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NEW STRATEGIES AND NEW CHALLENGES FOR PESTICIDE STUDIES: HOW TO COMBINE THE PRESERVATION OF OUR ENVIRONMENT AND SUSTAINABLE AGRICULTURE?

Pesticide contamination patterns in Montagu's harrier (Circus pygargus) chicks

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Abstract
Biomonitoring of persistent pesticides in birds of prey has been carried out for decades, but few studies have investigated their relevance for the monitoring of non-persistent pesticides. Herein, we determined the contamination patterns of multiple pesticides in Montagu's harrier (*Circus pygargus*) chicks in an intensive farming area of southwestern France. Blood samples from 55 chicks belonging to 22 nests in 2021 were assessed for 104 compounds (herbicides, fungicides, insecticides, safeners and synergists). All chicks had at least one herbicide in their blood, and half had at least two compounds. The 28 compounds detected comprised 10 herbicides, 12 fungicides, 5 insecticides and 1 synergist. Mixtures in blood were predominantly composed of herbicides, and six chicks presented a mixture of the three pesticide classes. The most prevalent compounds were sulcotrione (96% of chicks), tebutam (44%) and chloridazon (31%), of which the latter two had been banned in France for 19 and 3 years, respectively, at the time of sampling. Most compounds are considered non-acutely toxic, but sulcotrione is potentially carcinogenic, mutagenic and reprotoxic, raising questions about the effects on the health of nestlings. Biomonitoring of multiple pesticides through Montagu's harrier chicks in agroecosystems is clearly relevant because it reflects the general pattern of agricultural pesticide use in the study area. It also raises questions about exposure pathways in chicks, and further investigations are needed to disentangle the roles of dietary routes and maternal transfer for the established pesticide contamination patterns.

Keywords Biomonitoring · Farmland bird · Fungicide · Herbicide · Insecticide · Multiresidue pesticide analysis

Introduction

Agricultural intensification in the 1950s led to the massive use of chemical inputs as fertilisers and pesticides (Chamberlain et al. 2000; Stanton et al. 2018). Pesticides are now found in all compartments of agroecosystems including soils, earthworms, bees, nectar, small mammals and birds, years after being banned in some cases (Wintermantel et al. 2020; Fritsch et al. 2022; Pelosi et al. 2022; Fuentes et al. 2023). These products are the main drivers of declining farmland bird populations and have been associated with human diseases (Xu et al. 2022; Rigal et al. 2023). In a *One*

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Health context, determining pesticide contamination levels in wild species could facilitate estimation of human exposure to such contaminants (García-Fernández et al. 2020). In this regard, biomonitoring, which consists in the monitoring of the quality of an environment through wildlife, is crucial. In fact, biomonitoring of contaminants using bird species has been carried out for decades (Newton et al. 1993; Becker et al. 1994; Dauwe et al. 2002; Bustnes et al. 2007). Although most research has focused on heavy metals and persistent pollutants in seabirds and raptors (Albert et al. 2019; Helander et al. 2008; Crosse et al. 2012), attention is now being paid to biomonitoring of non-persistent pesticides—those not belonging to persistent organic pollutants (POPs)—in birds (Humann-Guilleminot et al. 2019, 2021; Badry et al. 2022; Fuentes et al. 2023). Birds have multiple advantages that make them valuable candidates as bioindicators; their biology and ecology are well known; they occupy various positions in the food chain, and they are easier to sample using non-lethal methods than many other taxa that may need to be destroyed (Becker 2003; García-Fernández et al. 2020). Among the different sampling methods, feathers and deserted eggs have been widely used as they constitute non-invasive methods (Becker et al. 1994, 2003; Burger and Gochfeld 1997; Espín et al. 2016). However, feathers may reflect past contamination from another site because contaminant deposition into feathers occurs as they grow (Espín et al. 2016), and eggs only reflect contamination of part of a population, namely breeding females (Pacyna-Kuchta 2023). More recently, blood sampling has received much attention because it reflects short-term exposure to contaminants (Espín et al. 2016), addressing one of the drawbacks of using birds as bio-indicators: their high mobility that may not reflect local environmental contamination (Becker 2003). Moreover, since only a small amount of blood is required for analyses, it allows the sampling of any individual from a population throughout the year. Together, these characteristics make blood a highly efficient matrix for biomonitoring (Espín et al. 2016; Pacyna-Kuchta 2023).

Birds of prey are particularly interesting as bio-indicators as they occupy high positions in trophic chains, making them vulnerable to biomagnification of contaminants (DesGranges et al. 1998; Voorspoels et al. 2007). Their populations have suffered severe declines since the 1960s due to acute poisoning by rodenticides, as well as the effects of POPs such as DDT (Ratcliffe 1967; Furness et al. 1989; Newton and Wyllie 1992; Fremlin et al. 2020). Various studies have highlighted their potential for biomonitoring programmes at large scales, most notably in Europe (Gomez-Ramirez et al. 2014; Badry et al. 2020; González-Rubio et al. 2021). Montagu's harrier (*Circus pygargus*) is a valuable candidate for pesticide biomonitoring because this raptor is a specialist predator of agroecosystems. Individuals nest on the ground in cereal crops, exposing eggs and chicks to pesticides present in the culture. The chicks are altricial, which exposes them to contaminants through contact with the soil and crops, through the air and through the diet during the ~ 4 weeks they spend in the nest before fledging. Parents mainly bring common voles (*Microtus arvalis*) to their chicks, alongside orthopteran insects and passerine birds as alternative prey (Salamolard et al. 2000), which can themselves be contaminated with pesticides. Although adult Montagu's harriers may be exposed to pesticide contamination in their wintering areas, chicks are mostly naïve in terms of pesticide contamination, except in cases of maternal transfer of certain molecules since females may detoxify themselves through egg-laying (Mineau 1982). Even so, contamination patterns in chicks are expected to largely reflect exposure to pesticide contamination in their local environment. The aim of the present study was thus to investigate to what extent Montagu's harrier chicks in an intensive farming area were contaminated by pesticides through blood sampling to specifically reflect their recent contamination.

