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A negative association between bromadiolone exposure and nestling body condition in common kestrels: management implications for vole outbreaks†

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Abstract

BACKGROUND: Vole outbreaks have been extensively described, along with their impacts on humans, particularly in agricultural areas. The use of rodenticides is a common legal practice to minimise crop damage induced by high vole density for biocidal use. However, rodenticides can have negative direct and indirect impacts on non-target species that feed on voles. We studied whether the use of a second-generation anticoagulant rodenticide (SGAR), bromadiolone, can be detected in the blood of fledglings of wild common kestrels *Falco tinnunculus* in two areas of central Spain, exploring its possible indirect effects.

RESULTS: We found that 16.9% of fledglings had a detectable concentration of bromadiolone in their blood, with an average concentration of 0.248 ± 0.023 ng mL^{-1}. Fledglings with bromadiolone in their blood, regardless of the concentration, had 6.7% lower body mass than those without detectable bromadiolone.

CONCLUSION: The use of bromadiolone was detectable in the blood of alive non-target species. Detected bromadiolone in blood may reduce the body condition of nestlings, potentially reducing their fitness. The source of bromadiolone found in nestlings needs to be determined in future studies to derive accurate management advice. However, we urge the discontinuation of official SGAR distribution to farmers and their use in agrarian lands to minimise damage of voles on crops, particularly where common kestrels breed, and encourage the use of alternative effective practices.

Supporting information may be found in the online version of this article.

Keywords: wild populations; rodenticide; raptors; voles; poisoning

1 INTRODUCTION

Variation in population abundances is commonly described in the animal kingdom. The causes of these variations have been extensively described, discussed and explained from different perspectives for different species. Particular attention has been paid to those species that have an impact on humans, mainly those whose fluctuations can represent a threat to public health[1] or result in financial impacts, either positive or negative, depending on the species.[2,3] Rodents in general, and microtines in particular, have been blamed for crop damage and sanitary problems associated with their population outbreaks. Policies are applied to minimise their impacts, sometimes leading to negative side effects on the biodiversity of agroecosystems.[4,5] Rodenticide use is a common practice to minimise vole damage on croplands during vole outbreaks, despite the potential impact that these toxicants may have on non-target species. The potential transfer of rodenticides to non-target species shifts an otherwise financial conflict to a more complex arena in which ecological, conservation and legal issues arise.[4,6] Particularly relevant are the problems caused by second-generation anticoagulant rodenticides (SGARs), such as

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bromadiolone. These problems often arise from secondary poisoning of species that prey on voles, particularly raptors.[7]

SGARs show greater acute toxicity and higher capacity to bioaccumulate than first-generation anticoagulant rodenticides (FGARs); it has been described that the liver half-life of SGARs can be 10 times longer than that of FGARs in nocturnal raptors (100 days and 11.7 days respectively).[8,9] These undesired effects have led to management strategies for controlling the spatiotemporal use of these rodenticides by farmers to reduce their environmental impacts.[10,11] SGARs are widely used both as biocides in more urbanised areas and as pesticides in croplands, increasing the exposure to bromadiolone for non-target species.[12,13] The European Union (EU) has recently implemented a new legal framework for their use in open land, so baits must be buried or disposed of within rodents' burrows, in an attempt to reduce exposure to other taxa.[14] Given that this is a recently implemented policy (in 2013), the evaluation of its usefulness in reducing secondary poisoning or exposure of raptors is scarce[12] (for buried baits, see Coeurdassier *et al.*[10]). The effects of these pesticides or transmission to offspring during the breeding season are also poorly understood. It is known that a sublethal dose of rodenticides can produce clotting abnormalities and haemorrhaging, which may increase the likelihood of death from other causes.[15] While the accumulation of bromadiolone in tissues, especially in the liver of dead animals, is well documented, particularly in Spain,[16] the presence of bromadiolone (or other SGARs) in the bloodstream of alive predators or the transfer to chicks in the nest have been less studied.[17] If this does occur, a potential effect on the body condition of affected individuals could also be expected, as the effects of ARs on blood parameters and liver malfunctioning are well described.[18] There is little information about the association between bromadiolone concentration in blood and body condition in wild predators. Only one study, to the best of our knowledge, has reported significant negative correlation between brodifacoum exposure and body mass and size in barn owl chicks,[19] supporting this idea. Additional information is needed about this subject, because bromadiolone is used in croplands as pesticide and as biocide in some countries of the EU, including Spain,[14] in order to control vole outbreaks to minimise their damage to croplands, and where predators, in turn, prey on voles. Thus, regardless of the use of bromadiolone either as pesticide or biocide, its potential influence on non-target species and its association with individual condition is little explored.

