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Risk assessment of pesticide seed treatment for farmland birds using refined field data

Ana Lopez-Antia*, Jordi Feliu, Pablo R. Camarero, Manuel E. Ortiz-Santaliestra and Rafael Mateo

Instituto de Investigación en Recursos Cinegéticos (IREC) CSIC-UCLM-JCCM, Ronda Toledo s/n, 13071 Ciudad Real, Spain

Summary

1. Due to reductions in winter food resources, newly sown cereal seeds have become a key component of many bird species' diets, but these seeds are often treated with pesticides that may cause toxic effects. To complete an appropriate risk assessment, data on treated seed toxicity need to be combined with information about the risk of exposure of birds in the field and the factors that modulate such exposure.

2. We studied the abundance of pesticide-treated seeds available for birds in the field, the pesticides and their concentrations in treated seeds, and the bird species observed in the field that were feeding on these pesticide-treated seeds. The exposure of red-legged partridge to treated winter cereal seeds was characterized through the analysis of crop and gizzard contents of hunted individuals (n = 189). Moreover, we measured the contribution of cereal seeds in the autumn–winter diet of partridges in order to assess the potential risk of exposure to pesticide-treated seeds.

3. Density of treated seeds on the soil surface after sowing ($11{\cdot}3 \pm 1{\cdot}2$ seeds m^{-2} in the centre of field and $43{\cdot}4 \pm 5{\cdot}5$ seeds m^{-2} in the headlands) was enough to provide, in an area between 6 and 50 m^2, doses of six active ingredients above those indicating acute (i.e. a dose capable of killing 50% of individuals of a sensitive species) and / or chronic (no observed effect level) toxicity.

4. Up to 30 bird species were observed consuming treated cereal seeds in recently sown fields. Corn bunting was identified as an appropriate focal passerine species for the risk assessment of pesticide-treated seeds.

5. We found that treated seeds were an important route of pesticide ingestion for red-legged partridge; pesticide residues (six fungicides and two insecticides) were found in 32·3% of crops and gizzards. Cereal seeds represented more than half ($53{\cdot}4 \pm 4{\cdot}3$%) of total biomass consumed by partridges from October to February.

6. *Synthesis and applications*. The field exposure data combined with previous studies about the toxicity to partridges of using pesticide-treated seeds point to an unacceptable risk of this practice to farmland birds. Our results suggest that the prophylactic use of pesticide-coated seeds should be avoided, with the approval of this treatment considered on a case-by-case basis and accompanied with specific measures to minimize risks of adverse effects on avian communities.

Key-words: agriculture, focal species, fungicides, high tier assessment, insecticides, pesticides, red-legged partridge, seed coating, treated seeds

Introduction

In Europe, changes in the agricultural policy designed to increase crop production have happened since the middle of the last century, leading to the decline of populations of many farmland birds (Donald, Green & Heath 2001; Stoate *et al.* 2001). Reductions in winter food resources, due to the replacement of overwinter cereal stubble and fodder crops by winter-sown cereal, is an important factor to explain these declines (Siriwardena *et al.* 2000, 2007).

*Correspondence author. E-mail: ana.lopezantia@uclm.es

These changes in land use have resulted in changes in birds' diet, making winter cereal a key component of many species diet (Browne & Aebischer 2003; Robinson 2004; Perkins, Anderson & Wilson 2007). Cereal seeds used for sowing are often treated with pesticides that are ingested by birds along with the seed and may cause toxic effects (Prosser & Hart 2005), which, depending on the targeted function and the degree of effect, can compromise survival or reproduction, and therefore affect population dynamics (Mineau & Whiteside 2013).

Seed treatment allows for placing the pesticide exactly where it is needed and reduces the risk of exposure for farmers. However, pesticide-treated seeds may represent an ecological trap for birds and mammals; sown seeds are often not properly buried and spillages occur frequently, so seeds can remain accessible for granivorous vertebrates that use them as a food source, especially in periods of shortage of other food sources (i.e. autumn and winter). The ingestion of treated seeds results in the ingestion of large amounts of pesticides in a short time. In the last century, seed coating, especially with insecticides, was estimated to be responsible for up to 50% of cases of lethal poisoning of wildlife caused by approved pesticides in European countries (De Snoo, Scheidegger & De Jong 1999).