Materials and methods

Study area

The Long-Term Socio-Ecological Research Zone Atelier Plaine & Val de Sèvre (LTSER ZAPVS) is a 435 km^2 area of intensive farming located in southwestern France (46°11′N, 0°28′W). The ZAPVS landscape is mainly composed of winter cereal crops (41.5%) and other dominant crops including sunflower (10.4%), corn (9.6%) and oilseed rape (8.3%) based on average cover for 2009 – 2016 (Fig. 1) (Bretagnolle et al. 2018). Meadows and urban areas represent approximately 13.5% and 9.8% of the study area, respectively (Bretagnolle et al. 2018). Organic crop plots in the ZAPVS accounted for ~ 18% of the surface in 2021. Organic farming practices in the area comply with European legislation (Regulation EU 2018/848 of the European Parliament and of the Council of 30 May 2018).

Model species

Montagu's harriers have been monitored since 1994 in the ZAPVS. In this site, their mean productivity is 2.05 fledglings per breeding attempt, mainly depending on the availability of their main prey, common voles (Salamolard et al. 2000; Arroyo et al. 2004), although females can lay up to eight eggs (Arroyo et al. 1998). The incubation period lasts 29 days, and chicks hatched asynchronously are reared until they fledge at the age of 30 – 35 days (Arroyo et al. 2004). Males ensure food provisioning of incubating females and chicks, and females join in with provisioning later in the

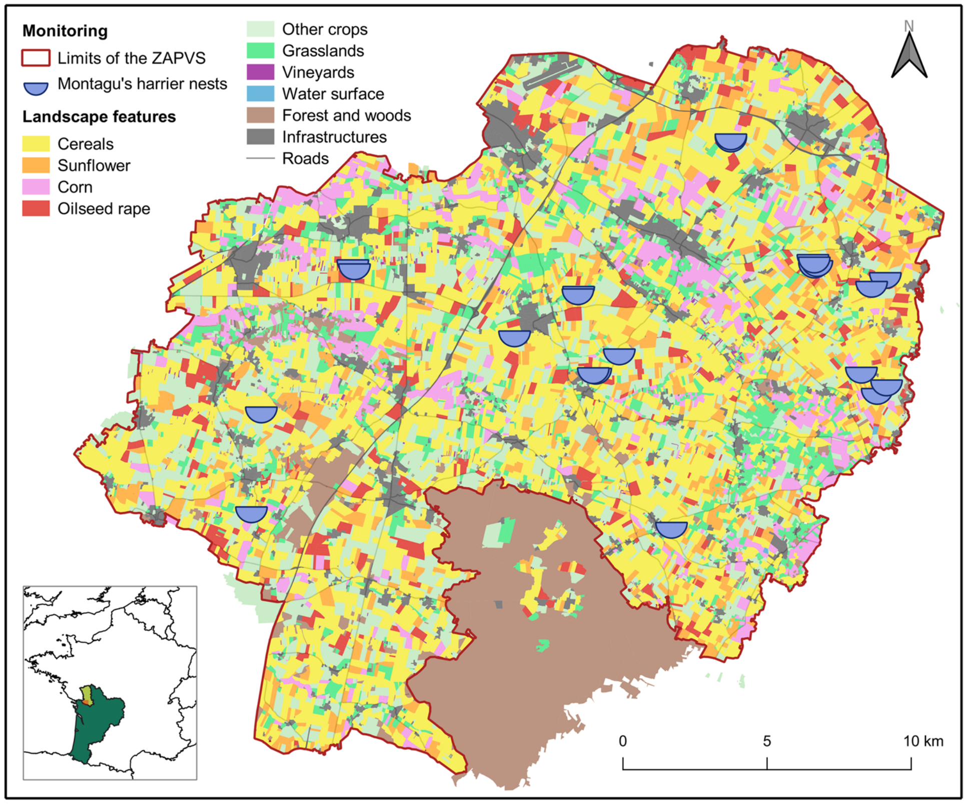

Fig. 1 Localisation of the 22 *Circus pygargus* nests monitored and landscape composition of the Zone Atelier Plaine & Val de Sèvres (ZAPVS) in 2021

rearing period (García and Arroyo 2005). Home ranges during the breeding season can stretch up to 100 km^2, although foraging ranges in the study area are generally ~ 14 km^2 (Salamolard 1997; Guixé and Arroyo 2011).

