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Quantification of potential exposure of gray partridge (*Perdix perdix*) to pesticide active substances in farmlands



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HIGHLIGHTS

- 71% of clutches and 13% of coveys are exposed to active substances.
- Partridge clutches/coveys are mostly exposed to 32/3 substances.
- Fungicides (53%), herbicides (25%), and insecticides (16%) dominate.
- Some substances have the potential to present a risk for bird reproduction.
- Complex patterns of exposure emerge from bird habitat use and farming practices.

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ABSTRACT

Estimating exposure of wild birds to plant protection products is of key importance in the risk assessment process evaluating their harmful potential.

In this paper, we propose an ecologically-relevant methodology to estimate potential exposure to active substances (ASs) of a farmland focal bird, the gray partridge *Perdix perdix*. It is based on bird habitat use of fields at the time of pesticide applications. It accounts for spatio-temporal heterogeneity at population and landscape scales.

We identify and quantify the potential exposure to 179 ASs of 140 clutches during pre-laying, laying, and incubation phases, and of 75 coveys. The data come from a large scale field study combining radiotelemetry and a farmer survey. They were collected in 12 different representative sites.

The proportion of clutches potentially exposed to a given chemical was $\geq 5\%$ for 32 ASs; prothioconazole and epoxiconazole ranking first. 71% of clutches were potentially exposed to ≥ 1 AS and 67% to ≥ 2 ASs. Mixtures involved 2 to 22 ASs. They emerged from commercial formulations, tank mixtures, bird habitat use, and combinations. ASs were fungicides (53%), herbicides (25%), and insecticides (16%) used on a variety of crops in April–June, when ground-nesting birds are breeding. The European Food Safety Authority conclusions report a long-term first-tier toxicity-to-exposure ratio (TER_{1t}) < 5 for 11 out of 19 documented ASs, and higher-tier TER_{1t} < 5 for 5 out of 10 ASs. This suggests a potential risk for bird reproduction in farmlands.

Globally 13% of coveys were potentially exposed to 18 ASs during the first month (1–4 coveys per AS).

The use of our field data in future research and risk assessment is discussed.

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1. Introduction

Evaluation of the reproductive risk of plant protection products (hereafter termed “pesticides”) to wild birds is part of the requirements

Abbreviations: AS, active substance; NO(A)EL, no observed (adverse) effect level; TER_{1t} , long-term toxicity-to-exposure ratio.

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to register active substances (ASs) (Regulation 2009/1107/EC). Risk assessment combines toxicity and exposure characterization, in the so-called “long-term toxicity-to-exposure ratio” (TER_{1t}) (Crocker, 2005; EFSA, 2009; Hart and Thompson, 2005; Mineau, 2005; Shore et al., 2005). Toxicity estimates are the lowest no-observed-effect concentrations from standard laboratory tests. Exposure is an estimated theoretical exposure, calculated for different AS/crop and bird scenarios (EFSA, 2009). Exposure is mainly based on diet, and modeled using AS application rate, residue levels in food items and daily food intake rates. In the first-tier assessment, exposure is estimated for a fictive species. A series

of refinements have been proposed in exposure calculations in higher-tier assessment to gain ecological realism, including habitat use, dietary composition, and other behavioral aspects (Crocker, 2005; EFSA, 2009). A phase-specific reproductive assessment (Bennett et al., 2005; Shore et al., 2005) is an additional refinement option recommended in a case-by-case approach (EFSA, 2009). Indeed, the birds and their embryos may differ in their sensitivity to ASs depending upon the phases of reproduction. Furthermore, only a proportion of birds/embryos may be exposed. However, such realistic calculation is data-intensive, needing spatio-temporal data both of bird habitat use and pesticide use. This issue can be overcome by using models (Roelofs et al., 2005). This convenient tool, that can be applied to a series of species/pesticides, requires, however, detailed data on pesticide use (USGS for United States; Engelman et al., 2012), that may not be available depending upon the member states. It is of key importance to identify and quantify exposure of some focal bird species to pesticides. This provides post-authorization safety information to assess population effects – which is challenging (Köhler and Triebkorn, 2013). This issue seems especially essential for bird conservation. Indeed some recent studies (Geiger et al., 2010; Gibbons et al., 2015; Hallmann et al., 2014; Mineau and Whiteside, 2013) suggest that pesticide use would be more involved in the ongoing decline of farmland birds (PECBMS, 2014) than previously reported.

In this context, we collected field data to identify and quantify the potential exposure of farmland birds to ASs. Data were retrieved from a large scale auto-ecological study on the gray partridge *Perdix perdix* (Bro et al., 2013), with a focus on non intentional effects of pesticide use on mortality (Millot et al., 2013, in press) and reproduction. The study was carried out in France in 12 sites representing a range of environmental conditions in order to ensure a robust data set.

The gray partridge is both a typical farmland bird (Aebischer and Kavanagh, 1997) and a representative focal species (Andrade et al., 2012; EFSA, 2009; Petersen, 2013). Its numbers have dramatically declined during the XXth century (BirdLife International, 2013; Kuijper et al., 2009; ECBMS, 2013). This species is highly exposed to pesticides. Birds forage in crops (Birkan and Jacob, 1988; Green, 1984). Adults have an opportunistic omnivorous diet. They feed on leaves, buds, crop grains, weed seeds of wild and cultivated species and a variety of invertebrates. Chicks feed almost exclusively on invertebrates during their first two weeks of life (Bro and Ponce-Boutin, 2004; Green, 1984; Potts, 2012). Three quarters of clutches are laid in crops, mainly in winter cereals but also in peas, sugar beets, potatoes or alfalfa (Bro et al., 2000, 2013).

The study was conducted in France, where agriculture is one of the most intensive in Europe, both in terms of yields and tonnages of pesticides used (FAOSTATS, 2014). The annual production of wheat ranged between 35 and 40 million tons in 2010–2012, with mean yields ranging between 6.2 and 7.6 t/ha.