In Spain, 12 chemicals are currently registered for cereal seed treatment (MAGRAMA 2015a), 11 fungicides and 1 insecticide (see Table S1 in Supporting information). The neonicotinoid insecticide imidacloprid, one of the most used insecticides in the world (Jeschke *et al.* 2011), has proven to be highly lethal to some birds at the dose used for seed treatment (Lopez-Antia *et al.* 2015a). Fipronil is another widely used insecticide for seed treatment (i.e. maize and sunflower); its effects on reproduction have been studied on zebra finch *Taeniopygia guttata* V. (Kitulagodage, Buttemer & Astheimer 2011) and red-legged partridge *Alectoris rufa* L. (Lopez-Antia *et al.* 2015b). Recently, the European Union declared a moratorium on the use for seed coating of the most important neonicotinoids (that can, nevertheless, still be used for treatment of winter cereal; Regulation 485/2013) and fipronil (Regulation 781/2013) due to their toxicity on pollinators. The risk of seeds treated with these insecticides to granivorous birds was recognized as requiring urgent attention. Toxic effects on birds have also been reported for the most used fungicides, like dithiocarbamates and triazoles. These compounds are proven or suspected to be endocrine disruptors (reviewed in McKinlay *et al.* 2008) and to imbalance the antioxidant system, which may end up in disturbances of the immune system or the reproductive function (Lopez-Antia *et al.* 2015a). However, to complete an appropriate risk assessment, these data on treated seed toxicity need to be combined with information about the risk of exposure of birds in the field and the factors that modulate such exposure.

We hypothesized that toxicological benchmarks for health effects could be achieved by birds that use treated seeds as a food source. Therefore, we aimed at characterizing the exposure of farmland birds to pesticide-coated seeds in the wild, and at estimating the risk of poisoning of animals as a consequence of such exposure. With this purpose, we structured the study in three parts: (i) we characterized the availability of pesticide-coated seeds in the field through the study of the abundance of treated seeds in cereal fields, the used pesticides and the concentration found on seeds. We also considered variables that influenced seed abundance, such as the drilling and post-drilling techniques (Davis 1974; Pascual *et al.* 1999b; De Snoo & Luttik 2004), the field section (Murton & Vizoso 1963; Tamis *et al.* 1994; De Snoo & Luttik 2004; Prosser & Hart 2005) and the presence of accidental spills of pesticide-treated seeds (Prosser & Hart 2005; EFSA 2009). (ii) We recorded which bird species were found feeding on seeds along with the frequency at which each species was detected. The collected information was useful to select an appropriate focal species (a species that actually occurs in the crop when the pesticide is being used). (iii) We estimated the exposure of a known focal species, the red-legged partridge, to pesticide-treated winter cereal seeds; we analysed the crop and gizzard content of hunted partridges to identify ingested pesticides and estimate the level of exposure. We also studied partridges' diet composition during sowing time in order to elucidate how sown cereal seeds contributed to the diet relative to other diet items.

Materials and methods

AVAILABILITY OF PESTICIDE-TREATED SEEDS FOR BIRDS AFTER SOWING

The availability of sown seeds was studied in Spain at fields of the Northern and Southern Plateaus. In the Northern Plateau, we sampled fields from the provinces of Soria, Segovia and Valladolid. In the Southern Plateau, we sampled fields around Tablas de Daimiel National Park (province of Ciudad Real), a continental wetland added to the Montreux record in 1990 and considered an UNESCO Biosphere Reserve and Special Protection Area under EC directive 92/43/CEE. This wetland is particularly important for breeding and wintering waterfowl and is surrounded by agricultural areas dominated by cereal crops, vineyards and olive groves. In both the Northern and Southern Plateaus, barley is the predominant winter cereal (from 59% to 67% of the surface occupied by cereal fields) followed by wheat (17–29%), oat (13–14%), rye (1–5%) and triticale (0·1–1%; MAGRAMA, 2009).

Sampling in the Northern Plateau was performed in four fields sown in October 2012 with wheat. Sampling around Tablas de Daimiel was performed in two fields sown in October 2012 with barley, and in 42 fields sown in autumn 2014 (from October to December) with one of the following cereals: wheat (3), oat (8), barley (29) and triticale (2). For Tablas de Daimiel fields sampled in 2014, we recorded sowing date, sowing technique (sowing using a fertilizer + harrowing, precision drilling, and precision drilling + rolling) and weather conditions (rain from sowing to sampling days, both included). According to farmers, sowing technique is generally selected as a function of the type crop that was formerly present in the field. We determined seed availability on the soil surface within the 48 h following sowing by placing a squared frame (1 m^2) and counting the visible seeds. In each field, we took five measures from the field centre and four measures from the headland (i.e. at a maximum distance of 15 m from the field edge). The location of the first measure in each field section was random, and the following measures were taken every 15 m. We did not make any systematic spill search, but when a spill was detected, we estimated the number of seeds in the spill; these spill counts were, however, excluded from the calculations of seed densities in the field centre or in the headlands. We collected a sample of sown seeds from each field for pesticide analysis. Finally, in order to study for how long seeds remained on the soil surface until disappearance, we monitored the number of seeds in nine newly sown headland plots in nine different fields during 28 days. We selected headland plots to minimize the impact of repeated visits to the crop.

