Ecology Letters

WILEY

ECOLOGY LETTERS

SYNTHESIS OPEN ACCESS

Neonicotinoids Impact All Aspects of Bird Life: A Meta-Analysis

Elke Molenaar[1] | Wolfgang Viechtbauer[2] | Janske van de Crommenacker[3] | Sjouke A. Kingma[1]

[1]Behavioural Ecology Group, Department of Animal Sciences, Wageningen University and Research, Wageningen, The Netherlands | [2]Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, The Netherlands | [3]Vogelbescherming Nederland, Zeist, The Netherlands

Correspondence: Elke Molenaar (elke.molenaar@wur.nl) | Sjouke A. Kingma (sjouke.kingma@wur.nl)

Received: 6 May 2024 | **Revised:** 26 August 2024 | **Accepted:** 2 September 2024

Editor: Gabriele Sorci

Funding: The authors received no specific funding for this work.

Keywords: bird | clothianidin | imidacloprid | meta-analysis | neonicotinoid | pesticide | thiamethoxam

ABSTRACT

Worldwide, bird populations are declining dramatically. This is especially the case in intensely used agricultural areas where the application of neonicotinoid insecticides is thought to—unintendedly—cause a cascade of negative impacts throughout food webs. Additionally, there could be direct (sub-) lethal impacts of neonicotinoids on birds, but to date there is no comprehensive quantitative assessment to confirm or rule out this possibility. Therefore, we use a meta-analytical approach synthesising 1612 effect sizes from 49 studies and show that neonicotinoids consistently harm bird health, behaviour, reproduction, and survival. Thus, in addition to reduced food availability, the negative direct effects of exposure to neonicotinoids likely contribute to bird population declines globally. Our outcomes are pivotal to consider in future risk assessments and pesticide policy: despite localised bans, the metabolites and residues of neonicotinoids remain present in the environment and in birds and will thus have long-lasting direct effects on both the individual and the population levels.

Neonicotinoid insecticides are the world's most widely used insecticides, but their use is increasingly connected with a long and concerning list of environmental effects (Goulson 2013; Humann-Guilleminot et al. 2019; Morrissey et al. 2015). Since their introduction in the 1990s, neonicotinoid application in agriculture and livestock production, home garden settings, and pet protection increased substantially because of their unique mode of action and effectivity: the seven available neonicotinoid compounds together protect plants against sucking and chewing insects from at least nine taxonomic groups (Frank and Tooker 2020). Neonicotinoids, which are structurally similar to nicotine, act as an agonist on insect nicotinic acetylcholine receptors and disrupt the functioning of the central nervous system, leading to acute paralysis and death of insects (Jeschke et al. 2011; Thompson et al. 2020). Unlike highly toxic organochlorine insecticides that were commonly used in the past (such as DDT), neonicotinoids were initially presumed relatively harmless to non-target organisms (Thompson et al. 2020) as they have a lower affinity to bind to vertebrate receptors, and residues in blood and organs were rapidly cleared (Bean et al. 2019; Thompson et al. 2020). Yet, particular chemical characteristics of neonicotinoids (i.e., high water solubility and long half-lives in soils), make that many terrestrial and freshwater ecosystems are unintendedly contaminated and organisms inhabiting those environments are to some level exposed to these substances or their metabolites (Hladik, Main, and Goulson 2018). Indeed, neonicotinoids may influence non-target organisms (including species of mammals, birds, fish, molluscs, crustaceans, and annelids; Goulson 2013; Wood and Goulson 2017) and whole food webs more than initially expected (Tooker and Pearsons 2021).

1 | Do Neonicotinoids Cause Global Bird Population Declines?

The declines in bird populations observed in recent decades are especially pronounced in intensely used agricultural areas (Li, Miao, and Khanna 2020). The correlation of bird population declines with geographic (Hallmann et al. 2014) and temporal (Li, Miao, and Khanna 2020) patterns of neonicotinoid use indicate an effect of neonicotinoids, but the actual causality of this association, as well as the potential mechanisms, are still debated (Goulson 2013; Moreau et al. 2022; Walters 2013). For instance, while reduced insect availability as a result of neonicotinoid usage has been suggested to lead to insufficient food availability for insectivorous birds (Hallmann et al. 2014; Tooker and Pearsons 2021), population declines are also common in a number of granivorous, frugivorous, and carnivorous bird species (Lees et al. 2022; Moreau et al. 2022). This suggests that neonicotinoids may—despite initial arguments against this possibility—directly affect the health of birds exposed to polluted food, water, air, or soil, thereby impacting their behaviour, reproduction, and survival, and thus, ultimately, population persistence regardless of diet.

To date, there is no comprehensive analysis using all information currently available in the literature about the direct effects of neonicotinoids on birds. Such an assessment is essential because birds not only consume neonicotinoids directly through ingestion of coated seeds and plant material containing neonicotinoids but it has now also become clear that neonicotinoids dissolve in water and soil, leading to long-lasting and intense pollution at large continental scales (Frank and Tooker 2020; Thompson et al. 2020). Half-lives of neonicotinoids are typically short—for the most commonly used neonicotinoids the time it takes to break down to half its original amount (i.e., DT_{50} or half-live) ranges from 4.7 to 40.3 days in water and 3 to more than 1000 days in soil (Hladik, Main, and Goulson 2018). However, under certain conditions they can persist in soils for almost 19 years—highlighting that the extent of neonicotinoid pollution is far greater than first assumed (Bonmatin et al. 2015; Goulson 2013; Hladik, Main, and Goulson 2018; Tooker and Pearsons 2021). Perhaps as a consequence, residues of neonicotinoid compounds are detected in feathers and plasma samples of a variety of wild bird species (such as sparrows, blackbirds, owls, swifts, gulls, and terns; Distefano et al. 2022; Graves, Meese, and Holyoak 2023; Humann-Guilleminot et al. 2019, 2021), which in turn illustrates that the susceptibility to neonicotinoids is independent of diet, behaviour, and habitat. Importantly, similar as for DDT (which is known to cause bioaccumulation and reproductive failure in a multitude of species; Fry 2021), neonicotinoids accumulate in birds, including those high up the food chain (i.e., birds of prey and owls) (Humann-Guilleminot et al. 2021; Taliansky-Chamudis et al. 2017). To address whether and how neonicotinoid exposure can be linked to the observed bird declines, we systematically quantified, using an extensive meta-analytic model of experimental exposure studies, whether and which aspects of performance (health, behaviour, reproduction, or survival) are affected by direct neonicotinoid exposure.

2 | Approach to Provide an Objective Estimation of Effects

From 50 published papers that experimentally assessed the effects of neonicotinoids (i.e., acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam) on 12 bird species from different families (see Figure S2), we computed the standardised mean difference (SMD) (i.e., the standardised difference in the mean of birds in the control group and birds that were experimentally exposed to neonicotinoids) for each of the reported performance measures. To assess the impact of neonicotinoids on birds, we categorised the effect sizes into four performance classes (i.e., health, behaviour, reproduction, or survival). Rather than using the (many) actual specific performance measures ($k = 275$), this categorisation allows for larger statistical power and thus more robust meta-analyses (Van Kleunen, Weber, and Fischer 2010; Winfree et al. 2009). More specifically, the performance measures were clustered within these classes based on the experimental design and the ultimate assessed performance measure where effects act on: for example, 'body mass' and 'weight gain' fell into the 'health' category, but if adults are exposed to neonicotinoids and hatchlings have a lower body mass, we categorise that as 'reproduction' and not 'health'.

