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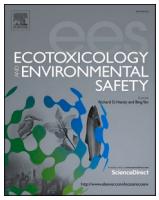

Levels of pesticide residues in the blood of ortolan buntings (*Emberiza hortulana)*, skylarks *(Alauda arvensis)* and yellowhammers *(Emberiza citrinella)* from Sweden

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ABSTRACT

Bird declines in European farmland have been linked to pesticide-driven food depletion and chronic exposure to pesticides well below levels causing acute toxicity. Yet, the extent of bird contamination by plant protection products remains largely unknown, partly because existing biomonitoring methods require relatively large blood volumes. Here, we developed a novel blood microsampling procedure (8 μl) combined with LC-MS/MS screening of 104 pesticides to determine exposure in nestling yellowhammers *Emberiza citrinella* (N = 5) and skylarks *Alauda arvensis* (N = 40) as well as adult ortolan buntings *Emberiza hortulana* (N = 21), species that have declined in European farmland over the last 60 years. Sampling was performed in Sweden from 2014 to 2016. Pesticide exposure was widespread: residues were detected in half of the nestlings (mean ± sd: 0.49 ± 0.07). Among individuals that tested positive for any pesticide, the mean number detected was 2.01 ± 0.49, with up to seven pesticides in a single nestling. Concentrations of individual pesticide residues in blood were relatively low compared to other studies (0.02–50 ng/ml) and were restricted to fungicides (N = 8) and herbicides (N = 5) in nestling skylarks and yellowhammers. Our results also provide the first evidence that exposure of migratory ortolan buntings to insecticide chlorpyrifos outside Sweden can be detected in the blood samples on their Swedish breeding grounds (7/10 tested positive; 0.25–0.56 ng/ml). These findings have key ecological and conservation implications, highlighting the need for and feasibility of, continued monitoring of pesticide exposure and its effects on non-target species in agro-ecosystems.

1. Introduction

Growing evidence indicates that the current use of pesticides on farmland in Europe and North America contributes to biodiversity loss and may pose risks to human health (Boatman et al., 2004; Paul et al., 2023). Despite policy efforts to reduce these risks, pesticides continue to harm many non-target species and ecosystem services (Mineau and Whiteside 2013; Woodcock et al., 2017). A large-scale study that considered 13 agronomic variables across eight European countries found that insecticides and fungicides had the most consistent negative effect on biodiversity, including farmland birds (Geiger et al., 2010). This aligns with recent trends showing steeper bird population declines in areas with higher neonicotinoid use (Hallmann et al., 2014; Li et al., 2020) or general pesticide use (Rigal et al., 2023). The main mechanism behind these patterns in birds is often assumed to be indirect (Potts, 1986; Burn, 2000). Pesticide use keeps populations of a wide range of weeds and invertebrates at much-reduced levels over large agricultural areas. This limits the food supplies for many ground foragers and invertebrate feeders in crop fields and adjacent non-crop habitats, thereby compromising their body condition, offspring survival and productivity (Van Dijk et al., 2013; Goulson, 2014).

Pesticides can harm vertebrates even in concentrations well below those causing acute toxicity (Crosby et al., 2015; Gibbons et al., 2015). Moreover, individuals carrying multiple residues during sensitive developmental stages may be at increased risk of additive or synergistic effects, as seen when prochloraz enhances organophosphate toxicity (Thompson, 1996; Laetz et al., 2009). Detection of legacy pesticides further indicates that past applications still feed into current food webs,

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prolonging ecological risks. Such chronic, low-level and multiple-compound exposure can reduce growth, condition, migration and later breeding success of birds (Lopez-Antia et al., 2016; Eng et al., 2019), helping to explain population-level declines (Rondeau et al., 2014; Moreau et al., 2021, 2022). Yet, any direct lethal effects on population numbers that may exist could be close to negligible if they do not add to natural losses or are offset by reduced losses from other causes (Newton, 1998).

The use of pesticides on farmland in Scandinavia is relatively low compared with other European regions (Rundlöf et al., 2012) and may thus not have an equal role in the observed bird declines. Sweden and Finland are among the few countries with quantities of sold pesticides below 1 kg per hectare (ha) of utilised agricultural area. The Netherlands, Belgium, Italy, Portugal, Spain, Germany, France and Slovenia all have amounts of pesticides sold per hectare above 2 kg/ha (range: ca. 2–6 kg/ha; Eurostat, 2017; KEMI, 2023). Pesticides used in Sweden are mostly herbicides (85 %; including haulm destructors and moss killers) and fungicides (10 %). At present, we do not know how these figures translate into pesticide residue levels in the blood of birds, nor what these levels represent in terms of toxicity.

