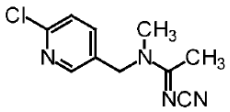
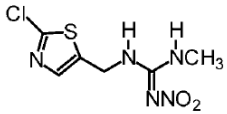
## Supplementary material

**Table S1.** Examples of studies that used different matrices to quantify neonicotinoid insecticides in free-living birds from different trophic levels. Molecules quantified are ACE = acetamiprid, CLO = clothianidin, DINO = dinotefuran, IMI = imidacloprid, NIT = nitenpyram, THIA = thiacloprid, and TMX = thiamethoxam.

| **References** | **Species** | **Feeding behavior** | **Matrix used** | **Molecules detected** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ACE | CLO | DINO | IMI | NIT | THIA | TMX |
| This study | *Circus pygargus* | Carnivorous | Blood | | ✓ | ✓ | | ✓ | ✓ | ✓ |
| | *Emberiza cirlus* | Omnivorous | Blood | | ✓ | ✓ | | ✓ | ✓ | ✓ |
| | *Luscinia megarhynchos* | Omnivorous | Blood | | ✓ | ✓ | | ✓ | ✓ | ✓ |
| | *Perdix perdix* | Granivorous | Blood | | ✓ | ✓ | | ✓ | ✓ | ✓ |
| Roy & Chen, 2023 | *Tympanuchus cupido* | Omnivorous | Feces, Liver, Carcasses | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | *Tympanuchus phasianellus* | Omnivorous | Feces, Liver, Carcasses | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Distefano et al., 2022 | *Ichthyaetus melanocephalus* | Piscivorous | Feathers | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | *Thalasseus sandvicensis* | Piscivorous | Feathers | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| Graves et al., 2022 | *Agelaius tricolor* | Granivorous | Carcasses | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Badry et al., 2021 | *Accipiter gentilis* | Carnivorous | Liver | | ✓ | | ✓ | | ✓ | |
| | *Accipiter nisus* | Carnivorous | Liver | | ✓ | | ✓ | | ✓ | |
| | *Haliaeetus albicilla* | Carnivorous | Liver | | ✓ | | ✓ | | ✓ | |
| | *Milvus milvus* | Carnivorous | Liver | | ✓ | | ✓ | | ✓ | |
| | *Pandion haliaetus* | Piscivorous | Liver | | ✓ | | ✓ | | ✓ | |
| Humann-Guilleminot et al., 2021 | *Tachymarptis melba* | Insectivorous | Food boluses, Plasma | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | *Tyto alba* | Apex predator | Feathers | ✓ | ✓ | | ✓ | | ✓ | ✓ |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bishop et al., 2020 | *Archilochus alexandri* | Nectarivorous | Cloacal fluid | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | *Archilocus colubris* | Nectarivorous | Cloacal fluid | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | *Calypte anna* | Nectarivorous | Cloacal fluid | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | *Selasphorus calliope* | Nectarivorous | Cloacal fluid | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | *Selasphorus rufus* | Nectarivorous | Cloacal fluid | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| Lennon et al., 2020 | *Alectoris rufa* | Granivorous | Liver, Bp | | ✓ | | | | | |
| | *Columba palumbus* | Herbivorous | Liver | | ✓ | | | | | |
| | *Perdix perdix* | Granivorous | Liver, Bp | | ✓ | | | | | |
| | *Phasianus colchicus* | Granivorous | Liver | | ✓ | | | | | |
| Graves et al., 2019 | *Archilochus alexandri* | Nectarivorous | Feathers, Carcasses | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | *Calypte anna* | Nectarivorous | Feathers, Carcasses | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Humann-Guilleminot et al., 2019 | *Passer domesticus* | Granivorous | Feathers | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| Bishop et al., 2018 | *Calypte anna* | Nectarivorous | Fecal pellets, Cloacal fluid | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | *Selasphorus rufus* | Nectarivorous | Fecal pellets, Cloacal fluid | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| Byholm et al., 2018 | *Pernis apivorus* | Insectivorous | Blood | ✓ | | | ✓ | | ✓ | |
| Ertl et al., 2018 | *Colinus virginianus* | Herbivorous | Liver | ✓ | ✓ | | ✓ | | | ✓ |
| Hao et al., 2018 | *Zonotrichia leucophrys* | Granivorous | Plasma | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| MacDonald et al., 2018 | *Meleagris gallopavo* | Granivorous | Liver | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Taliansky-Chamudis et al., 2017 | *Bubo bubo* | Apex predator | Blood | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Bro et al., 2016 | *Perdix perdix* | Granivorous | Eggs | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Berny et al., 1999 | Pigeons and partridges | Granivorous | Liver, Gizzard | | | | ✓ | | | |