Using a state-of-the-art meta-analytical model that allowed us to account for heterogeneity and the dependency in multiple estimates extracted from the same sample of birds, we found that exposure to neonicotinoids strongly deteriorates bird performance overall (Hedges' $g = -0.39$, 95% CI LB = −0.49, UB = −0.29, $k = 1566$; Figure 1), confirming our prediction that exposure to neonicotinoids impacts birds directly.

To determine what aspects of performance are responsible for the overall impact of neonicotinoids, we tested the separate effects on 'health' (e.g., physiological aspects, haematological values, measures of oxidative stress), 'behaviour' (e.g., consumption rates, migratory behaviour), 'reproduction' (e.g., reproductive physiology, egg characteristics and offspring production), and 'survival' (adult and chick mortality). Strikingly, and in contrast to the effects of DDT that mainly constrains reproduction (Fry 2021), this analysis revealed that neonicotinoids impact all these important aspects of birds' life. For the performance measure 'health', we calculated 1269 effect sizes, of which the majority (73%) is negative (i.e., 932 effect sizes), 21 are neutral and 316 are positive; for 'behaviour', we have 56 effect sizes, of which 50 are negative (89%), 5 are neutral and 1 is positive; for 'reproduction' 185 of the 241 effect sizes are negative (77%), 16 neutral and 40 positive. First, with regards to health, neonicotinoids caused many symptoms related to poisoning, for example, immune suppression, body weight loss, vitamin deficiency, and anaemia, with an overall negative effect on the birds' health status (Hedges' $g = -0.37$, 95% CIs: −0.48 to −0.26, $k = 1269$, $p < 0.0001$). Although health deficiency symptoms varied in strength, most negative effects were very clear and pronounced (932 of the 1269 effects (73%) were negative, Figure 1). There are exceptions in which exposure to neonicotinoids lead to seemingly positive health effects. For example, Lopez-Antia et al. (2015) found higher levels of carotenoids and vitamins in partridge eggs after exposure to imidacloprid,

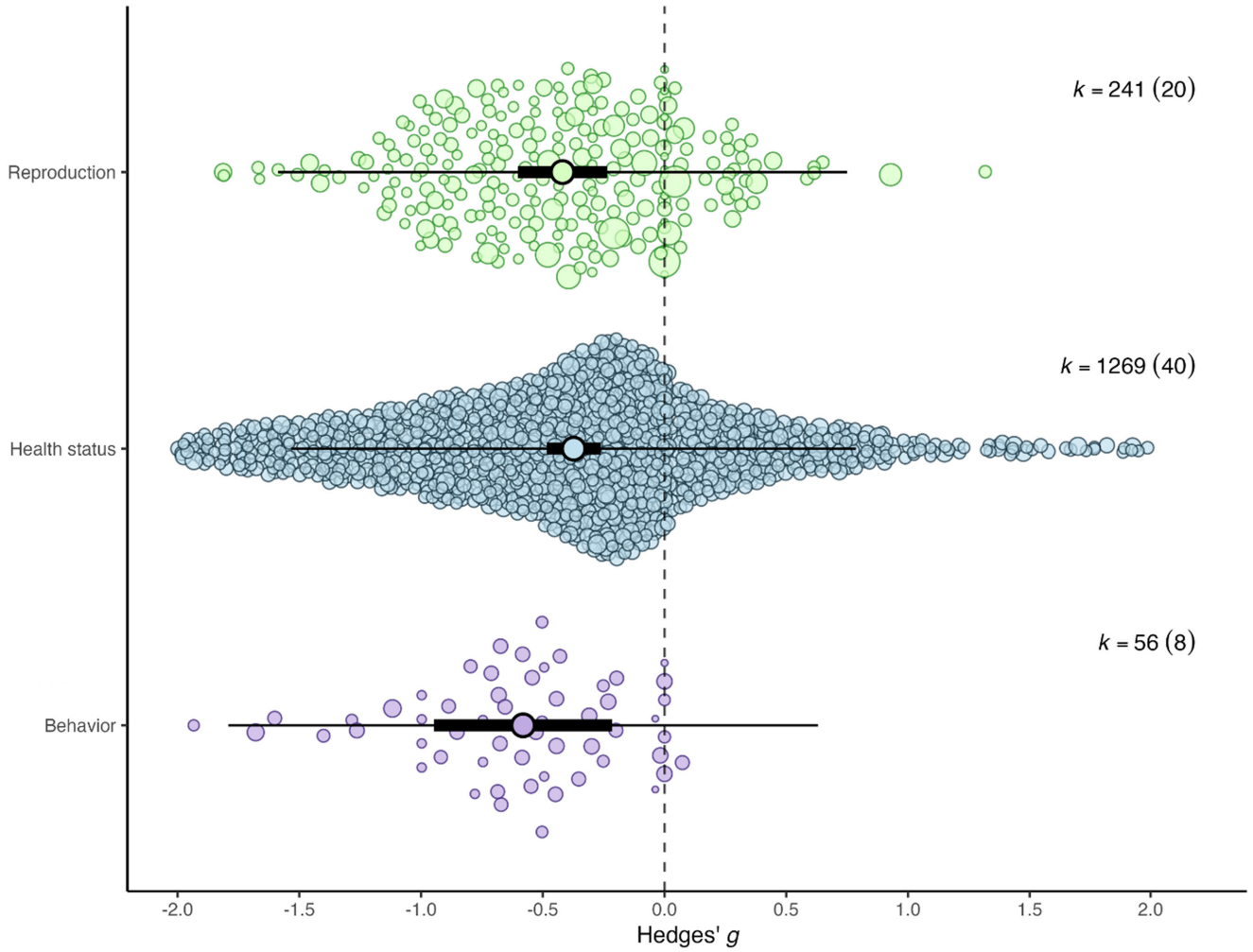

FIGURE 1 | The effects of experimental neonicotinoid exposure on bird reproduction, health, and behaviour. Data points are Hedges' *g* values (with circle size reflecting precision (1/SE)). The mean is bold and extends with the 95% CIs in bold horizontal lines, and the prediction interval in thin lines. The dashed vertical line at zero indicates no difference in effect between the exposed group and the non-exposed control group. Negative and positive effect sizes are indicative of a negative and positive effect of neonicotinoid exposure respectively. '*k*' represents the number of effect sizes, with in parentheses the number of studies. For all three performance measures, the overall effect (bold horizontal lines) does not overlap zero, indicating that neonicotinoids significantly impact all these measures of bird performance.