This paper is the first of a series analyzing our field data in a “step-stair” approach. It has two main objectives. First, propose a field methodology, scientifically robust and ecologically relevant, to better characterize bird exposure at the population level. The approach combines (i) an intensive radiotracking survey of breeding females, (ii) a farmer questionnaire to record pesticide use, and (iii) a spatio-temporal analysis using a GIS to cross-check bird habitat use and pesticide application. Second, quantify clutch and chick potential exposure to a series of actual ASs. Correlations between exposure and endpoints related to demographic parameters and egg characteristics will be reported later, as well as modeling refinements.

2. Materials and methods

2.1. Study sites

The study was carried out in 12 sites located in north-central France (Fig. 1A). The total area reached ca. 14,500 ha. Main crops were winter wheat and winter barley, and, to a lesser extent, rapeseed, sugar beets

and pastures (Fig. 1B, Bro et al., 2013). Agriculture was intensive with high crop yields – 6.5–9.5 T of winter wheat/ha (Agreste, 2012a). Habitat features (crop fields, hedgerows, woods, copses, set-aside plots, roads) were reported on a GIS using a nomad PDA-GPS (Trimble JunoSB, D3E Electronique – France/Software Windows mobile 6 and ArpentGIS).

The sites were typical of French cereal ecosystems where wild populations of gray partridges still occur (Agreste, 2012b; Bro and Crosnier, 2012). Spring partridge density and the reproductive success varied from 5 to 60 pairs/km², and from 2.4 to 10.5 offspring/female in summer, respectively (Bro et al., 2013). Chick survival rate at 6 weeks was estimated to 0.4–0.6 (Bro et al., 2013).

2.2. Clutch and covey monitoring

We carried out a radiotracking survey of gray partridges in spring and summer 2010–2011. Captures were made from late February to late March. 467 females were tagged with a necklace radio-transmitter (RI-2DM, Holohil Ltd, Canada). Handling time did not exceed a few minutes. Tag weight was <2.5% of the bird body mass, which limits potential adverse effects (Bro et al., 1999). Tags were equipped with a motion-sensitive mortality circuit to discern survival. The status (alive, dead or “missing”) and the locations of the birds were determined twice a day. Locations were recorded on a PDA-GPS.

Incubation was detected when a female was located at the same place for consecutive days. 281 clutches were detected (>2500 eggs laid). Clutches that failed during laying were likely to be undetected. Nest location was recorded using an averaged GPS measure in order to guide clutch searches. Clutch fate was identified to the appearance of eggs or eggshells. Hatching was identified by pipped eggshells, predation by the presence of scattered empty eggshell fragments, farming practices to compressed eggs and desertion to pooled intact cold eggs. The number of eggs laid was determined, when possible, according to the number of eggs, eggshells and chorionic membranes. All eggs were collected. Intact or compressed ones were broken in the laboratory to determine whether they were infertile or contained a dead embryo. When an embryo was observed, its stage of development was determined using reference standards (Bro et al., 2013).

Coveys were monitored by locating the females and by detecting the presence of offspring, when possible.

2.3. Identification of pre-laying, laying, incubation, and brooding phases

We determined the incubation, laying, and pre-laying phases for each clutch by back-dating the beginning of incubation and egg-laying from hatching/failure date (all chicks hatch within few hours).

- We considered 24 days for incubation when clutches hatched (Birkan and Jacob, 1988). We used the estimate of the development stage of the oldest embryo when clutches failed. Incubation duration could not be determined for failed clutches when only eggshell fragments were found or when all intact eggs were rotten. Incubation was set to 2 days in case of “infertile” eggs (*i.e.*, infertile eggs or embryo disks ≤ 2 days; Bro et al., 2013), except when daily locations of the female provided reliable information about incubation duration.
- The laying duration was calculated as the number of eggs laid multiplied by the mean laying rate (1.5 day/egg; Birkan and Jacob, 1988). Laying duration could not be determined when clutches were predated and only few eggs were retrieved (unknown clutch size). A minimum laying duration was therefore calculated using available data.
- We used 15 days for the pre-laying duration. It corresponds to the yolk formation phase in the domestic hen (Griffin et al., 1984; Sauveur and de Reviers, 1988). In our case, the pre-laying phase of a clutch corresponds to yolk formation of the first eggs laid.