**Table S2.** Main characteristics, legislative status, and toxicological profiles of neonicotinoids. These are nicotinic acetylcholine receptor (nAChR) competitive modulators, and the site of action is indicated in the column "Mode of Action". The year of prohibition of use in Europe and France is given for each molecule, and NA = "Not applicable" if the molecule is not considered as a phytopharmaceutical product (PPP) in Europe. The $DT_{50}$ (Detection Time 50% = time to detect a 50% decrease in pesticide concentration) ranges provided show the minimum and maximum values from general literature or field studies and can vary greatly depending on the sources (for more details see Lewis et al., 2016). Model species corresponds to the organism for which the $LD_{50}$ (Lethal Dose 50% = amount of pesticide killing 50% of the test animals) was obtained: *Colinus virginianus* (C.V.), *Coturnix japonica* (C.C.J.), *Anas platyrhynchos* (A.P.), and *Serinus canaria* (S.C.). Data were compiled from the Pesticide Properties DataBase (PPDB) of the University of Hertfordshire (http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm accessed date 15 November 2022; Lewis et al., 2016). Prohibition years in Europe and France were obtained from legislative texts (available at https://eur-lex.europa.eu/homepage.html and https://www.legifrance.gouv.fr/ accessed date 15 November 2022).

| | Use | Main crops | Prohibition in Europe/France | Emergency authorization (France) | $DT_{50}$ range (days) | Bird $LD_{50}$ ($mg \cdot kg^{-1}$) | Model species | Mode of Action |
|---|---|---|---|---|---|---|---|---|
| **Acetamiprid**  | Insecticide | vegetables, fruit, cotton, ornamentals | No/2018 | No | 3 | 98 | A.P. | Systemic with translaminar activity, stomach and contact action. |
| **Clothianidin**  | Insecticide | corn, rice, orchards | 2018/2018 | No | 13.3–1386 | 430 | C.V. | Translaminar and root systemic activity. |

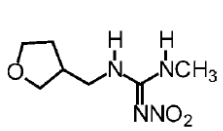
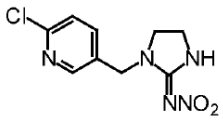
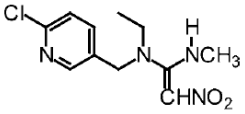
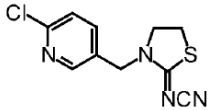
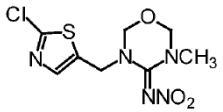
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Dinotefuran**  | Insecticide Veterinary substance | vegetables, fruit, turf, rice | NA | No | 50–100 | 2000 | C.C.J. | Systemic with contact and stomach action. |
| **Imidacloprid**  | Insecticide Veterinary substance | turf, rice, cereals, maize, potatoes, sugar beet | 2018/2018 | Yes | 174 | 31 | C.C.J. | Systemic with contact and stomach action. |
| **Nitenpyram**  | Insecticide Veterinary substance | glasshouse crops, rice | NA | No | 1–15 | 1124 | A.P. | Systemic with translaminar activity, stomach and contact action. No long-term activity. |
| **Thiacloprid**  | Insecticide | winterwheat, rape, seed, sugar beet, legumes | 2020/2018 | No | 5.95–16.8 | 35 | S.C. | Contact and stomach action with some systemic properties. |
| **Thiamethoxam**  | Insecticide | vegetables, turf, sod farms, landscape plants, ornamentals | 2018/2018 | Yes | 7–72 | 576 | A.P. | Broad spectrum, systemic with contact and stomach action. |

**Table S3.** Limits of detection (LOD), limits of quantification (LOQ), multiple reactions monitoring (MRM) transitions, and retention time (rt) for each compound (two MRM transitions were monitored, product ion Q was used for quantification, and product ion q was used for qualification).

