Plant protection products and farmland Galliformes: new concerns for an old issue?

Overview and perspectives of research

Elisabeth BRO
French National Hunting & Wildlife Institute (ONCFS)
Research Department
Sedentary small game team

Decline of farmland birds in Europe


Grey partridge
Red-legged partridge
Common quail
Ring-necked pheasant

Forest (N=34 sp.)
Farmland (N=39 sp.)
Causes of bird declines
a diversity of threats

Assessment of the population status & threats of all bird species at the European level
(reporting under Article 12 of the European Union « Birds » Directive)

Use of pesticides* may contribute to ongoing bird declines

* Plant Protection Products

BirdLife International (2015)

(Geiger et al., Basic & Applied Ecology, 2010)
Data: 2007, farmland birds, 8 countries in Europe

(Hallmann et al., Nature, 2014)
Data: 2003-2010, 15 passerine species, the Netherlands

(Halie, thesis, 2016)
Data: 1994-2012, northern bobwhite, Texas

L. Armand

Use of pesticides* may contribute to ongoing bird declines

Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland

Declines in insectivorous birds are associated with high neonicotinoid concentrations

Impact of Neonicotinoid Insecticide Use on Northern Bobwhite (Colinus virginianus) in Texas

(Alberto, Halie)
Controversy on relative importance of factors


Pesticide Acute Toxicity Is a Better Correlate of U.S. Grassland Bird Declines than Agricultural Intensification

Hill et al.
20 May 2014

Habitat Availability Is a More Plausible Explanation than Insecticide Acute Toxicity for U.S. Grassland Bird Species Declines

Mineau & Whiteside
20 February 2013

→ interdependencies of the predictor variables in correlative studies
→ lack of full mechanistic evidence (cf. Boatman et al. 2004)

Complex issue, little documented in the field
Use of pesticides as part of overall agriculture modernisation

80-100 years ago

First pesticides were dangerous chemicals

Late XIXth – early XXth centuries
Protection of crops with
- sulfuric acid (herbicide)
- arsenic salts (insecticide)
- organic mercury (fungicide)

- acute poising events of birds but little public attention

Bain et al. (2001); Baldi & Farago (2007); Ewald & Aebischer (2009); Rattner (2009); etc.
Toxicity of active substances to birds: an old issue

Emergence of public concern

- humans were using powerful & persistent chemical pesticides before knowing the full extent of their potential harm to the whole biota.

*(organochlorine and organophosphate insecticides)*

(Sept. 1962)

Multiple routes of exposure

application of a substance on a crop

direct contact

diet

grit

soil

volatility

air

solubility

water

drink
Unintentionnal effects of pesticide use
from obvious to subtle effects

application of a substance on a crop

indirect effects

- lethal effects
  (acute, subacute, chronic)
- sublethal effects
  (visible, invisible)

Direct effects

- application of a substance on a crop
- weeds/seeds
- invertebrates

% decrease in productivity

Unintentionnal effects of pesticide use
indirect effects

Evidence for the indirect effects of pesticides on farmland birds

<table>
<thead>
<tr>
<th>Species</th>
<th>Effect of pesticides</th>
<th>Effect on nest success</th>
<th>Time of year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fruit availability</td>
<td>1st winter</td>
<td>1st summer</td>
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<tr>
<td>Black-billed</td>
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<td>Pinyon Juncos</td>
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<td>American Dippers</td>
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<tr>
<td>Yellow-rumped</td>
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<tr>
<td>Yellowhammers</td>
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<tr>
<td>Blue-winged</td>
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<tr>
<td>White-rumped</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Red-winged</td>
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</tr>
</tbody>
</table>

11/65

12/65
Unintentionnal effects of pesticide use

**Indirect effects**

- Application of a substance on a crop
- Chick growth & development
- Body condition
- Survival rate
- Population change

<table>
<thead>
<tr>
<th></th>
<th>Intensive farm</th>
<th>Other farms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-insecticide years</strong> (before 1989)</td>
<td>30 ± 3 %</td>
<td>27 ± 2 %</td>
</tr>
<tr>
<td><em><em>Insecticide</em> years</em>* (1989-1994)</td>
<td>22 ± 5 %</td>
<td>34 ± 3 %</td>
</tr>
</tbody>
</table>