The general aim of this study is to provide Swedish data that can serve as reference values for pesticide residue levels in ground-foraging birds typically associated with farmland in Sweden. This may help to identify trends in pesticide exposure and toxicological risks to birds in Sweden and across Europe. We determined the prevalence and concentrations of 104 pesticides in the blood of nestling yellowhammers Emberiza citrinella and skylarks Alauda arvensis as well as adult ortolan buntings Emberiza hortulana during their breeding season. This is relevant, as these species have undergone declines to varying degrees in Sweden and other parts of Europe during the last 60 years (Ottvall et al., 2009). They all feed on seeds but also insects, which in turn may feed on crops that are treated with various pesticides. The exposure of birds to pesticides can be expected to depend on their migratory behaviour, age (nestling, adult) and habitat selection. Yellowhammers are exposed to pesticides mostly on their breeding and wintering grounds in Sweden, but skylarks and ortolan buntings are also exposed on their passage and wintering ranges across Europe and Sub-Saharan Africa (ortolan bunting only). This implies that skylarks and particularly ortolan buntings might be exposed to higher toxicological risks from a larger variety of pesticides, including insecticides such as chlorpyrifos, which was still authorised in 20 European union (EU) member states during this study between 2014 and 2016 except for Sweden and seven other partners (EU pesticides database, 2023). We therefore tested specifically for the prevalence of this neurotoxic pesticide in blood samples from ortolan buntings. We also assessed the effect of variation in pesticide use on the number and level of pesticide residues in the blood of our study species across conventional fields where pesticides are used and compared these to individuals of the same species nesting in organic fields and forest clear-cut areas where pesticides are not used.

2. Methods

2.1. Study area and species

This study was carried out primarily in the Kvismaren valley situated approximately 15 km SE of Örebro, central Sweden (WGS84: 59°11'22.7"N 15°23'41.2"E). This region is dominated by spring- and autumn-sown cereal, potato and grass silage fields under both organic and conventional management. To study the incidence of 104 pesticide residues (100 compounds or 4 compounds using two different methods) (see below, Table 1) in birds in this area, we sampled blood from 40 nestling skylarks and five nestling yellowhammers found in 17 and 3 nests, respectively, during spring 2014, 2015 and 2016 (Table 2). We collected blood samples from nine skylarks (from six nests) in organic fields and 31 skylarks (from 11 nests) in conventional fields at Kvismaren. One yellowhammer nest was situated adjacent to a conventional field, and two other nests were associated with organic fields. The skylarks originated from nests situated in autumn- and spring-sown cereal fields only. Nestlings were collected from opportunistically found nests at the age of 5–8 days close to fledging in May and June. To minimise disturbance, nest visits and handling of all nestlings per nest never lasted for longer than 30 min. In 2014 and 2015, we also obtained 21 blood samples from male ortolan buntings on conventional farmland close to the Kvismaren valley (N = 12) and on forest clear-cuts (N = 9) in Västerbotten, northern Sweden (WGS84: 63°46'48.2"N 19°50'43.5"E) (Table 2). Ortolan buntings were caught with mist nets in their breeding territories shortly after arrival from their wintering grounds in May.

2.2. Blood sampling technique

Microsamples of blood were collected in K_2EDTA-coated glass capillaries with an exact volume of 8 μl (Product number 173313, Vitrex Medical A/S, Herlev, Denmark) according to the capillary microsampling (CMS) technique, originally developed for exposure measurement in rodent studies in early drug development (Jonsson et al., 2012; Korfmacher et al., 2015). The large vein under the wing (brachial vein) was penetrated with a cannula, and from the small blood drop formed, an 8 μl sample was collected using the exact volume capillary. To do this, the capillary was held against the blood drop and filled end-to-end by capillary force. After being filled with blood, the capillary was placed in a small plastic tube (1.1 ml twist cap, Micronics, Lelystad, the Netherlands) and placed on ice in a cool bag. Any remaining blood flow was stopped by gently pressing a clean paper tissue against the blood vessel. When returned from the field, samples were frozen at −20 °C, pending transport to the analytical laboratory and chemical analysis. Nestlings were banded and gently put back in the nest. Great care was taken to avoid leaving any traces on the ground or in the crop revealing the position of the nest to visual hunters such as hooded crow *Corvus corone*.

2.3. Blood analysis and pesticides

We tested for the prevalence of in total 104 pesticides and plant growth regulators (fungicides, N = 36; herbicides, N = 48; insecticides, N = 17; biocides, N = 1; plant growth regulators, N = 2), including some of their degradation products (e.g., 2,6-dichlorobenzamide, BAM), in blood samples from nestling skylarks and yellowhammers and breeding ortolan buntings (Table 1). Only 70 of these substances (67 %) were approved by the European Commission during this study (see EU pesticides database, 2023), and 55 (53 %) were authorised by Sweden. Unfortunately, some of the pesticides used in the study area (herbicides: florasulam, fluroxipyr, fluroxipyr-methyl, MCPA; insecticide: tau-fluvalinate) were not included in our analysis. Ten (conventional farmland, N = 5 and clear-cut, N = 5) out of 21 adult ortolan buntings were tested for the neurotoxic organophosphate insecticide chlorpyrifos using a separate analytical method (GC—MS method, see below). During the study period, chlorpyrifos was authorised in 20 EU member states except for Sweden (EU pesticides database, 2023). As of 2020, chlorpyrifos was banned throughout the EU. However, it is still commonly used in ortolans winter grounds in sub-Saharan Africa. Following intake, some chlorpyrifos may, due to its lipophilic properties (Log P = 4.7), be distributed to fatty tissues and may therefore be detectable in the blood of adult birds in their Swedish breeding grounds.