USE OF SOWN FIELDS BY BIRDS

A total of 89 bird censuses were performed in 23 of the studied fields around Tablas de Daimiel shortly after finishing sown seed sampling (from October to December). Each census covered an area of approximately four hectares per field and was conducted during 30 min between 08·30 and 10·30 h. All the species feeding in the sown field were counted and the feeding behaviour recorded. Finally, only species for which we visually confirmed that were picking seeds or shoots up during the censuses were included in the final data base and considered for statistical analysis.

EXAMINATION OF CROP AND GIZZARD CONTENTS OF HUNTED RED-LEGGED PARTRIDGES

The determination of pesticides in crop and gizzard contents (hereafter referred as digestive content), along with partridge diet, was based on the analysis of 189 upper digestive tracts donated by hunting associations and coordinated by the Spanish Royal Federation of Hunters. Partridges were hunted between 24 October 2010 and 28 February 2011 and came from 51 locations from seven provinces in Spain: Burgos (18 locations), Valladolid (9 locations), Soria (2 locations), Madrid (4 locations), Toledo (2 locations), Murcia (10 locations) and Lleida (6 locations) (see Table 3; Fig. S1). At each location, we obtained the percentage of the territory used for winter cereal cultivation from land-use data provided by the Spanish Ministry of Agriculture, Food and Environment (MAGRAMA 2015b; see Table 3). Agriculture is the dominant land use in most of the localities where partridges were hunted. The average (±standard deviation) percentage of territory used for winter cereal cultivation was 45·0 (±31·9)%. Samples were frozen by hunters and shipped to our laboratory, where digestive tracts were opened and the content was weighed. We also determined the percentage of each food category (i.e. green plant material, weed seeds, winter cereal seeds, insects and others) in the total biomass content.

PESTICIDE ANALYSIS IN SOWN SEEDS AND DIGESTIVE CONTENT OF PARTRIDGES

The determination of pesticides in sown seeds and digestive contents was performed by LC-MS. We determined a total of nine active ingredients plus piperonyl butoxide, a synergist added to some fungicide formulations. About one gram of sample was extracted with 5 mL of acetonitrile during 1 min of vortex, 5 min of sonication and 1 min again of vortex. The extract was filtered through a nylon syringe filter of 0·2 μm and was analysed as described in Lopez-Antia *et al.* (2013) with some modifications. Pesticides were detected using positive and negative ions monitored with the following MM-ESI source settings. Nebulizer pressure was set at 35 psi, drying gas flow was 8 L min^{-1}, drying gas temperature was 250 °C, vaporizer temperature was 200 °C, capillary voltage was 3500 V in positive and 3000 V in negative, and charging voltage was 1000 V for both. The monitored ions for each pesticide along with the retention time, the fragmentation voltage for each ion and the obtained recoveries for each chemical are shown in Table S2. Obtained recoveries varied between 78·8% and 140·1%. Figure S2 shows the chromatograms of the monitored compounds in both voltage modes. Stock solutions of pesticide standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Calibration curves were performed with concentrations of the three pesticides ranging from 0·25 to 2 μg mL^{-1} in acetonitrile. Detection limits were between 0·18 ng g^{-1} for metalaxyl and 36·7 ng g^{-1} for thiram (Table S2).

DATA ANALYSIS

To identify which factors influenced the number of surface seeds found in a field we performed linear mixed models that included the field identification as a random effect and the sowing technique, type of cereal and rain events (binomial) along with their interactions as factors, and the days from sowing as a covariate. These parameters were subsequently removed from the models, with the drop-one method, when found to be non-significant. The number of surface seeds were log(x + 1)-transformed to obtain a normal distribution of the model residuals. Normality of the model residuals was checked by Kolmogorov–Smirnov tests. Differences in the number of surface seeds between the field centre and the headlands of the fields around Tablas de Daimiel were analysed with a paired t-test. The disappearance of seeds in the nine monitored plots over time was adjusted to a probit model in order to analyse mean time to disappearance.

In the case of digestive content data, we performed general linear models (GLM) with the percentage and the amount (g) of cereal seeds in digestive content as the dependent variables and the province as a factor to detect geographical differences in cereal seed ingestion. The percentage of cereal seeds in the digestive content was square-root-transformed and the amount was log (x + 1)-transformed. In order to analyse which environmental factors influenced the occurrence of pesticides in the digestive contents, we ran multiple regressions using the amounts (g) and percentages of each type of food found in crops and gizzards as explanatory variables. The influence of the percentage of the territory used for winter cereal cultivation on the presence or absence of pesticides in the digestive contents of partridges hunted at each site was analysed with a binary logistic regression.