Sampling procedure

In 2021, professional ornithologists located and visited Montagu's harrier nests. The locations of nests were recorded on a geographical information system (GIS; QUANTUMGIS 3.22.16; QGIS Development Team 2023; Fig. 1) using coordinate data. Nests were visited twice before eggs hatched and every week after hatching. At 15 days old (second visit), chicks were banded with an aluminium ring with a unique code provided by the Museum National d'Histoire Naturelle de Paris (France) and sexed according to the colour of their iris, grey for males and brown for females (Leroux and Bretagnolle 1996). At 26 ± 2 days old (fourth visit), chicks were carefully caught and released at the nest to collect blood samples. From each chick, a 50 µL blood sample was collected in an Eppendorf tube by puncturing the brachial vein using a sterile needle and heparinised capillaries. Samples were placed in a cooler (0 – 5 °C), transported to the laboratory and stored at – 20 °C until further analyses. A total of 55 chicks from 22 nests were sampled between late June and early August 2021 with no sex ratio bias observed (26 males and 29 females; binomial test $p = 0.53$).

Multiresidue pesticide analysis

Whole blood samples (i.e., red blood cells and plasma) were analysed following the method developed by Rodrigues et al. (2023). Plant protection products (PPPs) are commonly referred to as pesticides, but they are composed of at least one active ingredient (herbicide, fungicide or insecticide) in a mixture with synergists (increasing the actions of pesticides) or safeners (improving herbicide selectivity towards weeds rather than crop plants). The analytical method employed allowed the detection and quantification of 104 compounds, mainly active molecules (i.e., pesticides) among the most used in France, including one synergist (piperonyl butoxide) and one safener (benoxacor). Hereafter, 'pesticide' refers to all compounds searched, including safeners/synergists.

Blood samples were thawed and weighed before extraction, and 10 µL of a carbendazim-d4 solution at 1 mg L^{-1} was added to each blood sample as an internal standard to monitor both the liquid–liquid extraction and purification steps. Carbendazim-d$_4$ was then quantified in each sample to ensure extraction recovery. For all samples, the average recovery for carbendazim-d4 was calculated, and no variation greater than 15% was observed, which was considered acceptable. Each sample was mixed with 2 mL dichloromethane and ethyl acetate (1:1) and homogenised by vortexing for 1 min followed by three rounds of sonication for 10 min each time. After each sonication, samples were centrifuged for 5 min, and supernatants were pooled and gently evaporated under a fume hood until reaching a final volume of 500 µL. The resulting extract was stored at − 20 °C until pesticide level analyses were conducted by liquid chromatography coupled to tandem mass spectrometry (LC/MSMS) and gas chromatography coupled to tandem mass spectrometry (GC/MSMS) using multiple reaction monitoring (MRM) for quantification. A blank and a standard were injected every ten blood samples to check for carryover and system contamination or variability.

For more volatile compounds, GC/MSMS analyses were conducted using an automatic thermal desorption system (ATD 350, PerkinElmer Corp., Norwalk, CT, USA) connected to a Trace 1300 GC coupled to an ITQ 900 mass spectrometer (Thermo Scientific, Illkirch-Graffenstaden, France). Compounds desorbed by ATD were separated on a Macherey–Nagel OPTIMA XLB capillary column (30 m × 0.25 mm i.d; 0.25-µm film thickness) with helium as the carrier gas at a constant flow of 1.2 mL min^{-1}. Spectra were obtained in electron impact ionisation (EI) mode at an electron energy of 70 eV.

For less volatile pesticides, LC/MSMS analyses were performed using a TSQ Quantum Access Triple Quadrupole Mass Spectrometer (Thermo Scientific) in heated positive electrospray ionisation (HESI +) mode, coupled with a Thermo Accela 1250 pump and a Thermo Combi Pal autosampler (Thermo Scientific). A Nucleodur C18 Pyramid column (150 mm × 3 mm, 3 µm i.d.) was employed for gradient mode analyses using a mobile phase of water and acetonitrile, both containing 0.1% formic acid.

For both LC/MSMS and GC/MSMS, prior to injection, samples were dopped with an added mixture of internal standards (trifluralin-d_{14}, nitrophenol-d_4, 2,4-D-d_3 and pendimethalin-d_5) used for quantification to avoid any potential instrument variations. Concentrations were thus calculated using calibration from commercialised standards of analytical purity using an internal standard method. Calibration was performed in triplicate by spiking both a blank matrix and a non-matrix. Responses showed good linearity, with correlation coefficients of ≥ 0.98 using a linear regression model. The matrix effect was assessed by comparing the standard deviations of the slopes for calibrations with and without the matrix, and no variation greater than 15% was observed.

Multiresidue analysis involved the detection and quantification of 104 pesticide molecules in MRM detection mode for both instrumentations. For each analyte and internal standard, two MRM transitions were monitored for each target compound; the first product ion was used for quantitation, and the second product ion was used for qualification. The ion ratio was also monitored for all transitions. Limit of detection (LOD) and limit of quantification (LOQ) were determined as three and ten times the ratio of the average noise height on either side of a known amount of a compound's peak to the peak height, respectively. The aim was to establish the minimum peak height necessary to distinguish a compound's peak from surrounding noise. LODs and LOQs for all detected compounds are listed in Table 1. For further details concerning the analytical method, refer to Rodrigues et al. (2023).