which the authors suggest is a compensatory mechanism during yolk formation, and therefore a sign of stress. In another study, Tokumoto et al. (2013) found an increase in body mass gain after clothianidin exposure: this seems a positive effect but in reality, this effect could be attributable to an impaired liver function. Thus, the few positive effects have likely arisen from compensatory mechanisms (Lopez-Antia et al. 2015) and negative feedback loops rather than a true positive effect. This is in line with the finding that most studies show a negative effect of neonicotinoids on birds (in total 1167 of 1566 effect sizes (75%) are negative). Second, exposure to neonicotinoids led to a reduction in behavioural performance (Hedges' $g = -0.58$, 95% CIs: −0.94 to −0.22, $k = 56$, $p = 0.0021$, Figure 1) such as reduced rates of food consumption (Addy-Orduna, Brodeur, and Mateo 2019), neurobehavioral abnormalities (Franzen-Klein et al. 2020), and reduced migratory activity (Eng, Stutchbury, and Morrissey 2019). Negative effects on health and behaviour are probably the consequence of accumulation of toxic compounds or physiological damage causing immediate or persistent effects: for example, birds that were in a migratory state and received a dose of imidacloprid became unable to orient post-dosing but recovered after 2 weeks (Eng, Stutchbury, and Morrissey 2017). In another example, health effects (a significant decrease in dopamine levels) persisted up to 3 weeks after administering imidacloprid to Japanese quails (Rawi, Al-Logmani, and Hamza 2019). These overall negative impacts on many aspects of both behaviour and health status may well explain why reproduction and survival—aspects ultimately responsible for population persistence—appear to be significantly impacted by neonicotinoids. Our analyses reveal that reductions in proxies of reproduction (such as reproductive hormone levels, eggshell thickness (Lopez-Antia et al. 2013, 2015), egg laying rates (Hoshi et al. 2014), sperm densities (Humann-Guilleminot et al. 2019), and secondary sexual characters (Pandey, Tsutsui, and Mohanty 2017)) were responsible for the significant negative effect of neonicotinoids on reproduction overall (Hedges' $g = -0.42$, 95% CIs: −0.60 to −0.24, $k = 241$, $p < 0.0001$, Figure 1). Moreover, to disentangle if studies that

mimicked exposure through food (as they most likely do in natural settings) found a similar effect, we ran a separate model for 14 studies in which birds had neonicotinoids incorporated into their diet (i.e., all other studies administered neonicotinoids through oral gavage or injected eggs directly). Similar as the overall result, we found a negative effect of neonicotinoid exposure through food (Hedges' $g = -0.37$, 95% CIs: −0.64 to −0.10, $k = 288$, $p = 0.0102$). Lastly, all 12 studies investigating mortality rates of birds exposed to neonicotinoids either showed no or an elevated mortality rate (with a significant negative effect overall: Log Risk Ratio = −0.34; 95% CIs: −0.58 to −0.09, $k = 46$, $p = 0.012$, Figure 2). These results emphasise the toxicity of the substances, and strikingly, mortality effects in some cases manifested within hours after exposure (Addy-Orduna, Brodeur, and Mateo 2019)—but only after 8 days in others (Tokumoto et al. 2013). Combined with the previous analyses, this indicates that acute effects as well as more chronic or accumulative effects of neonicotinoids can be detrimental via pathways that reduce the health and behaviour of individual birds. In summary, we provide an objective estimation of effects through our meta-analysis of experimental studies and show that exposure to neonicotinoids impact every facet of a birds' life: generic adverse effects are found on all levels, where negative effects on health and behaviour result in substantial impact on reproduction and survival (Gibbons, Morrissey, and Mineau 2015; Pisa et al. 2021). In addition to overall effects, our data suggest that these effects are similar regardless of what type of neonicotinoid was used: there were significant negative effects for thiamethoxam (Hedges' $g = -0.48$, 95% CIs: −0.67 to −0.30, $k = 542$, $p < 0.0001$, Figure 3), thiacloprid (Hedges' $g = -0.66$, 95% CIs: −1.03 to −0.30, $k = 21$, $p = 0.0007$, Figure 3) and imidacloprid (Hedges' $g = -0.34$, 95% CIs: −0.45 to −0.22, $k = 939$, $p < 0.0001$, Figure 3). Acetamiprid and clothianidin were only used in two and three studies respectively, which may explain why their effects were not significant despite the overall effect sizes being comparable to those of other compounds (i.e., acetamiprid: Hedges' $g = -0.18$, 95% CIs: −0.59 to 0.23, $k = 19$, $p = 0.38$, Figure 3; clothianidin: Hedges' $g = -0.35$, 95% CIs: −0.79 to 0.09, $k = 45$, $p = 0.12$, Figure 3).

3 | Impacts on Birds That Are—and Will Be—Exposed to Neonicotinoids

Our conclusions are based on a synthesis of results obtained from mainly laboratory studies, raising the question how well

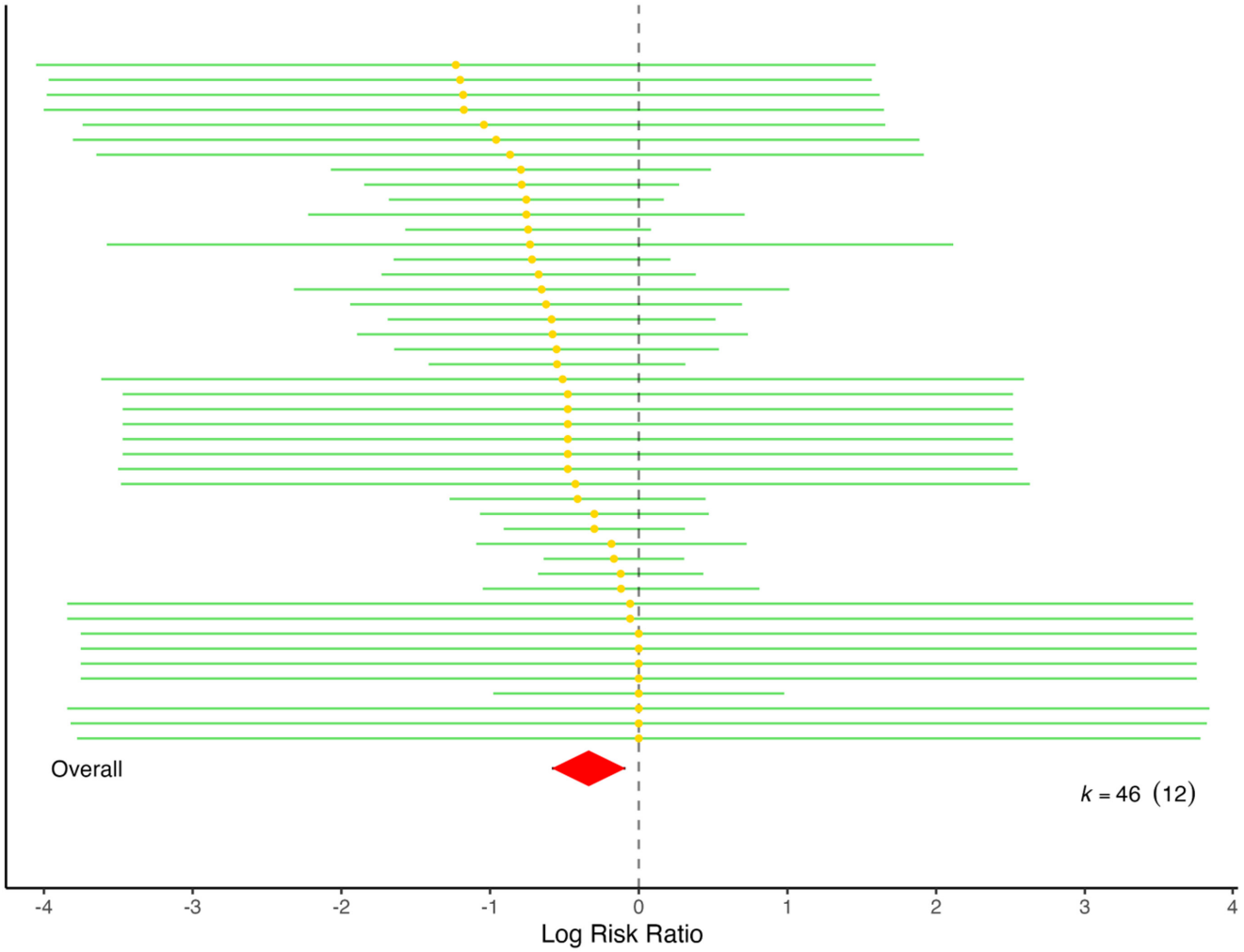

FIGURE 2 | The effects of neonicotinoid exposure on the survival probability of birds. The mean Log Risk Ratios (LogRR) of non-exposed control groups and neonicotinoid exposed groups on survival probability of birds is indicated using yellow dots, with in green bars the 95% CIs. A negative value indicates a mortality cost of exposure. In total, 12 studies reported effects on survival, with $k = 46$ effect sizes.

