A systemic large-scale assessment of risks from pesticide use for different organism groups in the United States of America and Germany based on a labeled property graph

by

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Overview of publications

The cumulative dissertation is based on the following three publications:

- Bub, S., Wolfram, J., Stehle, S., Petschick, L. L., & Schulz, R. (2019). Graphing ecotoxicology: the MAGIC graph for linking environmental data on chemicals. *Data*, *4*(1), 34. https://doi.org/10.3390/data4010034
- Schulz, R., Bub, S., Petschick, L. L., Stehle, S., & Wolfram, J. (2021). Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops. *Science*, *372*(6537), 81-84. https://doi.org/10.1126/science.abe1148
- Bub, S., Wolfram, J., Petschick, L. L., Stehle, S., & Schulz, R. (2023). Trends of total applied toxicity in German agriculture. *Environment Science & Technology*, *57*(1), 852-961. https://doi.org/10.1021/acs.est.2c07251

Table of contents

1 List of abbreviations

CAS	Chemical Abstracts Service
EFSA	European Food Safety Authority
EPA	United States Environmental Protection Agency's
EU	European Union
InChI	International Chemical Identifier
MAGIC	Meta-Analysis of the Global Impact of Chemicals
RTL	Regulatory Threshold Level
SMILES	Simplified Molecular Input Line Entry Specification
TAT	Total Applied Toxicity
US	United States of America
USGS	United States Geological Survey

2 Introduction

Ecotoxicology is the science that researches effects of toxicants on biological entities. Following the famous toxicological principle formulated 1538 by von Hohenheim, known as Paracelsus, thereby generally all chemicals are able to act as toxicants[1]. Unlike human toxicology that focuses on toxic effects on individuals and populations of one species, Homo sapiens, ecotoxicology is not constrained in its scope of biological entities. It is interested in toxic effects on individuals and populations of any species (excluding humans), and on communities and entire ecosystems (Walker et al., 2012; Köhler & Triebskorn, 2013; Newman 2014). One example of where the ecological foundation of ecotoxicology manifests itself are indirect effects, which are effects on biological entities that are not directly caused by chemicals but instead are mediated by ecological interactions and environmental conditions (Walker et al., 2012). With this large scope, ecotoxicology is an inter- and multidisciplinary science that links chemical, biological and environmental knowledge.

With millions of species and at least 100,000 chemicals that potentially interact with them in the environment (Wang et al., 2021), ecotoxicology has a large ground to cover. Among these sheer numbers, there are some groups that are of special importance regarding their potential environmental impact. Pesticides are one group of chemicals that have a large, if not the largest, ecotoxicological relevance: they are toxic for biological entities, sometimes in very low concentrations[2], and they are used in large amounts and globally (Bernhardt et al., 2017). The high toxicity of pesticides, much higher than that of most other groups of chemicals, is a result of their intended use: they are designed to reduce detrimental effects of, e.g., insects, plants or fungi on agriculture by controlling respective populations, often, and in the sense of their

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[1] In his work *Sieben Defensiones*, von Hohenheim responses to his critics: *"Wenn ihr jedes Gift recht auslegen wollt, was ist, das nit Gift ist? Alle Dinge sind Gift, und nichts ist ohne Gift; allein die dosis machts, daß ein Ding kein Gift sei."*

[2] "Concentration" is used throughout the text as a general measure of substance mass per medium, simplifying the commonly in ecotoxicology applied distinction of terms by medium, such as "concentration" for mass per volume, "dose" for mass per mass bodyweight or food, "rate" for mass per area, etc.

Latin name, through induced lethality (Walker et al., 2012). However, they act not specific enough to be toxic only for the intended species that are considered pests, but also show toxicity towards species living in habitats next to pesticide-treated areas. The widespread agricultural use of pesticides, on the other hand, is a result of their work-and-cost-efficiency for securing yields, but also results in exposure of ecosystems at a global scale (Sharma et al., 2019). In summary, pesticides can be abstractly seen as toxicity intentionally applied to agricultural areas, unintentionally also exposing organisms in non-agricultural areas to toxicity.

The risks of pesticide use for ecosystems have led major jurisdictions, like the United States of America (US) and the European Union (EU), to enact elaborated regulatory processes that require a registration of pesticides prior use (EFSA, 2013; EPA, 2011; Stehle & Schulz, 2015b). A by-product of these registration processes are regulatory threshold levels (RTL) which can be used for scientific risk analysis outside the regulatory process (Stehle & Schulz, 2015a). The RTL for an organism group is basically derived from the most sensitive effect concentrations found in standardized toxicity tests for species representative for the group, multiplied by a safety factor, although specifics differ among regulatory processes. Conceptually, they mark the threshold that separates environmental concentrations associated with acceptable risk (concentrations below the RTL) from concentrations associated with unacceptable risk (concentrations above the RTL).

Due to the high degree of procedural standardization in the derivation of RTLs, they have been found as a good measure to make the toxicities of different pesticides comparable, and they were employed in a series of studies to characterize environmental pesticide concentrations (e.g., Stehle & Schulz, 2015a; Stehle et al., 2018; Wolfram et al., 2018; Wolfram et al., 2021; Schulz et al., 2021, also, in Appendix B; Bub et al., 2023, also, in Appendix C). RTL reflect, for instance, that insecticides show regulatory unacceptable concentrations towards fish between 3 ng/L (deltamethrin, a pyrethroid) and 110 mg/L (imidacloprid, a neonicotinoid), a range of nine orders of magnitude. At the same, imidacloprid is very toxic to pollinators (RTL of 1.52 ng/organism), while more than 95% of all of the insecticides, with regulatory

unacceptable concentrations among insecticides ranging as high as 1,6 mg/organism, indicating a toxicity six orders of magnitude lower than that of imidacloprid.

At large-scales, ecotoxicology deals with pesticide impacts on a national (e.g., Bub et al., 2023; Douglas & Tooker, 2015; Hallmann et al., 2014; Schulz et al., 2021; Stehle et al., 2019; Wolfram et al., 2018), continental (Wolfram et al., 2021) or the global scale (Stehle & Schulz, 2015a; Stehle et al., 2018). This maximization of considered scale is in line with the general tendency of ecotoxicology towards larger scales, but generally requires new methodological and conceptual approaches. Historically, individual chemicals and groups of chemicals have been identified that mark, caused by their immense release into the environment, main disruptors of processes in the Earth system, like greenhouses gases for the climate change, chlorofluorocarbons for the depletion of the atmosphere's ozone layer, dichlorodiphenyl-trichloroethane and other organochlorides for bioaccumulation in food webs and declines in bird populations, etc., but for other phenomena, like declines in biodiversity or numbers of insect species (Outhwaite et al., 2020; Seibold et al., 2019; Vörösmarty et al., 2010), the active part of chemical pollution is only understood to a much lesser extent. There are indicators that pesticides may play a major role