Results

At least one herbicide was detected in all Montagu's harrier chicks, half had at least two compounds in their blood, and one nestling had 16 (Fig. 2). Twenty-eight different compounds were detected (concentrations > LOD) in blood samples: 10 herbicides, 12 fungicides, 5 insecticides and 1 synergist (piperonyl butoxide; Table 1). Of these, 26 were quantified (concentrations > LOQ). Ten of the pesticides detected were banned for sale and use before sampling in 2021, among which tebutam and flusilazole were banned > 10 years ago (Table 1). Fifteen of the 28 compounds were considered non-acutely toxic because their birds' oral 50% lethal dose (LD50 = quantity of pesticide killing 50% of test animals) was > 2000 mg kg^{-1}, below the level needed to place them in acute toxicity hazard categories according to EC Regulation No. 1272/2008 (Table 1 and Table S1 and S2 in Supplementary Information). Five substances—carbetamide, propyzamide, dimoxystrobin, sulcotrione and cyproconazole—were considered carcinogenic, mutagenic and reprotoxic based on the nomenclature including hazards to the aquatic environment and human health (Order of 22nd December 2022) (Table S2). The distribution of different classes of compounds (herbicides, fungicides, insecticides and synergists) among chicks is depicted in Fig. 3. Of the 55 nestlings, 36% had at least one fungicide, 18% had at least one insecticide and 9% had the synergist piperonyl butoxide. For 28 nestlings (50%), the mixture of pesticides was composed of only herbicides, and 40 (73%) had a mixture dominated by herbicides. Mixtures combining herbicides, fungicides and insecticides were found in six chicks, of which one also had piperonyl butoxide (Fig. 3a). In terms of concentrations in blood, the distribution of the four types of compounds varied but herbicides remained predominant for 50 nestlings (91%), while fungicides and insecticides exceeded half of the total concentration for three and one chicks, respectively (Fig. 3b). Occurrences, concentration ranges and means ± standard deviations for each compound are listed in Table 2. The most frequent compounds detected were three herbicides: sulcotrione, tebutam and chloridazon, in 53 (96%), 24 (44%) and 17 (31%) nestlings, respectively (Table 2). Sulcotrione had the highest concentrations in blood with a maximum of 3184.67 pg mg^{-1}.

Table 1 Main properties, analytical methods (GC = ATD-GC–MS/MS; LC = LC–MS/MS) and limit of detection (LOD) and quantification (LOQ) in pg mg^{-1} for the 28 compounds detected in the blood of Montagu's harrier (*Circus pygargus*) chicks. Compounds are ordered by pesticide type, then alphabetically. The ban corresponds to prohibition years in France obtained from legislative texts (https://www.legifrance.gouv.fr/) accessed on 16 November 2023. DT50 (detection time 50% = time to detect a 50% decrease in pesticide concentration) ranges were obtained from field studies (for more details, see Lewis et al. 2016). Model species correspond to birds for which the oral LD50 (lethal dose 50% = quantity of pesticide killing 50% of test animals) was obtained: *Colinus virginianus* (Cv), *Coturnix japonica* (Cj), *Anas platyrhynchos* (Ap) and *Serinus canaria* (Sc). Log *P* corresponds to the log of the partition coefficient and measures the lipophilicity of molecules (the larger the value, the more lipophilic). Main crops, DT50, bird LD50, model species and log *P* were compiled from the Pesticide Properties DataBase (PPDB) of the University of Hertfordshire (http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm) accessed on 16 November 2023 (Lewis et al. 2016). Main crops of application were indicated if present in our study area and in line with plant protection product (PPP) guidelines (https://ephy.anses.fr/; accessed 16 November 2023). *NA* not applicable when not considered an active substance of PPPs in Europe