Variation in population abundance of common voles (*Microtus arvalis*) has been described in central[20] and north-western Spain,[21] in grassland and agricultural areas respectively. Maximum abundances seem to be higher in agricultural areas, where several outbreaks have been described in the last few years, with vole population density reaching levels higher than 1000 voles ha^{-1} in optimal habitats,[22] resulting in important crop damage.[6] In an attempt to mitigate crop damage, anticoagulant rodenticides (ARs) have often been provided by the regional administration to farmers in north-western Spain to reduce vole density during or before outbreaks.[5,21] Since 2008 and up to 2014, bromadiolone has been the officially provided rodenticide in several areas of north-western Spain.[5] SGARs are toxic to all vertebrates, and can result in the accumulation of residues with additive toxicity.[23] The combination of high toxicity, non-specificity and long biological half-life in liver tissue leads to significant exposure potential for predators of rodents.[24] The death of affected rodents by bromadiolone intake is slow, as shown in the house mouse (*Mus musculus*), which can cope with acute bromadiolone intake from 2 up to 6 days,[25] increasing the susceptibility of predators to secondary poisoning by eating contaminated prey.[26,27] During our study period, between the months of April and June 2014, bromadiolone baits were provided to farmers by the regional government (coated cereal grains) in several areas of Castilla y León.

In this paper, we explore whether bromadiolone can be transmitted to offspring of common kestrels (*Falco tinnunculus*) and its association with individual body mass and condition. The common kestrel is a small-sized raptor widely distributed in Europe. They prey on a wide variety of prey items, depending on location, season and availability. However, voles are the main prey when present in high abundance,[28–30] making them an ideal species to study the potential effects of SGARs. In north-western Spain, common voles are the key prey, varying from an average 48% of the total biomass (range 5.7–82.6%; Navarro-Lopez J, private communication)[29] to nearly 80% of prey during periods of high vole abundance.[30] The abundance of kestrel breeding was increased to assess their potential impact in minimising vole damage to crops, an issue that is not tackled in this study by any means. We examined whether the use of bromadiolone can be detected in the blood of kestrel nestlings, and to find out whether there was an association between bromadiolone exposure and body condition. It was expected that bromadiolone concentration and prevalence in blood of kestrel nestlings would be higher in the area where bromadiolone was distributed by regional government the year we carry out this study..

2 MATERIALS AND METHODS

2.1 Study area and monitoring

The study was carried out in two agricultural areas located 53 km apart in north-western Spain, around the villages of Boada de Campos and Capillas (BC hereafter, Palencia, 41° 58′ N, 4° 52′ W) and Villalar de los Comuneros (VC hereafter, Valladolid, 41° 32′ N, 5° 08′ W – see supporting information Figs S1 and S2). Blood sampling was performed in May–June 2014 (BC: 26 May–10 June; VC: 2–10 June). In BC and VC, there were 94 and 100 nest boxes available for kestrels, respectively, over around 2000 ha each (see maps in supporting information Fig. S1). During the study period, 127 nest boxes were occupied (BC: $n = 64$; VC: $n = 63$), 104 of them successfully producing at least one offspring (BC: $n = 53$; VC: $n = 51$). In these two areas, experimental nest-box provisioning was carried out to increase the abundance of kestrel breeding, facilitating nestling blood sampling.[30] Given the prey preference of kestrels for common voles, we chose two study areas for two reasons: firstly to increase the robustness of our results, and secondly to potentially increase the variance in bromadiolone concentration in kestrel broods, as bromadiolone was provided to farmers by the regional government in one of our study areas. The regional government provided bromadiolone in 2009 and 2014 in BC and in 2008 in VC, both before the beginning of the kestrel breeding season. It is key to highlight that the bromadiolone distribution by the regional government does not necessarily mean use by farmers, a fact that we did not assess in this study and for which we only have incidental data (see supporting information Figs S1 and S2). We did not quantify bromadiolone use in our study areas, having only occasional