The 8-microliter liquid blood samples were analysed using two different analytical methods, either protein precipitation with acetonitrile followed by liquid chromatography connected to tandem mass spectrometry, LC—MS/MS (1260 LC-system with 6460 MS detector, Agilent, Santa Clara, USA; Jansson and Kreuger, 2010), covering 100 compounds, or liquid—liquid extraction followed by gas chromatography with mass spectrometric detection, and negative chemical ionization using methane as reagent gas, GC—(NCI)MS (7890 A GC with HP-5MS column and 5975 MS detector, Agilent, Santa Clara, USA), four

Table 1

We tested for the prevalence of 104 pesticides and plant growth regulators (Typ: F = fungicides, N = 36; H = herbicides, N = 48; I = insecticides, N = 17; B = biocides, N = 1; PGR = plant growth regulators, N = 2), including some of their degradation products (D; e.g., 2,6-dichlorobenzamide, BAM), in blood samples from nestling skylarks and yellowhammers and breeding ortolan buntings. Bold = used by farmers at the study site. Red=detected. EU=approved by the European Union. SW=Authorised in Sweden, 1 = yes, 0 = no. Study period 2014–2016. * LOD (method limit of detection) estimated from calibrating samples in human blood and from quality control (QC) samples in starling blood (N = 4) and human blood (N = 4), for GC-MS QC data only in starling blood (N = 3).