Type, compound	Ban (year)	Main crops	DT50 range (days)	Bird LD50 ($mg\ kg^{-1}$)	Model species	Log *P*	Method	LOD	LOQ
Herbicide									
Bifenox	No	Cereals	8.3 – 32.1	> 2000	Cv	3.64	GC	0.0012	0.0038
Carbetamide	No	Alfalfa, vegetables	8	> 2000	Cv	1.78	LC	0.0053	0.0176
Chloridazon	Yes (2018)	Beets	3 – 105	> 2000	Cv	1.19	GC	0.0213	0.0709
2,4-MCPA	No	Cereals, meadows, linseed	25	377	Cv	− 0.81	GC	0.1579	0.5263
Mecoprop-P	No	Cereals	21	> 500	Ap	− 0.19	GC	0.0500	0.1667
Metamitron	No	Beets	11.1	1302	Cj	0.85	GC	0.0577	0.1923
Oxadiazon	Yes (2018)	Grass	90 – 330	> 2150	Cv	5.33	GC	0.0086	0.0286
Propyzamide	No	Oilseed rape	13.9 – 271.3	6578	Cj	3.27	GC	0.0021	0.0071
Sulcotrione	No	Corn, linseed	10.8 – 89.7	> 1350	Ap	− 1.7	LC	0.0021	0.0071
Tebutam	Yes (2002)	Oilseed rape	60	> 5000	Ap	3	GC	0.0526	0.1754
Fungicides									
Boscalid	No	Cereals, sunflowers, linseed, peas, vegetables, fruits, vineyards	196 – 312.2	> 2000	Cv	2.96	GC	0.0005	0.0016
Carbendazim	Yes (2014)	Cereals, sunflowers, peas, beets, vineyards, soybeans	20 – 40	> 2250	Cv	1.48	LC	0.0042	0.0140
Cyproconazole	No	Cereals, beets, grass, vineyards	62.1 – 501.2	94	Cv	3.09	GC	0.0192	0.0639
Cyprodinil	No	Cereals, fruits	11 – 98	> 500	Ap	4	GC	0.0011	0.0036
Difenoconazole	No	Cereals, corn, vegetables,	20 – 265	> 2150	Ap	4.36	GC	0.0359	0.1196
Dimethomorph	No	Fruits, vegetables, vineyards	34 – 54	> 2000	Cv	2.68	GC	0.0072	0.0242
Dimoxystrobin	No	Wheat, oilseed rape	2 – 39	> 2000	Cv	3.59	GC	0.0038	0.0128
Epoxiconazole	Yes (2019)	Cereals, beets	52 – 226	> 2000	Cv	3.3	LC	0.0027	0.0091
Flusilazole	Yes (2008)	Cereals, beets, oilseed rape, fruits	63 – 240	> 1590	Ap	3.87	GC	0.0144	0.0481
Myclobutanil	No	Grass, fruits, vineyards	9 – 66	510	Cv	2.89	GC	0.0214	0.0714
Prochloraz	No	Cereals, oilseed rape, fruits, grass	28.6 – 245	662	Cv	3.5	GC	0.0170	0.0568
Quinoxyfen	Yes (2019)	Cereals, grapes, cucurbits, tomato	13 – 190	> 2250	Cv	5.1	GC	0.0048	0.0161
Insecticides									
Bifenthrin	Yes (2019)	Ornamentals, sports fields, lawns	65 – 125	1800	Cv	6.6	GC	0.0035	0.0116
Clothianidin	Yes (2018)	Corn, sorghum, fruits	13.3 – 1386	430	Cv	0.90	LC	0.0103	0.0344
Cypermethrin	No	Cereals, oilseed rape, vegetables, beets, fruits, grassland	9.3 – 31.2	> 9520	Ap	5.55	GC	0.0013	0.0042
Indoxacarb	No	Corn, vegetables, fruits	4.9 – 7.5	73.5	Cv	4.65	GC	0.0069	0.0231

Table 1 (continued)

Type, compound	Ban (year)	Main crops	DT50 range (days)	Bird LD50 (mg kg^{-1})	Model species	Log *P*	Method	LOD	LOQ
Thiacloprid	Yes (2018)	Beets, corn, vegetables	5.95 – 16.8	35	Sc	1.26	LC	0.0014	0.0048
Synergist									
Piperonyl butoxide	NA	NA	13	> 2250	Cv	4.75	GC	0.0004	0.0015

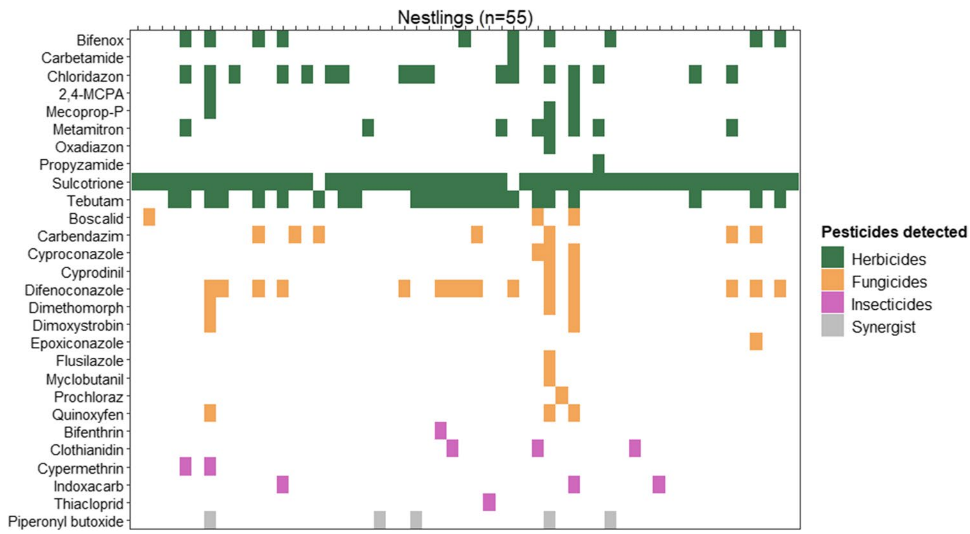

Fig. 2 Pesticide contamination patterns in *Circus pygargus* nestlings. Columns correspond to nestlings and rows correspond to compounds. Each coloured cell corresponds to a detected compound

Discussion

The present study revealed a general contamination to pesticides of Montagu's harrier chicks, and although only 27% of compounds searched for were detected, all chicks were contaminated. Herbicides and fungicides were most abundant in chicks (22 of 28 compounds), with three herbicides (sulcotrione, tebutam and chloridazon) detected at the highest occurrences, and difenoconazole was the most abundant fungicide. Most substances found in chicks were considered non-toxic based on acute toxicity hazard classification. Following this classification, thiacloprid had the highest level of acute toxicity, although the concentration measured in chick's blood did not exceed 0.25% of the substance's LD50. Nonetheless, sulcotrione, the most prevalent substance detected, is classified in category 4 for acute toxicity and is considered carcinogenic, mutagenic and reprotoxic, which raises questions about the consequences of this contamination for chicks' health.

