nature portfolio

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Last updated by author(s): Jan 18, 2025

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	We searched the Web of Science Core Collection, MEDLINE, SciELO Citation Index and KCI-Korean Journal Database (1900 to present) using the Boolean search string based on the "TOPIC" searching: ["pesticide" OR "insecticide" OR "fungicide" OR "herbicide"] AND ["non-target*" OR "carnivor*" OR "saprophag*" OR "saprovor*" OR "omnivor*" OR "bee" OR "pollinat*" OR "pollen*" OR "predat*" OR "parasitoid" OR "wasp" OR "mammal" OR "amphibi*" OR "reptil*" OR "bird" OR "fish" OR "mollus*" OR "invertebrate" OR "vertebrate" OR "herbivore" OR "arthropod" OR "plant" OR "dicot*" OR "monocot*" OR "alga*" OR "microorganism" OR "bacteria" OR "fung*"] were screened for relevance. Means, standard errors (or standard deviations) and sample sizes of the selected variables could be extracted from tables, figures, the main text or supporting information. This literature search was initiated in November 2012, although finalized in May 2022.
Data analysis	R version 4.1.0 was used for all statistical analyses. The R package 'metafor' 3.4-0 was used for performing meta-regression and analysis of publication bias. All code for this analysis is available on Zenodo (https://zenodo.org/records/14245304). In summary, meta-regression was performed to evaluate whether the effects of pesticide use on various taxonomic groups could be explained by the different moderator variables and their interactions. This was done using multilevel mixed-effects meta-regression models. We used usual large-sample approximation to compute the sampling variances. The original effect size difference log response ratios were employed as the response variable to fit the models. Taxonomic groups (i.e., animals, plants and microorganisms) were treated as fixed effects along with the moderator variables (i.e., pesticide class, study types, climatic zone, exposure medium, pesticide type, and number of added pesticide application rates). To account for between-pesticide differences, we used the active ingredient pesticide identity as a random effect in the models. Although not considered in the main results, the included studies were also analysed in subsets (e.g., as higher resolution taxonomic groups) to better understand the effects within these various strata (see Supplementary Methods). For all meta-analyses we accounted explicitly for phylogenetic effects for species where published phylogenies were available

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data and code used in this analysis are available on Zenodo (https://doi.org/10.5281/zenodo.14683219).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

This study undertakes a meta-analysis on biodiversity effects of pesticides on plants, animals and microorganisms by integrating 20,212 estimates of pesticide effects reported from 1,705 experimental studies conducted at locations across the globe. These studies collectively measure effects for insecticides, fungicides and herbicides. The included studies encompass laboratory and field experiments from temperate and tropical climatic zones, both in aquatic and terrestrial systems. We classified the effects on multicellular animals (vertebrates and invertebrates), plants and microorganisms based on change in growth, reproduction, behavior (animals only) as well as other relevant biomarkers (e.g., enzymatic activities, metabolic reaction and plant photosynthesis). To ensure that our results are meaningful under field-realistic application rates, we restricted our analysis to a sub-set of effect sizes where pesticide application rates were at or below recommended (e.g. product label) application rates. To test for application rate-dependent effects on non-target organisms (i.e. how responses increased with increasing rate of application), we used a meta-regression to relate effect sizes to increased pesticide application rates. We use meta-regression approaches to test the hypotheses that: (i) pesticides (insecticides, fungicides and herbicides) suppress growth, reproduction, and behavioural traits; ii) metabolic biomarkers of non-target organisms may be up- or down-regulated in response to pesticides, signalling a physiological perturbation; (iii) identified pesticide effects are consistent between taxonomic groups, experimental types, climatic zones, modes of exposure (terrestrial or aquatic), and broad pesticide classes (insecticides, fungicides or herbicides); and (iv) the effects also hold under realistic terrestrial and aquatic exposure scenarios. Overall, our synthesis comprehensively shows that insecticides, fungicides and herbicides have broad-scale detrimental effects on all groups of non-target organisms tested. Pesticides consistently decreased growth and reproduction across all taxonomic groups, while also eliciting behavioral responses in animals and perturbing multiple endpoints linked to metabolic or physiological status. Given the ubiquitous use of pesticides worldwide, estimated at about 4.1 million tons of active ingredient applied in 2015 alone, these findings emphasize the potential for prevalent non-target impacts relating to widespread pesticide use. Specifically, these results suggest that pesticide use has the potential to result in wider perturbation of ecological communities and functions than previously thought.

Research sample

We followed the PRISMA protocols for study selection and inclusion in the systematic meta-analysis. We collected data from Web of Science, finally we collected 20,212 observations from 1,705 studies to study the effects of pesticides (including insecticides, herbicides and fungicides) on non-target organisms (i.e., animals, plants and microorganisms and their taxonomic groups).