* Broad-spectrum organophosphate insecticide (dimethoate)

E.g.
- Rands (1985)
- Sotherton & Robertson (1990)
- Aebischer & Potts (1998)
- Southwood & Cross (2002)

Unintentionnal effects of pesticide use

**Lethal effects**

- Application of a chemical on a crop
- Monitoring surveys of Wildlife
  - *Wildlife Incident Investigation Scheme* (WISS, UK)
  - *Surveiller pour Agir network* (SAGIR, Fr)
- Direct effects
  - Lethal effects (acute, subacute)
Unintentionnal effects of pesticide use
lethal effects

Acute oral LD$_{50}$ (mg/kg of body weight) of some active substances for grey (♀) and red-legged (♂) partridges

Grolleau & Caritez (1986)

< 10 (very highly toxic)
< 50 (highly toxic)


Experimental measurement of acute oral toxicity (LD$_{50}$)

Dose that is lethal to 50 % of a batch of laboratory animals exposed to a single oral administration

OECD internationally accepted standard method
http://www.oecd.org/chemicalsafety/testing/1836220.pdf
Species sensitivity distribution (SSD) methodology

Multi-species criterion to assess toxicological hazard on avian biodiversity

Mineau et al. (2005)

Hazardous dose for birds (HD₅₀)

Mineau et al. (2005)

Unintentionnal effects of pesticide use

lethal effects

Example
Mortality due to carbofuran application on maize in North America in the 1980s: 17 – 91 million birds annually
Mineau et al. (2001), Mineau (2005)

- carbamate cholinesterase-inhibiting (AChE) insecticide
- used as granules on the soil at seedling

- LD₅₀: 0.71 mg / kg bw (Anas platyrhynchos)
- LC₅₀: 1.6 mg / kg bw / day
- HD₅₀: 0.21 mg/kg

Unintentionnal effects of PPP use
sublethal effects

application of a chemical on a crop

direct effects

sublethal effects
(visible, invisible)

vulnerability

other mortality factors
(predation, disease)
Unintentional effects of PPP use
sublethal effects

application of a chemical on a crop

direct effects

→ adverse health effects
  • genetical
  • immunological
  • neurological
  • developmental
  • reproductive
  in the contaminated individual and/or its progeny

Reproductive effects
of pesticide/pollutant contamination
in birds

• sex-ratio skew
• breeding behavior
  • egg desertion
  • decreased defense of territories
• supernormal clutch size (female-female pair)
• lower clutch size
• delayed egg-laying
• feminization of male embryos
• eggshell thinning (crushed eggs)
• embryo deformities & mortality (GLEMEDS)

Reproductive Effects in Birds Exposed to Pesticides and Industrial Chemicals
D. Michael Fry
Department of Environ. Science, University of California, Davis, California

References
• Fox et al. (1978)
• Bauer (1985)
• Dabbert et al. (1996)
• Fry (1995, REVIEW)
• Hoffman & Eastin (1982)
• Kamata et al. (2010)
• etc.
Detected sublethal effects in birds

**Laboratory & semi-natural experiments**

<table>
<thead>
<tr>
<th>Adult</th>
<th>Clutch/Egg</th>
<th>Chick</th>
</tr>
</thead>
<tbody>
<tr>
<td>• gonad histology / sperm production (Grote et al. 2008, Hoshi et al. 2014)</td>
<td>• egg characteristics – size, eggshell thickness, etc. (Lopez-Antia et al. 2013)</td>
<td>• immune system (Lopez-Antia et al. 2015a, b)</td>
</tr>
<tr>
<td>• breeder quality (sexual ornaments) (Lopez-Antia et al. 2013)</td>
<td>• fertility (Lopez-Antia et al. 2013)</td>
<td></td>
</tr>
</tbody>
</table>

Do they occur in operational conditions?