Previous studies reported the contamination of wild birds with boscalid, cypermethrin, difenoconazole, indoxacarb, oxadiazon, sulcotrione, thiacloprid and piperonyl butoxide as detected here (Millot et al. 2015; Varagiya et al. 2021; Rial-Berriel et al. 2021; Fernández-Vizcaíno et al. 2022; Movalli et al. 2023). However, these studies used different biological matrices (organs such as gizzards or livers); thus, the comparison with our results is senseless given the different temporal patterns of pesticide distribution in the different biological tissues (Espín et al. 2016). Only a limited number of studies screened the same compounds we detected here in blood of wild birds. In Montagu's harriers from Germany, 2,4-MCPA was also detected in blood of two nestlings at 1.3 and 1.8 pg μL^{-1} (which is equivalent to pg mg^{-1} of blood considering a density of ~ 1) which is a thousand times lower than the minimal concentration found here (Badry et al. 2022). Twenty-one of the 28 compounds detected here were also found in fledglings of the European Kingfisher (*Alcedo atthis*) sampled the same year in western France (Musseau et al. 2023). Sulcotrione was notably found at high occurrence as well (95% of sampled birds) and at similar average concentrations (1277 pg mg^{-1} in Musseau et al. 2023*vs* 1112 pg mg^{-1} here). For 12 compounds, the average concentrations found in Montagu's harrier nestlings were higher than those found in the European Kingfisher. Cyproconazole, difenoconazole and flusilazole have been found in plasma of blackbirds (*Turdus merula*) with maximal concentrations of 0.212, 0.157 and 0.016 pg mg^{-1}, respectively (Angelier et al. 2023) *vs* 148, 1214 and 138 pg mg^{-1} in Montagu's harrier blood. Clothianidin