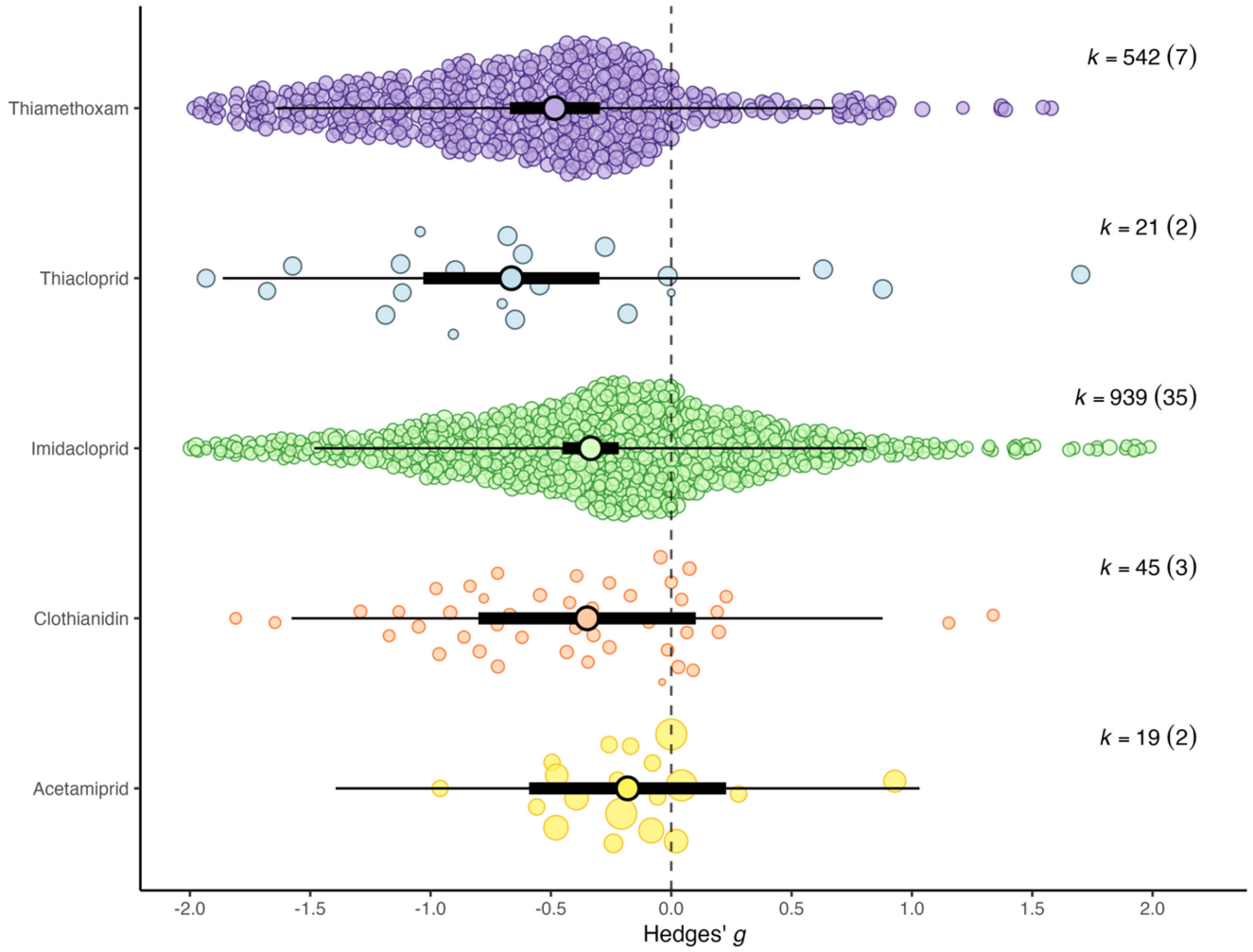

FIGURE 3 | Impact of different neonicotinoid compounds. Orchard plot of the mean effect size (Hedges' *g*) of differences between the untreated controls and neonicotinoid treatment effects of five different neonicotinoid substances. A negative value for *g* (<0) is indicative of a negative effect, whereas a positive value (>0) for *g* indicates a positive effect. The dot represents the mean, and the bold bars around the means represent the 95% confidence interval (CI), and the thin bars represent the prediction interval (PI). If the bars do not cross the vertical line, a mean effect size is significantly different from zero. In the parentheses on the right, we respectively report the number of effect sizes + the number of studies.

these overall conclusions translate to situations and effects in the wild and across species. Several lines of evidence suggest that the found effects indeed underlie the severe declines of many bird species in nature and especially of those in areas with high application rates of neonicotinoids (Mineau and Palmer 2013). First, while many included studies were performed on domesticated fowl and quails, the effects were also present in free-living songbirds (Eng, Stutchbury, and Morrissey 2019). Indeed, we did not find any between-species variance or a phylogenetic signal in our dataset (Table S3), meaning that all included species, across the avian phylogeny (Figure S2) were affected negatively by neonicotinoids. Second, a frequently encountered criticism of ecotoxicological risk assessment studies (Topping, Aldrich, and Berny 2020) is that they may not reflect field-realistic conditions and are largely dependent on the method, quantity, or type of compound used. While we encourage more field studies to be performed, several arguments illustrate that methodological issues are unlikely to explain our results and that the found effects are likely representative of the effects in nature. Although we find that higher doses of neonicotinoids within studies yield stronger negative effects (Figure S4), the highest administered dosage was often based on realistic concentrations consumed by birds in the wild. Doses were, for example, determined by both the concentrations of neonicotinoids that are applied to seeds used in agriculture (e.g., wheat, canola, or corn) and the consumption level of seeds by birds in the wild (Mineau and Palmer 2013; Roy and Coy 2020). Importantly, one study (Lopez-Antia et al. 2015)—that used an application rate corresponding to the recommended levels following Spanish regulations—found the dose killed all exposed adult partridges (*Alectoris rufa*) within 21 days. Moreover, field studies illustrate that birds consume enough seeds to cause sub-lethal effects (Roy and Coy 2020) and that, in fact, even a single corn kernel coated with neonicotinoids may kill a small songbird (of 15–50 g) (Mineau and Palmer 2013). Finally, unlike in the included exposure experiments with single compounds, neonicotinoids are nearly always applied in combination with other conventional pesticides such as herbicides and nematicides (Lehmann et al. 2017; Montiel-León et al. 2019; Sultana et al. 2018) to increase functionality. Indeed, studies investigating pesticide concentrations in wild birds usually detect the presence and residues of several different compounds within one bird (Fuentes et al. 2023; Hladik, Main, and Goulson 2018; Zhao et al. 2020).