Current regulatory background

**In Europe**

**Regulation 2009/1107/CE**

http://eur-lex.europa.eu/legal-content/

- placimg of plant protection products on the market
Public policies in Europe

European Directive 2009/128/EC

framework for Community action to achieve the sustainable use of Plant Protection Products

national action plans

https://ec.europa.eu/food/plant/pesticides/sustainable_use_pesticides

Exposure of farmland birds to plant protection products

The grey partridge as a case study

Elisabeth BRO
Florian MILLOT
Anouk DECORS

Hunter Associations

with the contribution of H. Devillers (INRA), A.K. Saxena (CDRI, India)
Partnership

« PeGASE » study

« M6P » study

Partners

Scientific collaboration

Funding

The grey partridge as case study

• Typical farmland bird

• Nests on the ground

• 75% of clutches laid in crops (mainly winter wheat) – in France

• Feeds on cultivated and weed plants (sprouts, leaves, buds, seeds) and invertebrates.
Study sites

% cereals in arable land (2010)

- 0.0 - 6.5
- 6.5 - 21.6
- 21.6 - 35.7
- 35.7 - 50.4
- 50.4 - 100.0

12 sites (∗)

- 14,500 ha
- intensively cultivated

occurrence area of the grey partridge (wild populations)

Field survey (2010-2011)

1. Radiotacking survey
   (529 adults, 281 clutches, 75 coveys)

2. Analysis of eggs

Status & localisation recorded twice daily in spring & summer

3. Farmer’s survey

   - 142 farmers
   - ca. 1000 fields
   - ca. 6500 ha
Use of Plant Protection Products
(arable land in France)

large diversity of active substances (ASs)
(186 applied between 1st March – 31st August 2010-2011 / ca. 32 crops)

winter wheat : 39 herbicides / 8 insecticides / 27 fungicides
(until 18 substances for one field: 9 H / 2 I / 7 F)

treatments coincide with laying (& incubation) period

Potential exposure of partridges / eggs / chicks to active substances

Potential exposure = presence on / near treated fields at the time of treatments
(EFSA, 2009)

To which extent birds are actually exposed to these substances?

radiotracking
partridge habitat use

fields in home range

farmer’s survey
crop treatments

<table>
<thead>
<tr>
<th>Active substance</th>
<th>farmer’s survey</th>
<th>crop treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td></td>
<td></td>
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<td>d</td>
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<tr>
<td>e</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Potential exposure of partridges / eggs / chicks to active substances**

<table>
<thead>
<tr>
<th></th>
<th>Breeders (n=529)</th>
<th>Clutches (n=140)</th>
<th>Coveys (n=75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% exposed ≥ 1 AS:</td>
<td>100%</td>
<td>71%</td>
<td>13%</td>
</tr>
<tr>
<td>Nb ASs:</td>
<td>157</td>
<td>108</td>
<td>18</td>
</tr>
<tr>
<td>% main ASs:</td>
<td>86 (≥10)</td>
<td>32 (≥5%)</td>
<td>-</td>
</tr>
<tr>
<td>fungicides:</td>
<td>28% (mainly azoles)</td>
<td>53% (mainly azoles)</td>
<td>67%</td>
</tr>
<tr>
<td>herbicides:</td>
<td>51%</td>
<td>25%</td>
<td>28%</td>
</tr>
<tr>
<td>insecticides:</td>
<td>11%</td>
<td>16%</td>
<td>-</td>
</tr>
<tr>
<td>growth regulators:</td>
<td>8%</td>
<td>6%</td>
<td>-</td>
</tr>
</tbody>
</table>

Potential exposure to complex mixtures (up of several tens of active substances)

Unpublished result

**Potential exposure of partridges / eggs / chicks to active substances**

**Surrogate**

Unpublished result

Breeders: $R^2 = 91\%$
Clutches: $R^2 = 77\%$

→ statistics of usage as a semi-quantitative surrogate
### Multi-residues analyses

**Bro et al. (2016)**  
**Millot et al. (2015)**

<table>
<thead>
<tr>
<th>94 carcasses</th>
<th>139 failed eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>(over 261 dead birds)</td>
<td>(52 successful clutches)</td>
</tr>
</tbody>
</table>

| analytical methods: |  
|---------------------|---------------------|
| GC/MS & HPLC... | GC&LC/MS-MS |

|Nb compounds: |  
|--------------|----------------|
| 60 | 505 |

| limit of quantification: |  
|-------------------------|-----------------------|
| (mg/kg) |          |
| 0.01 – 0.50 | 0.005 - 0.01 |