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information from farmers (supporting information Figs S1 and S2). These two study areas are part of an experimental programme of nest-box provisioning for common kestrels and barn owls.[30] Nest boxes were visited regularly from the laying to the fledging period (on average 3–4 times) to determine laying date, clutch size and number of fledglings. An additional visit after hatching allowed us to estimate the age of offspring (Martínez-Padilla J, unpublished data; linear regression from 1171 measurements of wing length of 140 nestlings of known age from hatching to fledging, $F_1 = 201.24$, $P < 0.001$, $R^2 = 0.932$: age = wing length*0.150 + 1.111), just before they fledged.[31,32] At an average age of 27.6 days (range 18–34 days), 1–3 nestlings were randomly chosen from each nest (average 2.8, range 1–3) and measured (tarsus length to the nearest 0.01 mm), weighed (to the nearest g) and sexed by morphological assessment.[28] Date of measurement was the Julian date of sampling. As a proxy of body condition we considered body mass corrected by tarsus length, a widely used index in birds that informs about the relative body mass that an individual has in relation to its size.[33] Structural size, i.e. tarsus length, is complete in this species at the time of bird sampling, reducing growth-biased estimates of our proxy of body condition. We took 1 mL of blood from the brachial vein, which was kept refrigerated at 5 °C during the field work, and kept frozen at −20 °C from the end of the day until the lab analyses were performed.

2.2 Bromadiolone analysis

The analysis of bromadiolone in blood was carried out using a modification of a method described previously for liver analysis.[16] Specifically, 400 μL of whole blood was transferred to a Teflon-capped 10 mL glass tube and 50 μL of coumachlor (0.05 ng μL^{-1}) was added as an internal standard. Then, 3 mL of a solvent mixture of dichloromethane:acetone (70:30) and 2 g of anhydrous sodium sulphate (Prolabo, Leuven, Belgium) were added. The homogenate was vortexed for 10 min, horizontally shaken for 5 min and sonicated for 5 min. The extract was centrifuged at 1048 × *g* for 5 min, and the supernatant was transferred to another glass tube. The extraction was repeated with 3 mL of the solvent mixture, and the supernatant obtained was pooled with the previous one. The solvents were evaporated under N_2 flow, and the dry extract was reconstituted in 200 μL of a mixture (50:50) of acetonitrile with formic acid (0.1%) and H_2O with formic acid (0.1%) and transferred to a chromatography vial. Bromadiolone analysis was performed by liquid chromatography coupled to mass spectrometry with an electrospray ionisation source (LC-ESI-MS),[18] but here the chromatographic conditions of analysis consisted of an elution gradient of two solvents [A: H_2O with formic acid (0.1%); B: acetonitrile with formic acid (0.1%)]. The initial conditions were 25% A and 75% B, reaching 0% A and 100% B at 8 min and returning to the initial conditions by 9 min. The column was then stabilised under the initial conditions until 10 min before the next sample injection. The flow rate was 1.2 mL min^{-1}. ARs were detected using negative ion monitoring with the following MM-ESI source settings: nebuliser pressure was set at 40 psi, drying gas flow was 7 L min^{-1}, drying gas temperature was 250 °C, vaporiser temperature was 150 °C, capillary voltage was 2000 V, charging voltage was 1000 V and fragmentation voltage varied among compounds (supporting information Table S1). Four ions previously selected for each compound by means of analysis of complete scanning and flow injection analysis of sequences (FIAS) of AR standards were monitored in SIM mode. Calibration curves were performed with concentrations ranging from 0.24 to 8 ng mL^{-1} of methanol. Blanks were analysed regularly with each batch of samples. The recovery of the analytical procedure was calculated with six replicates of red-legged partridge (*Alectoris rufa*) blood (0.2 mL) spiked with 1 ng of bromadiolone (5 ng mL^{-1}). Recovery for bromadiolone with the extraction method and LC-MS analyses described here was 97% (RSD 14.3%). The limit of detection (LOD) of bromadiolone in blood was established at 0.01 ng mL^{-1}, based on the signal-to-noise ratio (3:1) of the measurements (ICH Expert Working Group, 2005).