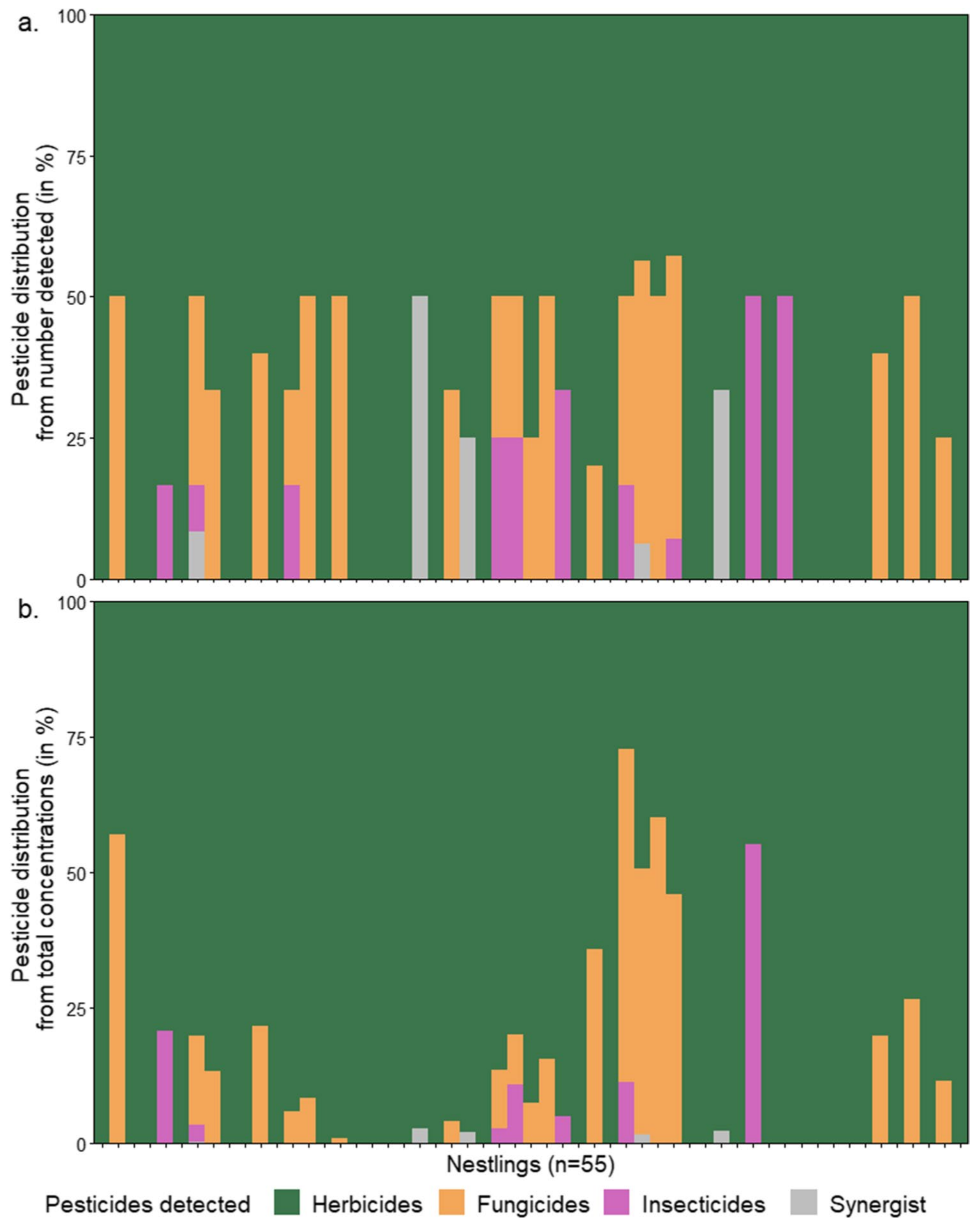

Fig. 3 Distribution of pesticide classes in blood samples of *Circus pygargus* nestlings according to their **a** number detected and **b** concentrations in pg mg^{-1}. One stacked bar corresponds to one nestling. When compounds were detected but not quantified, concentrations were estimated to be equal to the LOQ divided by 2

and thiacloprid have also been found at lower concentrations in blood of birds of different trophic levels within our study area and elsewhere (Rial-Berriel et al. 2020; Fuentes et al. 2023 and references therein). Differences in the sensitivity of the analytical methods used in the mentioned studies influence the occurrence and concentrations reported; comparisons should thus be considered with caution (except Fuentes et al. 2023; Musseau et al. 2023 and the present study).

Contamination of Montagu's harrier chicks was mostly herbicides and fungicides, indicating heavy use of these classes of pesticides in the study area and/or higher exposure of chicks to these classes due to specific ecological factors of this raptor species. Indeed, Montagu's harriers nest on the ground in cereal crops, which are dominant in the study area and mainly treated with fungicides and herbicides in France (DRAAF 2017). The large quantities of herbicides and fungicides bought into the study area support their heavy use, despite some mismatches between the amounts purchased and detection in nestlings; for example, propyzamide was bought in large amounts around nests but detected in only one nestling (Table 2 and Figure S1 in Supplementary Information). In fact, the quantities of substances bought and applied may vary according to the concentrations of active ingredients in PPPs, the application guidelines for PPPs (quantity to apply per hectare) and the proportions of different crop types surrounding nests. For instance, a higher proportion of corn crops locally may

Table 2 Compounds detected in the blood of 55 *Circus pygargus* chicks belonging to 22 nests, ordered by occurrence (highest to lowest). Means, standard deviation (SD) and minimum and maximal values were obtained from concentrations quantified in pg mg^{-1} in chicks' blood (i.e., values < LOD were excluded from calculations)

Compound	Detection (number of chicks)	Percentage	Mean ± SD	Min–max
Sulcotrione	53	96.36	1111.78 ± 540.67	312.42–3184.67
Tebutam	24	43.64	64.62 ± 49.77	13.81–180.24
Chloridazon	17	30.91	121.02 ± 125.90	35.96–563.10
Difenoconazole	15	27.27	240.02 ± 317.74	31.68–1213.55
Bifenox	10	18.18	< LOQ	-
Metamitron	8	14.55	25.01 ± 28.29	3.28–88.34
Carbendazim	7	12.73	97.50 ± 81.21	0.216–258.29
Piperonyl butoxide	5	9.09	36.30 ± 22.48	12.60–70.67
Boscalid	3	5.45	1369.09 ± 819.48	791.50–2307.00
Clothianidin	3	5.45	929.30 ± 1268.76	189.92–2394.32
Cyproconazole	3	5.45	70.04 ± 69.82	13.65–148.13
Dimethomorph	3	5.45	241.49 ± 94.27	163.33–346.19
Indoxacarb	3	5.45	< LOQ	-
Mecoprop-P	3	5.45	799.35 ± 369.40	409.93–1144.79
Quinoxyfen	3	5.45	39.93 ± 38.73	9.73–83.59
Cypermethrin	2	3.64	204.34 ± 46.88	171.19–237.49
Cyprodinil	2	3.64	41.34 ± 42.91	11.00–71.68
Dimoxystrobin	2	3.64	171.79 ± 16.87	159.86–183.72
2,4-MCPA	2	3.64	2020.12 ± 176.57	1895.27–2144.98
Bifenthrin	1	1.82	18.46	-
Carbetamide	1	1.82	29.75	-
Epoxiconazole	1	1.82	51.05	-
Flusilazole	1	1.82	137.63	-
Myclobutanil	1	1.82	142.12	-
Oxadiazon	1	1.82	71.68	-
Prochloraz	1	1.82	1292.89	-
Propyzamide	1	1.82	339.07	-
Thiacloprid	1	1.82	87.48	-

result in higher application of sulcotrione, whereas more beet crops might lead to greater use of metamitron, irrespective of the amounts bought at a larger scale. Nonetheless, the general pattern of pesticide use in the study area is reflected in the contamination of Montagu's harrier chicks. Furthermore, some compounds appear to be ubiquitous in the agroecosystem; boscalid, cyproconazole, prochloraz and thiacloprid have also been detected in soils, earthworms and small mammals in the study area (Pelosi et al. 2021; Fritsch et al. 2022).

