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POISONING OF REINTRODUCED RED KITES (*Milvus milvus*) IN ENGLAND

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Abstract

Programmes to reintroduce predatory birds are resource intensive and expensive, yet there are few long-term studies on the health of these reintroduced birds following release. A total of 326 red kites (*Milvus milvus*) were released at four sites in England between 1989 and 2006 as part of efforts to reintroduce this species to England and Scotland, resulting in the establishment of several rapidly expanding populations in the wild. Detailed post-mortem examinations were carried out on 162 individuals found dead between 1989 and 2007, involving both released and wild-fledged birds. Toxicological analysis of one or more compounds was performed on 110 of the 162 birds. Poisoning was diagnosed in 32 of these 110 kites, 19 from second generation anticoagulant rodenticides, 9 from other pesticides and six from lead. Criteria for diagnosing anticoagulant rodenticide poisoning included visible haemorrhage on gross post-mortem examination *and* levels of anticoagulant rodenticide exceeding 100 ng/g, but levels were elevated above 100 ng/g in a further eight red kites without visible haemorrhages, suggesting poisoning may have occurred in more birds. The anticoagulant rodenticides difenacoum and bromadiolone were the most common vertebrate control agents involved during this period. Poisoning of red kites may be slowing their rate of population recovery and range expansion in England. Simple modifications of human activity, such as best practice in rodent control campaigns, tackling the illegal use of pesticides and the use of non-toxic alternatives to lead ammunition, can reduce our impact on red kites and probably other populations of predatory and scavenging species.

Key words: birds of prey; scavenger; reintroduction; pathology; toxicology; anticoagulant rodenticide; pesticide; lead

Introduction

Red kites (*Milvus milvus*) are opportunistic, primarily scavenging birds of prey that were once common throughout the United Kingdom (UK) but were lost from England and Scotland by the late 19th century. Misconceptions about the threat they posed to game birds and livestock with consequent persistent persecution reduced the numbers of red kites to fewer than ten breeding pairs restricted to Wales by the 1930s (Carter 2007). In the late 1980s, following assessment under the IUCN guidelines (IUCN 1987), the decision was taken to reintroduce red kites to England and Scotland. Most of the birds for release at the first English release site (Chiltern Hills in southern England) were collected as four to six week old young from nests in Spain. The nestlings were subsequently captive reared in the UK for six to eight weeks with minimal human contact and soft-released into the wild. Once red kites had become established in the first release area, young were collected from this location and released in Northamptonshire, Yorkshire and Gateshead (Carter 2007). A total of 326 red kites were released at four sites in England between 1989 and 2006. To minimize the risks of introduction of alien parasites and to safeguard the welfare of the birds, red kites were clinically examined and screened for

infectious agents prior to release. All red kites that died during captive rearing and those that were found dead in the wild were examined post-mortem. Reintroduction of the red kite in England has proven successful with rapid population growth and range expansion (Balmer et al. 2013). Estimates based on sample surveys and national rates of population increase suggest that there are over 3,000 pairs in England, representing more than 10% of the global population.

Although restoration of raptor populations through reintroduction has been attempted with several species, there are relatively few long-term studies involving the monitoring of health of populations established by reintroduction and translocation programmes. Love (1988), Bainbridge et al. (2003) and Leighton et al. (2008) carried out post-release monitoring of individual species of predatory birds, but there have been few detailed long term pathological studies attempting to determine the threats to released populations, with the notable exception of the California condor, *Gymnogyps californianus* (Rideout et al. 2012; Kelly et al. 2015). Red kites have been released into habitat substantially modified by people since it was last occupied and there is a need to gather information on the main threats facing these birds.

Previous work on reintroduced red kites in England identified lead poisoning through ingestion of lead pellets in prey items in 14% of 44 red kites examined (Pain et al. 2007). Secondary exposure to anticoagulant rodenticides (Walker et al. 2008a) and metabolic bone disease (Pain et al. 2007) have also been highlighted as risks to red kites in Britain. In this paper, we (*i*) describe the findings of pathological and diagnostic examinations on reintroduced red kites found dead in England in a long term study between 1989 and 2007; (*ii*) analyse the epidemiology of the toxicities which occurred post-release; and (*iii*) discuss the significance of these findings for the sustainability of both the current English red kite population and potential future raptor reintroductions.