2.3 Statistics

We first ran a set of models to test the influence of sex, area, date of measurement and age of chicks on bromadiolone concentration. We ran these same models separately considering prevalence (whether or not bromadiolone was detected in individuals), concentration of bromadiolone including all individuals (regardless of whether they had detectable levels or not, assigning a zero value below the detectability threshold – denoted hereafter as C_{all}) and concentration of bromadiolone considering only individuals that had detectable levels of bromadiolone (individuals with a bromadiolone concentration of >0.01 ng mL^{-1} – denoted hereafter as C_{pos}) as dependent variables. We ran generalised linear mixed models with a binomial distribution of errors[34] or a negative binomial distribution of errors[35,36] when prevalence or C_{all} was the dependent variable respectively. We ran general linear mixed models with a normal distribution of errors[34] when C_{pos} was the dependent variable.

In a second set of models, we explored the influence of bromadiolone concentration in blood on nestling body condition. We ran two sets of general linear mixed models with normal distribution of errors in R[34] for two dependent variables: body mass and nestling body condition. When body condition was analysed, body mass was included as a dependent variable and tarsus length as a covariate to correct for size of the individual, as previously suggested.[37] As explanatory variables we considered date of measurement, age of nestlings, sex and area. Variables were removed from the model following a backward stepwise procedure. All categories of bromadiolone concentration were considered as explanatory variables separately (prevalence, C_{all} or C_{pos}). Thus, we constructed three models to evaluate factors affecting bromadiolone exposure of chicks and another three to assess factors related to body condition. As several siblings were sampled from the same brood, the 'nest' was included in all models as a random variable.

Finally, in a third model we explored body mass at the level of nests that had at least one nestling with detectable levels of bromadiolone in comparison with those that did not have any. We built a general linear model where average body mass of the nest was the dependent variable, presence of bromadiolone was a factor (1: at least one nestling with a detectable concentration of bromadiolones; 0: none of the nestlings had a detectable concentration of bromadiolone), sex ratio of the brood was a covariate (to control for the sex ratio variation, because females are heavier than males) and, finally, study area as a factor.

3 RESULTS

We sampled a total of 112 chicks (BC: $n = 69$; VC: $n = 43$) (Table 1) from 40 nests (BC: $n = 25$; VC: $n = 15$), representing a subsample of 23.3% of fledglings (BC: 30.4% of 227 fledglings; VC: 17.0% of 253) and 38.5% of successful nests (BC, 47.2%; VC: 29.4%) of both populations. We detected bromadiolone in 16.9% of sampled nestlings (19 out of 112), and in ten nests at least one

Table 1. Bromadiolone residues in the blood of wild nestlings of common kestrels in two areas of north-western Spain (BC and VC – see Section 2 for additional details). Sample sizes are shown in brackets. Despite the fact that all values were worked out using individual as a sample unit, for informative purposes, we have included sample size at individual (n_{ind}) and nest (n_{nst}) levels, along with the range (r_g: lowest–highest) of values of bromadiolone concentration at the individual level. C_{all} and C_{pos} refer to all individuals regardless of their bromadiolone concentration or only individuals with positive levels of bromadiolone concentration respectively (see Section 2 for further details)