---

### Multi-residues analyses

**Bro et al. (2016)**  
**Millot et al. (2015)**

- 29% of the 94 carcasses contaminated
- 5 ASs actually used:
  - **insecticides**: tefluthrin, cyfluthrin, cypermethrin
  - **herbicides**: linuron, s-metolachlor
- concentrations: 0.01 – 0.50 mg/kg
- old ASs: atrazine, lindane, carbofuran
46% of the 52 clutches contaminated

- 9 ASs currently used:
  - **fungicides**: difenoconazole, tebuconazole, cyproconazole, fenpropidin, prochloraz
  - **insecticides**: lambda-cyhalothrin, thiamethoxam/clothianidin
  - **herbicides**: bromoxynil, diflufenican

- Concentrations: < LoQ – 0.34 mg/kg

- Old ASs: DDT (Σ isomers), HCH (α,β,δ isomers), Fipronil (sulfone), Heptachlor (epoxyde)

- No relation with egg/embryo status

*One could have expected a higher level of contaminations & higher number of substances*
Residues analyses
Interpretation of results

1. Potential exposure ≠ contamination

- Application of a substance on a crop
- Soil
- Air
- Water
- Volatility
- Solubility

Plant metabolism
Biodegradation
Photo degradation
Hydrolysis

Move

No transfer to the eggs (or not to all eggs)

2. A contaminant may be not detected

⇒ 2.1. Due to excretion / metabolisation

Metabolisation

Storage - muscles - fat ...

Transfer to eggs

Excretion

Concentration of the contaminant

- Contamination of a laying female
- Digestive system
- Liver
- Eggs

Time (days)

Limit of detection

39/65

40/65
Residues analyses
Interpretation of results

2. A contaminant may not be detected
⇒ 2.2. Due to methodological aspects

<table>
<thead>
<tr>
<th>appropriate sample (= ‘biological matrix’)</th>
<th>no</th>
<th>Not Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>• bird – liver/crop-gizzard/muscle/fat</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>• egg – yolk/white/embryo/eggshell and timing</td>
<td>no</td>
<td>Not Detected</td>
</tr>
<tr>
<td>stability (storage conditions)</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>concentration ≥ Limit of Detection</td>
<td>yes</td>
<td>Detected</td>
</tr>
</tbody>
</table>

A non-positive result is non conclusive.

By no means, it is ‘negative’.
Currently, data of field contaminations can not be interpreted
1- lack of (available) detailed benchmark data
2- exposure level & nature → contamination level
3- experimental exposure scenarios not always field realistic
### Biological significance of contaminations?

**Lipophily and persistence**

**Lipophily (Log(P))**

- **≥ 5**
- **3-5**
- **< 3** (regulatory value)

**Persistance (biodegradability)**

- **Months**
- **Weeks**
- **Days**

<table>
<thead>
<tr>
<th>ACTIVE SUBSTANCE</th>
<th>BREEDER (n=529)</th>
<th>CLUTCH (n=140)</th>
<th>COVEY (n=75)</th>
<th>Log(P)</th>
<th>Biodegradability</th>
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<tbody>
<tr>
<td>Bro &amp; Devillers (2014)</td>
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<tr>
<td>Epoxiconazole</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothioconazole</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Chlormequat chloride</td>
<td>+++</td>
<td></td>
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<tr>
<td>Boscalid</td>
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<td>+++</td>
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<tr>
<td>Prochloraz</td>
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<td>++</td>
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<td>Propiconazole</td>
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<td>Chlorothalonil</td>
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<td>++</td>
<td>+</td>
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<tr>
<td>Cyproconazole</td>
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<tr>
<td>lambda-Cyhalothrin</td>
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<td>Tebuconazole</td>
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<tr>
<td>Fenpropidin</td>
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<td>Deltamethrin</td>
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<td>Linuron</td>
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<td>Fipronil</td>
<td>-</td>
<td>-</td>
<td></td>
<td>3,75</td>
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</tbody>
</table>