Materials and methods

Free-living red kites in England that were reported dead or dying between 1989 and 2007 were submitted for post-mortem examination. Most carcasses were refrigerated prior to examination, others were stored frozen. Red kites were aged as follows: birds found in the vicinity of the nest area with traces of down feathers, with tail feathers that had not yet fully grown and with blood visible in shafts of the growing feathers, usually younger than eight weeks of age, were described as '*Nestlings*' (Carter 2007). Birds that had pale breast feathers, with a white line on the upper surface of the wing formed by pale tips on the wing coverts, between eight weeks and approximately 12 months of age were described as '*Juveniles*'. Mature birds with full adult plumage lacking juvenile traits were classed as '*Adults*'.

All carcasses were examined according to a standard post-mortem examination protocol. Firstly, a full body ventro-dorsal radiograph was taken (42 kV, 0.02 mAs) and examined for abnormalities of shape, size and bone density. Standard morphometrics included body weight, wing length and tibiotarsal length. Body condition was assessed according to the tissue cover in the pectoral area as poor, normal or good. External lesions were recorded. All internal organs were examined, both superficially and on their cut surface. Samples

were taken from any lesions found. Samples of the feathers, subcutaneous fat deposits, pectoral musculature, liver, kidney, brain and femur were collected in glass jars and stored frozen till submission for contaminant analysis. The remains of the carcasses were stored frozen for possible future examination.

Analysis of chemical residues in body tissues was undertaken when gross post-mortem findings were suggestive of poisoning. Examples of such findings were: (*i*) kites with internal haemorrhages but no associated trauma, potentially indicative of poisoning by anticoagulants, (*ii*) birds in good physical condition that had apparently died suddenly without evidence of trauma, haemorrhage or infectious disease, potentially indicative of (non-anticoagulant rodenticide) poisoning. The non-anticoagulant pesticides that were tested for included a range of pesticides that are typically associated with vertebrate poisoning incidents (organochlorines, carbamates, organophosphates, metaldehyde, alphachloralose, strychnine); different analyses were targeted depending on supporting information and history of pesticide poisonings in the area. Hereafter, all non-anticoagulant rodenticides and non-lead toxic chemicals are described collectively as 'other pesticides.' When anticoagulant or other poisoning was suspected, liver, kidney and crop and/or gizzard contents were collected in glass vials, stored frozen and subsequently analysed using standardised analytical methods (Brown et al. 2005). Wet weight limits of detection were: 5 ng/g for anticoagulant rodenticides (bromadiolone, difenacoum, flocoumafen and brodifacoum); 200 ng/g for organophosphates, carbamates and strychnine; 400 ng/g for organochlorine; 800 ng/g for alphachloralose and 4000 ng/g for metaldehyde. These were typical of the detection limits of the time, as described by Brown et al. (2005), although higher than those that may be achieved currently using improved analytical equipment.

In addition to investigation of suspected poisonings, a further 20 red kite carcasses were analysed for liver residues of the second generation anticoagulant rodenticides (SGARs) bromadiolone, difenacoum, flocoumafen and brodifacoum, as part of small scale studies to assess exposure to these compounds (for example Walker et al., 2007, Walker et al., 2008a). A larger number of kites (n=87) were analysed for liver (n=44) and bone (n=86) lead (Pb) residues as part of a study to examine the risk from ingesting Pb shot in this species (Pain et al. 2007). Analytical methodologies and detection limits are given in Shore et al. (2003a) and Pain et al. (2007).