Although 'synergetic effects' (joint pesticide usage exerting interactive damages and intensifying effects) are, as far as we are aware, still untested (Cedergreen 2014; Rizzati et al. 2016), this arguably causes even more pronounced effects in field-realistic settings than we report here. Taken together, these arguments highlight that neonicotinoid exposure causes widespread (sub)lethal effects on a variety of bird families and that the impact of neonicotinoids might, in fact, be more severe and extensive in wild populations than presented in this analysis.

While diets of different bird species are variable, and some bird species may therefore be more susceptible to neonicotinoid exposure than others (Lennon et al. 2019), detrimental long-term effects are perhaps not restricted to birds directly consuming treated seeds. Real-life exposure to neonicotinoids not only occurs through ingestion of neonicotinoid-coated seeds but also from contact with contaminated plants, soil, or water, and consumption of contaminated food such as insects, fish, or pollen (Frank and Tooker 2020; Walters 2013). Worryingly, recent studies suggest that repeated application leads to the accumulation of neonicotinoids in soils, only reaching a plateau after four to 6 years (Zhang et al. 2023). Moreover, neonicotinoids do not easily break down: despite the bans of neonicotinoids in 2013 and 2018 (Bass and Field 2018), the concentration of three banned compounds was similar or higher in wild birds than before the ban (Fuentes et al. 2023). Neonicotinoids appear to persist and accumulate in surviving birds and are present in those high up in food chains, such as bird of prey that predate on them (Humann-Guilleminot et al. 2021; Taliansky-Chamudis et al. 2017).

Ultimately, depending on the strength of exposure via these routes, the severity of the impact on birds may not only lead to direct mortality, reduced reproductive success, and delayed sublethal health effects as shown here but may also make birds increasingly more vulnerable to other threats and stressors such as predator presence, low food availability, and limited food-finding capacity. With the current knowledge that many terrestrial and freshwater ecosystems are unintendedly contaminated (Hladik, Main, and Goulson 2018), it becomes clear that there is a large potential for cascading effects throughout food webs. Thus, the increasingly toxic load of ecosystems (Frank and Tooker 2020) is a major point of concern because the general presence and persistence of neonicotinoids can for a long-time cause sub-lethal effects that act slowly and chronically in many non-target organisms, birds and biodiversity in general.

4 | Conclusions

With this meta-analysis, we provided an objective estimation of the effects of neonicotinoid exposure on birds and compared the effects among bird species and performances parameters. Our meta-analysis shows consistent negative effects of neonicotinoid exposure on various levels of performance of birds. We argue that in addition to decreases in food availability (Hallmann et al. 2014; Møller et al. 2021), these direct effects are responsible for at least part of the extensive bird declines globally (Goulson 2014; Li, Miao, and Khanna 2020). Unintended consequences of widespread neonicotinoid exposure are of great concern to non-target organisms, such as birds, and may have far-reaching consequences. In addition to the complexity of proving a causal effect of neonicotinoid exposure on wild bird population declines, there is the difficult task ahead of disentangling the contributions of indirect, direct, and delayed effects. These are the next steps needed to determine from which contaminated resources and exposure routes birds are most likely to be negatively affected. Until then, however, we argue that our results, along with others (Hallmann et al. 2014; Tooker and Pearsons 2021; Wood and Goulson 2017; Zhang et al. 2023), should stimulate caution in adopting and using established and new neonicotinoids.

5 | Materials and Methods

5.1 | Literary Search and Inclusion Criteria

We performed a systematic literature search using keyword searches in the 'Web of Science Core Collection' (1990 to present), finding relevant papers reporting the effects of experimental studies testing the effect of neonicotinoids on birds until 25 April 2022. We used the PECO framework to develop our search query, which distinguishes four elements: Population, Exposure, Comparator, and Objective (Morgan et al. 2018). Using derivatives from two of the four PECO elements (population and exposure), our full search term was: ('Bird*' OR 'Avifauna' OR 'Chick*' OR 'Nestling*' OR 'Hatchling*' OR 'Vogel*' OR 'Vertebrate*') AND ('Neonicotinoid*' OR 'Imidacloprid*' OR 'Thiamethoxam*' OR 'Clothianidin*' OR 'Acetamiprid*' OR 'Nitenpyram*' OR 'Thiacloprid*' OR 'Dinotefuran*').

Our search rendered 315 papers, to which another 10 studies were added through cross-referencing of included studies (see Supporting Information). One duplicate was removed, and one paper was not included as we did not get access. To be included in the meta-analysis, studies needed to (1) be conducted on birds; (2) expose birds to a type of neonicotinoid (e.g., imidacloprid, thiamethoxam, clothianidin, acetamiprid, or thiacloprid) either in the field or the laboratory; (3) report effects on both a control (non-exposed) and exposed group that contain data on means, standard deviation or error, and sample sizes, or other convertible statistics (see Table S2a); and (4) study the effects on performance, falling into one of the following four categories (i.e., performance types): behaviour, health status, reproduction, or survival (see below). We identified a total of 50 papers that adhered to all inclusion criteria and overall extracted 1962 effect sizes to be included in the meta-analysis (full reference list in Supporting Information; PRISMA diagram, Figure S1).

5.2 | Data Extraction

From the studies that met our inclusion criteria, we collected the mean and statistical variation (SD or SE) of the untreated control and the neonicotinoid exposed group, and their respective sample sizes to calculate effect sizes. If these values were unavailable, we used either the χ^2, *t*-value, or *F* statistics (Table S2a). To complete or use the data of some studies, the authors were asked for the original data (see Source Data). In case no answer was received, the data were either excluded or only partially included. If only figures were available, data were extracted using the WebPlotDigitizer version 4.6 (https://apps.automeris.io/wpd/).

From each study, we also collected the details of the following moderators: the performance type studied, the type of neonicotinoid that was used (e.g., imidacloprid, thiamethoxam, clothianidin, acetamiprid, or thiacloprid), the bird species, the dose in which the neonicotinoid was applied, the percentage of the maximum dose used within a study (e.g., concentration level; this only differs within a study in case multiple groups of birds were exposed to different concentrations), and the percentage of the maximum time point taken (this only differs within a study in case birds were measured multiple times; this was set to 100% in case a single dose or time point dose was used; see Source Data file). Given the vast variety of aspects of performance measures that were reported (275 different response variables were identified; a full list is provided in Supporting Information), we classified the effects into four performance types (behaviour, health status, reproduction, and survival) to conduct a robust meta-analysis (Van Kleunen, Weber, and Fischer 2010; Winfree et al. 2009). Variables were categorised based on the experimental design and the life stage where effects were measured: for example, if adults were exposed to neonicotinoids and hatchlings showed a lower body mass, we categorised that as 'reproduction' and not 'health status'.