Name	CAS-No	Type	EU	SW	Method	LOD* (ng/ml)	Relative recovery %	% RSD	QC level (ng/ml)	Name	CAS-No	Type	EU	SW	Method	LOD* (ng/ml)	Relative recovery %	% RSD	QC level (ng/ml)
acetamiprid	135410–20–7	I	1	1	LC-MS	0.016	96.1	4.7	1.04	isoproturon	34123–59–6	H	0	0	LC-MS	0.05	99.1	3.6	1.04
alachlor	15972–60–8	H	0	0	LC-MS	2	102.9	4.4	5.22	linuron	330–55–2	H	0	0	LC-MS	0.2	99.8	6.0	1.04
amidosulfuron	120923–37–7	H	1	1	LC-MS	1	72.4	18.9	1.04	mandipropamid	374726–62–2	F	1	1	LC-MS	0.2	96.4	6.4	1.04
amisulbrom	348635–87–0	F	1	1	LC-MS	10	87.7	17.4	26.03	metalaxyl	57837–19–1	F	1	0	LC-MS	0.05	99.5	2.4	1.04
atrazine	1912–24–9	H	0	0	LC-MS	0.1	95.2	3.9	1.04	metamitron	41394–05–2	H	1	1	LC-MS	0.5	88.3	7.8	1.04
atrazine-desethyl	6190–65–4	H (D)	0	0	LC-MS	0.5	92.3	7.3	1.04	metazachlor	67129–08–2	H	1	0	LC-MS	0.02	98.7	3.0	1.04
atrazine-desisopropyl	1007–28–9	H (D)	0	0	LC-MS	5	81.5	22.9	5.22	methabenzthiazuron	18691–97–9	H	0	0	LC-MS	0.05	98.3	3.7	1.04
azoxystrobin	131860–33–8	F	1	1	LC-MS	0.02	94.7	2.1	1.04	methiocarb	2023–65–7	I	1	0	LC-MS	0.2	94.5	9.5	1.04
BAM (2,6-dichlorobenzamide)	2008–58–4	H (D)	0	0	LC-MS	1	110.6	12.6	1.04	metolachlor	51218–45–2	H	0	0	LC-MS	0.2	78.9	12.1	1.04
bifenox	42576–02–3	H	1	1	LC-MS	10	92.4	21.2	26.03	metrafenone	220899–03–6	F	1	1	LC-MS	0.05	81.8	12.3	1.04
bitertanol	55179–31–2	F	0	0	LC-MS	2	90.8	20.6	5.22	metribuzin	21087–64–9	H	1	1	LC-MS	0.5	93.5	3.5	5.22
boscalid	188425–85–6	F	1	1	LC-MS	1	102.1	2.2	5.22	metsulfuron-methyl	74223–64–6	H	1	1	LC-MS	0.065	86.0	6.4	1.04
carbendazim	10605–21–7	F(D)	0	0	LC-MS	0.2	92.4	4.2	1.04	oxadiazon	19666–30–9	H	0	0	LC-MS	0.2	76.5	13.2	1.04
carbofuran	1563–66–2	I	0	0	LC-MS	0.05	98.9	1.8	1.04	penconazole	66246–88–6	F	1	1	LC-MS	0.2	87.2	18.3	1.04
carfentrazone-ethyl	128639–02–1	H	1	1	LC-MS	0.2	81.3	10.4	1.04	pendimethalin	40487–42–1	H	1	0	LC-MS	1	77.2	16.5	5.22
chlorfenvinphos	470–90–6	I	0	0	LC-MS	0.2	83.3	8.5	1.04	phenmedipham	13684–63–4	H	1	1	LC-MS	0.2	84.6	12.1	1.04
chloridazon	1698–60–8	H	0	0	LC-MS	0.2	90.9	4.4	1.04	picoxystrobin	117428–22–5	F	0	0	LC-MS	0.065	91.0	8.2	1.04
clomazone	81777–89–1	H	1	1	LC-MS	0.05	100.4	4.9	1.04	pirimicarb	23103–98–2	I	1	1	LC-MS	0.05	93.2	2.3	1.04
clothianidin	210880–92–5	I	0	0	LC-MS	0.5	99.1	4.5	5.22	prochloraz	67747–09–5	F	1	0	LC-MS	0.2	92.5	12.9	5.22
cyanazine	21725–46–2	H	0	0	LC-MS	0.2	102.2	10.0	1.04	propamocarb	24579–73–5	F	1	1	LC-MS	0.02	87.4	6.0	1.04
cyazofamid	120116–88–3	F	1	1	LC-MS	0.2	107.9	6.1	1.04	**propiconazole**	60207–90–1	F	1	1	LC-MS	1	93.9	7.2	5.22
cybutryne (irgarol)	28159–98–0	B	0	0	LC-MS	0.2	92.0	7.6	1.04	propyzamide	23950–58–5	H	1	1	LC-MS	0.2	84.4	13.7	1.04
cycloxydim	101205–02–1	H	1	1	LC-MS	1	125.9	21.1	5.22	prosulfocarb	52888–80–9	H	1	1	LC-MS	0.2	97.3	13.0	5.22
cyflufenamid	180409–60–3	F	1	1	LC-MS	0.2	79.0	14.1	1.04	**prothioconazole-desthio**	120983–64–4	F(D)	1	1	LC-MS	0.25	100.5	7.9	1.04
cyprodinil	121552–61–2	F	1	1	LC-MS	0.5	83.4	6.9	1.04	pymetrozine	123312–89–0	I	1	1	LC-MS	0.2	84.0	22.2	5.22
desmedipham	13684–56–5	H	1	1	LC-MS	1	83.3	13.0	5.22	**pyraclostrobin**	175013–18–0	F	1	1	LC-MS	0.065	93.1	12.8	1.04
dichlorvos	62–73–7	I	0	0	LC-MS	1	91.8	15.6	1.04	pyroxsulam	422556–08–9	H	1	1	LC-MS	0.2	109.4	6.5	1.04
difenoconazole	119446–68–3	F	1	1	LC-MS	1	78.9	12.2	5.22	quinmerac	90717–03–6	H	1	1	LC-MS	1	98.3	6.2	1.04
diflufenican	83164–33–4	H	1	1	LC-MS	0.5	91.0	6.8	5.22	quinoxyfen	124495–18–7	F	0	0	LC-MS	0.2	82.1	19.6	5.22
dimethoate	60–51–5	I	1	0	LC-MS	0.2	93.5	3.9	1.04	rimsulfuron	122931–48–0	H	1	1	LC-MS	0.5	80.3	10.1	1.04
diuron	330–54–1	H	1	0	LC-MS	0.2	107.4	4.7	1.04	silthiofam	175217–20–6	F	1	1	LC-MS	0.2	91.9	7.7	1.04
epoxiconazole	135319–73–2	F	1	0	LC-MS	5	102.0	4.1	5.22	simazine	122–34–9	H	0	0	LC-MS	0.2	89.6	4.0	1.04
ethofumesate	26225–79–6	H	1	1	LC-MS	0.5	101.9	6.7	1.04	spiroxamine	118134–30–8	F	1	0	LC-MS	0.2	100.0	6.6	1.04
fenpropidin	67306–00–7	F	1	1	LC-MS	0.5	165.2	10.3	1.04	sulfosulfuron	141776–32–1	H	1	0	LC-MS	0.5	75.3	16.6	1.04
fenpropimorph	67564–91–4	F	1	1	LC-MS	1	117.5	15.7	5.22	terbuthylazine	5915–41–3	H	1	0	LC-MS	0.2	91.9	4.7	1.04
florasulam	145701–23–1	H	1	1	LC-MS	5	96.5	3.5	5.22	terbuthylazine-desethyl	30125–63–4	H (D)	1	0	LC-MS	0.2	96.2	3.6	1.04
fludioxonil	131341–86–1	F	1	1	LC-MS	0.2	85.1	16.3	1.04	terbutryn	886–50–0	H	0	0	LC-MS	0.5	103.3	3.3	5.22
flufenacet	142459–58–3	H	1	0	LC-MS	0.05	99.7	5.6	1.04	thiacloprid	111988–49–9	I	1	1	LC-MS	0.02	97.8	4.3	1.04
fluopicolide	239110–15–7	F	1	1	LC-MS	0.5	90.8	4.8	1.04	thiamethoxam	153719–23–4	I	0	0	LC-MS	0.2	89.8	11.5	1.04
flupyrsulfuron-metyl	144740–53–4	H	0	0	LC-MS	0.2	71.2	19.0	1.04	thifensulfuron-methyl	79277–27–3	H	1	1	LC-MS	1	91.1	10.7	1.04
flurprimidol	56425–91–3	PGR	0	0	LC-MS	0.5	104.8	11.1	1.04	thiophanate-methyl	23564–05–8	F	1	1	LC-MS	0.2	97.7	2.5	1.04
flurtamone	96525–23–4	H	0	0	LC-MS	0.05	98.6	3.4	1.04	tolclofos-methyl	57018–04–9	F	1	1	LC-MS	2	83.6	23.3	26.03
flusilazole	85509–19–9	F	0	0	LC-MS	0.5	91.4	11.0	1.04	tri-allate	2303–17–5	H	1	0	LC-MS	2	64.0	16.6	5.22
flutriafol	76674–21–0	F	1	0	LC-MS	0.5	96.8	1.8	1.04	**tribenuron-methyl**	101200–48–0	H	1	1	LC-MS	0.1	104.5	2.8	1.04
foramsulfuron	173159–57–4	H	1	1	LC-MS	1	83.6	10.4	5.22	trifloxystrobin	141517–21–7	F	1	1	LC-MS	0.02	81.2	15.8	1.04
fuberidazole	3878–19–1	F	0	0	LC-MS	0.2	91.2	3.3	1.04	triflusulfuron	126535–15–7	H	1	1	LC-MS	0.05	64.5	1.7	1.04
hexazinone	51235–04–2	H	0	0	LC-MS	0.05	97.8	2.3	1.04	trinexapac	95266–40–3	PGR	1	1	LC-MS	1	110.8	8.6	1.04
hexythiazox	78587–05–0	I	1	1	LC-MS	0.2	53.8	15.0	5.22	triticonazole	131983–72–7	F	1	1	LC-MS	1	102.0	7.6	1.04
imazalil	35554–44–0	F	1	1	LC-MS	1	128.7	8.6	26.03	aldrin	309–00–2	I	0	0	GC-MS	20	97.6	18.7	104
imidacloprid	138261–41–3	I	1	1	LC-MS	0.5	90.6	4.1	1.04	chlorpyrifos	2921–88–2	I	1	0	GC-MS	0.2	102.7	19.3	10.3
indoxacarb	173584–44–6	I	1	1	LC-MS	0.5	81.3	14.7	5.22	gamma-HCH (lindane)	58–89–9	I	0	0	GC-MS	2	114.1	29.7	10.3
iodosulfuron	185119–76–0	H	1	1	LC-MS	1	78.7	10.8	1.04	vinclozolin	50471–44–8	F	0	0	GC-MS	0.5	99.3	17.1	5.2