	BC (Boada de Campos and Capillas)			VC (Villalar de los Comuneros)			Total
	Females ($n_{ind} = 30$; $n_{nst} = 18$)	Males ($n_{ind} = 30$; $n_{nst} = 20$)	Unknown ($n_{ind} = 9$; $n_{nst} = 6$)	Females ($n_{ind} = 22$; $n_{nst} = 12$)	Males ($n_{ind} = 20$; $n_{nst} = 12$)	Unknown ($n_{ind} = 1$; $n_{nst} = 1$)	($n_{ind} = 112$; $n_{nst} = 40$)
Prevalence	($n_{ind} = 2$; $n_{nst} = 2$) 6.6%	($n_{ind} = 7$; $n_{nst} = 4$) 23.3% *Total BC*	($n_{ind} = 2$; $n_{nst} = 1$) 22.2% *15.9%*	($n_{ind} = 5$; $n_{nst} = 3$) 22.7%	($n_{ind} = 3$; $n_{nst} = 2$) 15.0% *Total VC*	($n_{ind} = 0$; $n_{nst} = 0$) 0.0% *18.6%*	($n_{ind} = 19$; $n_{nst} = 10$) 16.9%
C_{all} (ng mL^{-1})	($n_{ind} = 30$; $n_{nst} = 18$) 0.007 ± 0.001	($n_{ind} = 30$; $n_{nst} = 20$) 0.247 ± 0.045 *Total BC*	($n_{ind} = 9$; $n_{nst} = 6$) 0.135 ± 0.045 *0.128 ± 0.015*	($n_{ind} = 22$; $n_{nst} = 12$) 0.647 ± 0.015	($n_{ind} = 20$; $n_{nst} = 12$) 0.232 ± 0.138 *Total VC*	($n_{ind} = 1$; $n_{nst} = 12$) 0.000 ± 0.000 *0.439 ± 0.067*	($n_{ind} = 112$; $n_{nst} = 40$) 0.248 ± 0.023
C_{pos} (ng mL^{-1})	($n_{ind} = 2$; $n_{nst} = 2$) 0.102 ± 0.072 r_g: 0.014–0.189	($n_{ind} = 7$; $n_{nst} = 4$) 1.059 ± 0.400 r_g: 0.182–2.130 *Total BC*	($n_{ind} = 2$; $n_{nst} = 1$) 0.607 ± 0.429 r_g: 0.311–0.903 *0.803 ± 0.242 r_g: 0.014–2.130*	($n_{ind} = 5$; $n_{nst} = 3$) 2.848 ± 1.274 r_g: 0.243–6.550	($n_{ind} = 3$; $n_{nst} = 2$) 1.550 ± 0.895 r_g: 0.950–2.088 *Total VC*	($n_{ind} = 0$; $n_{nst} = 0$) 0.000 r_g: – *2.362 ± 0.835 r_g: 0.243–6.550*	($n_{ind} = 19$; $n_{nst} = 10$) 1.459 ± 0.334 r_g: 0.014–6.550

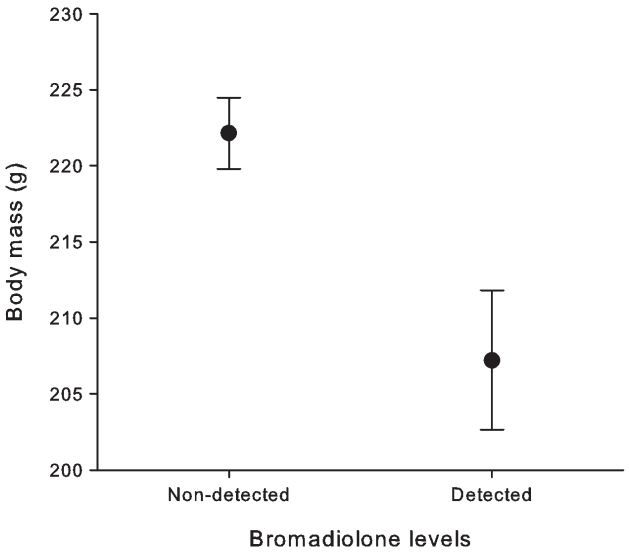

Figure 1. Mean ± SE of body mass of nestlings of common kestrels in relation to prevalence of bromadiolone.