This dissertation contributes to the research of large-scale risks of pesticide use, and of large-scale ecotoxicology in general, in several ways (Figure 1). In Chapter 2, it presents a labeled property graph, the MAGIC graph (Meta-Analysis of the Global Impact of Chemicals graph), as a solution to the methodological issues that arise when increasing amounts of data from more and more sources are combined for analysis (Bub et al., 2019; also, in Appendix A). The MAGIC graph is able to link chemical information from different sources, even if these sources use different nomenclatures. This enables analyses that incorporate toxicological data, like thousands of RTLs (for different organism groups and jurisdictions) for hundreds of pesticides, and information on pesticide use and chemical classes. The MAGIC graph is implemented in a way that allows it to be organically extended by additional chemical, biological and environmental data, and eventually scaled to all chemicals of environmental interest.
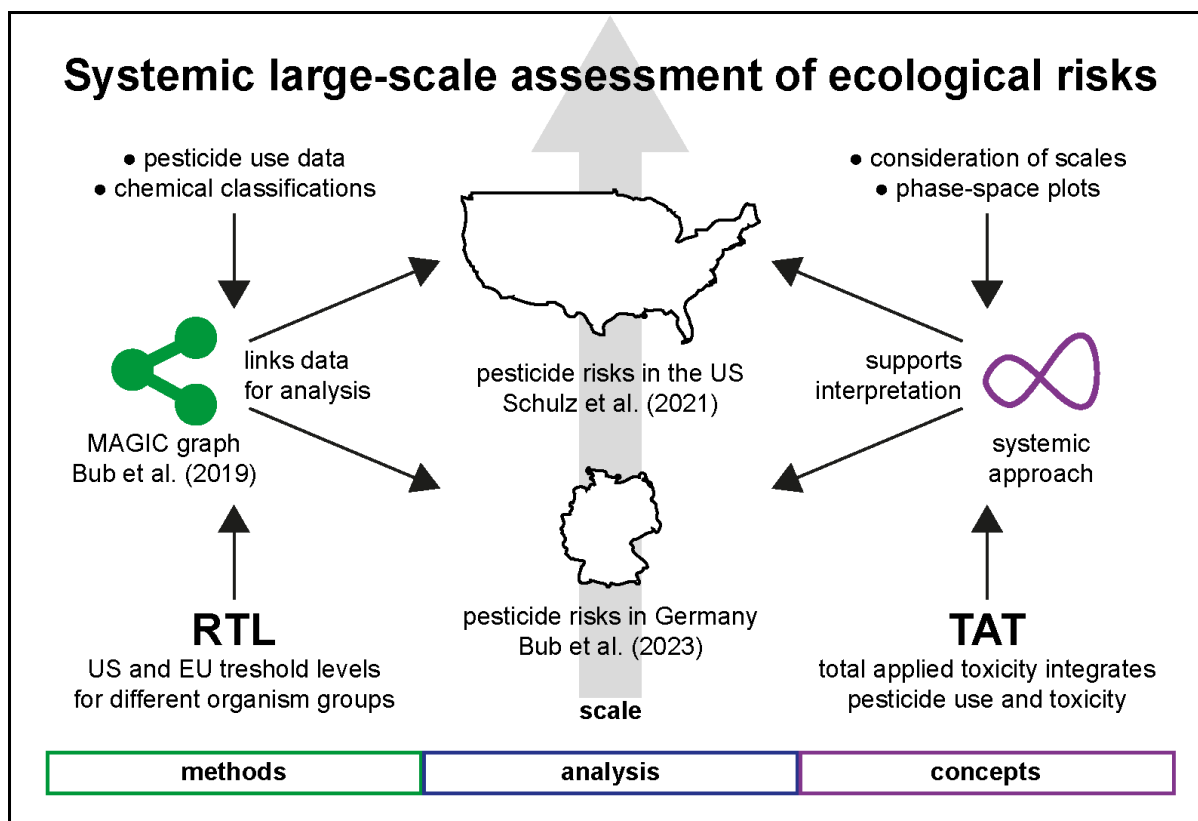
7

Figure 1. Overview over this cumulative dissertation thesis. Journal articles Bub et al. (2019), Schulz et al. (2021) and Bub et al. (2023) can be also found in Appendix A, B and C, respectively.

Chapter 3 shows, how the combination of the linked pesticide data with a systemic consideration of pesticide use supports the interpretation of pesticide risks in the US (Schulz et al., 2021; also, in Appendix B). This systemic approach includes a new measure, the total applied toxicity (TAT), which integrates used pesticide amounts and pesticide toxicities, and the consideration of pesticide use as a complex system whose state and evolution can be visualized in phase-space plots. The combination of the described methods and concepts led to a novel view on pesticide risks in the US and can provide a framework for future ecotoxicological research at large scales.

Chapter 4 displays the results of the methods and concepts of the US pesticide risk analysis applied to Germany (Bub et al., 2023; also, in Appendix C). A pesticide risk analysis of Germany is of special importance in the context of the EU's goal to drastically reduce pesticide risks (European Commission, 2020) and Germany being one of the important agricultural

8

producers in the EU. A comparison of the results for Germany to those for the US did also allow to evaluate the impact of scale and differing RTLs, information that can help other ecotoxicological large-scale assessments. Chapter 5 adds a conclusion and an outlook.

3   Database for large-scale evaluations of chemicals

A problem that occurs during the seemingly simple task of collating chemical data from different sources is the use of different denominators by the various data providers. For ecotoxicology, at least the 100,000 chemicals are of environmental interest (Wang et al., 2021), but the use of concurring chemical nomenclatures in is a long-known issue that impedes immediate data collation (Heller et al., 2013; Wiswesser, 1968). To identify, for which of these chemicals which kind of information is available, chemical denominations among various data sources must be harmonized. Within the available sources, a chemical substance can be denominated using one (of often many) trivial, brand or scientific names. Alternatively, its identity can be specified by a data provider using one of the many available chemical registries. For instance, the Chemical Abstracts Service (CAS) is an early computerized provider of widely used registry numbers for chemicals (*CAS Registry System*, 1978). Other ways of identifying chemicals are directly based on the molecular structure of chemicals, like the Simplified Molecular Input Line Entry Specification (SMILES) or the International Chemical Identifier (InChI; Heller et al., 2013). Related to the differences in how the same chemical is denominated using different systems, is the problem of differing scopes of what is named at all, e.g., only distinct compounds or also mixtures, and the ecotoxicological relevant distinction of different levels of specificity of chemicals (Smith, 2009; Stehle & Schulz, 2015a). Other domains, like biology, show similar difficulties as described here for chemicals.