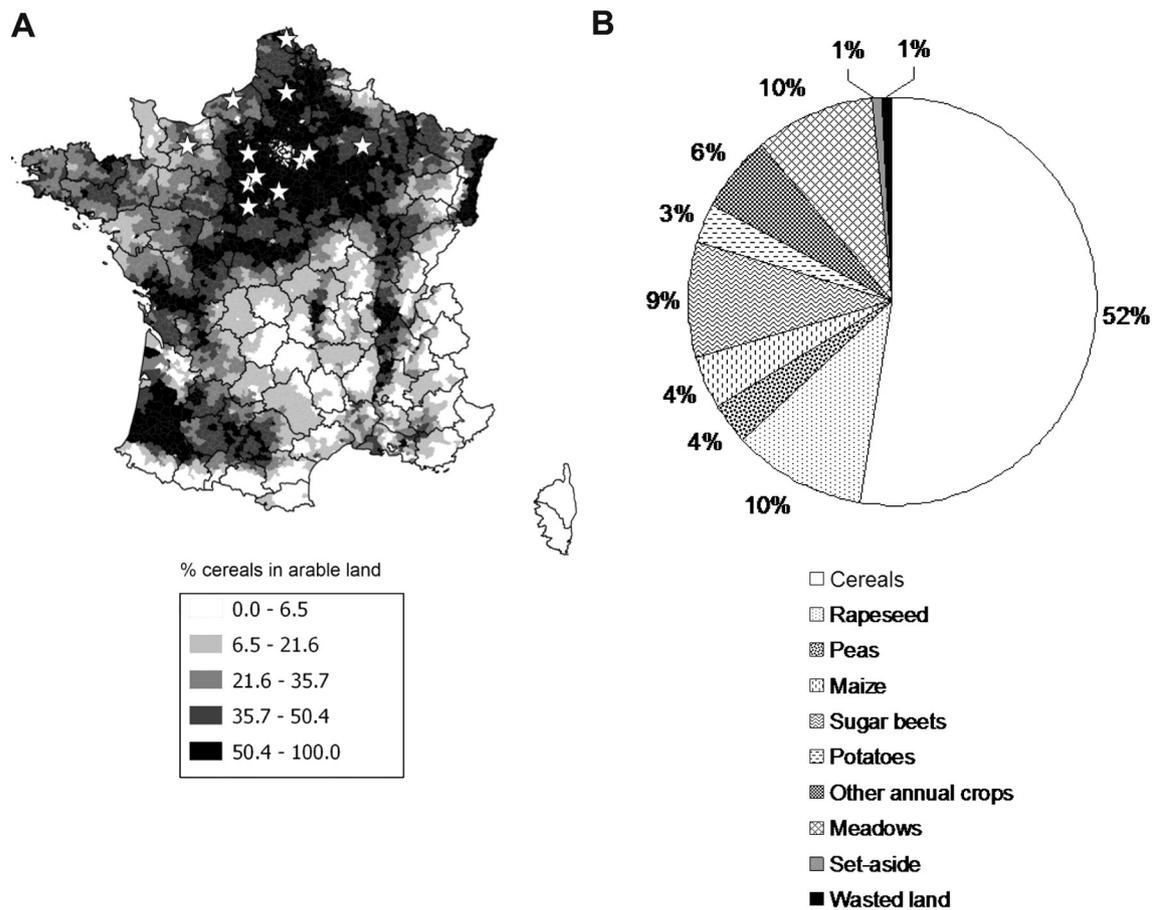


Fig. 1. A. Geographical location of the 12 study sites (★) and proportion of cereals in the arable land in 2010 in France (Agreste, 2012a); B. mean crop cover over the study sites.

The dates of the first weeks of life of chicks (the brooding phase) were determined from the date of hatching.

2.4. Pesticide use

We conducted a survey of farmers to record their operations at the field level. 142 farmers collaborated to the study and provided the following data: trade formulation of the pesticides used, tank mixtures, dates of application, and doses used (see table headers in Fig. 2). Data were collected for ca. 1000 fields and a total area of ca. 6500 ha. Data were encoded to ensure their anonymity. ASs were identified from trade formulations using the E-PHY database of the French Ministry in charge of farming (<http://e-phy.agriculture.gouv.fr/>).

We recorded a total of 179 ASs used between 1st of March and 31st of August 2010 and 2011, over the 317 ASs listed in the French AGRITOX database in early 2013 (<http://www.agritox.anses.fr/php/fiches.php>, access on 29/01/2013). We focused on organic pesticides. We did not include inorganic fungicides, additives and biological agents.

For each AS, we recorded its Chemical Abstracts Service (CAS) registry number, chemical family, and main use type (e.g., fungicide, herbicide, insecticide, molluscicide, growth regulator, bird repellent) following the pesticide manual (MacBean, 2012) and the pesticide properties database (PPDB, <http://sitem.herts.ac.uk/aeru/ppdb/en/>).

2.5. Potential exposure of clutches and coveys to active substances

We considered that a female (and then her eggs or her chicks) was potentially exposed to a pesticide if the area where it was tracked overlapped with treated fields. Field margins (e.g., lanes, grassy banks, hedges) that could have been incidentally sprayed were not considered *per se* for the estimation of potential exposure,

but they were included in the area where the female was tracked. The “area” corresponded to the convex envelope of daily locations. The envelope was determined using Quantum GIS software (QGIS version 1.8.0. “Lisboa”). We used the envelope rather than only the fields where the female was actually located the days when a pesticide was applied because we claim that it was more ecologically relevant. Indeed, females were geo-located twice a day, which is probably not enough to capture in detail the whole daily habitat use. Envelopes were assumed reliable given the high sedentary nature of female partridges (Reitz, 2009).

Then, we listed the ASs spread on each treated field using farmer's questionnaires (Fig. 2). Following the recommendations of Bennett et al. (2005) and Shore et al. (2005), we assessed separately the exposure of clutches for the incubation, laying, and pre-laying phases, and of chicks during brooding. Given the high mortality rate of chicks (Bro et al., 2013), we estimated their potential exposure to ASs for their four first weeks of life separately. Given the results, we also estimated their potential exposure to ASs for their whole first month of life.

To quantify the potential exposure to a given AS, we calculated the proportion of females (i.e., clutches and coveys) that were potentially exposed to this AS.

From our data, it was possible to determine whether potential exposure to multiple ASs issued from commercial formulations, tank mixture, emerged from bird habitat use, or from their combination.