For the purposes of the present paper, red kites were grouped according to pathological findings, the principal ones being poisoning, trauma, metabolic bone disease and infectious disease, using the criteria below (each kite might be included in more than one group):

Attribution of poisoning – Poisoning was sub-defined into three categories: anticoagulant rodenticide poisoning, 'other pesticides' poisoning, and lead poisoning. Birds that had internal haemorrhage without associated trauma and summed liver SGAR (bromadiolone, difenacoum, flocoumafen and brodifacoum) concentrations of 100 ng/g wet weight or more, a range considered to be potentially toxic in barn owls *Tyto alba* (Newton et al. 1999), were classed as anticoagulant rodenticide poisoning cases. Some kites had signs of

haemorrhage without associated trauma, but were not diagnosed as poisoned by SGARs because liver SGAR residues were either below 100 ng/g wet weight or information on liver residues was lacking. Birds that died in good physical condition without evidence of trauma, haemorrhage or infectious disease and in which alphachloralose, strychnine, mevinphos, carbofuran, aldicarb or bendiocarb was detected at likely toxic concentrations were classed as 'other pesticide' poisoning cases. Lead poisoning was attributed as a cause of death in birds with a liver lead concentration >15 mg/kg dry weight (Pain et al. 2007), meaning only those birds with liver samples analysed could be included in this category. In a small number of cases, the threshold for classification as being poisoned was exceeded for more than one toxicant.

Attribution of physical trauma – This category included (*i*) suspected electrocution cases, (where birds had been found under power lines, in good physical condition with scorching of the feet and/or feathers), (*ii*) shooting incidents (where [fragments of] lead shot was found embedded in the tissues or where a bullet tract in the tissues was evident), (*iii*) vehicle collisions (where birds had been found on a road or railway track and the extent of trauma was consistent with high velocity impact), and (i*v*) other trauma of unknown cause.

Attribution of metabolic bone disease – Metabolic bone disease was suspected if, on radiography, there was thinning of the cortices of the long bones with coarse trabeculation of the medulla and reduced mineralisation and/or on gross post-mortem examination the long bones were pliable.

Attribution of infectious disease – This category includes cases with pathological changes of infectious aetiology.

Other – Pathological findings that could not be placed in any of the above categories were grouped under this term.

The Rayleigh test for circular distributions, within the circular package in R version 3.3.3 (R Development Core Team 2017) was used to ascertain whether the seasonal data followed a uniform distribution (Zar 1974). Although SGARs vary in their toxicity (Erickson and Urban 2004; Lasseur et al. 2007), given the number of multiple residues, concentrations of individual compounds were summed for statistical analysis. A Mann-Whitney U test was used to test the significance of the difference in summed SGAR levels between birds diagnosed with rodenticide poisoning compared to those in the other categories, while a Fisher's exact test was used to analyse the significance of seasonality affecting the probabilities of SGAR exposure and poisoning.

Results

Between 1989 and 2007, 162 red kites (including 15 nestlings, 65 juveniles, 65 adults) were examined post-mortem (Table 1). Fourteen of these birds were found showing signs of illness in the wild and were hospitalised for up to 73 days prior to death or euthanasia. The age class of 17 red kites and the sex of 57 red kites could not be established, mostly due to decomposition or severe trauma.