The MAGIC graph is an endeavor to represent ecotoxicological knowledge for large-scale analysis in a labeled property graph (Bub et al., 2019; also, in Appendix A), a type of data representation that is known to be performant for linked data (Vicknair et al., 2010). Conceptually, labeled property graphs (Robinson et al., 2015) represent information as nodes that are linked by relationships. Properties of various data types can be attached to nodes and relationships for further specification. Relationships have a single, user-defined type, and an arbitrary number of labels can be attached to each to group nodes together in sets.
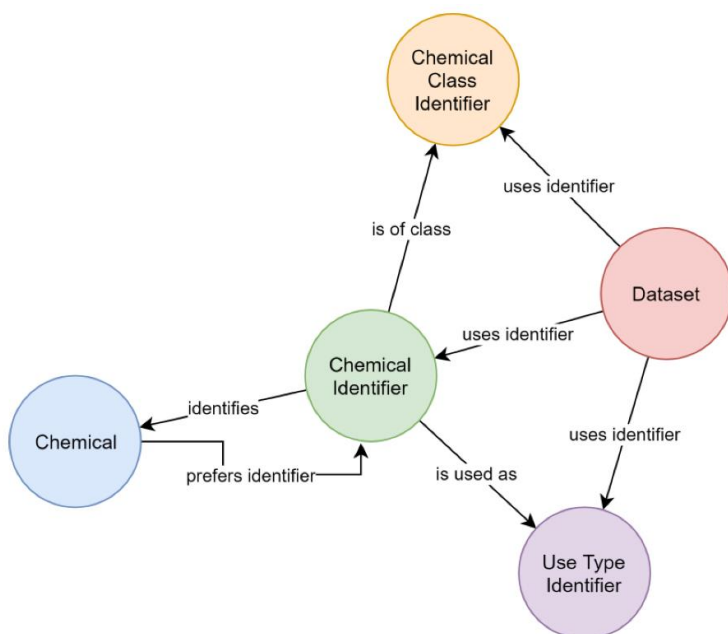
Figure 2. Schema of the "Meta-analysis of the Global Impact of Chemicals" (MAGIC) graph, depicting typed relationships (arrows) between labeled nodes (circles). From Bub et al. (2019).

The MAGIC graph defines an extendable set of relationship types, labels and properties that allow describing important ecotoxicological entities, like chemicals, their names, and chemical classes, as well as the relationships between these entities (Figure 2). Its initial goal is to provide means to link data from existing databases of ecotoxicological relevance, which use different chemical denominations of different specificity. Such databases include the United States Environmental Protection Agency's (EPA) ECOTOX Knowledgebase (EPA, n.d.) that provides a large set of records on aquatic and terrestrial toxicity tests, including effect concentrations for more than 11,000 chemicals; the European Food Safety Authority's (EFSA) Chemicals Hazards Database (OpenFoodTox; Kovarich et al., 2022) that provides data used during the official registrations of chemicals in the European Union for more than 3,000 chemicals; the US National Water Quality Monitoring Council's Water Quality Portal (National Water Quality Monitoring Council, n.d.) that provides water quality parameters, including physical, chemical (for more than 3,000 chemicals), and biological characteristics in US waters, collated from samples of more than 400 agencies; the United States Geological Survey's (USGS) Estimated Annual Agricultural Pesticide Use dataset (Baker, 2016) that

provides estimates on use amounts of pesticides (more than 400 chemicals) at the county level; the Pesticide Action Network's PesticideInfo database (Pesticide Action Network, n.d.) that provides summary information on pesticides and other chemicals (more than 6,000 chemicals), including chemical classification and use type.

With the MAGIC graph, the linkage of chemicals between databases increases considerably compared to a relational join (Figure 3). This is especially true for links between data sources that are based on chemical names, e.g., links between EPA's ECOTOX database (identifies chemicals by name and CAS registry number) and USGS's use dataset (only chemical names). The multitude of realized possibilities to name chemicals, and even differences in spelling, result in generally unsatisfying linkages with naive relational joins based on chemical names. Some databases employ both, chemical names and CAS registry numbers, e.g., EPA's ECOTOX database and EFSA's OpenFoodTox database. In such cases, links based on registry numbers have considerably higher success rates (Fig. 1). However, although CAS registry numbers act as a superordinate naming scheme that supposedly should result in a success rate of 100%, this is not the case. One of the reasons why CAS registry numbers may not match among data sources is that chemicals can have multiple CAS registry numbers. Among them is always only one valid number, but for historic reasons, the same chemical could have been assigned different numbers over time. If data sources do not update their identifiers when registry numbers for a chemical change, it might occur that the same chemical appears under different CAS registry numbers in different data bases.
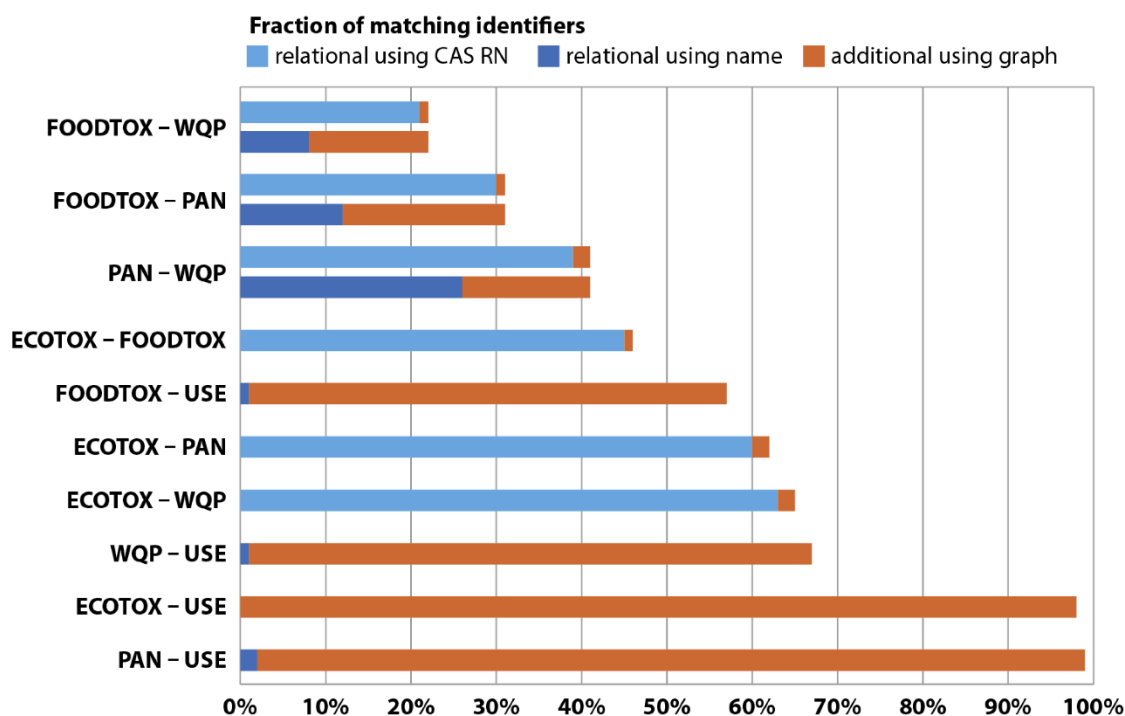
Figure 3. Linkage of chemical data relative to a theoretical 100%-maximum for different databases. Complete linkage presumes that all chemicals listed in the smaller database are contained in the larger one. Linkage with a relational approach, where only CAS registry numbers (light blue) or same-spelling chemical identifiers match (dark blue), is compared to the additional gain with the graph approach (orange). From Bub et al. (2019).