### Biological significance of contaminations?

**Acute oral toxicity**

<table>
<thead>
<tr>
<th>ACTIVE SUBSTANCE</th>
<th>BREEDER (n=529)</th>
<th>CLUTCH (n=140)</th>
<th>COVEY (n=75)</th>
<th>LD50 (mg/kg bw)</th>
<th>HD5 (mg/kg)</th>
<th>TER 1</th>
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</thead>
<tbody>
<tr>
<td>Bro &amp; Devillers (2014)</td>
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<td>++</td>
<td></td>
<td>&gt; 703</td>
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<td></td>
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<td>+</td>
<td>&gt;151</td>
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</table>

**Risk assessment (TER 1)**

- **≤ 1**
- **1-5**
- **5-10**
- **>10** (regulatory value)

= - = : missing value
Ecological risk assessment: the *Toxicity-to-Exposure ratio* (TER) methodology

**Toxicity**
- experimental measure (toxic dose, e.g. DL<sub>50</sub>)

**Field exposure**
- estimated through modelling

Diet exposure:
- dose applied
- residues in food items

**Trigger value / « unacceptable »** (10 for lethality, 5 for reprotoxicity)

**Different scenarios:**
- Types of toxicity (acute – DL<sub>50</sub>; dietary – CL<sub>50</sub>; chronic – NOEL)
- Routes of exposure (diet, inhalation, dermal contact)
- Species (diet, weight), crops / applications / doses

**Tiered approach** (TER1 vs. Refined TER)

---

Biological significance of contaminations?

*Reproductive toxicity*

<table>
<thead>
<tr>
<th>ACTIVE SUBSTANCE</th>
<th>POTENTIAL EXPOSURE</th>
<th>REPRODUCTIVE TOXICITY</th>
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<tbody>
<tr>
<td></td>
<td>BREEDER (n=529)</td>
<td>CLUTCH (n=140)</td>
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<tr>
<td>Epoxiconazole</td>
<td>+++</td>
<td>+++</td>
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<td>Propiconazole</td>
<td>+++</td>
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<td>Chlorothalonil</td>
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<td>Propiconazole</td>
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</tbody>
</table>

**NO(A)EL (mg/kg bw/d)**

- **≤ 10**
- **[10-25]**
- **> 25**

**Risk assessment (TER 1)**

- **≤ 1**
- **[1-5]**
- **> 5** (regulatory value)

**Bird value**

- **- : missing value**

---

Experimental measurement of reproductive toxicity (NOEC) methodolog)

No Observed (Adverse) Effect Concentration

CONTROL

Concentration in food

NO(A)EC = long-term dietary/reproductive toxicity (mg a.s. / kg of food)
Maximum concentration in a long-term dietary exposure that has no (adverse) effect on bird reproduction (egg-laying, hatchability, chick body condition)

OECD internationally accepted standard method
http://www.oecd.org/chemicalsafety/testing/oecguidelinesfortheTestingofchemicals.htm

test No. 206. Avian Reproduction Test (04 Apr 1984)

Sources of data
Databases, reports and papers

PPDB, Herfordshire Univ.
JMPR, FAO & WHO
TOXNET

Peer reviews, EFSA
AGRITOX & EPHT
ANESE

data papers

etc.
Substances registered on the European list of potential endocrine disruptors (released early July 2017)

QSAR (Quantitative Structure - Activity Relationship) modelling

In silico experiment testing the binding affinity of 46azole substances on the active site of aromatase

sex differentiation in embryos
sexual behaviour in males
Biological significance of contaminations? Endocrine disruption

Binding affinity of azole chemicals \([\text{fungicides (↓)} + \text{medicines}]\) on the active site of the aromatase enzyme in 4 species

Good inter-species correlation \((R^2 = 82\% – 86\%)
\)

- **extrapolation**

Saxena et al. (2016)