We calculated the estimated daily intake (EDI) of pesticides due to ingestion of treated seeds in red-legged partridges. We calculated EDI considering a daily food intake of 25 g (Lopez-Antia, Ortiz-Santaliestra & Mateo 2014) and a mean body weight of 400 g. We considered two percentages of cereal seeds in biomass of diet corresponding to the mean and maximum values observed in our study (53·4 and 89·3%, respectively, see Results).

The theoretical pesticide concentrations in treated seeds were obtained from recommended application data (MAGRAMA 2015a) and used to calculate EDI. We compared the obtained EDI values with the following threshold values:

1. Acute hazardous dose 5% (HD_5). This value was obtained from Mineau *et al.* (2001). It corresponds to the dose of pesticide (mg kg_{bw}^{-1}) estimated to lead to 50% of mortality in a species on the top 5% of a species sensitivity distribution model (i.e. more sensitive than 95% of the other bird species).

2. Chronic NOEL. The highest dose level at which no effects were seen (mg kg_{bw}^{-1} day^{-1}) after a long-term exposure. These values were obtained from the EFSA scientific reports for each pesticide (http://www.efsa.europa.eu/en/pesticides/pesticidessc-docs.htm). When more than one value was provided (for the same or for different species), we calculated the mean of all the values.

Results

AVAILABILITY OF PESTICIDE-TREATED SEEDS IN RECENTLY SOWN FIELDS

Seed availability was influenced by the sowing method and the location within the field (Table S3). There were more seeds on the surface in the headland (43·4 ± 5·5 seeds m^{-2}) than in the field centre (11·3 ± 1·2 seeds m^{-2}) (t_{151}=−4·67, P < 0·001; Table S3). Surface seed density was influenced by the sowing technique in the field centre but not in the headlands. In the field centre, fields drilled and then rolled presented more surface seeds than fields just drilled ($F_{2,24·7}$ = 3·6, P = 0·04; Table S3). The type of cereal (wheat, oat, barley and triticale seeds), sowing date, time elapsed from sowing until sampling (0–7 days), or rainfall occurring before the sampling did not account for differences in seed density. We detected some spills on the roads near the fields (where the seed-drills are filled) or in the headlands (where seed-drills turn). We have not taken into account these spills when calculating the mean number of seeds remaining on the soil surface (Table S3), but they contained between 142 and 10 000 seeds m^{-2} (n = 8).

Six pesticides, mostly fungicides, were detected in the seeds collected from recently sown fields (Table 1). 55·2% of the seed samples contained detectable levels of pesticides, with tebuconazole being the most frequently found active ingredient.

Daily monitoring of selected plots revealed that times to disappearance (and 95% confidence intervals) of 10%, 50% and 90% of seeds were 2·45 (0·24–4·01), 10·93 (9·77–12·14) and 19·41 (17·61–21·90) days, respectively (Fig. 1). The initial number of seeds on the surface (ranging from 420 to 14 seeds m^{-2}) did not correlate with the days elapsed until complete disappearance.

USE OF SOWN FIELDS BY BIRDS

During the sowing period, 30 species were observed feeding on sown cereal seeds (Table S4). Corn bunting *Miliaria calandra* L. was the species most frequently observed during censuses (41·5%), whereas magpie *Pica pica* L. was the one found in a higher number of sampled fields (69·6%). Considering the fields with presence of each species, greylag goose *Anser anser* L. was the most abundant species, with a mean density of 32 individuals per hectare. Besides cereal seeds, greylag goose and common crane *Grus grus* L. were observed later ingesting cereal shoots. No dead birds were found during the censuses.

PARTRIDGES' CROP AND GIZZARD CONTENT ANALYSIS

We detected pesticide residues in the digestive content of 32·3% of the analysed red-legged partridges (Table 2), including insecticides in a 3·7% of the analysed samples and fungicides in a 29·6%. As observed in seeds, tebuconazole was the most frequently detected pesticide (19·1% of the samples). The digestive contents in which detectable amounts of fungicides were detected always contained winter cereal seeds. However, none of the digestive contents in which detectable amounts of insecticides were detected contained winter cereal seeds (Table 2).

The geographical distribution of the analysed digestive contents showed that Burgos was the province with the highest pesticide prevalence (56·7%; Table 3, Fig. S1). In most samples (55 out of the 61 positive samples), we found a single pesticide, but we also found five samples

Table 1. Presence of pesticides and synergists in seed samples in the field after autumn sowing in Spain (n = 67)

		Pesticide concentration (ng g^{-1})			
Chemical	*N* of positive samples (%)	Mean	SD	Minimum	Maximum
Fungicides					
Triticonazole	5 (7·5)	4735	7039	48·78	16 554
Fludioxonil	1 (1·5)	2985			
Tebuconazole	20 (29·9)	3183	4097	42·4	14 724
Difenoconazole	6 (9·0)	14 814	9836	1161	24 433
Insecticides					
Fipronil	1 (1·5)	25			
Synergists					
Piperonyl butoxide	4 (6·0)	272	21	256	296