Table 2
Number of blood samples taken from different species and in different years and regions of Sweden.

Species & sample	2014, central	2014, northern	2015, central	2016, central	Sum
Skylark, nestlings	6		12	22	40
Yellowhammer, nestlings	5				5
Ortolan Bunting, adult birds	10	9	2		21
Sum	21	9	14	22	66

compounds determined. The LC—MS/MS and GC—MS instrumental methods are developed for the determination of pesticides with present or a historical use in Swedish agriculture and are the basis for the monitoring program of pesticides and some of their degradation products in surface water in Sweden (Kreuger, 1998; Boye et al., 2019). These methods, accredited for the analysis of water samples, were modified to enable the analysis of extracts from blood microsampling. The complex composition of whole blood limited the GC—MS method to early eluting compounds (chlorpyrifos, lindane (gamma-HCH), vinclozolin and aldrin). High-boiling compounds such as tau-fluvalinate and other pyrethroids, typically included in water analysis, could not be analysed with sufficient quality and were therefore excluded from this study. Each blood sample was analysed using either the LC—MS/MS or the GC—MS method. To enable determination with both methods, two separate blood samples are needed, i.e. 16 µl in total. A detailed description of the two sample preparation methods is presented in Supplemental material 1. Table 1 lists all compounds analysed by each method, along with their application, authorization status (at the time of the study), method detection limit (LOD), recovery, and precision.

2.4. Statistical analysis

We used generalised linear (mixed) models to estimate the probability of nestlings having detectable pesticides in their blood, the magnitude of pesticide concentrations and the difference in these metrics between conventional and organic sites. To estimate the prevalence of nestlings with at least one detectable pesticide in their blood, we used a binomial regression where the number of 'trials' was the total number of nestlings that were tested in each nest, and the number of 'successes' was the number of nestlings with pesticides in each nest. To calculate the average number of different pesticides that were present in each nestling, we used Poisson regression, with nest as a random factor. For the mean concentration of all pesticides in the blood, we used a gamma distribution regression (identity link) with nest as a random factor. Where we wanted to compare potential differences in pesticide prevalence between nestlings from conventional and organic fields (or between breeding ortolan buntings from conventional fields in central Sweden and clear-cut areas in northern Sweden), we used a categorical variable in the model to distinguish between the two groups. Models were implemented in R (R Core Team, 2019) using the Bayesian modelling platform JAGS (Plummer, 2003). We used a Bayesian framework in the modelling to allow us to easily calculate the magnitude of between-group differences and the probability of the direction of any effects. Thus, values reported are the means and their associated standard deviations of the posterior distributions, the 95 % credible intervals and where differences between groups are calculated, the posterior distribution of the differences between groups. Model fit for all analyses were checked using posterior predictive checks (Hooten and Hobbs, 2015), with no models indicating overdispersion. Values below LOD were treated as zeros. The complete data set with complementary information about detection limits (LOD) for the different detected substances is provided in Supplemental material 2.