offspring had detectable levels of bromadiolone (VC: $n = 3$; BC, $n = 7$). The average of bromadiolone concentration considering only individuals with positive values (C_{pos}) was 1.46 ± 0.33 ng mL^{-1} (Table 1), and the concentration of bromadiolone considering all individuals (C_{all}) was 0.25 ± 0.02 ng mL^{-1} (Table 1).

We did not find any influence of sex, area, date of measurement or nestling age on prevalence (sex: $F = 0.235$, $P = 0.571$; area: $F = 0.001$, $P = 0.980$; age: $F = 0.029$, $P = 0.987$), C_{all} (sex: $z = -0.22$, $P = 0.823$; area: $z = -0.30$, $P = 0.763$; age: $z = 1.44$, $P = 0.150$) or C_{pos} (sex: $F_{1,6.00} = 1.17$, $P = 0.320$; area: $F_{1,5.55} = 0.831$, $P = 0.399$; age: $F_{1,6.76} = 2.042$, $P = 0.197$) (Table 1). Interactions between the explanatory variables were not significant in any combination ($P > 0.214$).

We found that body mass and body condition were statistically and significantly lower in chicks with bromadiolone residues than in chicks without bromadiolone residues (Fig. 1; prevalence – Table 2), but there was no correlation with C_{all} or C_{pos}. Specifically, chicks with detectable levels of bromadiolone weighed 207.2 ± 4.6 g, and those that did not have detectable bromadiolone in their blood weighed 222.1 ± 2.3 g. The difference in weights suggests that individuals that had detectable levels of bromadiolone were 6.7% lighter than those that did not have detectable bromadiolone in their blood. Any other variable or interaction between variables considered was not statistically significant (Table 2). Our results suggest that there is no statistically significant effect of presence of bromadiolone on average body mass at the nest level ($F_{1,34} = 1.54$, $P = 0.222$; sex ratio: $F_{1,34} = 7.81$, $P = 0.008$; area: $F_{1,34} = 0.01$, $P = 0.897$). Thus, the average body mass of the nestlings within a nest in which at least one chick had detectable levels of bromadiolone was not statistically different from the average body mass of nestlings in nests in which none of the nestlings had detectable levels of bromadiolone.

4 DISCUSSION

Our results confirm that bromadiolone use leads to the secondary exposure of common kestrels in croplands. The presence of bromadiolone in chick blood of kestrels, irrespective of concentration, partially explained the occurrence of lower body mass and poorer

Table 2. Associations between body mass and body condition of nestlings of wild common kestrels in relation to the bromadiolone concentration in their blood. Statistically significant terms are shown in bold