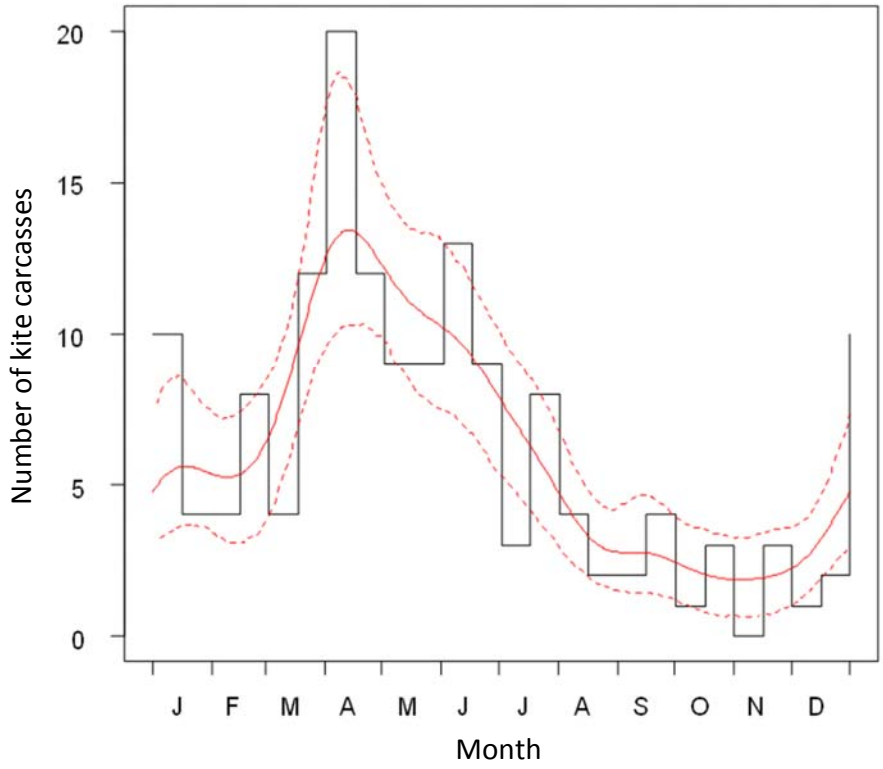
Table 1 Breakdown of age class and sex of the 162 examined red kites from the reintroduction programme in England 1989-2007, together with information on the number analysed for lead, second generation anticoagulant rodenticides (SGARs) or other pesticides. Some individuals were analysed for more than one set of compounds.

	Nestlings	Juveniles	Adults	Unknown age class	Total
Male	3	19	25	5	52
Female	2	19	30	2	53
Unknown	10	27	10	10	57
Total	**15**	**65**	**65**	**17**	**162**
Compounds analysed for:					
Lead	10	33	32	12	87*
SGARs	3	16	23	1	43
'Other pesticides'	1	8	15	1	25

*87 birds analysed for lead in total: 86 bone samples, 44 liver samples.

The date on which the carcass had been found was known for 147 red kites. Although red kites were found dead throughout the year, there was a significant (Rayleigh test $z=0.425$, $p < 0.0001$) non-uniformity in the distribution of records, with 77% found dead in the first half of the year, between January and June, and 50% in the peak breeding period of April, May and June (Fig. 1). This pattern was found in all age and sex groups, although it was weaker and non-significant in juveniles ($p=0.08$; in all other cases $p < 0.0002$).

Fig. 1 Seasonality of red kite carcasses received at ZSL during the study period (1994-2007). **Solid black line shows the absolute number of carcasses received per two week period, while solid and dotted red lines represent the fitted circular kernel and 95% confidence interval**



In all, poisoning was attributed to 32 (20%) of the 162 red kites (Table 2), including anticoagulant rodenticide poisoning in 19 red kites, other pesticide poisoning in nine kites and six with lead poisoning (two birds were categorised as having two types of poisoning). Evidence of metabolic bone disease (n=2; 1%), physical trauma (n=46; 28%), and infectious disease (n=29; 18%) were each found in a minority of birds. In 24% (n=39) of the red kites no pathological findings that provided indication of the cause of death could be identified.

Table 2 Pathological findings in 162 red kites found dead during a reintroduction programme in England 1989-2007 based on pathological examination and diagnostic tests. Note that birds could be classified in multiple categories.