The MAGIC graph provides a methodological solution for providing complex and linked ecotoxicological knowledge to large-scale analyses, for example chemical monitoring data, chemical or biological taxonomies, or effect data. Augmented with other, differently structured data, for instance geoinformation that is available in high resolution for large extents, the linking of already available information in a graph database, as done by the MAGIC graph, can act as a profound base for large-scale ecotoxicology. The MAGIC graph has so far been successfully applied to link chemicals, among others, for evaluation of pesticide risks in the US (Schulz et al., 2021; also, in Appendix B) and in Germany (Bub et al., 2023; also, in Appendix C), to model regulatory threshold levels of pesticides (Petschick et al., 2019) and to compare various chemical databases (Heinemann et al., 2020).

4 Systemic aspects of pesticide use and toxicity

A fundamental insight of ecotoxicology is that toxicities among chemicals towards the same group of organisms differ greatly. That is even true within groups of similar chemicals, for instance, insecticides, where the RTLs for aquatic invertebrates span multiple orders of magnitude (Schulz & Stehle, 2015a). Schulz et al. (2021; also, in Appendix B) highlight that this insight is strikingly often ignored in public discussions about pesticide use, and even in the ecotoxicological discourse. Trends in pesticide use are instead often based solely on the amounts of pesticides used, more specifically, often on the total mass of pesticides used, e.g., within a country and a year (e.g., Douglas & Tooker, 2015; Ewald et al., 2015; Lamberth et al., 2013; Larsen & Noack, 2017; Osteen & Fernandez-Cornejo, 2013).

In Schulz et al. (2021), a new measure, the TAT, was introduced, that divides the used amounts of pesticides by their RTLs for different organism groups before summing them up, and that so incorporates both used amounts and toxicities into a single measure. The TAT so provided a measure to analyze trends in pesticide use and their potential environmental effects in the US over the last 25 years (Figure 4 B-D,F-H,J,K). For instance, decreasing TATs for mammals (Figure 4C) and birds (Figure 4D) indicate that these organism groups may face decreasing burden by pesticide use, whereas increasing TATs for aquatic invertebrates (Figure 4F), pollinators (Figure 4G) and terrestrial plants (Figure 4J) indicate potentially increasing burden to these organism groups. With applied pesticide amounts being determined by market situation, pest pressure and other dynamic factors, but toxicity being a substance-specific property covering many orders of magnitudes, changes in TAT are often induced by changes in the composition of the set of pesticides used.
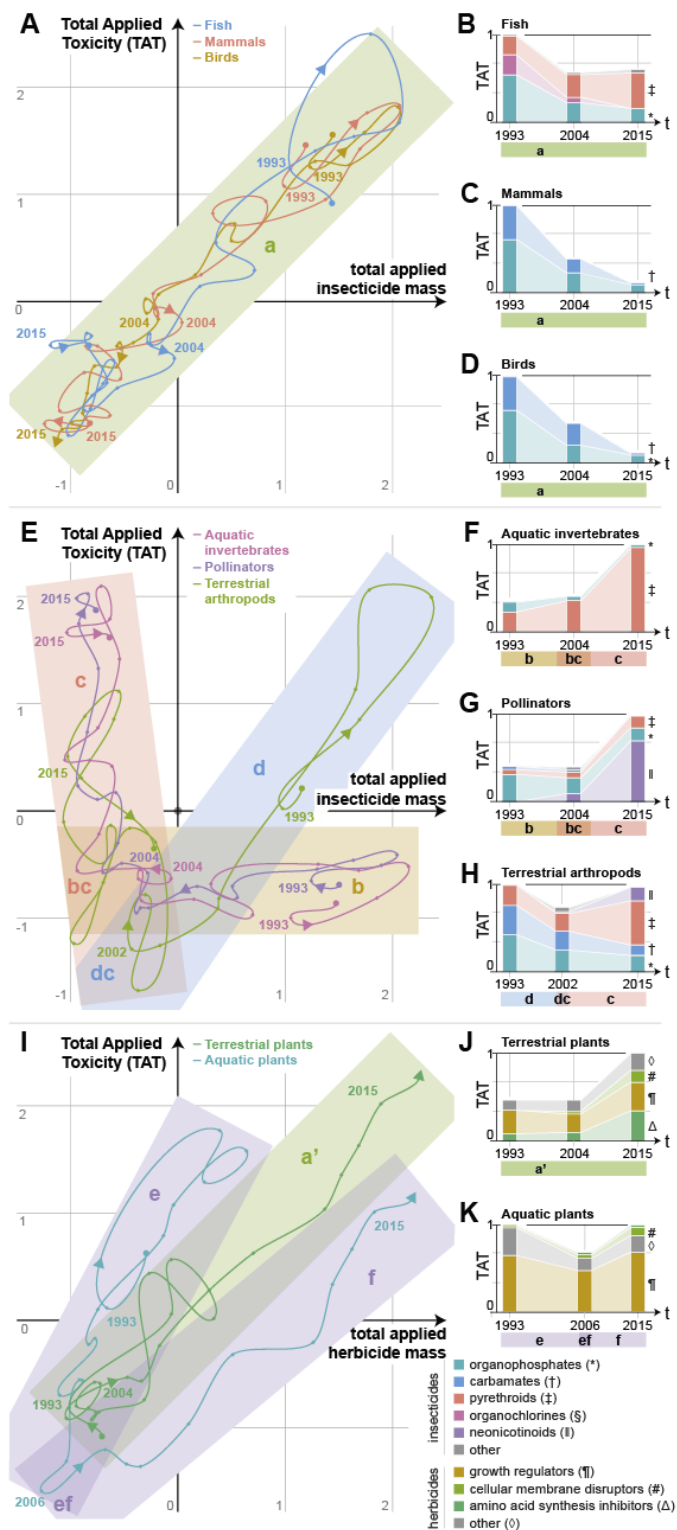
Figure 4. Temporal trends in total applied pesticide amount (i.e., mass) versus total applied toxicity (TAT) and main pesticide classes contributing to applied toxicity during a 25-year period for different nontarget species groups. Phase-space plots (both axes z-score normalized) for vertebrates (A), invertebrates (E) and plants (I) with highlighted areas indicating separate phases (a to f). Contribution of major pesticide classes in selected periods (three-year averages; phases a-f shown as horizontal time bars): organophosphorus and carbamate insecticides in vertebrates (B-D), pyrethroids in aquatic invertebrates (F), neonicotinoids in pollinators (G), and herbicides in plants (J, K). From Schulz et al. (2021).