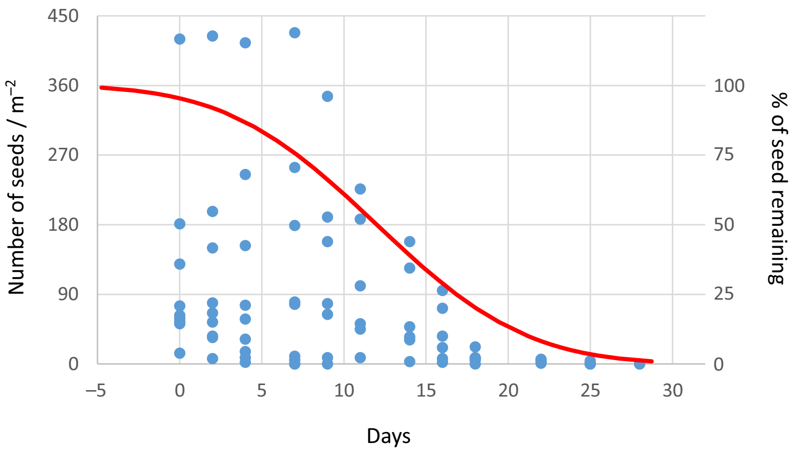

Fig. 1. Evolution of the number of seeds in the nine sampled headland plots during the 28 sampling days (circles, left scale). The probit function (line, right scale) shows the temporal evolution of the estimated percentages of seeds remaining in the field; the adjustment of the probit function was significant ($\chi^2 = 3076{\cdot}37$, 77 d.f., $P < 0{\cdot}001$).

Table 2. Pesticides found in the digestive contents of wild partridges. For each pesticide, the following information is provided: number of samples in which it was detected (*N*+), percentage of the total samples analysed in which it was detected (%+), mean (and SD) concentration, maximum concentration (Max) and the food categories found in the digestive contents

			Digestives (*N* = 189)					
Use	Chemical family	Chemical	*N*+	%+	Mean (μg g^{-1})	SD	Max (μg g^{-1})	Digestive content*
Insecticide	Neonicotinoids	Imidacloprid	6	3·1	0·082	0·031	0·128	GPM; I; Ot
	Phenyl pyrazoles	Fipronil	1	0·5	0·007		0·007	GPM; WS
Fungicide	Azoles	Flutriafol	4	2·1	0·035	0·013	0·046	WCS; I
		Triticonazole	11	5·7	0·010	0·003	0·0146	WCS; GPM; WS
		Tebuconazole	36	19·1	0·421	1·650	9·313	WCS; GPM; WS; Ot
		Difenoconazole	4	2·1	1·038	1·834	3·790	WCS; GPM; Ot
	Dithiocarbamates	Thiram	2	1·0	0·177	0·177	0·261	WCS; GPM
		Fludioxonil	2	1·0	0·030	0·022	0·046	WCS; Ot
Synergist		Piperonyl butoxide	2	1·0	0·221	0·116	0·303	WCS

*Type of food: green plant material (GPM); weed seeds (WS); winter cereal seeds (WCS); insects (I); others (Ot).

that contained two pesticides and one that contained three pesticides (Fig. S1).

Winter cereal seeds represented a 53·4% of the biomass found in the digestive content of red-legged partridges (mean ± value: 2·4 ± 0·3 g) (Table 3). Significant differences among provinces were observed for both the percentage ($F_{6,104} = 3{\cdot}7$, $P = 0{\cdot}002$) and the amount (g) ($F_{6,104} = 3{\cdot}5$, $P = 0{\cdot}003$) of cereal seeds in digestive content. The occurrence on partridges' digestive contents of barley and wheat was very similar (17% and 20%, respectively) based on the visual identification of the entire seeds. Other observed food items were sunflower seeds, maize and olives. Olives and maize were quite important in Lleida, where they represented 26·8% of ingested biomass and were present in 39·1% of the digestive contents. The presence of pesticides in the digestive content of partridges was not explained by the amount of any type of food in the digestive content but was positively explained by the percentage of cereal seeds in total biomass of the digestive content ($\beta = 0{\cdot}92 \pm 4{\cdot}28$, Wald = 3·9, d.f. = 1, $P = 0{\cdot}04$) and was also positively explained by the percentage of the territory used for winter cereal cultivation in each location ($\beta = 0{\cdot}10 \pm 0{\cdot}05$, Wald = 4·1, d.f. = 1, $P = 0{\cdot}04$).