2.6. Reproductive toxicity of active substances to birds

We compiled data on reproductive toxicity for each AS: the no-observed (adverse)-effect level (NO(A)EL) reported in the European

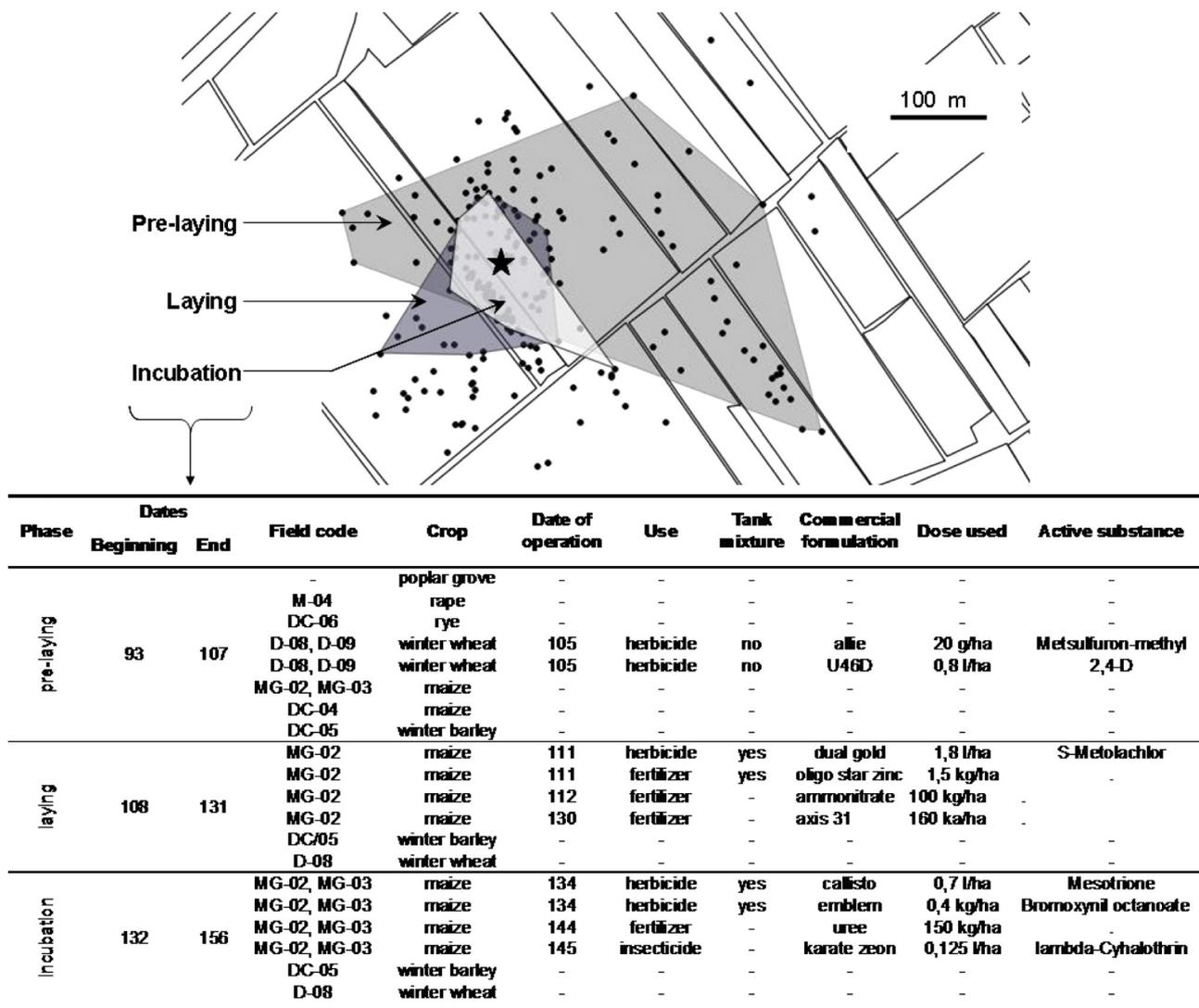


Fig. 2. Identification of the fields frequented by a breeding female (•: daily locations) during pre-laying, laying, and incubation phases. Gray polygons represent the successive activity ranges. The black star indicates the location of the nest. Dates are provided as the number of calendar days. Example of partridge n°1089, young bird in first reproduction, clutch laid in winter barley, 16 eggs laid (15 hatched, 1 infertile), hatching 21/06/2011.

Food Safety Authority (EFSA) conclusions on the pesticide peer review (<http://www.efsa.europa.eu/en/publications.htm>), when available.

We also recorded bird first-tier TER_{it} for insectivorous, herbivorous, and granivorous bird scenarios as well as higher-tier TER_{it} when available in the reports.

3. Results

3.1. Potential exposure of clutches to single active substances

Partridge clutches (n = 140 clutches, >1600 eggs) were globally exposed to 108 ASs during pre-laying, laying, and incubation phases over a total of 179 ASs used between 1st March and 31st August 2010 and 2011 (84 herbicides, 4 herbicide safeners, 58 fungicides, 20 insecticides, 7 plant growth regulators, 2 molluscicides, 2 acaricides, 1 anti-sprouting, and 1 bird repellent. Some ASs were also defoliants.) (Table 1, Appendix A). 71.4% of the clutches were exposed to at least one AS. The proportion of clutches potentially exposed to a given AS was ≥ 5% for 32 ASs. These “top” ASs were mainly fungicides (n = 17, 53.1%). Herbicides represented 25% of top ASs, insecticides 15.6% and growth regulators 6.2%.

Top ASs were all used both years, on at least 8 study sites out of 12, and by 17–72% of the farmers. Thus, they can be regarded as commonly used ASs, from this statistical point of view. All but 5 were reported to be used on cereals, which represent the main crop cover and the preferred nesting habitat of the species in France.

3.2. Correspondence between use of active substances and bird breeding phenology

The main period of use of top ASs was from early April to early/mid-June (Table 1). Spring to mid-summer is also the time of laying, incubation, hatching, and brooding of the gray partridge (Table 1, Appendix B) and other ground nesting birds (Appendix B). So, there is a large spatio-temporal correspondence between bird breeding and pesticide use. This matching includes a large number of ASs and an array of wildlife species.