In meta-analyses that compare two experimental groups, the standardised mean difference (SMD) is often used as the effect size measure of choice, to make comparison and synthesis of effects possible across studies that used a variety of different response variables (Borenstein et al. 2021; Nakagawa et al. 2022; Nakagawa and Santos 2012; Viechtbauer 2010). For each performance measure, this was computed as the difference between the group means standardised by the (pooled) standard deviation of the two groups, and then correcting these values for their slight positive bias, resulting in Hedges' *g* values (Harrison 2011; Hedges and Olkin 2014). The effect sizes were computed in such a way that a negative value is always indicative of an adverse effect of exposure to a neonicotinoid, compared to the control (e.g., a lower body weight is indicative of a negative effect as lower body weights are generally associated with a poorer health and energy status (Pandey, Tsutsui, and Mohanty 2017); yet, a higher value for growth retardation is also indicative of a negative effect, as this means there were more developmental defects after exposure to imidacloprid (Hussein et al. 2014)), while a positive value indicates a positive effect following exposure (Supporting Information). Additionally, we calculated the effect size variance, which is based on the sample size of both the treatment and control group (Hamman et al. 2018) (Table S2a). In few cases in which reported data for a 'behaviour', 'health status', or 'reproduction' measure represented proportions (e.g., proportion of total number of eggs that hatched), and not means based on continuous response variables, we estimated the corresponding standardised mean difference by calculating the log odds ratio from the proportions and converting this into Hedges' *g* (formulas in Table S2b). On the other hand, data falling into the 'survival' class always represent proportions (i.e., mortality, either of the birds themselves or their chicks or embryos) and for ease of interpretation were therefore analysed separately by computing the log risk ratios (logRR): negative values indicate a lower risk of survival in the exposed group compared to the control group. A single measure (Lopez-Antia et al. 2013) of survival listed as 'mean chick survival time' did not fit in either class and was therefore excluded; this exclusion was conservative since this study reported a negative effect of neonicotinoids on survival.

Many of the 50 papers reported the effects of neonicotinoids on a wide variety of response variables, exposed birds to different doses and compounds, and/or measured effects at varying time points. We accounted for arising dependencies and continuing time series in our meta-analytical model as described below.

5.3 | Multivariate Meta-Analysis

Multivariate meta-analysis models extend standard meta-analytic models to the case where one is analysing multiple and possibly correlated outcomes, such as in this dataset (Hong et al. 2020; Jackson, Riley, and White 2011). We accounted for dependencies by constructing an appropriate variance–covariance matrix of the estimates by assuming a correlation of rho = 0.6 for effect sizes from the same study belonging to the same performance type and a correlation of rho = 0.4 for the correlation among effect sizes belonging to different performance types. We used cluster-robust inference methods (also known as 'robust variance estimation' (RVE)) in the final model to double-check our results. The difference between the model-implied variance–covariance matrix and the variance–covariance matrix obtained via RVE was very minor, confirming that our working model is reasonable. In addition, some studies used multiple exposure groups (e.g., different dosages and/or neonicotinoid types), which were compared against a common control group. The dependency among the corresponding estimates (due to the reuse of information from the common comparator) was also accounted for in the variance–covariance matrix. Finally, some studies measured the response variable at multiple timepoints. To account for this, we assumed a continuous-time autocorrelation structure among the corresponding estimates, with an autocorrelation coefficient of phi = 0.97, using the percent of total time as the time variable (hence, $0.97^{100} \approx 0.05$ represents the correlation between two estimates that are maximally apart and hence this maximal time difference is also the assumed 'effective range' for the temporal correlation).

In addition, the model included random effects for studies, exposure group (i.e., neonicotinoid that was used) within studies, performance type within studies, performance measure within performance type within studies, and a random effect at the level of the individual estimates. We also examined whether it was necessary to account for phylogenetic relatedness between species in the context of this model (Cinar, Nakagawa, and Viechtbauer 2022). Using the *ape* package in R (Paradis, Claude, and Strimmer 2004), we imported the tree we created through birdtree.org (Jetz et al. 2012). After computing the branch lengths, we created the corresponding correlation matrix based on a Brownian model of evolution. Two random effects for species were then added to the model, one assuming that effects from different species are correlated according to this correlation matrix (to capture any phylogenetic signal) and one assuming uncorrelated random effects (to capture pure species heterogeneity). We found that accounting for phylogenetic relatedness and species-level heterogeneity did not significantly improve the fit of the model and therefore excluded these random effects from the rest of the analyses.

The strength of effect sizes varied substantially between studies, with some studies yielding Hedges' *g* values that were exceedingly

large. For four studies that reported SD values, the resulting effect sizes were too large to represent biologically realistic values; therefore, we (conservatively) assumed that the authors had reported SEs instead (asterisk in Source Data). However, for other studies, it was not as clear why some effect sizes were so large. To reduce the potential of such effects driving the overall results, we therefore examined the impact of excluding extreme effect sizes by refitting the model when including only Hedges' *g* values that fell, in absolute value, below a certain threshold. We assessed the impact of all possible threshold values between 2 and the largest observed effect size. The specific threshold value had no noteworthy impact on the estimated overall effect or its significance, but the size of the variance components decreased with stricter thresholds (Figure S3a,b). We therefore set the threshold to 2, trimming away 344 effect sizes (total number of effect sizes for the SMD: 1910 to 1566), which was conservative, since the vast majority (95%) of the effect sizes that were excluded by this choice were negative.

To assess whether the effects of neonicotinoids differed for the various performance types, per neonicotinoid type, or as a function of the relative dose or time, we fitted a series of models where we added moderator variables (i.e., performance type, pesticide, % of max concentration, and % of max time) to the base model (res8 to res12 in Table S3; see Figures S4 and S5 for results). To detect if publication bias was present in our dataset, we ran an extended regression-based model to examine asymmetry (Nakagawa et al. 2022). However, since the standardised mean differences are correlated with the standard errors, we did not use the standard errors in this test, but rather the sample sizes of the exposed and control groups. This test yielded no indication of asymmetry and thus publication bias.

All models were fitted using restricted maximum likelihood (REML) estimation, using the rma.mv() function from the *metafor* package (Viechtbauer 2010) in R (version 4.2.2). A detailed description of the model selection process is found in Table S4.

We also measured the effect of neonicotinoids on bird survival. This meta-analysis was restricted to effect sizes calculated from proportions only (i.e., log risk ratios). We again fitted the model with a nested structure but had to simplify the model due to the smaller size of this part of the dataset ($k = 46$, $n = 12$). Here, we assumed a correlation of rho = 0.5 for multiple estimates from the same study and only added study identity and effect size ID as random effects (res 12, Table S4).

Author Contributions

E.M. and S.A.K. conceptualised the study and all authors participated in the design. E.M. collected the data. E.M. and S.A.K. did a quality control of the data. E.M. and W.V. conducted the analyses. W.V. conducted the model evaluation. E.M. and S.A.K. led the writing of the first draft. All authors contributed to discussions and production of the manuscript.

Acknowledgements

We thank the scientific council of Vogelbescherming Nederland for elaborate discussions on the content, Jouke Altenburg, Frans van Alebeek, and Gretchen Wagner for comments on the manuscript, and Laura Addy Orduna, Margaret Eng, Xu Peng, Barnett A. Rattner, and Shafia Tehseen Gul for providing unpublished data or clarification of published data.

Data Availability Statement

The data, complementary documents and code for the analyses and figures are available on Dryad at: https://doi.org/10.5061/dryad.12jm63z5f.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ele.14534.