We have used the mean (53·4%) and maximum (89·3%, corresponding to Valladolid province) percentages of winter cereal consumption by red-legged partridges during sowing periods to calculate the EDI of pesticides approved for cereal seed treatment in Spain. In the most conservative case (i.e. taking the mean percentage of winter cereal consumption into account), acute HD_5 and chronic NOEL would be exceeded by the EDI in three and six pesticides, respectively. If we consider the less conservative case (using the maximum percentage of winter cereal consumption), the EDI will be above the acute HD_5 and chronic NOEL for four and six pesticides, respectively (Table 4).

Discussion

Our results show that the use of pesticide-coating treatments in winter cereal seeds poses a risk for farmland birds, which can ingest toxic doses of pesticides when feeding on these seeds during the sowing season. This risk was confirmed by a geographically wide analysis of digestive contents of red-legged partridges, 32·3% of which showed detectable levels of different pesticides.

Table 3. Pesticide prevalence, total food biomass and contribution of different food items in the digestive contents of red-legged partridges hunted in Spain

Province	% Territory for WC cultivation (mean ± SE)*	Sample (*n*)	With food (*n*)	With pesticides (*n*)	Pesticide prevalence (%)[†]	Biomass (g) (mean ± SE)	Type of food (%)[‡] (mean ± SE)				
							WCS	WS	GPM	Insects	Other
Madrid	26·7 ± 4·9	20	6	2	10	6·3 ± 2·9	44·4 ± 19·1[ab]	14·0 ± 14·0	41·7 ± 20·1	0·0 ± 0·0	0·0 ± 0·0
Toledo	52·6 ± 9·8	3	2	0	0	7·1 ± 4·8	50 ± 50[ab]	49·6 ± 49·6	0·4 ± 0·4	0·0 ± 0·0	0·0 ± 0·0
Burgos	61·9 ± 3·0	60	43	34	56·7	3·4 ± 0·5	60·7 ± 6·4[b]	20·8 ± 5·3	9·6 ± 3·7	1·4 ± 1·4	7·4 ± 3·7
Valladolid	74·1 ± 6·4	17	14	3	17·6	4·5 ± 0·5	89·3 ± 7·13[c]	3·6 ± 3·6	3·6 ± 1·9	0·0 ± 0·0	3·6 ± 3·5
Soria	36·0 ± 9·9	7	6	1	14·2	3·9 ± 1·5	47·2 ± 18·5[ab]	22·2 ± 16·5	30·5 ± 16·3	0·0 ± 0·0	0·0 ± 0·0
Lleida	25·6 ± 5·3	30	23	14	46·7	4·5 ± 0·7	42·1 ± 10·1[ab]	1·3 ± 1·3	30·0 ± 8·6	0·0 ± 0·0	26·8 ± 8·2
Murcia	9·9 ± 0·6	52	17	7	13·5	2·3 ± 0·7	26·5 ± 10·3[a]	18·6 ± 9·0	30·8 ± 10·2	5·9 ± 5·9	12·4 ± 6·5
Overall		189	111	61	32·3	4·5 ± 0·5	53·4 ± 4·3	14·5 ± 2·9	19·0 ± 3·2	1·4 ± 1·0	10·8 ± 2·6

[a,b,c]Different letters indicate differences between localities in the ratio of winter cereal seeds. WC, winter cereal; WCS, winter cereal seeds; WS, weed seeds; GPM, green plant material.
*The mean % of the territory used for winter cereal cultivation in each province was calculated with the values of % of territory used for winter cereal cultivation of each locality where partridges were hunted within that province.
[†]Pesticide prevalence in each province was calculated over the total sample, independently of the presence of food items.
[‡]Relative to the total biomass of the examined digestive content.

Table 4. Estimated daily intake doses of pesticides by red-legged partridges considering the mean (53·4%) and maximum (89·3%) percentage of winter cereal seed in diet biomass

Chemical	Application rate (mg kg_{seed}^{-1})	Daily exposure (mg kg_{bw}^{-1}) for 53·4% seeds in diet	Daily exposure (mg kg_{bw}^{-1}) for 89·3% seeds in diet	Acute HD_5* (mg kg_{bw}^{-1})	Chronic NOEL[†] (mg kg_{bw}^{-1} day^{-1})
Copper oxychloride	900	30·04[b]	50·17[a,b]	49·95	16·88
Mancozeb	300	10·01	16·72	710·95	54·4
Maneb	1400	46·72[b]	78·05[b]	345·34	6·7
Thiram	1750	58·41[a,b]	97·56[a,b]	36·81	9·6[‡]
Metalaxyl	1017	33·94	56·70	89·09	900
Fludioxonil	20	0·67	1·11	208·12	37·2
Difenoconazole	60	2·00	3·34	207·13	9·71
Flutriafol	62·5	2·08	3·48	481·7	35·8
Tebuconazole	375	12·51[b]	20·91[b]	347·3	5·8
Triticonazole	50	1·67	2·79	232·29	19·5
Propiconazole	277	9·24	15·44	296·8	300
Imidacloprid	700	23·36[a,b]	39·02[a,b]	8·4	19·35
Fipronil	1250	41·72[a,b]	69·69[a,b]	1·47	0·88

[a,b]These values are higher than the acute HD_5 or chronic NOEL, respectively. bw, body weight.
*Acute HD_5 is the amount of pesticide estimated to cause 50% of mortality in a species more sensitive than 95% of all bird species. Data obtained from Mineau *et al.* (2001).
[†]No observed effect level. Mean of all the values provided (for the same species or for different species) for long-term applications. Data obtained from EFSA scientific reports.
[‡]Datum from the US EPA, not available from EFSA.