Pathological findings	Number of red kites
Anticoagulant rodenticide poisoning	19
Haemorrhage without associated trauma but not diagnosed as rodenticide poisoning	7
'Other pesticides' poisoning	9
Lead poisoning	6
Physical trauma	46
Metabolic bone disease	2
Infectious diseases	29
Other pathological changes	46
No pathological changes detected	39

In all, of the 43 red kites analysed for SGAR residues (Table 1), 32 (74%) contained one or more detectable liver SGAR residues. SGAR poisoning (in which haemorrhages were observed without associated trauma and summed SGAR liver residues were ≥ 100 ng/g) was attributed to 19 of these birds; two were nestlings, five were juveniles and 12 were adults. For birds in which the liver residue of the predominant individual SGAR was ≥100 ng/g wet weight, the mean (± SE) concentration was 366 ng/g ± 232 (range 140-780 ng/g, n=6) for difenacoum, 188 ng/g ± 102 (range 100-406, n=9) for bromadiolone and 308 ng/g ± 133 (range 169-489, n=4) for brodifacoum. Nine of the 19 birds had multiple SGAR residues in the liver with non-predominant compounds present in concentrations <100 ng/g.

Haemorrhaging without associated trauma was observed in an additional seven red kites, of which four were not tested for anticoagulant rodenticides, two were tested and no liver SGAR residues were detected, and in the remaining bird, a residue of only 16 ng/g bromadiolone was detected. In contrast, summed liver SGAR residues >100 ng/g were detected in a further nine red kites which were not diagnosed as SGAR poisoning cases because either detected haemorrhages were associated with trauma (n=4) or no haemorrhages were found (n=5). In the latter cases, this was either because there were no haemorrhages or detailed examination was hindered; for example, bromadiolone (109 ng/g), difenacoum (47 ng/g) and flocoumafen (62 ng/g) were detected in the remains of one red kite but it had been scavenged to such an extent that it was not possible to search for macroscopic pathological changes in all organs. Three more birds without visible haemorrhages had detectable SGAR residues, but with summed liver residues <100 ng/g.

The summed SGAR residues in the group that was diagnosed with rodenticide poisoning were significantly higher than in those without visible haemorrhages or that had haemorrhages associated with trauma (Mann-Whitney U test, W= 365.5, P=0.048; Table 3).

Table 3 Liver SGAR concentrations (ng/g ww) in red kites, *Milvus milvus,* with detectable liver residues between 1989 and 2007.

	Diagnosed as poisoned by rodenticides[1]	Not diagnosed as poisoned by rodenticides[2]
Number of kites tested	19	13
Mean (±SE) summed SGARs (ΣSGARs)	294 ± 170	192 ± 131
Median (Inter-Quartile Range) ΣSGARs	242 (181-431)	180 (84.5-234)
Range ΣSGARs	100-780	16-526
Mean ±SE (range) no. SGARs in the liver	1.6 ± 0.7 (1-3)	2.0 ± 1.0 (1-3)

[1]Red kites with liver ΣSGARs residues ≥100 ng/g and haemorrhaging without associated trauma.
[2]Red kites with detectable SGARs but not diagnosed as poisoned (no haemorrhages detected (n=8), haemorrhaging with associated trauma (n=4) and haemorrhage without trauma but ΣSGARs <100 ng/g (n=1).

Because rodenticide exposure is known to vary seasonally in other species (Shore et al. 2003a), we expected seasonal variation in the proportion of submission of kites that had been exposed to SGARs, i.e. with detectable residues. However, no significant difference was found between seasons (peak season of April-May versus off-peak) in the proportion of red kites that were exposed to ($p=0.71$) or poisoned by ($p=1$) SGARs. This may reflect a lack of power in the analysis as sample sizes were small or indicate there was a lack of seasonal variation in exposure.

Of the 25 red kites analysed for 'other pesticides', this category of poisoning was attributed to nine red kites (four juveniles, three adults and one of unknown age; Table 4). Mevinphos was detected in five of these birds, and alphachloralose, bendiocarb, carbofuran and strychnine were each detected in single individuals. A further three red kites (one juvenile and two adults) were in poor body condition (excluding them from 'other pesticide' poisoning diagnosis), and were without signs of haemorrhage or infectious disease. Aldicarb, alphachloralose, and carbofuran were detected in one each of these birds. Multiple 'other pesticides' were not detected in any individuals. However, of these 12 red kites containing 'other pesticides', five had liver residues of SGARs (including three with summed SGARs of >100 ng/g). Tissues from a further eight red kites that had suddenly died without signs of trauma, infectious disease or internal haemorrhage were submitted for toxicological analysis and were negative for 'other pesticides.'