References

Addy-Orduna, L. M., J. C. Brodeur, and R. Mateo. 2019. "Oral Acute Toxicity of Imidacloprid, Thiamethoxam and Clothianidin in Eared Doves: A Contribution for the Risk Assessment of Neonicotinoids in Birds." *Science of the Total Environment* 650: 1216–1223.

Bass, C., and L. M. Field. 2018. "Neonicotinoids." *Current Biology* 28: R772–R773.

Bean, T. G., M. S. Gross, N. K. Karouna-Renier, et al. 2019. "Toxicokinetics of Imidacloprid-Coated Wheat Seeds in Japanese Quail (*Coturnix japonica*) and an Evaluation of Hazard." *Environmental Science & Technology* 53: 3888–3897.

Bonmatin, J.-M., C. Giorio, V. Girolami, et al. 2015. "Environmental Fate and Exposure; Neonicotinoids and Fipronil." *Environmental Science and Pollution Research* 22: 35–67.

Borenstein, M., L. V. Hedges, J. P. Higgins, and H. R. Rothstein. 2021. *Introduction to Meta-Analysis*. Chichester, UK: Wiley.

Cedergreen, N. 2014. "Quantifying Synergy: A Systematic Review of Mixture Toxicity Studies Within Environmental Toxicology." *PLoS One* 9: e96580.

Cinar, O., S. Nakagawa, and W. Viechtbauer. 2022. "Phylogenetic Multilevel Meta-Analysis: A Simulation Study on the Importance of Modelling the Phylogeny." *Methods in Ecology and Evolution* 13: 383–395.

Distefano, G. G., R. Zangrando, M. Basso, et al. 2022. "The Ubiquity of Neonicotinoid Contamination: Residues in Seabirds With Different Trophic Habits." *Environmental Research* 206: 112637.

Eng, M. L., B. J. Stutchbury, and C. A. Morrissey. 2019. "A Neonicotinoid Insecticide Reduces Fueling and Delays Migration in Songbirds." *Science* 365: 1177–1180.

Eng, M. L., B. J. M. Stutchbury, and C. A. Morrissey. 2017. "Imidacloprid and Chlorpyrifos Insecticides Impair Migratory Ability in a Seed-Eating Songbird." *Scientific Reports* 7: 15176.

Frank, S., and J. Tooker. 2020. "Neonicotinoids Pose Undocumented Threats to Food Webs." *Proceedings of the National Academy of Sciences* 117: 22609–22613.

Franzen-Klein, D., M. Jankowski, C. L. Roy, et al. 2020. "Evaluation of Neurobehavioral Abnormalities and Immunotoxicity in Response to Oral Imidacloprid Exposure in Domestic Chickens (*Gallus Gallus Domesticus*)." *Journal of Toxicology and Environmental Health. Part A* 83: 45–65.

Fry, D. M. 2021. "Reproductive Effects in Birds Exposed to Pesticides and Industrial Chemicals." *Environmental Health Perspectives* 103: 165–171.

Fuentes, E., A. Gaffard, A. Rodrigues, et al. 2023. "Neonicotinoids: Still Present in Farmland Birds Despite Their Ban." *Chemosphere* 321: 138091.

Gibbons, D., C. Morrissey, and P. Mineau. 2015. "A Review of the Direct and Indirect Effects of Neonicotinoids and Fipronil on Vertebrate Wildlife." *Environmental Science and Pollution Research* 22: 103–118.

Goulson, D. 2013. "An Overview of the Environmental Risks Posed by Neonicotinoid Insecticides." *Journal of Applied Ecology* 50: 977–987.

Goulson, D. 2014. "Pesticides Linked to Bird Declines." *Nature* 511: 295–296.

Graves, E. E., R. J. Meese, and M. Holyoak. 2023. "Neonicotinoid Exposure in Tricolored Blackbirds (*Agelaius tricolor*)." *Environmental Science and Pollution Research* 30: 15392–15399.

Hallmann, C. A., R. P. Foppen, C. A. Van Turnhout, H. De Kroon, and E. Jongejans. 2014. "Declines in Insectivorous Birds Are Associated With High Neonicotinoid Concentrations." *Nature* 511: 341–343.

Hamman, E. A., P. Pappalardo, J. R. Bence, S. D. Peacor, and C. W. Osenberg. 2018. "Bias in Meta-Analyses Using Hedges'd." *Ecosphere* 9: e02419.

Harrison, F. 2011. "Getting Started With Meta-Analysis." *Methods in Ecology and Evolution* 2: 1–10.

Hedges, L. V., and I. Olkin. 2014. *Statistical Methods for Meta-Analysis*. Orlando: Academic press.

Hladik, M. L., A. R. Main, and D. Goulson. 2018. "Environmental Risks and Challenges Associated With Neonicotinoid Insecticides." *Environmental Science & Technology* 52: 3329–3335.

Hong, C., J. Zhang, Y. Li, E. Elia, R. Riley, and Y. Chen. 2020. "A Regression-Based Method for Detecting Publication Bias in Multivariate Meta-Analysis" ArXiv Preprint ArXiv200204775.

Hoshi, N., T. Hirano, T. Omotehara, et al. 2014. "Insight Into the Mechanism of Reproductive Dysfunction Caused by Neonicotinoid Pesticides." *Biological & Pharmaceutical Bulletin* 37: 1439–1443.

Humann-Guilleminot, S., S. Clément, J. Desprat, Ł. J. Binkowski, G. Glauser, and F. Helfenstein. 2019. "A Large-Scale Survey of House Sparrows Feathers Reveals Ubiquitous Presence of Neonicotinoids in Farmlands." *Science of the Total Environment* 660: 1091–1097.

Humann-Guilleminot, S., S. Laurent, P. Bize, A. Roulin, G. Glauser, and F. Helfenstein. 2021. "Contamination by Neonicotinoid Insecticides in Barn Owls (*Tyto alba*) and Alpine Swifts (*Tachymarptis melba*)." *Science of the Total Environment* 785: 147403.

Hussein, M., V. Singh, P. Gupta, B. Yadav, and A. K. Singh. 2014. "Developmental and Biochemical Effects of Imidacloprid on Chick Embryos." *Journal of Anatomy Sciences* 22: 12–17.

Jackson, D., R. Riley, and I. R. White. 2011. "Multivariate Meta-Analysis: Potential and Promise." *Statistics in Medicine* 30: 2481–2498.

Jeschke, P., R. Nauen, M. Schindler, and A. Elbert. 2011. "Overview of the Status and Global Strategy for Neonicotinoids." *Journal of Agricultural and Food Chemistry* 59: 2897–2908.

Jetz, W., G. H. Thomas, J. B. Joy, K. Hartmann, and A. O. Mooers. 2012. "The Global Diversity of Birds in Space and Time." *Nature* 491: 444–448.

Lees, A. C., L. Haskell, T. Allinson, et al. 2022. "State of the World's Birds." *Annual Review of Environment and Resources* 47: 231–260.

Lehmann, E., N. Turrero, M. Kolia, Y. Konaté, and L. F. De Alencastro. 2017. "Dietary Risk Assessment of Pesticides From Vegetables and Drinking Water in Gardening Areas in Burkina Faso." *Science of the Total Environment* 601: 1208–1216.