3.3. Potential exposure of clutches to several active substances

A mixture is defined here as a combination of several ASs to which birds/clutches were potentially exposed, either simultaneously (commercial formulations, tank mixture) or sequentially (bird

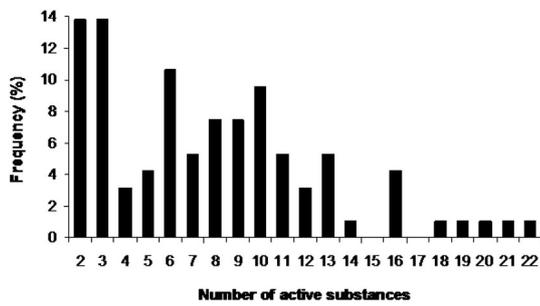


Fig. 3. Frequency of clutch potential exposure to mixtures according to the number of active substances involved. Data from global exposure, 94 clutches exposed to mixtures.

habitat use). 67.1% of clutches were potentially exposed to mixtures, involving 2 up to 22 ASs (Fig. 3). For illustration purpose of mixture diversity, 10 and 12 different combinations were recorded for 2-AS

and 3-AS mixtures (13 clutches in both cases). However, combinations of some ASs were more frequently recorded (Table 2); ASs involved were those associated with the highest proportion of clutches potentially exposed (Table 1). Detailed data are provided in supporting information (Appendix C) for readers who would like to estimate the frequency of a given mixture.

3.4. Causes of potential exposure to several active substances

Mixtures may result from the use of co-formulations, tank mixtures and/or habitat use, as well as their combination. Because we cannot detail all existing ones, we describe hereafter three contrasting examples of 2-AS mixtures.

- 1) The exposure to both prothioconazole and epoxiconazole was recorded as relatively frequent (25 clutches). They are two widely used fungicides applied on several crops, among which cereals. One formulation among the 15 recorded with prothioconazole

Table 2

Description of a sample of mixtures involving 2, 3 or 4 active substances, and associated frequency (94 clutches exposed to mixtures).

Active substance 1	Active substance 2	Active substance 3	Active substance 4	Frequency																																																											
Prothioconazole (n = 48, 51%)	Epoxiconazole (n = 25, 27%)	Boscalid (n = 18, 19%)	Chlormequat chloride	(n = 7, 7.4%)																																																											
			Cyproconazole	(n = 5, 5.3%)																																																											
			Ethephon	(n = 8, 8.5%)																																																											
			Fenpropidin	(n = 5, 5.3%)																																																											
			Fluoxastrobin	(n = 6, 6.4%)																																																											
			Prochloraz	(n = 8, 8.5%)																																																											
			Propiconazole	(n = 8, 8.5%)																																																											
			Spiroxamine	(n = 6, 6.4%)																																																											
			Tebuconazole	(n = 8, 8.5%)																																																											
			Prochloraz (n = 15, 16%)	Azoxyastrobin	Boscalid	Chlorothalonil	Cypermethrin	Fluoxastrobin	lambda-Cyhalothrin	Lenacil	Metconazole	Phenmedipham	Propiconazole	Tebuconazole	Trifloxystrobin																																																
																Epoxiconazole (n = 10, 11%)	Boscalid	lambda-Cyhalothrin	Picoxystrobin	Prochloraz	Propiconazole	Tebuconazole																																									
																							lambda-Cyhalothrin (n = 9, 9.6%)	Propiconazole	Prochloraz	Azoxyastrobin	Cyproconazole	Lenacil	Phenmedipham	Tebuconazole																																	
																															Prochloraz (n = 10, 11%)	Azoxyastrobin	Cyproconazole	Lenacil	Phenmedipham	Tebuconazole																											
																																					Boscalid	Prochloraz	lambda-Cyhalothrin	Propiconazole																							
																																									Chlormequat chloride (n = 7, 7.4%)	Boscalid	Prochloraz	lambda-Cyhalothrin	Propiconazole																		
Fluoxastrobin	Prochloraz	Epoxiconazole																																												Boscalid	Fenpropidin	Prothioconazole	Cyproconazole	Propiconazole													
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incorporated both ASs. Only two cases resulted from tank mixture, and 23 cases from habitat use.

- 2) The exposure to prothioconazole and fluoxastrobin, two fungicides widely used on cereals, was also recorded for 25 clutches. The use of two co-formulations including these two ASs was recorded. In this situation, the double exposure resulted from commercial formulations (24 cases), habitat use (13 cases), both commercial formulation and habitat use in 12 cases, and to tank mixture in only one case.
- 3) The exposure to both epoxiconazole and prochloraz, two fungicides commonly used on cereals, has been recorded for 20 clutches. No co-formulation including these two ASs was recorded in our questionnaire. This mixture resulted from tank mixture in 16 cases, habitat use in 15 cases, and both in 11 cases.

So the cause of mixture occurrence depended upon the ASs that were considered. As illustrated above, no generalization is possible and a specific extraction of data should be done to quantify a specific situation in a one-AS or a one-mixture approach. However, mixtures involving a high number of ASs clearly resulted from a combination of tank mixture of several co-formulations and bird habitat use. Small fields and scattered fields are two characteristics that are likely to increase the number of ASs in mixtures both because farmers have their own sanitary programs and because birds are likely to frequent a high number of fields. As an example to illustrate the high complexity of mixtures *in situ*: the bird identified 2010-14/61-513-1 experienced a potential exposure to 16 different ASs (10 fungicides, 3 herbicides and 3 growth regulators) within 15 days by frequenting 6 different fields from late March to early May. This bird experienced two successive exposures to epoxiconazole in two days. Five ASs were applied on a winter barley the same day following a tank mixture of two commercial co-formulations of fungicides and plant growth regulators. Such mixture was roughly equally due to the use of commercial co-formulations and to bird habitat use.

3.5. Potential exposure of coveys to active substances

13.3% of coveys ($n = 75$) were potentially exposed to ASs. Chicks were globally exposed to 18 ASs during their first month of life (Table 1, Appendix A). ASs were mainly fungicides (66.7%) and insecticides (27.8%). Defoliants accounted for 5.5%.