Table 4 Other (non-anticoagulant rodenticide) pesticides detected in red kites found dead between 1989 and 2007 in England.

Other Pesticide	Number of cases		Tissue sample analysed	Level detected (mg/g) Range given when n>1
Aldicarb	1*		Crop/gizzard contents	2
Alphachloralose	1*		Kidney	25
	1		Crop/gizzard contents	500
Bendiocarb	1		Crop/gizzard contents	4.1
Carbofuran	2	1*	Crop/gizzard contents	2.8
		1	Crop/gizzard contents	4.8
Mevinphos	5		Crop/gizzard contents	3-39
Strychnine	1		Crop/gizzard contents	400

*Aldicarb, alphachloralose and carbofuran were each detected in one red kite that had poor body condition (and thus were not classed as pesticide poisoning cases).

Liver Pb levels greater than 15 mg/kg dry weight (dw), compatible with death due to lead poisoning, were detected in six red kites, as previously described by Pain et al. (2007). 'Other pesticides' were also detected in two of these six birds. One of these birds was found dead in good body condition with recently ingested food in the oropharynx and gizzard, and mevinphos (3 mg/g) was detected in gizzard contents. This bird was categorised under both lead poisoning and with 'other pesticides' poisoning (Table 2). The second was found to have respiratory and renal haemorrhages, and bromadiolone levels in the liver were 270 ng/g ww; this bird was categorised under both lead poisoning and SGAR poisoning in Table 2. A further 11 red kites had lead bone concentrations of between 30.3 and 187.5 mg/kg dw lead, and bone lead concentrations greater than 30 mg/kg dw have been detected in birds which have died from lead poisoning (Pain et al. 2007). In one of these 11 birds, lead shot was found (in the oral cavity only) at post-mortem examination.

Discussion

In this long term study of reintroduced red kites, poisoning was attributed as the cause of death in 20% (n=32) of 162 birds. High summed second generation anticoagulant rodenticide (SGAR) levels (>100 ng/g) were found in a further nine birds, and other pesticides probably contributed to the death of another three birds examined. Only a subset of the red kites examined in this study had tissue residues analysed for toxins (44 for lead concentration in liver, 25 for 'other pesticides' and 43 for SGARs, see Table 1) and although this sample was biased towards birds where poisoning was suspected, it is likely that other birds were poisoned but went undetected. Some carcasses were too decomposed to detect haemorrhages accurately and additionally it is known that birds can die from SGAR poisoning with haemorrhage detected on histopathology that is not visible on gross post-mortem examination (Rattner et al. 2011). Therefore, it is likely that the true percentage of birds that were poisoned was greater than 20% in total.

Difenacoum and/or bromadiolone residues of >100 ng/g were detected in 15 of 19 red kites diagnosed as having been poisoned by SGARs. This is consistent with other studies

showing that difenacoum and bromadiolone are the most commonly detected anticoagulant rodenticides in predatory birds and mammals in Britain that were sub-lethally exposed (Walker et al. 2008b; Shore et al. 2003b; Shore et al. 2006). The data in the present study, and those from the UK Wildlife Incident Investigation Scheme (Barnett et al. 2007), suggest that these are the compounds that are also responsible for most of the poisonings of red kites and other wildlife. Difenacoum and bromadiolone are the two most widely used SGARs in the UK (Dawson and Garthwaite 2002). Brodifacoum is less commonly used and was detected in only four anticoagulant rodenticide poisoning cases. Flocoumafen was detected in only one bird.