---

Biological significance of contaminations? Endocrine disruption

Devillers et al. (2016)
**Biological significance of contaminations?**

*Post-market data*

**Number of breeders potentially exposed to the substance**
(N ≥ 30)

HDs < 50 mg/kg

% of mortality within 10 days after potential exposure

Millot et al. (2016)

© E. Bro & Devillers (2017)
More information & data

Bro et al. (Science of the Total Environment - STOTEN, 2015) (open access)

Millot et al. (Ecotoxicology and Environmental Safety - EEF, 2015)

Bro et al. (Environmental Science and Pollution Research - ESPR, 2016) (open access)

Saxena et al. (SAR and QSAR in Environmental Research – SQER, 2015) (open access)

Devillers et al. (SAR and QSAR in Environmental Research - SQER, 2015)

Limits and contributions of the study

→ Field & modelling data for a wide number of substances

- information to the regulators on the level of concern of substances to birds (post-market data)
- data to prioritise substances for further works (experiments, modelling)
  - chlormequat chloride, cypermethrin, tefluthrin, cyfluthrin, cyproconazole, epoxiconazole, metconazole, fenpropidin, lambda-cyhalothrin, diflufenican
- field data (residue concentrations) to calibrate experimental studies

conclusive results required
  (cause-to-effect relationships at the individual / population levels)
  ↓
  more research
1. Field exposure/contamination

Residue analyses

➢ To document contaminations in the field
  • species / matrix (droppings, liver, blood, egg)
  • substances
  • concentrations
  • farming background

• Bro et al. (2015)
• Millot et al. (2015)
• Espin et al. (2016)
• Turaga et al. (2016)
• Halim (2016)
• Mateo et al. (2016)
• Corcellas et al. (2017)

2. Effects on individuals

Experimental studies ➢ cause-to-effects relationships

⚠ Field realistic exposure/contamination

Laboratory (in vitro)

➢ in ovo injection

➢ effects on:
  • embryo development & deformities
  • hatchability
  • chick behaviour, body condition & survival

/ window of exposure, low concentration, mixtures

• Gary et al. (2001)
• Kitulagodage et al. (2011)
• Bhashkar et al. (2012)
• Gobeil et al. (2017)
2. Effects on individuals

**Experimental studies ➔ cause-to-effects relationships**

Field realistic exposure/contamination

- **Laboratory (in vitro)**
- **Captivity (in vivo)**
  - short / long term dietary exposure
  - effects on:
    - health (physiology, immunity...)
    - reproduction (clutch size, egg-laying rate, egg characteristics, fertility, hatchability, chick survival rate...)
  - chronic & low-dose exposure
  - maternal transfer to eggs

© François Mougeot (IREC, Spain)

- Lopez-Antia et al. (2013–16)
- Grote et al. (2008)
- Hoshi et al. (2014)
- Garg et al. (2004)
- Adhikari et al. (2014)
- Kitulagodage et al. (2011)

**Perspectives of research**
Perspectives of research
3. Impact on populations

Field survey (e.g. quantification of mortality)
correcting for carcass disappearance (scavenging) and search efficiency

Identification of risk factors

Florian Millot (ONCFS, France)
Ralf Barfknecht (BAYER, Germany)
imidacloprid (neonicotinoid insecticide)

Perspectives of research
3. Impact on populations

Correlative analysis of field data
(pesticide use – population trend / abundance)

Long-term / nationwide database of pesticide use/sale:

- Geiger et al. (2010)
- Hallmann et al. (2014)
- Mineau & Whiteside (2013)
- Hall et al. (2014)
- Halie (2016)

Spurious correlations (confounding effects)
spatial/temporal replicates
Perspectives of research

3. Impact on populations

Population modelling

- life cycle matrix models
- individual-based landscape models

![Diagram of population modelling](image)

Effects of a substance

- Sibling et al. (2005)
- Roelofs et al. (2005)
- Millot et al. (2015)

Perspectives of research

4. Weight-of-evidence

Synthesis of scientific literature

(one substance approach)

Framework to structure & evaluate the collective information

- strength of the evidence of a causal link (pesticide use → individual / population effects)

![Diagram of weight-of-evidence](image)

from Lopez-Antia et al. (2015)