3. Results

3.1. Skylark and yellowhammer nestlings

Twenty-two out of 40 skylark nestlings and one out of five yellowhammer nestlings exceeded the limit of detection (LOD, Supplemental material 2) for at least one pesticide or degradation product (BAM, carbendazim, prothioconazol-destio). We estimated that there was a 0.49 (SD: 0.07; 95 % CI: 0.35–0.63) probability of detecting at least one pesticide or metabolite in nestlings in the study area.

We detected residues from 13 out of 100 substances (LC—MS/MS method) at levels between 0.02 and 50 ng/ml (Table 1, Supplemental material 2). Of these 13 compounds, eight were fungicides and five were herbicides. None of the 14 insecticides included in the LC—MS/MS analysis of skylark and yellowhammer nestling blood were detected. The most frequently detected pesticides were the two fungicides fenpropimorph (showing the highest concentrations) and pyraclostrobin (used in the study area) and the herbicide tribenuron-methyl (used in the study area; Fig. 1). The maximum number of pesticide residues identified per nestling was seven (Fig. 2). However, on average, only 0.51 (SD: 0.25; 95 % CI: 0.14–1.08) pesticides were found per nestling when including individuals who tested negative for all pesticides. Among individuals who exceeded the LOD for at least one pesticide, the average number of pesticides found per nestling was 2.01 (SD: 0.49; 95 % CI: 1.11–3.05). The mean concentration of all pesticides per individual was 2.32 ng/ml (SD: 0.80; 95 % CI: 1.04 – 4.04).

We detected carbendazim in blood samples of one nestling yellowhammer and one skylark (Fig. 1). The use of this fungicide has been prohibited in Sweden since 1998, but it is widely used across the EU. However, it is also a degradation product of the fungicide thiophanate-methyl, which is authorised in Sweden. In addition, residues of BAM (2,6-dichlorobenzamide), a known degradation product of the herbicide dichlobenil (prohibited in the EU since 2008) and the fungicide fluopicolide (authorized in EU) were present in blood samples from three nestlings from the same nest on a conventional field in 2015 (Fig. 1). Among 55 substances authorised in Sweden, we detected 24 % (N = 13) in nestling skylarks and yellowhammers. To our knowledge, only 3 of these substances were used by farmers in the study area in 2016.

Breeding Ortolan buntings Seven out of ten individuals (proportion:

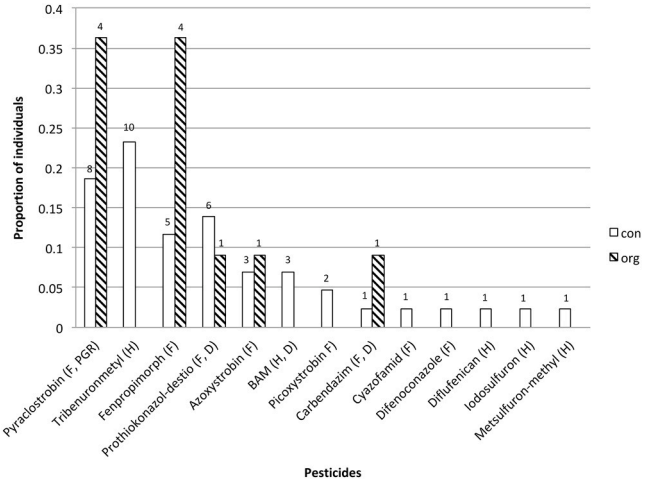

Fig. 1. Prevalence and relative frequency distribution of 13 pesticide residues (chlorpyrifos excluded; see method) detected in 24 out of 66 blood samples of nestling skylarks (N = 40), nestling yellowhammers (N = 5) and adult ortolan buntings (N = 21) sampled on their breeding sites in conventional (con) and organic (org) crop fields. One ortolan bunting that tested positive for azoxystrobin was sampled on a forest clearcut and classified as organic (F=fungicide; H=herbicide; D=degradation product; PGR=plant growth regulator).

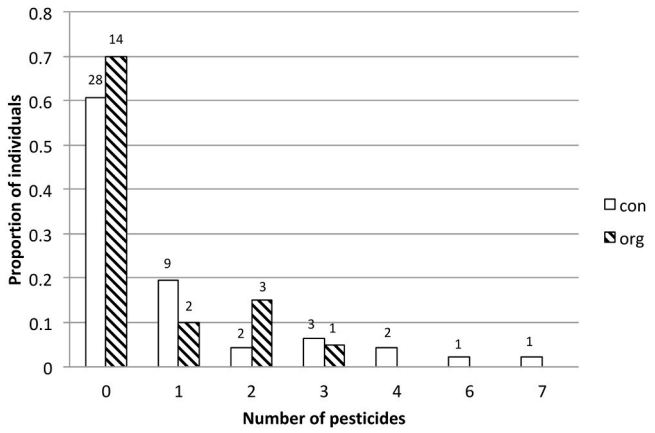

Fig. 2. Prevalence and relative frequency distribution of the number of pesticide residues detected in individual nestling skylarks (N = 40), nestling yellowhammers (N = 5) and adult ortolan buntings (n = 21) sampled on their breeding sites in conventional (con) and organic (org) crop fields. One ortolan bunting that tested positive for the fungicide azoxystrobin was sampled on a forest clearcut and classified as organic.