Our results show that liver summed SGAR concentrations were significantly greater in those kites that were diagnosed as poisoned by SGARs (Table 2), as might be expected. However, there was considerable overlap in the magnitude of residues between birds diagnosed with rodenticide poisoning and those excluded from this category (Table 2). This may in part be due to uncertainties in accurate diagnosis of SGARs as a cause of death and also because simple summing of the concentrations of individual SGARs obscures differences that exist between the compounds in their acute toxicity (Erickson and Urban 2004; Lasseur et al. 2007). Elevated levels of summed SGARs (>100 ng/g) were detected in five red kites in which haemorrhages could not be detected and this could be because the SGARs were not lethally toxic to these birds, or because haemorrhages may not always occur (or may be difficult to detect) with toxic levels of SGAR; in such cases, poisonings would be undiagnosed. Other studies on raptors in the UK, including tawny owls *Strix aluco* (Walker et al. 2008b), barn owls and kestrels *Falco tinnunculus* (Walker et al. 2010), red kites (Walker et al. 2008a) and buzzards *Buteo buteo* (Shore et al. 2006) have shown that, in addition to birds killed by ingesting anticoagulant rodenticides, a high proportion of birds found dead contain ostensibly non-lethal anticoagulant residues. There is uncertainty regarding the lethal level of residues of these compounds in raptors (Thomas et al. 2011) as there is likely wide inter-individual variability in susceptibility to rodenticide poisoning and no single threshold value has been identified as indicative of death. This makes it difficult to determine to which extent these lower levels of anticoagulant residues may have adverse effects on the health of these birds.

Red kites feed mainly on carrion (Barton and Houston 1994), and a study of the feeding behaviour of red kites in England showed that red kites regularly take food from confined spaces, including close to farm buildings; such behaviour is likely to increase their exposure to prey containing rodenticides (Ntampakis and Carter 2005). Exposure of scavenging raptors, like the red kite, to SGARs could be minimised through the use of less toxic and less persistent first generation rodenticides (such as warfarin) where rodents have not developed resistance to these compounds. However, the toxicity of some first generation compounds to raptors may have been underestimated (Rattner et al. 2011). Exposure can also be reduced by following best practice during rodent control campaigns including the legal requirement to carry out regular searches for dead rodents so that the bodies may be disposed of safely (http://www.thinkwildlife.org).

The other pesticides found in red kites during this study may have been used in illegal poison baits in order to control predators of livestock and game birds. These pesticides have no current approval as Plant Protection Products, although some, such as alphachloralose, could still be legally used at the time the birds in the present study were collected (Thomas et al. 1988; Pesticides Safety Directorate 2008). Some, such as mevinphos and carbofuran, can still be obtained either from sources outside of the UK or from material held illegally in stockpiles. There are schemes in place in the UK to detect the misuse and abuse of pesticides, such as the Wildlife Incident Investigation Scheme. However, gathering sufficient evidence to allow successful prosecutions for the illegal poisoning of wildlife is often difficult.

The ingestion and accumulation of harmful lead in red kites could be reduced by the use of alternatives to lead shot for shooting small game (mammals and birds), such as steel, tungsten-based or bismuth-tin which are readily available in the UK (Thomas 2014). There are already some regulations in place to prohibit the use of lead for shooting waterfowl and for shooting over many wetlands in England, but these regulations are limited and compliance is poor (Cromie et al. 2015). Unnecessary deaths of terrestrial predators and scavengers could be avoided if the scope of these regulations were extended or if the use of non-toxic alternatives were to be more widely adopted voluntarily.

The red kite population in England has increased rapidly following the reintroduction programme, in spite of deaths from anthropogenic poisoning. However, it has thus far been restored to only a small part of its former natural range in England. Anticoagulant rodenticide poisoning, illegal pesticide poisoning and lead poisoning, all of anthropogenic origin, may be slowing the recovery of red kites in England. Natural recolonisation from population centres established by reintroduction is potentially being limited by these unnecessary and preventable deaths, which amount to at least 20% of the birds found dead in this study. The red kite has been intensively monitored as part of the efforts to reintroduce it. As a result, dead red kites are more likely to be reported and the cause of death established through post-mortem examination and tissue analysis than for other species that may be affected by the same threats. Our work on the red kite has highlighted the poisoning threats that may be of wider importance to a range of predatory and scavenging species in England and elsewhere.

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