In the analysis of factors determining the availability of pesticide-treated seeds for birds, we found that the surface concentrations were 11·3 seeds m^{-2} in the field centre and 43·4 seeds m^{-2} in the headland. These concentrations are similar to those found in eastern England (<11 seeds m^{-2} in the field centre and 45 seeds m^{-2} in headlands; Pascual *et al.* 1999a) and lower to those found in the Netherlands (18·5–40 seeds m^{-2} in the field centre and 89 seeds m^{-2} in headlands; Tamis *et al.* 1994; De Snoo & Luttik 2004). This difference between headland and field centre can be due to a poorer soil condition (because of more intense traffic), double drilling, or unintentional spills of seeds at turning happening at the headlands (Tamis *et al.* 1994; Pascual *et al.* 1999a; De Snoo & Luttik 2004). Because headlands are the field sites most used by birds (Prosser & Hart 2005), seed densities left on the surface of the headlands must be taken into account when estimating the risk of pesticide-treated seeds on farmland birds. Other considered factors, except for rolling, did not influence the concentration of surface seeds.

We found up to 30 bird species foraging in cereal sown fields, with corn bunting, crested lark *Galerida cristata* L., magpie, Spanish sparrow *Passer hispaniolensis* T. and skylark *Alauda arvensis* L. being the species more frequently observed. These results suggest that corn bunting could be a good focal species to estimate the risk from the ingestion of cereal seeds treated with pesticides. This species forages almost exclusively (98%) on cereal seeds in winter (Robinson 2004; Perkins, Anderson & Wilson 2007). The appropriateness of corn bunting as focal species for cereal fields from southern Europe was also pointed by Dietzen *et al.* (2014) after evaluating several candidates. Moreover, small birds like corn buntings (bw: 38–55 g) tend to be more sensitive to acute poisoning by pesticides than large ones (Mineau *et al.* 2001). It is noteworthy that four of the five farmland bird species that were more frequently detected consuming recently sown cereal seeds present negative long-term population trends in Spain and/or Europe (EBCC, 2014; MAGRAMA, 2011; Table S4).

As far as we know, this is the first paper to use the digestive content of hunted birds to estimate the prevalence of pesticide ingestion, specifically through pesticide-treated seeds. Our results in this context show that winter cereal seeds are an important food source for wild red-legged partridges, but also an important route of pesticide ingestion during the sowing period. We confirmed the presence of pesticides in 32·3% of the analysed digestive contents. We must note, however, that sampling hunted partridges could lead to an overestimate of the prevalence of pesticide ingestion, as intoxicated animals could have been positively selected if their chances of being shot were greater as a result of pesticide ingestion. On the other hand, here we only monitored 9 out of the 13 pesticides approved for use as seed treatment in Spain at the time that partridges were hunted (only 12 currently after fipronil ban). The exposure to some other pesticides (i.e. maneb, mancozeb, propiconazole and copper oxychloride) remained unknown.

The prevalence of pesticide ingestion varied significantly between localities, but the site with the highest prevalence (Burgos, 56·7%) was not the site where cereal seeds constituted the most important portion of the diet (Valladolid, 89·3%). This could be due to differences among sites either in the use of treated seeds or in the applied pesticides, considering that we did not monitor four of the 13 used pesticides. In fact, we found two partridges from Valladolid with coloured seeds in their crops but we could not detect the used pesticide. In spite of this lack of correspondence between the sites with highest pesticide prevalence and with highest ingestion of cereal seeds, we found that the percentage of winter cereal seeds in the digestive contents, together with the percentage of area dedicated to winter cereal cultivation, were the main factors explaining the presence of pesticides in crops and gizzard contents. This relationship suggests that the use of pesticides for seed treatment is relatively uniform across the study sites and that spatial availability would be the main determinant of the risk of pesticide ingestion from treated seeds. Although barley is a predominant winter cereal in the area (MAGRAMA, 2009), its prevalence on partridges' digestive contents is very similar to that of wheat (17% and 20%, respectively). This could indicate a preference for wheat, but we should have more precise information about the availability of each cereal at the hunting sites to confirm such hypothesis that may have implications for risk assessment.