3.6. Reproductive toxicity to birds

The reproductive risk assessment scheme is based on the TER approach (EFSA, 2009). It comprises three tiers. The first step is a “screening step”. It uses an “indicator species” and worst-case assumptions regarding exposure. If a substance (and its associated use) does not pass the screening step ($TER_{it} < 5$), then the next step is the first-tier risk assessment. The TER_{it} is then calculated using a “generic focal species” and more realistic assumptions regarding exposure (mixed diet for example). Indicator and generic focal species are not real species but they are considered to be representative of all species potentially at risk. Figure 5 is the trigger value above which an AS is not considered to have unacceptable adverse effects on avian reproduction. If again this step is not successful, then the risk assessment is refined using a “focal species” that is a real species. This provides a greater degree of ecological realism.

We did not find an EFSA conclusion for 13 ASs. Eleven out of 19 documented top ASs are associated to a lowest first-tier $TER_{it} < 5$ (Table 3). These ASs are mostly fungicides (8 ASs out of 11), in particular triazoles (5 fungicides out of 8). However, some ASs used as herbicides, insecticides or plant growth regulators are also associated with a lowest first-tier $TER_{it} < 5$. Higher-tier TER_{it} calculated for some passerine species (or unspecified species) tend to confirm a risk for some bird species for 5 out of 10 ASs (Table 3).

4. Discussion

4.1. Field methodology

In this paper, we estimated a realistic direct and indirect (maternal effects) potential exposure of gray partridge clutches and offspring to pesticides and corresponding ASs. The method is based on individual bird ecology (daily crop habitat use) and farmer's practices (daily pesticide use on fields). Therefore it integrates the whole complexity of the natural system such as the different sources of spatial and temporal variability.

Four methodological feedbacks from this field study are worth to be shortly mentioned.

- 1) The method is associated with high financial, logistic and human costs. Data collection cost ca. 800 K€. Such intensive and extensive field study is thus difficult to replicate.
- 2) We encountered three practical difficulties that reduced the effective sample size for data analysis: 1) a high female mortality rate during the breeding season (50%; Bro et al., 2001, 2013), 2) a high clutch failure rate (50%; Bro et al., 2013) and 3) a lack of involvement of some farmers to the study.
- 3) The potential exposure of an animal to ASs can be assessed throughout its life. For example, in the light of the effects of imidacloprid on the reproduction of game birds observed in laboratory conditions (Gibbons et al., 2015; Lopez-Antia et al., 2013), exposure of birds to this insecticide should be estimated in the field, at least through coated seeds in autumn and spring. It would be also interesting to monitor the reproduction of surviving birds (Bro et al., 2010; Goulson, 2013; Guitart et al., 2010).
- 4) The method deals with ASs that are applied in fields. Those persistent in the soil or water (Goulson, 2013) are not taken into account.

4.2. Exposure statistics and risk of reproductive toxicity

We identified 32 top ASs to which $\geq 5\%$ of partridge clutches have been potentially exposed. They are commonly used, mainly during the breeding period of birds. Several of them are associated with a first-tier TER_{it} lower than the trigger value of 5 and a higher-tier TER generally calculated for passerines lower than or close to 5 (epoxiconazole, cyproconazole, tebuconazole, chlormequat, fenpropidion, metamitron). Such estimates suggest that some ASs have the potential to adversely impact the reproduction of some avian species. However, this hypothesis has to be tested for the gray partridge and consolidated with field data. For this purpose, we plan in further steps to 1. Refine the criteria to assess the toxicity of ASs by using (quantitative) structure-activity relationship ((Q)SAR) and quantitative structure-property relationship (QSPR) modeling (Devillers, 2004; Devillers and Devillers, 2009; Devillers et al., 2006, 2011; Saxena et al., 2014), 2. Relate these predicted activity and property data to several endpoints (clutch size, fertility and hatchability rates, embryo anomalies, eggshell thickness) by means of *in silico* approaches, 3. Perform residue analyses on eggs to examine actual vs. potential exposure, and 4. Calculate higher-tier TER_{it} for the gray partridge (see 4.7.).

4.3. Potential exposure to multiple active substances

It arose from field data that potential exposure of clutches to ASs is an emergent probabilistic event resulting from natural variation in spraying dates on crop fields, bird individual breeding calendar and habitat use. This is especially true for exposure to several ASs. Mixtures resulted from commercial co-formulations, tank mixtures, bird habitat use and farmland characteristics (field size, spatial organization of crops, sparsely located fields). The commercial strategy discounting pesticide packs and tank mixtures encouraged by fuel economy are further factors likely to increase the occurrence of mixtures. Most

Table 3
Reproductive toxicity to birds and estimates of long-term toxicity-to-exposure ratios for different risk assessment scenarios. Estimates are those reported in the EFSA conclusions. Data are provided for documented top active substances. They are ranked in decreasing order of frequency of clutch potential exposure. Lab species: *Colinus virginianus* (CV), *Coturnix. C. japonica* (CCJ), and *Anas platyrhynchos* (AP).