A systemic description of pesticide use is able to trace the impact of changes in applied mass and composition in more detail. Figure 4A,E,I show phase-space plots, a form of diagram originating from mechanical physics and a popular tool in system-theoretic visualizations that displays state and change of a system simultaneously (Nolte, 2010). Tracking the evolution of complex systems in phase-space plots can help identifying distinct phases of system development and distinguishing different kinds of system changes. In the case of pesticide use in the US, this reveals some additional insights for different species groups. Plotting the TAT for fishes, mammals and birds of different years against the total applied insecticide mass (preliminary analysis showed that no other group of pesticide had any influence on the TAT for these groups; both parameters were z-score transformed prior plotting to make them relatively comparable between groups) and connecting the data points of the individual years to represent the temporal system development in the applied mass-TAT space, shows that the system states (represented by the individual years) are bound to a single corridor in the phase space (Figure 4A). Numerical analysis shows that this single corridor has a width of less than 0.5, indicating a relatively low variability, a center line that intersects the coordinate system exactly in its origin and a slope of exactly 1. In conclusion, the TAT for the three vertebrate groups appears to be proportional to the applied masses of the pesticides that contribute to the respective TAT, which are organophosphates and carbamates for mammals and birds, and organophosphates, pyrethroids and neonicotinoids for fishes. The same seems to be the case for terrestrial plants (Figure 4I), only that the TAT here increases proportionally to the increased applied mass of some herbicides belonging to various chemical classes. Increased herbicide use is likely also caused by development of pesticide resistances (Gould et al., 2018), but is associated with detrimental effects (Morandin & Winston, 2005; Stenoien et al., 2016). The proportionality of mass and TAT does, however, not exclude changes in the composition of the applied pesticides. For instance, contribution of organochlorines to the fish TAT were continuously replaced by equally high contributions of pyrethroids, a group of pesticides that is, e.g., correlated with declining abundances of California Bay-Delta fishes (Fong et al., 2016).

16

A different picture emerges when considering the phase-space plots for aquatic invertebrates and pollinators (Figure 4E). Here, two different phases of system development can be discerned. In a first phase from 1992 to approximately 2004, changes in the applied insecticide mass (again, only insecticides contribute to the TAT of both invertebrate groups), more specifically, the decrease of the applied masses in this time, do not lead to changes in the TATs. In a second phase until 2016, the TATs increase considerably, despite further decreasing masses. The transition from the first into the second phase occurs within only a couple of years. Unlike it was the case for the vertebrate TATs, changes in the amounts of applied pesticides are, thus, not a good explanation for changes in the invertebrate TATs. Instead, structural change of the pesticide use regime, namely changes in the set of applied pesticides, delivers an explanation. Although phases and phase change occur concurrently for aquatic invertebrates and pollinators, the specifics of the observed patterns in both phase-spaces are different: the continuous replacement of high-volume organophosphates by pyrethroids (Stehle & Schulz, 2015a), which are much more toxic to aquatic invertebrates but used in smaller volumes, explain the initial steadiness and subsequent increase of the TAT for aquatic invertebrates despite decreasing applied masses. Pyrethroids are discussed as relevant risk contributors to aquatic invertebrates, but their high toxicity, low application rates and transient occurrence in surface waters make them hard to cover in monitoring campaigns (Stehle & Schulz, 2015a). They are, however, regularly found in biofilms (Mahler et al., 2020) and led even to resistances in amphipods (Weston et al., 2013). For pollinators, the replacement of organophosphates by neonicotinoids, a group highly toxic to bees (Stanley et al., 2015; Woodcock et al., 2017), leads to a similar, concurring phase shift. In conjunction with the increasing TAT for terrestrial plants, this could additionally stress these highly interlinked organism groups (Biesmeier et al., 2006). The phase shifts around 2004 hint at fundamental structural changes in the human-ecological system of agricultural pesticide use in the US at this time, but requires further evaluation for a more detailed understanding.

17

The results of Schulz et al. (2021) challenge the widespread view that insecticide risks in the US are declining because the applied amounts are decreasing. They show, following a systemic approach, that changes in the choice of insecticides, away from those especially toxic to vertebrates and towards those especially toxic to invertebrates, may be a major driver for the overall risks of the latter organism group.

5 Importance of system size for pesticide use and toxicity

The TAT provides a tool to assess potential risks of pesticide use at large scales for various species groups. It can be applied to any region for which the amounts of applied pesticides are known on a substance-level. This kind of data is available for an increasing, although still limited, number of countries. One of the countries for which use data in sufficient detail, i.e., per active ingredient, has recently become available, is Germany, and, for several reasons, it is interesting to study the TAT in Germany in more detail (Bub et al., 2023; also, in Appendix C): Germany is among the important agricultural producers in Europe; a comparison of the German TAT with the US TAT allows insights into the role that the differences in agriculture and pesticide use between the two countries play, but also allows studying scale-effects when considering that the German agriculture is much smaller than that of the US; finally, the EU has set ambitious goals to reduce environmental risks posed by pesticides (European Commission, 2020), and the TAT could help to track the accomplishment of these goals. Several studies discuss potential biodiversity effects of pesticide use for Germany (Geiger et al., 2010; Liess et al., 2020; Nationale Akademie der Wissenschaften Leopoldina et al., 2020; Schmitz et al., 2015).

Pesticide use in Germany forms a system of a much smaller size compared to the US, but it also differs in crop composition, field sizes, farming practices, and landscape context (Larsen et al., 2017; Sachverständigenrat für Umweltfragen, 2016). For instance, while there are about 400,000 tons of pesticides applied in the US every year, this figure is only about 30,000 tons, or 7.5%, in Germany. This might be one of the reasons, why TAT trends in Germany indicate a different, less clear, system behavior. Unlike in the US, where a clear identification of phases was possible for all of the six studied organism groups, it was not possible to identify phases for the TATs in Germany, although four of the eight studied organism groups showed significant trends. Other than in the US, the TAT trends in Germany are not constrained by well-defined regime boundaries, they rather show a high degree of freedom in the applied

19

mass-TAT phase spaces. As a consequence, there are also no apparent regime shifts in the German TATs.

The about one order of magnitude smaller scale of German pesticide use, compared to the US, increases the relative importance of interannual variance that is caused by smaller-scale factors. At smaller scales, pesticide use develops very dynamically, with changes in the preferred pesticides applied, and the space and time of applications. This applies especially to insecticides, which are applied only on small proportions of agricultural land, in response to certain pest pressures, but in regional clusters (Schulz et al., 2021). This could also be seen for the US if the TATs were calculated for individual counties instead of for the entire country. The larger the (spatial) scale of the considered pesticide use, the more these local effects of clustered TAT are averaged over time. Nevertheless, there are striking similarities between both countries: both show a significant and decreasing trend in the TAT for terrestrial vertebrates, and a significant and increasing trend for the TAT of terrestrial plants.