The higher exposure of chicks to herbicides and fungicides implies the persistence of these substances for several weeks or even months in crop plots. Indeed, application of PPPs to cereal crops generally takes place in winter but can be extended until May for fungicides, coinciding with the onset of the breeding period for Montagu's harriers. This may expose chicks on the ground to persistent compounds through contact with the soil and vegetation, or through ingestion of contaminated prey. The concomitant detection of 16 compounds including difenoconazole, metamitron and carbendazim in small mammals sampled in the study area (Fritsch et al. 2022) supports a dietary contamination route. Higher concentrations were found in raptor chicks than in small mammals (843-fold higher on average; Table S3), which suggests a potential biomagnification of these compounds up the trophic chain. For recently banned compounds, their presence in the blood of Montagu's harrier chicks can be rationally explained by the delay afforded to distributors and users of PPPs. For example, chloridazon, a substance banned in France in 2018 but detected in 30% of Montagu's harrier nestlings and in small mammals (Fritsch et al. 2022), was bought into the study area in 2020 (Figure S1 in Supplementary Information). This compound was purchased as a PPP mixture with quinmerac with an end date for distribution of June 2020 and an end date for use of December 2020. Thus, the application and persistence of this compound until chicks were raised during the summer of 2021 may be the origin of their contamination. However, regarding compounds that have been banned for a long time, their detection implies either fraudulent use or strong persistence in the environment. The persistence of a compound is generally established from its 50% detection time (DT50), the time taken to detect a 50% decrease in pesticide concentration under controlled conditions in either laboratory or field. For tebutam, the DT50 is 60 days in the field, meaning that this molecule is supposed to be naturally degraded within 2 months in the environment (Lewis et al. 2016). Based on our results, its rate of degradation would be much slower than predicted, which can be explained by the gap between *in natura* conditions and the conditions to establish the DT50 (Moreau et al. 2022), and plants would remobilise this contaminant from the soil 20 years after its ban, which seems quite unlikely. However, the detection of the banned compounds tebutam, chloridazon, epoxiconazole, quinoxyfen, bifenthrin and clothianidin in the blood of European Kingfisher's fledglings sampled the same year at ~ 100 km of our study area raises questions concerning the potential fraudulent use of such pesticides (Musseau et al. 2023).

If we discount the fraudulent use of legacy substances, their presence in Montagu's harrier chicks raises questions about the aforementioned exposure pathways (i.e., contact and diet). Thus, another contamination route might be the maternal transfer of pesticides. Indeed, if these substances are currently used in western African countries, where this raptor species overwinters, females may be exposed before arriving to their breeding site, then detoxify themselves through egg-laying. Maternal transfer of pollutants is a well-known process for persistent molecules and heavy metals (Mineau 1982; Van den Steen et al. 2009; Jouanneau et al. 2021). More recently, some studies demonstrated the maternal transfer of 'non-persistent' pesticides such as tebuconazole (Bellot et al. 2022). Lipophilic molecules are generally more prone to be excreted by females in the vitellus of their eggs (Fry, 1995). Flusilazole, a triazole fungicide just as tebuconazole, and tebutam have high and moderate lipophilicity, respectively (see log *P* values in Table 1), suggesting this contamination pathway should not be excluded. Further investigations on pesticide use in African countries and in migratory stopover areas are needed to assess maternal transfer of these pesticides in Montagu's harriers.

Regardless of the route of exposure, our study provides evidence that 'naïve' individuals such as Montagu's harrier chicks are contaminated with pesticide mixtures after only 4 weeks of life within crop plots. This highlights the ubiquity of pesticides in agroecosystems, including some that have been banned for many years. Although most studies consider the greater risk of adverse effects of insecticides on wildlife, our study also highlights, in line with previous studies, the need to consider herbicide and fungicide risks to non-target organisms into more details as these were the most prevalent compounds found here (Tassin de Montaigu and Goulson 2020). Besides, even if not discussed in the present study, there seems to be a quite large variability in contamination among nestlings, and further investigations are needed to determine if this could have implications for the use of Montagu's harrier chicks in biomonitoring schemes. Ongoing research on soils and earthworms in the study area should help to disentangle the origin and exposure routes for these contaminants. Moreover, dietary exposure could be investigated by analysing pesticides in food pellets collected at nests. Blood sampling of breeding adults and of younger nestlings would also be of great interest for studying the potential maternal transfer of pesticides *in natura*. Additionally, given the mixtures (16 compounds in one nestling) and the toxicity of some of the substances detected, further investigations are needed to shed light on the effects of pesticides on the life-history traits of chicks and adults. This would help to determine the consequences of pesticide exposure on the health of Montagu's harriers and eventually humans in a *One Health* framework.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by EF, AR, MM and KM. The first draft of the manuscript was written by EF. EF, AR and KM performed the writing. JM, VB and KM supervised, commented and edited on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The data that support the findings of this study are not openly available but are, however, available from the authors upon reasonable request.

Declarations

Ethical approval This study was conducted following the French guidelines for the ethical use of animals in research (APAFIS#18557–2019010822312199v2). Handling of Montagu's harriers was licensed by the Centre de Recherches sur la Biologie des Populations d'Oiseaux – Museum National d'Histoire Naturelle (license #1308).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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