Active substance	Reproductive toxicity to birds			First tier model				Higher tier refinement	
				Insectivorous bird		Herbivorous bird		Lowest value	
	Parameter	Value (mg/kg bw/day)	Lab species	TER _{it}	Crop (application rate)	TER _{it}	Crop (application rate)		
1 Prothioconazole ⁽¹⁾	NOEL	≥86	CV	“Unlikely”		10.4	Cereals (spray, 1 × 0.6 kg a.s./ha)	2.5	
2 Epoxiconazole	NOEL	1	CV	0.27	Cereals (2 × 0.125 kg a.s./ha)	64.5	Rape (spray, 1 × 0.6 kg a.s./ha)	0.27	skylark: 0.9; yellowhammer: 1.2
3 Boscalid	NOEL	24.1				0.37	Cereals (2 × 0.125 kg a.s./ha)		
6 Lambda-cyhalothrin ⁽²⁾	NOEL	2.7	AP	14	Wheat & barley (3 × 4.5 g a.s./ha)	Omnivorous lark: >28.9	Wheat & barley (3 × 4.5 g a.s./ha)	14	
7 Prochloraz	NOEL	>14.16	CV	2.44	Cereals (2 × 450 g a.s./ha)	3.01	Cereals (2 × 450 g a.s./ha)	2.44	skylark: 7.16; yellow wagtail: 11.3; herbivorous: 7
8 Fluoxastrobin	NOEL	151	CV	79.5	Cereals (2 × 200 g a.s./ha)	10.3	Cereals (2 × 200 g a.s./ha)	10.3	
9 Cyproconazole		6.6	CV	0.79	Cereals (2 × 100 g a.s./ha)	1.2	Cereals (2 × 100 g a.s./ha)	1.2	skylark: 6.9; yellowhammer: 5.7; yellow wagtail: 5.6
10 Tebuconazole	NOEL	5.8	CV	0.77	Cereals (2 × 0.25 kg a.s./ha)	0.94	Cereals–grass (2 × 0.25 kg a.s./ha)	0.5	insectivorous: 1.75 - 4.8; herbivorous: >5.3; granivorous: 1.5
11 Chlormequat chloride		54.8	CCJ	1.21	Cereals (1 × 1.5 kg a.s./ha)	2.06	Cereals (1 × 1.5 kg a.s./ha)	1.21	yellow wagtail: 4.19; large herbivorous: 5.62
12 Ethephon	NOEL	159	CCJ	11	Cereals (1 × 0.48 kg a.s./ha)			11	
13 Metconazole	NOEL	6.19		2.28	Cereals (2 × 0.090 kg a.s./ha)	2.78	Cereals (2 × 0.090 kg a.s./ha)	2.28	yellow hammer (weed seeds): 387; marsh warbler (foliar insects): 88.4
15 Fenpropidin	NOEL	14.6	CV	0.65	Cereals (1 × 0.75 kg a.s./ha)	1.1	Cereals (1 × 0.75 kg a.s./ha)	0.65	skylark: 0.29; yellowhammer: 0.29
16 Ethofumesate	NOEL	>406							
17 Fluroxypyr ⁽³⁾		40.1	AP	6.7	Cereals & maize & pasture (1 × 200 g/ha)	11	Cereals (1 × 200 g/ha)	6.7	
18 Metamitron	NOEL	81.5	CV	1.93	Beets (700, 1400 and 1400 g a.s./ha)	1.24	Beets (700, 1400 and 1400 g a.s./ha)	1.24	skylark: 1.6; yellowhammer: 4.6; yellow wagtail: 4.1
19 Azoxystrobin		1200	CV	16	Cereals (2 × 250 g a.s./ha)	20	Brassica (2 × 250 g a.s./ha)	16	
20 Cypermethrin ⁽⁴⁾		4.29	CV	1.91	Maize (1 × 0.0375 kg a.s./ha)	3.26	Maize (1 × 0.0375 kg a.s./ha)	1.91	herbivorous/maize: 8.96
				4.78	Cereals (2 × 0.015 kg a.s./ha)	5.81	Cereals (1 × 0.015 kg a.s./ha)		
				4.78	Peas (2 × 0.015 kg a.s./ha)	6.32	Peas (1 × 0.015 kg a.s./ha)		
21 Lenacil		100.4	CV	6.66	Beets (1 × 0.500 kg a.s./ha)	12.4	Beets (1 × 0.500 kg a.s./ha)	6.66	
22 Clopyralid	NOEL	118	AP	13	Rape & beets (1 × 0.300 kg/ha)	44	Cereals (1 × 0.150 kg/ha)	13	
						24	Rape & beets (1 × 0.300 kg/ha)		
27 Spiroxamine	NOEL	2.02	CV	0.2	Cereals ()	0.2	Cereals ()	0.2	quail: 8.23; lark: 8.65; yellow wagtail: 11.5
	NOEL	5.4							
31 Pirimicarb				9.85	Wheat (1 × 0.21 kg/ha)			9.85	

⁽¹⁾ lowest TER_{it} of metabolite: 2.5.

⁽²⁾ in gamma-Cyhalothrin EFSA conclusion.

⁽³⁾ lower TER_{it} values for Fluroxypyr-methyl.

⁽⁴⁾ TER_{it} data for zeta-Cypermethrin. The full dataset is provided in supporting information (Appendix D).

common ones combined several fungicides with a reproductive risk potential. The great diversity and complexity of mixtures highlight the importance to evaluate pesticides and some major combinations, and not only ASs and their metabolites. However data reflecting real-world exposure are lacking whereas it is pointed out as a crucial need (Kortenkamp et al., 2009).

4.4. Potential vs. actual exposure

We quantified the potential exposure of clutches and offspring to ASs. However, a series of factors may reduce real exposure such as the time actually spent in the treated crops, interception by crop foliage, evaporation, proportion of contaminated food items, degradation of residues, and the behavior of parents. Regarding the gray partridge, laying females only remain on their nests during a few hours per day to lay (Birkan and Jacob, 1988). They cover their eggs with plant material before leaving, which is likely to limit direct contamination. On the contrary, incubating females only leave their nests for short periods to feed and do not cover their eggs.

4.5. Routes of exposure

Different transfer processes are likely to occur during the successive phases of reproduction. They may be associated to different risks.

Pesticide exposure of females during egg formation, especially yolk formation, may result in the contamination of eggs, as a maternal effect. Indeed, in the domestic hen, some lipoproteins of the yolk are exclusively synthesized by the liver of the laying female (Sauveur and de Reviere, 1988). Thus lipophilic ASs may be included in the fatty content.