| Compound | Precursor ion | Q | q | rt (min) | LOD ($pg \cdot \mu L^{-1}$) | LOQ ($pg \cdot \mu L^{-1}$) |
|---|---|---|---|---|---|---|
| **Acetamiprid** | 223 | 99 | 126 | 6.24 | - | - |
| **Clothianidin** | 250 | 132 | 169 | 5.22 | 0.010 | 0.035 |
| **Dinotefuran** | 203 | 112 | 114 | 2.86 | 0.011 | 0.038 |
| **Imidacloprid** | 256 | 209 | 211 | 5.48 | - | - |
| **Nitenpyram** | 271 | 56 | 196 | 2.03 | 0.009 | 0.031 |
| **Thiacloprid** | 253 | 126 | 99 | 7.40 | 0.001 | 0.005 |
| **Thiamethoxam** | 292 | 181 | 211 | 4.35 | 0.012 | 0.042 |

**Table S4.** Comparison between studies that quantified neonicotinoids in blood of free-living birds. Molecules quantified are ACE = acetamiprid, CLO = clothianidin, DINO = dinotefuran, IMI = imidacloprid, NIT = nitenpyram, THIA = thiacloprid, and TMX = thiamethoxam. LOD = limits of detection and LOQ = limits of quantification; NA indicates the compounds' value was not provided by authors. Percentage of detection rounded to the nearest percent corresponds to the proportion of samples in which the neonicotinoid insecticide has been detected and is present in quantities greater than or equal to LOD or LOQ (specified in footnotes). [c] = mean or median concentration, SD = standard deviation, and IQR = interquartile range; by default mean ± SD are given (specified in footnotes when median ± IQR are given).