Lennon, R. J., N. J. B. Isaac, R. F. Shore, et al. 2019. "Using Long-Term Datasets to Assess the Impacts of Dietary Exposure to Neonicotinoids on Farmland Bird Populations in England." *PLoS One* 14: e0223093.

Li, Y., R. Miao, and M. Khanna. 2020. "Neonicotinoids and Decline in Bird Biodiversity in the United States." *Nature Sustainability* 3: 1027–1035.

Lopez-Antia, A., M. E. Ortiz-Santaliestra, F. Mougeot, and R. Mateo. 2013. "Experimental Exposure of Red-Legged Partridges (*Alectoris rufa*) to Seeds Coated With Imidacloprid, Thiram and Difenoconazole." *Ecotoxicology* 22: 125–138.

Lopez-Antia, A., M. E. Ortiz-Santaliestra, F. Mougeot, and R. Mateo. 2015. "Imidacloprid-Treated Seed Ingestion Has Lethal Effect on Adult Partridges and Reduces Both Breeding Investment and Offspring Immunity." *Environmental Research* 136: 97–107.

Mineau, P., and C. Palmer. 2013. The Impact of the Nation's Most Widely Used Insecticides on Birds Report of the American Bird Conservancy.

Møller, A. P., D. Czeszczewik, E. Flensted-Jensen, et al. 2021. "Abundance of Insects and Aerial Insectivorous Birds in Relation to Pesticide and Fertilizer Use." *Avian Research* 12: 1–9.

Montiel-León, J. M., G. Munoz, S. V. Duy, et al. 2019. "Widespread Occurrence and Spatial Distribution of Glyphosate, Atrazine, and Neonicotinoids Pesticides in the St Lawrence and Tributary Rivers." *Environmental Pollution* 250: 29–39.

Moreau, J., J. Rabdeau, I. Badenhausser, et al. 2022. "Pesticide Impacts on Avian Species With Special Reference to Farmland Birds: A Review." *Environmental Monitoring and Assessment* 194: 790.

Morgan, R. L., P. Whaley, K. A. Thayer, and H. J. Schünemann. 2018. "Identifying the PECO: A Framework for Formulating Good Questions to Explore the Association of Environmental and Other Exposures With Health Outcomes." *Environment International* 121: 1027–1031.

Morrissey, C. A., P. Mineau, J. H. Devries, et al. 2015. "Neonicotinoid Contamination of Global Surface Waters and Associated Risk to Aquatic Invertebrates: A Review." *Environment International* 74: 291–303.

Nakagawa, S., M. Lagisz, M. D. Jennions, et al. 2022. "Methods for Testing Publication Bias in Ecological and Evolutionary Meta-Analyses." *Methods in Ecology and Evolution* 13: 4–21.

Nakagawa, S., and E. S. Santos. 2012. "Methodological Issues and Advances in Biological Meta-Analysis." *Evolutionary Ecology* 26: 1253–1274.

Pandey, S. P., K. Tsutsui, and B. Mohanty. 2017. "Endocrine Disrupting Pesticides Impair the Neuroendocrine Regulation of Reproductive Behaviors and Secondary Sexual Characters of Red Munia (*Amandava amandava*)." *Physiology & Behavior* 173: 15–22.

Paradis, E., J. Claude, and K. Strimmer. 2004. "APE: Analyses of Phylogenetics and Evolution in R Language." *Bioinformatics* 20: 289–290.

Pisa, L., D. Goulson, E. C. Yang, et al. 2021. "An Update of the Worldwide Integrated Assessment (WIA) on Systemic Insecticides. Part 2: Impacts on Organisms and Ecosystems." *Environmental Science and Pollution Research* 28: 11749–11797.

Rawi, S. M., A. S. Al-Logmani, and R. Z. Hamza. 2019. "Neurological Alterations Induced by Formulated Imidacloprid Toxicity in Japanese Quails." *Metabolic Brain Disease* 34: 443–450.

Rizzati, V., O. Briand, H. Guillou, and L. Gamet-Payrastre. 2016. "Effects of Pesticide Mixtures in Human and Animal Models: An Update of the Recent Literature." *Chemico-Biological Interactions* 254: 231–246.

Roy, C. L., and P. L. Coy. 2020. "Wildlife Consumption of Neonicotinoid-Treated Seeds at Simulated Seed Spills." *Environmental Research* 190: 109830.

Sultana, T., C. Murray, S. Kleywegt, and C. D. Metcalfe. 2018. "Neonicotinoid Pesticides in Drinking Water in Agricultural Regions of Southern Ontario, Canada." *Chemosphere* 202: 506–513.

Taliansky-Chamudis, A., P. Gómez-Ramírez, M. León-Ortega, and A. García-Fernández. 2017. "Validation of a QuECheRS Method for Analysis of Neonicotinoids in Small Volumes of Blood and Assessment of Exposure in Eurasian Eagle Owl (*Bubo bubo*) Nestlings." *Science of the Total Environment* 595: 93–100.

Thompson, D. A., H. J. Lehmler, D. W. Kolpin, et al. 2020. "A Critical Review on the Potential Impacts of Neonicotinoid Insecticide Use: Current Knowledge of Environmental Fate, Toxicity, and Implications for Human Health." *Environmental Science. Processes & Impacts* 22: 1315–1346.

Tokumoto, J., M. Danjo, Y. Kobayashi, et al. 2013. "Effects of Exposure to Clothianidin on the Reproductive System of Male Quails." *Journal of Veterinary Medical Science* 75: 755–760.

Tooker, J. F., and K. A. Pearsons. 2021. "Newer Characters, Same Story: Neonicotinoid Insecticides Disrupt Food Webs Through Direct and Indirect Effects." *Current Opinion in Insect Science* 46: 50–56.

Topping, C. J., A. Aldrich, and P. Berny. 2020. "Overhaul environmental risk assessment for pesticides." *Science* 367: 360–363.

Van Kleunen, M., E. Weber, and M. Fischer. 2010. "A Meta-Analysis of Trait Differences Between Invasive and Non-invasive Plant Species." *Ecology Letters* 13: 235–245.

Viechtbauer, W. 2010. "Conducting Meta-Analyses in R With the Metafor Package." *Journal of Statistical Software* 36: 1–48.

Walters, K. 2013. "Data, Data Everywhere but We don't Know What to Think? Neonicotinoid Insecticides and Pollinators." *Outlooks on Pest Management* 24: 151–155.

Winfree, R., R. Aguilar, D. P. Vázquez, G. LeBuhn, and M. A. Aizen. 2009. "A Meta-Analysis of bees' Responses to Anthropogenic Disturbance." *Ecology* 90: 2068–2076.

Wood, T. J., and D. Goulson. 2017. "The Environmental Risks of Neonicotinoid Pesticides: A Review of the Evidence Post 2013." *Environmental Science and Pollution Research* 24: 17285–17325.

Zhang, X., Y. Huang, W. J. Chen, et al. 2023. "Environmental Occurrence, Toxicity Concerns, and Biodegradation of Neonicotinoid Insecticides." *Environmental Research* 218: 114953.

Zhao, G., F. W. Yang, J. W. Li, H. Z. Xing, F. Z. Ren, and G. F. Pang. 2020. "Toxicities of Neonicotinoid-Containing Pesticide Mixtures on Nontarget Organisms." *Environmental Toxicology and Chemistry* 39: 1884–1893.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.