac a few hours before death; 13 of these vultures (65 %) died and their necropsies revealed visceral gout and renal lesions as those observed in the vultures collected in the field.

Sharp population declines of oriental white-rumped and long-billed vultures continued between 2000 and 2004 in India and Pakistan (31, 32), and a high proportion (72%) of individuals found dead in India and Nepal had residues of diclofenac and visceral gout. A demographic simulation model showed that the observed rates of population decline in 2000-2004 could result from a small proportion of ungulate carcasses (0.277 %, i.e. 1 of each 360) contaminated with a lethal dose of diclofenac (31).

Overall, there is strong evidence of diclofenac causing mortality in individuals of the oriental white-backed vulture and other *Gyps* species (30, 32), but the link of the evidence gathered at individual level with the widespread population declines of vultures is finally suspected or

inferred. Thus, Shultz et al. [32] concluded that “*Taken together, our study and that of Oaks et al. [30] have found diclofenac residues and gout in vulture carcasses collected across most of the geographical extent of the documented declines in vulture populations. The high proportion of dead vultures with signs of diclofenac poisoning make it probable that this is the major cause of the rapid population declines reported to have occurred across the subcontinent*”.

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**Table S1.** A total of 657 red kites were confirmed as poisoned in Spain in 1995-2013 by toxicological analyses, i.e. 61.1 % out of a total of 1,075 dead individuals recorded as suspected to be poisoned in the same period. Major families of toxics are shown together with the probable cause of poisoning, i.e. intentional: when used to deliberately kill fauna, or accidental: when red kite poisoning is a collateral impact derived from other use of the chemical such as, for example, pest control (1). The approved uses of the chemicals (e.g. to control insects, rodents...) and the registration status as plant protection products (PPT) and biocides in the European Union (2, 3) are also provided.



	Dead kites	%	Probable cause	EU Registration status as PPT/Biocides
<b><i>INSECTICIDES<sup>a</sup></i></b>				
<b><i>Carbamates</i></b>				
Aldicarb	316	48.10	Intentional	Withdrawn in 2003 / NA
Benfuracarb	1	0.15	Intentional	Withdrawn in 2007 / NA
Carbaryl	1	0.15	Intentional	Withdrawn in 2007 / NA
Carbofuran	228	34.70	Intentional/Accidental <sup>b</sup>	Withdrawn in 2007 / NA
0.30Propoxur	2	0.30	Intentional	Withdrawn in 2002 / NA
<b><i>Organophosphates</i></b>				
Chlorfenvinphos	3	0.46	Intentional	Withdrawn in 2002 / NA
Chlorpyrifos	7	1.07	Intentional	Approved / NA
Demeton-S-methyl	8	1.22	Intentional	Withdrawn in 2002 / NA
Diazinon	2	0.30	Intentional	Withdrawn in 2007 / NA
Dimethoate	2	0.30	Intentional	Withdrawn in 2019 / NA
Fenamiphos	4	0.61	Intentional	Approved / NA
Fenitrothion	1	0.15	Intentional	Withdrawn in 2007 / NA
Fenthion	15	2.28	Intentional	Withdrawn in 2003 / NA
Malathion	8	1.22	Intentional	Approved / NA
Methamidophos	7	1.07	Intentional	Withdrawn in 2006 / NA
Methomyl	6	0.91	Intentional	Withdrawn in 2019 / NA
Monocrotophos	6	0.91	Intentional	Withdrawn in 2002 / NA
Parathion	3	0.46	Intentional	Withdrawn in 2001/NA
Other organophosphates	2	0.30	Intentional	
Unknown carbamates/organophosphates	10	1.52	Intentional	
<b><i>Organochlorines</i></b>				
Endosulfan	3	0.46	Intentional	Withdrawn in 2005 / NA
<b><i>RODENTICIDES</i></b>				
<b><i>Non-anticoagulant rodenticides</i></b>				
Strychnine	4	0.61	Intentional	Withdrawn in 2004 / NA
<b><i>Anticoagulant rodenticides (ARs)</i></b>				
Brodifacum	1	0.15	Accidental	Withdrawn in 2007 / Approved
Bromadiolone	4	0.61	Accidental/Intentional <sup>c</sup>	Approved / Approved
Difenacoum	2	0.30	Accidental	Approved / Approved
Flocoumafen	4	0.61	Accidental	Withdrawn in 2004 / Approved
Other ARs	7	1.07	Accidental	--
<b>TOTAL</b>	<b>657</b>	<b>100</b>		