0.70) tested positive for chlorpyrifos when analysed with the GC—MS method. In contrast, only two out of 21 ortolan buntings (0.1) tested positive for either one herbicide (tribenuron-methyl) or one fungicide (azoxystrobin) in the LC—MS/MS analysis.

3.2. Pesticide residues and land use

We found little evidence for possible differences (mean: 0.05 ± 0.17 SD; probability of conventional > organic = 0.6) in the probability of detecting at least one pesticide in the blood of skylark and yellowhammer nestlings between conventional (mean: 0.50 ± 0.08 SD) and organic fields (mean: 0.45 ± 0.15 SD). Similar results were obtained for the differences in the number of pesticides and concentration of pesticides in the blood samples collected from conventional and organic fields (Table 3).

For ortolan buntings, there was little evidence of differences in chlorpyrifos concentrations (0.07 ng/ml ± 0.09 SD) in the blood of breeding males from conventional fields (mean: 0.24 ng/ml ± 0.08 SD) compared to forest clear-cut areas (mean: 0.31 ng/ml ± 0.09) without pesticide treatment (Table 3).

4. Discussion

Our results show that nestling yellowhammers, skylarks and adult ortolan buntings in central and northern Sweden are widely exposed to pesticides. However, pesticide use is lower in Sweden than in much of the EU (Rundlöf et al., 2012), likely resulting in fewer detected compounds and lower blood concentrations than in birds from more intensively treated regions. Consistent with this, only about half of the 45 skylark and yellowhammer nestlings had detectable levels of at least one of the 100 pesticides screened by LC—MS/MS, with a mean of 0.51 pesticides per individual (SD: ± 0.25; including non-detects). Among nestlings with at least on pesticide above the limit of detection (LOD), the mean number of compounds per nestling was 2.01 ± 0.49. Residues were restricted to fungicides (N = 8) and herbicides (N = 5). In contrast, Fuentes et al. (2024) reported on average 5.6 pesticides (SD: ±1.52; > LOD) in blood samples from 35 Montagu's harrier *Circus pygargus* nestlings, based on screening for 116 pesticides in conventional cereal fields in southwestern France. In that study, the detected pesticides comprised similar numbers of fungicides (N = 4) and herbicides (N = 9) but also included insecticides (N = 5), which we did not detect in skylark and yellowhammer nestlings.

Table 3
Differences (Diff.) in the number and concentration (ng/ml) of pesticide residues in Skylark and yellowhammer nestlings from nests in conventional (Conv.) and organic (Org.) fields.

	Diff.	Conv.	Org.
Number of residues	0.21 ± 0.41	0.58 ± 0.31	0.37 ± 0.33
Mean concentration of residues	0.17 ± 0.54	2.63 ± 0.86	2.47 ± 0.87
Sum of concentrations of all residues	0.03 ± 0.79	4.10 ± 0.61	4.07 ± 0.79

Fuentes et al. (2024) did not find a relationship between pesticide levels and bird condition. At our study site, concentrations of individual pesticide residues and their metabolites in blood ranged from 0.02 to 50 ng/ml (Supplemental material 2) and were generally low compared to other studies. For example, organochlorine pesticide (OC) residues in blood plasma of various birds in India and Mexico range from 11.4 ng/ml (sum of concentration: ΣHCH; hexachlorocyclohexane) in white ibis Threskiornis melanocephalus to 286 ng/ml (ΣHCH) in sarus crane Grus antigone (Dhananjayan and Muralidharan, 2010) and up to 204.9 ng/ml (aldrin) in common ground dove Columbina passerina (Rivera-Rodréguesz et al., 2007). The only study from northern Europe reports neonicotinoid residues in the European honey buzzard, Pernis apivorus, with concentrations of imidacloprid and thiacloprid ranging from 0.009 to 0.031 ng/ml (Byholm et al., 2018), i.e. comparable concentrations to those observed for other compounds in our study.

Our results provide the first evidence that long-distance migratory ortolan buntings were exposure to the insecticide chlorpyrifos outside Sweden (Europe and/or Sub-Saharan Africa) prior to the 2020 EU-wide ban (EUR-lex, 2020), with residues still detectable on Swedish breeding grounds during our study period. However, chlorpyrifos continues to be widely applied in some non-EU European countries as well as North and Sub-Saharan Africa, particular in maize and cotton cultivation and in locust control (Theriault et al., 2020; Mazur and Alieksieieva, 2024). Consequently, long-distance migratory passerines such as the ortolan bunting are likely to remain exposed to chlorpyrifos on migration and in African wintering areas. Repeated exposure during energetically demanding stages during migration may have important conservation implications for the species showing a major population decline across Europe, with particularly steep declines in northern European populations (Jiguet et al., 2016). Eng et al. (2017) adds to these findings showing that short, sub-lethal exposure at field-realistic doses can disrupt migratory orientation and flight performance in seed-eating songbirds. However, assessing the effects of the low pesticide levels and exposure to multiple residues observed in our study species is beyond the scope of this work and requires long-term, standardized research. Moreover, our data may be biased toward lower contamination, as birds and nests with higher exposure could be underrepresented due to reduced survival.