Sampling strategy

With the aim of constructing a comprehensive database, we tried to collect as many as experiments that fulfill our criteria (described below). We searched the Web of Science Core Collection, MEDLINE, SciELO Citation Index and KCI-Korean Journal Database (1900 to present) using the Boolean search string described above in sampling strategy. In total, 887,482 papers were reviewed for relevance. Of this large number of initially identified papers, we undertook a rapid screening process that focused on the titles and abstracts to determine whether the study had measured a response variable (growth, reproduction, behaviour or biomarker) for non-target organisms in response to exposure to insecticides, fungicides or herbicides. Overall, our preliminary screening removed 878,772 papers. The remaining 8,710 (i.e., 8,710=887,482-878,772) papers were then screened in full to assess if they met the following criteria: a) the study included at least one comparison between a randomly allocated control group (without pesticide) and one or more treatment groups exposed to insecticides, fungicides, or herbicides; b) similarly to reduce within study heterogeneity for field experiments, control and treatment areas were required to have comparable non-pesticide agricultural practices, e.g. fertilizer, irrigation, soil tillage etc; c) treatment groups needed to included single insecticide, fungicide, or herbicide active ingredient at a time, so that multiple pesticide exposure experiments were discarded. This was done to avoid the risk of bias associated with study heterogeneity resulting from unexpected synergistic interactions between compounds; d) control and pesticide-treated groups were required to include the same non-target organisms for lab or field experiments; and finally; e) the measurements of control and pesticide-treated groups had to have been undertaken at the same spatiotemporal scale. These screening criteria resulted in 1,705 papers from which it was possible to extract effect sizes for inclusion in the meta-analysis. Data were extracted from the papers by N.F.W. and L.F. during which regular cross-checking was performed to ensure consistency in extracted effect sizes.

When a study included multiple application rates of pesticides, measurements with different application rates of pesticides were treated as independent observations. For studies that included more than one location, we considered these experimental observations separately. When numeric values were not provided directly, we extracted them from figures using the "GetData Graph Digitizer" software. However, where linear or non-linear relationships between pesticide application rates and one of these response

	indicators was presented in a figure, we extracted the values via fitted regression equations. When the pesticide-treated group was paired with the control group, we excluded multiple comparisons within a single study and selected different comparison data. As such, observations without pesticide use were considered as control groups, while those with different pesticide application rates were considered as the treatment groups. Where pesticides were water soluble, we selected pure distilled water as the control even when multiple control solvents had been present (e.g. ethanol). If pesticides were not water soluble, we ensured that the control and treatment group had the same solvent.
Data collection	First, we selected the papers through a search on the Web of Science, and then extracted the data from the papers. Second, we established three datasets: 1) the effects of pesticides including insecticides, fungicides and herbicides on the response variables (i.e., growth, reproduction, behavior and biomarker of animals including invertebrates and vertebrates and their taxonomic groups; 2) the effects of pesticides including insecticides, fungicides and herbicides on the response variables (i.e., growth, reproduction and biomarker of plants including dicotyledonous, monocotyledonous and spore-producing plants and their taxonomic groups); and 3) the effects of pesticides including insecticides, fungicides and herbicides on the response variables (i.e., growth, reproduction and biomarker of microorganisms including bacteria and fungi and their taxonomic groups). Third, we established a new dataset about the effects of pesticides (i.e., insecticides, fungicides and herbicides) on the response variables (i.e., animal growth, animal reproduction, animal behavior, animal biomarker, plant growth, plant reproduction, plant biomarker, microorganism growth, microorganism reproduction and microorganism biomarker), to ensure that our results are meaningful under field-realistic application rates (namely, we restricted our analysis to a sub-set of effect sizes where pesticide application rates were at or below recommended (e.g. product label) application rates). Finally to test for application rate-dependent effects on non-target organisms (i.e. how responses increased with increasing rate of application), we used a meta-regression to relate effect sizes to increased pesticide application rates. Nian-Feng Wan (mainly) and Liwan Fu recorded the data which were recorded in Excel.
Timing and spatial scale	The deadline for collecting data is May 2022 (searches were performed in Web of Science between November 2012 and May 2022). Searches included globally published studies but were limited to those published in English.
Data exclusions	When we collected the data, the data were excluded in this meta-analysis if they did not conform to the following criteria: 1) the study included at least one comparison between a control group (without pesticide) and one or more treatment groups exposed to insecticides, fungicides, or herbicides; 2) multiple pesticide exposure experiments were discarded so that each treatment group included only one insecticide, fungicide, or herbicide active ingredient at a time, which was done to avoid the risk of bias associated with unexpected synergistic interactions between compounds; 3) for field experiments, control and treatment areas were required to have similar non-pesticide agricultural practices (fertilizer, irrigation, soil tillage etc); 4) control and pesticide-treated groups were required to include the same non-target organisms for lab or field experiments; 5) the measurements of control and pesticide-treated groups had to have been done at the same spatiotemporal scale; and 6) when studies reported mean values across multiple sampling dates or years these were included as separate data points.
Reproducibility	The readers can extract the data from the 1,705 papers which were selected in this paper to reproduce the results. R code is provided to re-run all analyses
Randomization	We used a mixed-effects meta-regression to account for between-study, within-study and sampling variances.
Blinding	Not applicable. There were no human or animal research participant involved in this study.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study	
☒	☐	Antibodies
☒	☐	Eukaryotic cell lines
☒	☐	Palaeontology and archaeology
☒	☐	Animals and other organisms
☒	☐	Human research participants
☒	☐	Clinical data
☒	☐	Dual use research of concern

Methods

n/a	Involved in the study	
☒	☐	ChIP-seq
☒	☐	Flow cytometry
☒	☐	MRI-based neuroimaging