<sup>a</sup>Several insecticides are/were also acaricides and nematicides. <sup>b</sup>At least one case of insecticide poisoning was caused by the ingestion of passerines accidentally poisoned with microgranulated carbofuran (secondary poisoning). <sup>c</sup>Anticoagulant rodenticides usually appeared in accidental secondary poisonings, but in some cases egg baits prepared with bromadiolone against predators have been detected in Spain. NA: not apply.

## References

1. Berny, P. Pesticides and the intoxication of wild animals. *J. Vet. Pharmacol. Therap.* **30**, 93–100 (2007).
2. *EU Pesticides database* (2019). <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN>. Downloaded on 24 September 2019.
3. European Chemicals Agency (ECHA). *Information on biocides* (2019). <https://echa.europa.eu/information-on-chemicals/biocidal-active-substances>. Downloaded on 24 September 2019.

**Table S2.** Response and explanatory variables considered to assess the impact of wildlife poisoning on the abundance and distribution of the red kite breeding population in mainland Spain between 1994 and 2014.

<b>Variable</b>	<b>Description</b>	<b>Data source</b>
<i>Response variables</i>		
<b>Red kite presence</b>	Presence (1) of breeding red kites in 10x10-km UTM squares in the two considered censuses (1994 and 2014). Absence (0) indicates the species disappearance as breeder, i.e. present in 1994 but absent in 2014. The final dataset consisted of 167 presences and 107 absences.	(1, 2)
<b>Red kite abundance</b>	Changes in the abundance of red kite breeding pairs per 10x10-km UTM square between the two considered censuses (1994 and 2014). The dataset consisted of 274 squares.	
<i>Explanatory variables</i>		
<i>Wildlife poisoning</i>		
<b>Poisoned red kites</b> <b>Poisoned kites</b> <b>Poisoned raptors</b> <b>Poisoned dogs</b> <b>Poisoned animals</b>	Mean number of individuals per specie or group registered as poisoned in the Spanish database per km <sup>2</sup> at municipality level and per year in 1995-2013. All the variables were calculated by considering all the suspected episodes (subscript s), and only the episodes for which a toxic compound was identified by toxicological analyses (subscript c).	WWF Spain and SEO/BirdLife database (3)
<b>Poisoned baits</b>	Number of poisoned baits calculated as the previous variables (per km <sup>2</sup> and year and considering all and only the confirmed episodes).	
<i>Habitat characteristics</i>		
<b>Pastures</b> <b>Woody crops</b> <b>Dry crops</b> <b>Irrigated crops</b> <b>Forests</b>	Percentage (%) of change in surface of the considered land use per 10x10-km UTM square in 1994-2014. Negative values indicated loss and positive values increase of each considered land use.	(4)
<i>Other</i>		
<b>Breeding pairs 1994</b>	Number of red kite breeding pairs estimated per 10x10-km UTM square in 1994, standardized by subtracting the mean and dividing by the standard deviation.	(1)

**Table S3.** Explanatory variables retained by the best minimum models explaining changes in occupancy and abundance of breeding red kites per 10x10-km square between 1994 and 2014. The importance of each variable ( $\omega_i$ ) and its relationships with the response variable (sign of Z value) are shown. *P* and Z values of the Wald test were calculated for a full model including all the significant variables ( $P < 0.05$ ) retained within the set of best models ( $\sum \omega_m = 0.95$ ) used to obtain the best final model after filtering. Models were trained with the 70 % of the data (i.e. Training) and tested with the remaining 30 % (i.e. Test). Results when using all data in the models are shown also (i.e. Total). Pearson correlations show the robustness of the relationship between model predictions and the observed changes in the abundance of red kite breeding pairs per square between 1994 and 2014. AICc shows the range of AICc values of the best models (i.e. ( $\sum \omega_m = 0.95$ )). Subscript C: only the poisoning episodes for which a toxic compound was identified by toxicological analyses; subscript S: suspected poisoning episodes (see table S2). \* $P < 0.05$  for significant correlations. Sens.: sensitivity, Spec.: specificity. See main text for further details.