On the contrary, a contamination of eggs once laid would occur directly. Contact may come from air or vegetation (during spraying for example), or from contaminated feathers of the female if exposed when moving through treated vegetation. In these cases, eggs would be more likely to be contaminated by ASs that could penetrate the egg-shell and the chorionic membranes.

Chick exposure can occur through the remaining yolk the very first days of life, food (especially contaminated invertebrates) or through a direct air and dermal contact.

4.6. Exposure of farmland birds to pesticides: a need for data

Knowledge of bird exposure is an essential preliminary step to further investigate the overall potential impact of pesticides on avian reproduction (Engelman et al., 2012; Prosser and Hart, 2005). However, little is known in agricultural landscapes. Exposure of birds of prey – that benefit from dedicated surveys such as the Predatory Bird Monitoring Scheme in U.K. – to rodenticides is one exception (Christensen et al., 2012; Hughes et al., 2013; Ruiz-Suarez et al., 2014; Walker et al., 2008). As far as eggs are concerned, studies reporting an exposure to contaminants mainly deal with marine and fresh water birds, and birds of prey. Farmland birds are less documented, with however recent works such as those of Rüdél et al. (2011) and Eng et al. (2014) on the starling, the wood pigeon and the rook. However, most studies focus on given chemicals and residue analyses are often performed on a particular sample (i.e., unhatched eggs.) so, extrapolations to the whole population are difficult.

Global exposure may also be estimated using pesticide usage statistics as a proxy (Environment Canada, 2006; Sugeng et al., 2013) or using model outputs (Engelman et al., 2012; Pisani et al., 2008; Roelofs et al., 2005). To the best of our knowledge, no detailed national or regional statistics on pesticide use are available for France, contrarily to other countries such as U.K. (Food and Environment Research Agency) or USA (United States Geological Survey). The availability of our robust database for research purposes allowed us to fill this gap.

4.7. Field database

This work provides one of the few largest dataset dealing with European cereal ecosystems with regards to exposure of bird' clutches and coveys to a large number of actual ASs – and the underlying field data of partridge habitat use and pesticide use. Because our statistics are based on a high sample size for an avian field study (140 clutches from 126 females, 75 coveys) issuing from a variety of situations (12 study sites located in intensively cultivated farmlands, 2 years, 142 farmers), they are valuable and robust. We claim that these statistics can be extrapolated to several other ground nesting birds breeding in cultivated farmlands, based on similarities in the breeding phenology, nesting habitat use, and diet.

The dataset may be requested for research purposes.

- (1) Many studies carried out on pesticides focus on those that are extensively used and/or hazardous (e.g., Lopez-Antia et al., 2013) but exposure of wildlife is little known and quantified. In this context, the statistics of potential exposure reported herein may serve to target ASs for future research on pesticide effects on wild birds inhabiting cultivated farmlands. In particular they may refine risk ranking models (EFSA, 2012; Environment Canada, 2006; Sugeng et al., 2013) that use pesticide usage data as a proxy of exposure.
- (2) The gray partridge is likely to be another well documented case study together with the skylark (Roelofs et al., 2005; Topping and Odderskaer, 2004; Topping et al., 2005). Indeed, our data provide spatio-temporal parameter estimates required to refine TER_{it} models (Crocker, 2005; Roelofs et al., 2005) and to run sophisticated individual-based and/or landscape models (Sibly et al., 2005; Topping et al., 2005, 2010). The inclusion of effect data, such as egg and embryo characteristics and sex-ratio (work in progress) would refine even more such tools.

Calculating higher-tier TER_{it} using our gray partridge and farming field data will be highly beneficial for two reasons. First, it will provide risk assessment estimates for another focal species (see EFSA conclusions for fenpropimorph and bromuconazole, Appendix D). Focal species are commonly passerines, such as the omnivorous skylark (*Alauda arvensis*), the granivorous and insectivorous yellowhammer (*Emberiza citrinella*), and the insectivorous yellow wagtail (*Motacilla flava*), because of their high food intake rate (EFSA, 2009). However the calculation of refined TER_{it} for different species highlights an inter-species variability (Table 3) that would be worth to examine further. Second, refined TER_{it} will be estimated for an array of individuals and a TER_{it} of the population, based on the distribution of TER_{it} of individuals, could be built following Roelofs et al. (2005). As stated by these authors, this would be highly valuable for regulatory authorities since it would provide additional information about frequency and uncertainty, two key concepts necessary in decision-making.

5. Conclusion

In this paper, we propose a field methodology to identify and quantify the actual potential exposure of wild birds to ASs. The gray partridge was taken as a focal species.

Our results reveal that a high proportion of clutches is potentially exposed to a diversity of ASs and mixtures, mostly during egg formation. Chicks seem less potentially exposed to ASs. Over the 19 documented top ASs, 26% have a potential for a reproductive risk for birds (higher-tier TER_{it}). If individual adverse effects are confirmed, a population incidence might be expected.

The database is made available for research in avian ecotoxicology, in particular in risk assessment, and landscape modeling. The gray partridge is a good candidate to become a reference species of the holistic “weight-of-evidence” approach to increase our understanding of pesticide risks against farmland birds.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2015.03.073>.

Ethical standards

This study was conducted in accordance with the principles and specific guidelines of the French legislation for animal welfare and wildlife regulations (AP n°2009-014 and n°2010-013).

Conflict of interest

The authors declare that they have no conflict of interest. The field study was funded by participants and Europe (European Regional Development Fund- no. 32962 and no. 32936), France (Fonds national d'aménagement et de développement du territoire - no. 32956; Conseils Généraux 27, 41 and 76 - no. 850050068 and no. Arr/B954), hunter associations (Fédération Nationale des Chasseurs, Fondation François Sommer, Club de l'Epagneul Breton, Société Canine d'Ile-de-France), Agence Services & Paiement Limousin and Vermillon.

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