According to the literature, combinations of pesticide have, at least, an additive effect, and in some cases, especially those involving insecticides, the simultaneous exposure to several pesticides could act synergistically, with reported toxicity increases of up to 100-fold (Thompson 1996). In our study, we found up to three different pesticides within a single partridge and one of the mixtures included the insecticide imidacloprid. These combinations could be due to the ingestion of different food items treated with different products or to the ingestion of a food item treated with various products. The study of the toxicity of pesticide mixtures in wildlife is a very important issue as well as one of the most challenging tasks to tackle.

The calculation of the estimated daily intake of pesticides by red-legged partridges and the comparison of these values with the toxicological data found in the literature reveals a worrying situation for wild red-legged partridges and other farmland birds. Apart from insecticide exposures, which seemed to be unrelated to seed ingestion, thiram treated seeds could represent a risk of acute intoxication for partridges (>HD_5) and thiram, copper oxychloride, maneb and tebuconazole treated seeds could represent a risk of chronic intoxication (>NOEL). Moreover, tebuconazole, a product with a very low chronic NOEL, appeared in 19·1% of the analysed digestive contents. The chronic exposure of birds is likely to occur as autumn sowing season (long-cycle winter cereal) lasts from October to December and late winter sowing season (short-cycle winter cereal) occurs during late January and February, extending to the beginning of the breeding season. Moreover, here we found that treated seeds remained at high densities on the surface of fields during more than ten days after sowing, so risk assessment based on long-term exposures (ideally equivalent to the period of time during which coated seeds are available in the field) can be appropriate for this type of pesticide use.

This risk assessment is supported by the observed availability of pesticide-treated seeds in recently sown fields. Considering the mean density of unburied seeds (headland: 43·4 seeds m^{-2}; field centre: 11·3 seeds m^{-2}), we can estimate that partridges could obtain 2·17 g of cereal seeds m^{-2} at the headlands and 0·565 g of cereal seeds m^{-2} at the field centre. With these densities and an average daily food intake of 25 g per partridge, the amounts of pesticide calculated in Table 4 as daily exposure (for 53·4–89·3% of food biomass) could be achieved by one partridge in approximately 6–20 m^2 of headland or 24–50 m^2 of field centre, depending on the pesticide used.

APPLIED IMPLICATIONS

The present study demonstrates that the use of pesticide-treated seeds constitutes an important way of exposure of farmland birds to pesticides and that there exists a potential risk for these birds to suffer toxic effects from ingestion of treated seeds in the wild. Future management decisions in Europe, taken under an Integrated Pest Management (IPM) strategy (European Directive 128/2009/EC), should minimize the use of pesticides and limit it to those cases in which it is economically justified and no alternatives exist. Some of the pesticide seed treatments mentioned in this study are known to be used in a prophylactic way (Goulson 2013), which is not economically justified because the cost of the damage they prevent is lower than the total cost of the prophylactic protection (Furlan & Kreutzweiser 2015). The fact that most pesticide treatments of seed are not consistent with IPM strategies suggests that they should be avoided. Only a case-by-case identification of cases in which these treatments fit into the IPM principles should lead to its punctual approval. In these cases, specific treatments should be selected among those that pose the lowest risk to wildlife, with special consideration of side effects on granivorous animals that consume sown seeds.

When seed treatments are used, preventing birds from ingesting them would minimize risks. Some strategies like the use of repellents not necessarily prevent poisonings (Pascual, Hart & Fryday 1999c), as even if an initial aversion exists, birds can end up feeding on treated seeds if no alternative sources exist (Lopez-Antia, Ortiz-Santaliestra & Mateo 2014). Mulching crop fields with plastic foil, although avoids the access of birds to seeds, has negative effects on farmland bird populations (Skórka *et al.* 2013). Sown cereal seeds constitute an essential food source for many avian species in agricultural areas during autumn in winter (more than 50% of the ingested biomass according to our study). Even if preventing the access of birds to sown cereals were possible, a proper management of the habitat (e.g. providing non-cropped areas such headlands and fallow fields) would be necessary to guarantee that alternative food sources are available.

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Data accessibility

All data are archived in Dryad Digital Repository http://dx.doi.org/10.5061/dryad.kf245 (Lopez-Antia *et al.* 2016).

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Supporting Information

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

Fig. S1. Origin of partridges of which digestive contents were analysed with the results of the pesticides analysis.

Fig. S2. Chromatograms of the monitored compounds in both voltage modes.

Table S1. Chemicals currently approved for use as cereal seed treatment in Spain.

Table S2. ESI-MS parameters used for pesticide analysis and recovery.

Table S3. Number of seeds m^{-2} remaining in the soil surface in the field centre and headland depending on the sowing method.

Table S4. Bird species observed consuming seeds in cereal fields and their global long-term trends in Europe and Spain.