Variable	Coefficient	Importance ( $\omega_i$ )	Z	P				
<b>Occupancy best model</b>		<b>D<sup>2</sup>: 9.2 %</b>						
Intercept	0.463	--	2.613	0.009				
Poisoned dogsc	-3578.421	0.94	-2.238	0.025				
Breeding pairs 1994	0.344	0.82	1.895	0.058				
Poisoned red kitess	649.003	0.76	1.783	0.075				
Irrigated crops	0.036	0.72	1.641	0.101				
<i>AICc</i>	<i>Training</i>			<i>Test</i>			<i>Total</i>	
	<i>AUC</i>	<i>Sens.</i>	<i>Spec.</i>	<i>AUC</i>	<i>Sens.</i>	<i>Spec.</i>	<i>Sens.</i>	<i>Spec.</i>
245.48-251.87	0.71	70.2	65.4	0.60	52.8	75.9	61.5	70.7

Variable	Coefficient	Importance ( $\omega_i$ )	Z	P				
<b>Abundance best model</b>		<b>D<sup>2</sup>: 9.9 %</b>						
Intercept	0.036	--	0.589	0.556				
Forests	-0.026	1.00	-8.293	<0.001				
Irrigated crops	0.071	1.00	2.687	0.007				
Poisoned red kitess	-211.597	0.95	-2.404	0.016				
Poisoned dogsc	-1803.247	0.91	-2.693	0.007				
<i>AICc</i>	<i>Training</i>			<i>Test</i>			<i>Total</i>	
	<i>Pearson correlation</i>							
1221.18-1226.84	0.23*			0.26*			0.24*	

## References

1. Viñuela, J., Martí, R. & Ruíz, A. *El milano real en España. Monografía n° 6.* (SEO/BirdLife, 1999).
2. Molina, B. *El milano real en España. III Censo Nacional. Población invernante y reproductora en 2014 y método de censo.* (SEO/BirdLife, 2015).
3. Cano, C., De la Bodega, D., Ayerza, P. & Mínguez, E. *El veneno en España. Evolución del envenenamiento de fauna silvestre (1992-2013)* (WWF Spain & SEO/BirdLife, 2016).

4. CLC, CORINE Land Cover European Inventory 1990 and 2012.

<https://land.copernicus.eu/pan-european/corine-land-cover>. Last accessed 2<sup>th</sup> August 2019.

**Table S4.** Bold figures show animal species and species of raptors and carnivores for which the IUCN Red List includes poisoning (i.e. threat categories: 5.1.2. Unintentional effects (species is not the target), 5.1.3. Persecution/control and 9.3.3. Herbicides and pesticides) among the main threats for their global populations (*I*). The percentages represented by these species from the total and total threatened in the wild (i.e. included in the IUCN categories of “Vulnerable”, “Endangered”, “Critically Endangered”) within each considered group. <sup>a</sup>Raptors include species of orders Accipitriformes, Cathartiformes, Falconiformes and Strigiformes. <sup>b</sup>Order Carnivora.

IUCN Category	Number of species threatened by poisoning	
	Animals	Raptors <sup>a</sup> and carnivores <sup>b</sup>
Extinct (EX)	<b>16</b>	<b>4</b>
Extinct In The Wild (EW)	<b>4</b>	<b>0</b>
Critically Endangered (CR)	<b>226</b>	<b>16</b>
Endangered (EN)	<b>421</b>	<b>32</b>
Vulnerable (VU)	<b>417</b>	<b>45</b>
Near Threatened (NT or LR/nt)	<b>280</b>	<b>40</b>
Least Concern (LC or LR/lc)	<b>877</b>	<b>108</b>
Data Deficient (DD)	<b>234</b>	<b>0</b>
<b>TOTAL</b>	<b>2,475</b>	<b>245</b>
TOTAL Threatened (CR+EN+VU)	13,868 (7.67 %)	186 (50.00 %)
TOTAL Vertebrates	48,101 (4.98 %)	861 (28.46 %)
TOTAL Animal Kingdom	71,999 (3.44 %)	---

## References

1. *The IUCN Red List of Threatened Species. Version 2018-2.* (IUCN, 2019). <http://www.iucnredlist.org>