		Body mass				Body condition		
		Estimate (SE)	*F*	*P*		Estimate (SE)	*F*	*P*
Prevalence	**Sex**	**−23.019 (3.795)**	**36.794**	**<0.001**	**Body size**	**4.046 (1.245)**	**10.561**	**0.001**
	Age	−1.391 (0.939)	2.195	0.144	**Sex**	**−19.544 (3.777)**	**26.770**	**<0.001**
	Date	0.311 (0.449)	0.480	0.493	Age	−1.614 (0.911)	3.138	0.081
	Prevalence	**−12.316 (5.672)**	**4.716**	**0.032**	Date	0.164 (0.442)	0.340	0.712
	Area	−3.312 (4.893)	0.431	0.516	Area	−3.328 (4.776)	0.485	0.490
					Prevalence	**−13.129(5.477)**	**5.745**	**0.018**
C_{all}	**Sex**	**−23.679 (3.849)**	**37.841**	**<0.001**	**Body size**	**4.090 (1.262)**	**10.503**	**0.001**
	Age	−1.705 (0.933)	3.339	0.0732	**Sex**	**−21.171 (3.845)**	**30.303**	**<0.001**
	Date	0.197 (0.448)	0.193	0.662	**Age**	**−1.939 (0.908)**	**4.555**	**0.037**
	C_{all}	−1.815 (2.464)	0.572	0.4518	Date	0.049 (0.439)	0.012	0.911
	Area	−3.375 (4.974)	0.460	0.5022	Area	−3.368 (4.847)	0.482	0.491
					C_{all}	−2.252 (2.315)	0.946	0.333
C_{pos}	**Sex**	**−24.498 (7.388)**	**10.995**	**0.008**	**Body size**	**11.079 (3.049)**	**13.201**	**0.002**
	Age	0.207 (2.252)	0.008	0.928	Sex	−6.393 (6.788)	0.886	0.363
	Date	1.869 (1.270)	2.165	0.183	Age	−0.127 (1.590)	0.006	0.937
	C_{pos}	0.841 (2.553)	0.108	0.748	Date	0.519 (0.622)	0.697	0.420
	Area	−7.868 (17.542)	0.201	0.669	Area	−1.533 (7.989)	0.036	0.851
					C_{pos}	−0.159 (2.303)	0.004	0.946

body condition in nestlings. However, our results did not suggest a dose-dependent effect because the study was unreplicated and we did not control where and how much bromadiolone was used or when and how individuals ingested bromadiolone. In this study, the body mass of kestrel nestlings with a detectable concentration of bromadiolone in their blood was 207.2 ± 4.6 g (mean ± SE), a 6.7% lower weight than individuals without detectable bromadiolone in their blood. This suggests, at least indirectly, that offspring having detectable levels of bromadiolone had a relatively lower body size and poorer body condition.

Our results also suggest that body mass and condition of chicks with a detectable concentration of bromadiolone were lower than for those that did not have a detectable concentration of bromadiolone. Body mass and body condition are key life history traits in birds, particularly near the time of fledging. The maximum rate of mortality in birds is detected at the time that young individuals become independent.[38] Body condition is a key factor affecting mortality, with individuals in better condition having an increased probability of survival in the first year of life.[39] An increased risk of mortality will reduce the probability of recruitment in the population,[39] and therefore those factors that worsen body condition may reduce the probability of recruitment, negatively impacting on population viability. In fact, a reduction of about 5% in body mass in nestlings of another wild population of kestrels may reduce recruitment probability by 55% (from 0.22 to 0.10, unpublished and own data). Further studies are required to clarify the influence of bromadiolone use on bromadiolone concentration in nestlings and thus on their body mass, but our results suggest an incompatibility of the use of SGARs in areas where breeding of avian predators is favoured by providing nest-boxes.[30] The correlative nature of our study does not allow us to prove any causation link between concentration of bromadiolone and nestling body mass. Individuals that had detectable levels of bromadiolone might have reduced body condition owing to loss of appetite and haemorrhage.[40,41] This effect might be particularly relevant when low but continuous doses of bromadiolone are ingested, like the expected continuous potential ingestion of poisoned voles. On the other hand, individuals in lower body condition can be more exposed or more susceptible to the accumulation of bromadiolone. Exposure may perhaps be explained by a higher probability of parents choosing to hunt voles in bromadiolone-treated areas.