Another finding for Germany that resembles insights gained for the US is that a small number of pesticides already explains large parts of the TATs of the different organism groups (Figure 5). Which these pesticides are, is however different in the two countries. A systemic reason for these differences is the different structure of agriculture in the two countries and a different pesticide use therein. For instance, the lists of the top-5 TAT contributors contain for four of the eight considered organisms groups at least one fungicide, a group of pesticides that was not relevant for the TAT in the US at all. Fungicides are used in special cultures like vines, which have a relatively larger proportion in German agriculture than in the US. Fungicides dominate the TAT for soil organisms in Germany, an organism group that was, however, not assessed in the US study. Soil organisms are important for soil health (Maeder et al., 2002; Riedo et al., 2021), but are also prone to pesticide exposure (Gunstone et al., 2021; Topping et al., 2020). Another reason for the difference in the top-5 TAT contributors in the two countries are differences in the RTLs that were used to express the toxicity of individual pesticides based on the data of the respective regulatory processes. Taking into account that

the top-contributors to the TATs are often particularly toxic substances that are only used in comparably small amounts, that is, that the high TAT contribution of these substances is more determined by their toxicity than by their applied masses, this highlights the large sensitivity that large-scale ecotoxicological assessments have on values that were yielded at much smaller scales, and, thus, the necessity for a consistent determination of these values.
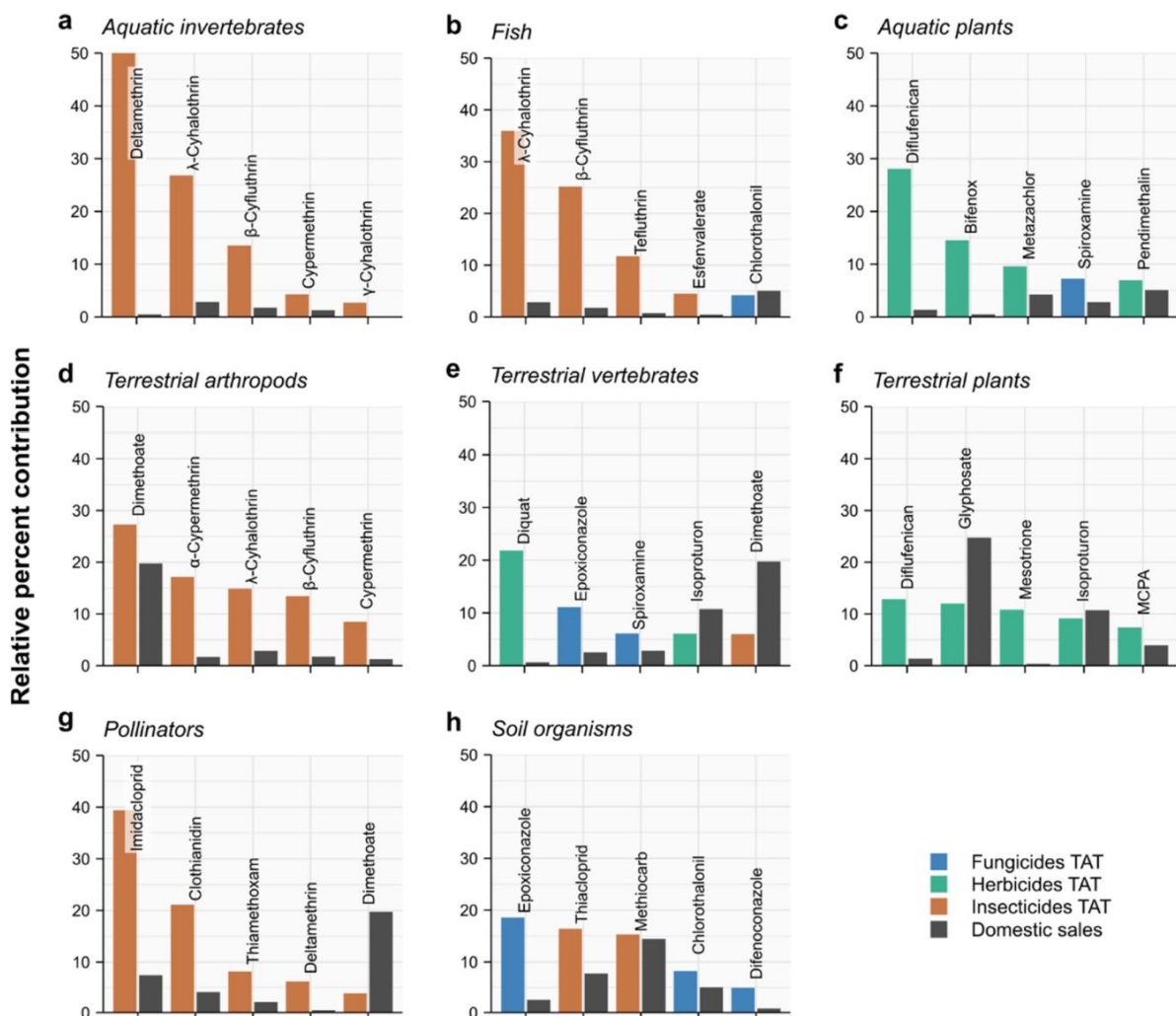

Figure 5. Relative contribution of individual pesticides to the TAT of (a) aquatic invertebrates, (b) fish, (c) aquatic plants, (d) arthropods, (e) vertebrates, (f) terrestrial plants, (g) pollinators and (h) soil organisms. Domestic sales refer to the sold mass of the pesticide relative to the total sold mass of its pesticide type. For display, the top five pesticides per species group regarding TAT contribution were selected. Percentages refer to the total of the years 1995-2019. From Bub et al. (2023).

The results of the US and German studies both point out the large potential risks originating from pyrethroids for aquatic invertebrates and, to a lesser extent, for terrestrial arthropods and fish, a group of substances that is often not identified as driving pesticide risks due to their low total of applied masses. In Germany, the largest observed TAT increase by a factor of 3 was observed for fish and has been be attributed to an increase in the usage of highly toxic pyrethroids. The role of pesticides for fish declines (Freyhof & Brooks, 2011; Müller et al., 2018; Wolter, 2001) is currently discussed (Stokstad et al., 2013), and the TAT indicates some importance of insecticide use in this context.

Like for the US, pesticide risks in Germany are often being assumed to decrease as applied masses decrease, whereas a consideration based on the TAT much more diverse picture. In the context of the ambitious EU goals to reduce pesticide risk in each member state, this finding challenges the proposed, mostly mass-based, indicator that is to be used for assessing this risk reduction. The comparison between the risk analysis of Germany and that of the US hinted at potential scale effects.

6 Conclusion

Ecotoxicology at large scales requires adapted methods and conceptual frameworks. Graph databases offer a manageable approach to link and semantically enrich data relevant for ecotoxicological risk analyses from different sources. Measures like the TAT are able to summarize rich and complex data into comprehensible proxies for assessing large-scale risks. System-theoretic conceptual frameworks facilitate the linkage of knowledge among different fields of research and enhances the interpretation of results.