| | **LOD/LOQ in original measurement units** | **LOD in pg·µL$^{-1}$** | **LOQ in pg·µL$^{-1}$** | **Species** | **% of detection** | **[c] ± SD or IQR (range)** | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | **ng·mL$^{-1}$** | **pg·mL$^{-1}$** | **pg·µL$^{-1}$** |
| This study[1] | | | | | | | | |
| CLO | - | 0.010 | 0.035 | *Emberiza cirlus* | 15 | - | - | 2.28 ± 1.55 (1.04-4.87) |
| | | | | *Common nightingale* | 8 | - | - | 0.05 ± 0.06 (0.009–0.093) |
| | | | | *Perdix perdix*[4] | 15 | - | - | 5.26 ± 4.10 (1.92-14.26) |
| | | | | *Circus pygargus* | 5 | - | - | 951.60 ± 1299.21 (194.48-2 451.78) |
| DINO | - | 0.011 | 0.038 | *Perdix perdix*[4] | 24 | - | - | 7.47 ± 3.89 (2.14-16.61) |
| NIT | - | 0.009 | 0.031 | *Perdix perdix*[4] | 91 | - | - | 20.25 ± 11.18 (1.24-43.53) |
| THIA | - | 0.001 | 0.005 | *Emberiza cirlus* | 100 | - | - | LOD < [c] < LOQ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Common nightingale* | 100 | - | - | LOD < [c] < LOQ |
| | | | | | *Perdix perdix*[4] | 2 | - | - | 0.07 |
| | | | | | *Circus pygargus* | 2 | - | - | 89.58 |
| TMX | | - | 0.012 | 0.042 | *Emberiza cirlus* | 12 | - | - | 2.59 ± 1.37 (1.61-4.56) |
| | | | | | *Common nightingale* | 4 | - | - | 0.06 |
| | | | | | *Perdix perdix*[4] | 7 | - | - | 7.16 ± 11.05 (1.23-23.73) |
| Humann-Guilleminot et al., 2021[2] | | | | | | | | | |
| ACE | | NA/0.01 ng·mL$^{-1}$ | NA | 0.01 | | 0 | 0 (0–0) | - | 0 (0–0) |
| CLO | | NA/0.05 ng·mL$^{-1}$ | NA | 0.05 | | 20 | 0.06 ± 0.11 (0–0.34) | - | 0.06 ± 0.11 (0–0.34) |
| IMI | | NA/0.05 ng·mL$^{-1}$ | NA | 0.05 | *Tachymarptis melba* | 0 | 0 (0–0) | - | 0 (0–0) |
| THIA | | NA/0.01 ng·mL$^{-1}$ | NA | 0.01 | | 0 | 0 (0–0) | - | 0 (0–0) |
| TMX | | NA/0.03 ng·mL$^{-1}$ | NA | 0.03 | | 0 | 0 (0–0) | - | 0 (0–0) |
| Lennon et al., 2020[1,3] | | | | | | | | | |
| CLO | | 0.15 ng·mL$^{-1}$/0.21 ng·mL$^{-1}$ | 0.15 | 0.21 | *Alectoris rufa* | 95 | 47.1 ± 382 (0.40–3200) | - | 47.1 ± 382 (0.40–3200) |
| | | | | | *Perdix perdix* | 83 | 1.80 ± 1.20 (0.60–3.00) | - | 1.80 ± 1.20 (0.60–3.00) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Byholm et al., 2018[1] | | | | | | | | |
| ACE | 0.6-7.5 pg·mL$^{-1}$/NA | 0.0006–0.0075 | NA | | 0 | - | < LOD | < LOD |
| IMI | 11-15 pg·mL$^{-1}$/39 pg·mL$^{-1}$ | 0.011–0.015 | 0.039 | *Pernis apivorus* | 40 | - | 8.9 | 0.0089 |
| THIA | 1.6-2.0 pg·mL$^{-1}$/1.3-6.3 pg·mL$^{-1}$ | 0.0016–0.0020 | 0.0013–0.0063 | | 70 | - | 15.43 ± 8.32 (12.00-31.00) | 0.01543 ± 0.00832 (0.012–0.031) |
| Hao et al., 2018[1] | | | | | | | | |
| ACE | 3.6 pg·mL$^{-1}$/NA | 0.0036 | NA | | 11 | - | 3.83 ± 0.10 (3.70-3.90) | 0.00383 ± 0.0001 (0.0037–0.00390) |
| CLO | 7.4 pg·mL$^{-1}$/NA | 0.0074 | NA | | 0 | - | < LOD | < LOD |
| DINO | 9.1 pg·mL$^{-1}$/NA | 0.0091 | NA | | 0 | - | < LOD | < LOD |
| IMI | 4.6 pg·mL$^{-1}$/NA | 0.0046 | NA | *Zonotrichia leucophrys* | 78 | - | 35.83 ± 42.88 (5.00-177.00) | 0.03583 ± 0.04288 (0.005–0.177) |
| NIT | 8.8 pg·mL$^{-1}$/NA | 0.0088 | NA | | 0 | - | < LOD | < LOD |
| THIA | 2.3 pg·mL$^{-1}$/NA | 0.0023 | NA | | 11 | - | 2.70 ± 0.27 (2.50-3.10) | 0.0027 ± 0.00027 (0.0025–0.0031) |
| TMX | 4.5 pg·mL$^{-1}$/NA | 0.0045 | NA | | 22 | - | 13.46 ± 9.58 (5.10-33.70) | 0.01346 ± 0.00958 (0.0051–0.0337) |
| Taliansky-Chamudis et al., 2017[1] | | | | | | | | |
| ACE | NA/2 ng·mL$^{-1}$ | NA | 2 | *Bubo bubo* | 0 | < LOD | - | < LOD |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CLO | NA/10 ng·mL$^{-1}$ | NA | 10 | 0 | < LOD | - | < LOD |
| DINO | NA/10 ng·mL$^{-1}$ | NA | 10 | 0 | < LOD | - | < LOD |
| IMI | NA/2 ng·mL$^{-1}$ | NA | 2 | 3 | 3.28 | - | 3.28 |
| NIT | NA/10 ng·mL$^{-1}$ | NA | 10 | 0 | < LOD | - | < LOD |
| THIA | NA/2 ng·mL$^{-1}$ | NA | 2 | 0 | < LOD | - | < LOD |
| TMX | NA/10 ng·mL$^{-1}$ | NA | 10 | 0 | < LOD | - | < LOD |

[1] Percentage of detection was obtained by including individuals with concentrations > LOD. [2] Percentage of detection was obtained by including individuals with values > LOQ. [3] Descriptive statistics given are median ± IQR. Descriptive statistics were obtained [4] for coupled data of *Perdix perdix* caught in 2020–2021 winter and 2021–2022 winter, [5] from online available dataset based on all sample sizes (including individuals with concentrations < LOD), or [6] based on data for sample size excluding individuals with concentrations < LOD.

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