To date long-term environmental monitoring of pesticides is often restricted surface water, groundwater, sediment and food (Moreau et al., 2022, Boye et al., 2019). We suggest ongoing monitoring of pesticides in blood samples of farmland birds using nonlethal and well-developed standardized methods to identify exposure trends and toxicological risks from pesticide use to farmland biodiversity in line with recommendation from Rodrigues et al. (2023). Such an approach is feasible through modern, highly sensitive mass spectrometry instrumentations combined with novel microsampling techniques such as capillary microsampling (CMS) that were used here. CMS is particularly suitable for monitoring small wild animals because it is fast and easily performed and allows for sampling and quantitative handling of very small blood volumes (e.g., 8 μl), thereby reducing the possible negative impacts of sampling efforts on animal welfare (refinement). The sampling strategy used in this study is labour-intensive, logistically demanding and requires highly trained personnel, making it costly and unreliable (in terms of sample yield) for long-term monitoring. A more feasible

approach would be to focus on species that can be sampled efficiently at bird observatories with standardised banding schemes or via nest-box based research (Eens et al., 2013; Eng et al., 2014),

Despite the small sample volume and the inability to pre-concentrate samples before analysis, the LC—MS/MS multimethod achieved an average detection limit of 0.77 ng/ml (range 0.016—10 ng/ml, see supplemental material 2). Both analytical methods developed for this study required the full 8 μl of blood. To analyse the same blood sample with both methods, two separate microsamples may be collected. Alternatively, the blood sample can be mixed with internal standard solution and split into two 4 μl subsamples, allowing analysis by both methods. However, this approach would affect detection limits, as maintaining the low dilution factor used in this study (8 μl blood to 80 μl final extract) would be challenging.

The GC—MS method was hindered by the complex composition of whole blood, limiting the analysis to early eluting, low-boiling compounds such as chlorpyrifos. For high- boiling compounds like the pyrethroid insecticides, the method lacked robustness and data quality. To improve results for these compounds, additional extract clean-up before GC—MS analysis is necessary, or alternatively, an LC—MS/MS method could be developed for their analysis in blood microsamples.

Our study shows that environmental monitoring is also important for pesticides prohibited in Sweden and the EU, as pesticides and their degradation products can persist in groundwater and soil, bioaccumulate, hitchhike on particles and thus travel long distances (carry-over effect). In line with this idea were the levels of chlorpyrifos in blood samples of ortolan buntings in our study; they were at least as high for birds breeding on clear cuts without pesticide use compared to farmland. Additionally, our study indicates that the use of pesticides at the local level (conventional fields) can expose nestlings also in nearby fields without the use of pesticides, presumably through wind drift and/or habitat use of parents at a scale larger than the field level. Hence, any positive effects of organic and pesticide reduced farming on farmland birds at the local scale (e.g. via increased invertebrate abundance) are likely to depend strongly on the surrounding landscape context (Winqvist et al., 2011, Hološková et al. 2025). The limited evidence for differences between conventional and organic fields (and clearcuts; see Table 1 and Figs. 1 and 2) may also be due to small samples sizes, the timing of pesticide applications, generally low exposure at our study sites and similar levels of exposure in the winter grounds outside Sweden.

Finally, our study highlights the fact that the origin of some pesticides in blood samples of nestling skylarks and yellowhammers remains unclear. For instance, we detected carbendazim and 2,6-dichlorobenzamide (BAM) in blood samples of nestling skylarks (Fig. 1). Carbendazim has been prohibited in Sweden since 1998 but was widely used across the EU during the time of the study, suggesting possible carry-over effects (see ortolan bunting above). However, carbendazim is also a degradation product of the fungicide thiophanate-methyl, which is authorized in Sweden. Similarly, BAM is a degradation product of dichlobenil and has been prohibited in the EU since 2008. BAM is known to be very persistent in both soil and groundwater and poses a potential environmental problem for the quality of drinking water production (Ellegard-Jensen et al., 2017). Possible harmful effects of BAM on birds are unknown.

Taken together, our findings offer the first reference values for pesticide residues in birds for Sweden. They show that even in landscapes with relatively low direct pesticide use, farmland birds can still be exposed to multiple residues through local spillover and long-distance transport. Future monitoring should target both current and legacy compounds and use sensitive, non-lethal techniques to better assess risks to avian biodiversity. Such systematic monitoring could be based on easily managed species, such as starlings breeding in nest boxes, to maximize sampling success and minimize labour and costs, while still providing high relevance for other species in the agricultural landscape.

Author contributions

All authors contributed to the study conception and design. Field work and blood sampling was coordinated by Jan Sondell. The pesticide residue methods were developed by Ove Jonsson who also analysed the blood samples. The statistical analysis was performed by Matthew Low. The first draft of the manuscript was written by Sönke Eggers and Ove Jonsson, and all authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

CRediT authorship contribution statement

Jan Sondell: Supervision, Project administration, Funding acquisition, Conceptualization. **Matthew Low:** Writing – review & editing, Validation, Formal analysis. **Ove Jonsson:** Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization. **Sönke Eggers:** Writing – original draft, Visualization, Validation, Supervision, Conceptualization.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee Linköping, Sweden (Linköpings djurförsöksetiska nämnd, 2014–03–14, Dnr 18–14).

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jan Sondell reports financial support was provided by Swedish Environmental Protection Agency. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.119502.

Data Availability

Data will be made available on request.

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