It could be argued that the low body condition of chicks in nests exposed to bromadiolone could indirectly reflect vole scarcity caused by the use of bromadiolone in crop fields. Our study cannot tackle this issue, as the quantification of the use of bromadiolone and vole abundance were not recorded (see supporting information Fig. S2). However, vole trapping carried out in the same areas during the study period detected a high vole density at the landscape level, maintained until summer, along with high kestrel productivity (unpublished data). Alternatively, poor body condition could be explained by breeding parents exposed to bromadiolone perhaps being impaired in their hunting abilities, resulting in reduced food provisioning to nestlings. It is clear that additional research is needed to understand the link between bromadiolone exposure and chick condition. To establish that the use of bromadiolone on crop fields is associated with the bromadiolone concentration in vole-feeding nestlings of avian predators, and to derive the potential influence on body condition, temporally and spatially replicated quantification of how and how much bromadiolone has been used in the field is required. We did not measure the use of bromadiolone, so the generalisation of our results has to be done carefully. If findings are validated in relation to the use of the compound in crops, the in-crop use of bromadiolone should be more restricted than is currently the case. In such a case, other management alternatives in the targeted crops should be scientifically assessed, including their cost/benefit balance in financial and ecological terms.

We cannot rule out the possibility that secondary exposure occurs in other vole predators, or even non-predator species. Other species feed on voles and some, such as weasels (*Mustela nivalis*) or barn owls (*Tyto alba*), are considered specialist predators on this species. Thus, we could expect similar effects in their offspring.

Similarly, secondary poisoning may occur in generalist predators with opportunistic diets, such as red kites (*Milvus milvus*) or buzzards (*Buteo buteo*), which often forage close to farm buildings and on agricultural lands.[42] The negative effect of SGARs on generalist species is reinforced by the fact that SGARs have been detected in 62.8% of 344 dead individuals of different species of raptors in Spain.[16]

4.1 Management considerations

A similar prevalence of bromadiolone in kestrel chicks was found in both areas where the study was carried out. As bromadiolone was provided only in one area the year of the study (BC), our results suggest that bromadiolone has been used in VC without official distribution. It is important to highlight that, while some crop damage occurred in the BC area where SGARs were officially provided to farmers, we did not find evidence of crop damage in the VC area (authors' unpublished results). Thus, we cannot rule out that SGARs can be used as biocides in villages, houses, gardens, barns or livestock shelters, potentially increasing the exposure of bromadiolone to breeding kestrels. The spatial and temporal distribution of its use are little known in our study area (but see supporting information Fig. S2) and are therefore beyond the scope of this study. Regardless of its use, our results confirm that bromadiolone concentration is detectable in the blood of non-target species during their development, and that this might reduce their body condition. Further research is required about how and to what extent the concentration levels we describe in this study can be accumulated in the liver of individuals, what might increase the persistence of bromadiolone in non-target species and the conservation implications beyond the time of use of SGARs.[13]

In conclusion, we confirm that bromadiolone can be detected in nestlings of common kestrels, most likely owing to the consumption of poisoned voles, the main prey for kestrels in the study area. We cannot rule out that the use of bromadiolone as biocide could partially explain our results, but given the previous use of bromadiolone as pesticide to control vole damage to crops, we consider the latter as a likely explanation. Further research is required to explore the effect that bromadiolone exposure of breeding females could have on the composition and quality of the eggs they lay. We also found that nestlings in worse body condition and with lower body mass had detectable concentration levels of bromadiolone, and this raises a conservation concern about the use of this rodenticide when bromadiolone is used to minimise the damage that voles cause to crops, even with restricted use or disposal within burrows. The provisioning of nest boxes to facilitate the breeding of common kestrels and barn owls hoping to reduce crop damage by rodents is common in some countries, such as Israel,[9] and this practice is also spreading in north-western Spain. The use of SGARs is particularly inappropriate in areas where breeding of avian vole predators is facilitated, as most of them are of conservation concern and protected by law. Therefore, we encourage the use of alternative management tools. Our results add to a growing body of evidence supporting the effects of these toxicants on wildlife.[43] The EU perhaps should consider additional restrictions for the use of bromadiolone, particularly in areas important for conservation of European agrarian biodiversity, such as agrarian lands in north-western Spain, the target of high financial inputs from conservation-related funds or agri-environmental subsidies from the EU.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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