The combination of a graph database approach with system-theoretic interpretations of the TAT has revealed insights into the dynamics of pesticide use in the US and Germany, and the increasing risk it imposes to different organism groups. Risks for non-target ecosystems may even increase more at a global scale than for the two studied countries due to the considerable expansion of cropland and pesticide use in many regions. Given the indicators for the detrimental impact of pesticide use, these large-scale developments urgently request for further research. However, in many regions, fundamental data, like pesticide use, are not available.

Ecotoxicology at large scales is needed more than ever. Processes show increasing dynamics at the global scale, some of which are concerning because they may exceed the adaptability of natural systems. At a global scale, these dynamics include increasing pesticide use (considering mass and toxicity) that co-occur with other stressors to ecosystems, like continuous land-transformation and degradation, or climate change, but eventually extends to an steadily increasing number of chemicals that are of potential environmental impact. Human actions are the main driver for these increasing dynamics, but the role that chemicals as a whole play at large scales is not yet entirely understood. Knowledge of this role, however, is necessary to adapt human action in order to mitigate, manage and reverse chemical risks and effects in the future.

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8   Declaration

I, the undersigned, author of this work, declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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9 Acknowledgements

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11 Appendices

Appendix A: Journal article "Graphing ecotoxicology" (Appendix A.pdf)

Bub, S., Wolfram, J., Stehle, S., Petschick, L. L., & Schulz, R. (2019). Graphing ecotoxicology: the MAGIC graph for linking environmental data on chemicals. Data, 4(1), 34. https://doi.org/10.3390/data4010034

Appendix B: Journal article "Applied pesticide toxicity shifts towards plants and invertebrates, even in GM crops" (Appendix B.pdf)

Schulz, R., Bub, S., Petschick, L. L., Stehle, S., & Wolfram, J. (2021). Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops. Science, 372(6537), 81-84. https://doi.org/10.1126/science.abe1148

Appendix C: Journal article "Trends of total applied toxicity in German agriculture" (Appendix C.pdf)

Bub, S., Wolfram, J., Petschick, L. L., Stehle, S., & Schulz, R. (2023). Trends of total applied toxicity in German agriculture. Environment Science & Technology, 57(1), 852-961. https://doi.org/10.1021/acs.est.2c07251

Graphing Ecotoxicology: The MAGIC Graph for Linking Environmental Data on Chemicals

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Abstract: Assessing the impact of chemicals on the environment and addressing subsequent issues are two central challenges to their safe use. Environmental data are continuously expanding, requiring flexible, scalable, and extendable data management solutions that can harmonize multiple data sources with potentially differing nomenclatures or levels of specificity. Here, we present the methodological steps taken to construct a rule-based labeled property graph database, the "Meta-analysis of the Global Impact of Chemicals" (MAGIC) graph, for potential environmental impact chemicals (PEIC) and its subsequent application harmonizing multiple large-scale databases. The resulting data encompass 16,739 unique PEICs attributed to their corresponding chemical class, stereo-chemical information, valid synonyms, use types, unique identifiers (e.g., Chemical Abstract Service registry number CAS RN), and others. These data provide researchers with additional chemical information for a large amount of PEICs and can also be publicly accessed using a web interface. Our analysis has shown that data harmonization can increase up to 98% when using the MAGIC graph approach compared to relational data systems for datasets with different nomenclatures. The graph database system and its data appear more suitable for large-scale analysis where traditional (i.e., relational) data systems are reaching conceptional limitations.

Dataset: The dataset can be found in Supplementary Materials, http://www.mdpi.com/2306-5729/4/1/34/s1.

Dataset License: CC-BY-SA

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1. Summary

The primary concern of ecotoxicology is the impact of chemicals on the environment [1]. To assess this impact at a large-scale, i.e., continental or global context, data of environmental concentrations, effects, use types or application rates have to be incorporated into a consistent structure. Today, science can rely on numerous databases providing these data (Table 1) for potential environmental impact chemicals (PEICs, e.g., pesticides, industrial chemicals, flame retardants, and solvents). However, the process of linking them takes significant harmonization efforts, even after a common semantic framework has been established, i.e., even after their integration into a coherent base. Among the most fundamental reasons hindering instant data linkage and affecting dimensions of the ecotoxicological data are differing nomenclatures and differing levels of specificity (see Table 2 for

examples). For instance, when linking data spatially, some problems typically arise around issues of specificity, i.e., data present at different spatial scales or resolutions, while linking data within chemical dimension is often impeded by the usage of different nomenclatures. For ecotoxicology, however, the specific interest lies in the chemical dimension, as it applies to all core data (Table 1).

Table 1. Core data, their dimensions and exemplary datasets, providing these data and being used in this study.

Type of Data	Dimensions	Example Datasets
Environmental Concentrations	Space, time, medium, chemical	WQP [1]
Biological effects	Species, medium, chemical	ECOTOX [2], FOODTOX [3]
Use types	Chemical	PAN [4], (WQP)
Application rates	Space, time, chemical	USE [5]

[1] National Water Quality Monitoring Council—Water Quality Portal (WQP) [2]. [2] U.S. EPA ECOTOX database (ECOTOX) [3]. [3] European Food Safety Authority—OpenFoodTox (FOODTOX) [4]. [4] Pesticide Action Network—Pesticide Database (PAN) [5]. [5] U.S. Geological Survey—Estimated Annual Agricultural Pesticide Use (USE) [6].

The usage of different nomenclatures is a well-known issue in chemistry [7,8]. Although a chemical compound is defined by its molecular structure, there is no exclusive convention for naming or identifying it. Instead, there are many concurring schemes based on two different approaches: naming chemicals based on their molecular structure (International Chemical Identifier (InChI) and Simplified Molecular Input Line Entry Specification (SMILES) [7,9]) or assigning arbitrary, yet unique, identifiers to them (e.g., Chemical Abstract Services registry number (CAS RN) and Distributed Structure-Searchable Toxicity substance identifier (DTXSID) [10,11]). In addition, many chemicals, such as pesticides, also have various other names (e.g., trivial, brand, and formulation names) that may also differ among languages (Table 2). The co-existence of these naming schemes results in a high number of synonyms making nomenclature an important issue, particularly when linking larger datasets. This issue further aggravates in analyses that operate in a trans-national or global context, consider many PEICs and require harmonizing many different data sources.

Table 2. Examples of problems occurring when linking data from different sources.

Field of Problem	Affected Dimension	Problem	Example
Nomenclature	Chemical	Different spellings	Lambda-cyhalothrin–cyhalothrin, lambda
		Different nomenclatures	Thiametoxam (trivial name)–153719-23-4 (CAS RN)
	Space	Different languages	United States of America–Estados Unidos de América
Specificity	Chemical	Different stereo-chemical information	Concentration of beta-cyfluthrin–threshold of cyfluthrin
	Space	Different spatial resolution	Concentration at GPS coordinate–use rate at county-level
	Time	Different temporal resolution	Concentration with date–use data in yearly resolution

Differing data specificity is also an issue when linking chemical data. Measured environmental concentrations or effect endpoints may be provided in different databases specifically for any kind of isomers, including data specific to enantiomers or diastereomers, or at the level of unique compound structures, ignoring stereo-compositions and, thus, including isomeric and racemic mixtures. Differing specificity, if not addressed, substantially hinders the integration of data from different sources for some of the ecotoxicologically most important groups of compounds, e.g., insecticides [12], that may act substantially differently based on their stereo-chemical composition [13].

For analyses that cover only relatively small sets of PEICs, problems of nomenclature and specificity can be handled manually by expert judgment. Knowledge of PEICs thereby allows constructing data analysis workflows that cover all deviations in chemical names and that reasonably span different levels of specificity. Larger analyses that cover several dozens, or more, of PEICs are often based on relational data representations [12]. In the case only two different naming schemes are involved, e.g., if only two data sources are linked, differing identifiers of the same chemical can still be resolved by establishing a synonym table. However, relational database joins are costly, and linking more than two different data sources by joining their chemical identifiers (e.g., chemical name) with synonym tables increases the processing complexity significantly, quickly reaching points where complex data analyses become cumbersome [14]. Moreover, resolving different levels of specificity within and among relational datasets requires sophisticated techniques that entail even more effort to develop and that can hardly be established without significant lack of performance [15]. At least when combining more than two data sources—a requirement of many ecotoxicological meta-analyses—relational data representations are suboptimal due to their constraints in performance and usability [15–17].

Labeled property graph databases represent an effective tool to address the aforementioned issues of extendibility, scalability, and flexibility [14,18,19]. Briefly, a labeled property graph consists of nodes (vertices) that are connected through relationships (directed edges) [19]. Both nodes and relationships can be labeled to distinguish functional roles and can be enriched with properties (see Appendix A for further details). In contrast to relational database systems, the number and type of relationships between entities is, thus, not strictly defined and allows linking information very flexibly [18,20]. This flexibility and the graph's emphasis on relationships appear well suited for the establishment of a synonym database that can also resolve hierarchical relationships [14,15]. Consequently, over the last years, graph databases have evolved as a technical alternative to the established relational database systems, featuring large-scale business (e.g., logistics, social media, and health management) and scientific applications (e.g., web science and sociology) where relational solutions become unfeasible [21,22]. However, even after an extensive literature review, we could not find any published approach that uses a graph for managing and analyzing data in ecotoxicology.

The aim of this study was to assess the usability of graph databases for large-scale ecotoxicological meta-analyses that integrate and link a wide range of relevant data (Table 1) and was conducted by the research group "Meta-Analysis of the Global Impact of Chemicals" (MAGIC). In addition, multiple ecotoxicologically-relevant databases were used to perform a data harmonization, using U.S. EPA Chemical Dashboard (CDDB) [11] as a synonym provider, demonstrating the method's applicability in a large-scale ecotoxicological scope and quantifying the method's advantages compared to relational joins. The data were subsequently reprojected into tabular form, granting easy accessibility to researchers and professionals. The Microsoft® Excel worksheet published with this data description summarizes the information that is currently contained in the MAGIC graph in a tabular format, while an up-to-date version of the MAGIC graph can be explored using our website (https://magic.eco; see User Notes). Harmonized data for 16,739 PEICs in the MAGIC graph contain information about unique identifiers (CAS RN and DTXSID), valid synonyms, respective chemical classes, use type classification and their inclusion in various databases.

We are positive that the MAGIC graph can serve as a reliable proof that, with graph databases, one already has a suited data integration tool at hand. With it already being actively integrated in large-scale risk analysis at the national level [23], the MAGIC graph will find further applications and become a central tool in trans-national or global risk analyses in the future (DFG SCHU 2271/6-2). The MAGIC graph is publicly available and will provide a continuously expanding feature set, allowing researchers to take advantage of graph database solutions.

2. Data Description

2.1. Database

The MAGIC graph contains 16,731 PEICs (see Microsoft® Excel worksheet for complete list). For these chemicals, chemical identifiers (n = 66,636) used by relevant datasets (Table 1) are stored and linked to the chemicals they identify. Each chemical has a preferred name for consistent creation of output. Use types and chemical classes, as provided by external datasets, are included in the MAGIC graph and linked with the chemical identifier used by the external dataset. The resulting schema (Figure 1) allows collecting chemical information over multiple databases regardless of the individually used identifiers by navigating the graph (Figure 2). Up-to-date contents of the graph can be retrieved using the website https://magic.eco (also see user notes).
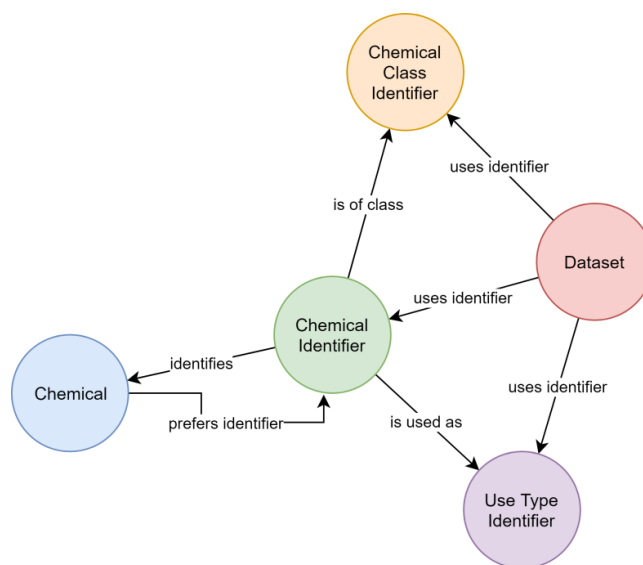

Figure 1. Schema of the "Meta-analysis of the Global Impact of Chemicals" (MAGIC) graph depicting typed relationships (arrows) between labeled nodes (circles).

Figure 2. Network map of the MAGIC graph. A subset of chemical identifiers is shown as green dots. Larger circles outlined in black represent the datasets included in the graph. Grey lines connect the datasets with chemical identifiers, and individual identifiers with chemicals (cyan circles, rarely visible).

2.2. Summary Microsoft® Excel Worksheet

Published with this data descriptor is a Microsoft® Excel worksheet that summarizes the content of the MAGIC graph. The columns of this worksheet are described in Table 3.

Table 3. Description of the submitted Microsoft® Excel worksheet.

Column	Description
Chemical	The preferred name of the chemical as derived from the CDDB. In most instances, the name given here equals the preferred name of the CDDB.
CAS RN	The currently valid Chemical Abstract Service registry number as given by the CDDB. Alternative CAS RNs, such as deleted numbers, are given under synonyms if they are used by at least one of the databases included in the MAGIC graph.
DTXSID	The substance identifier of the distributed structure-searchable toxicity database as provided by the CDDB
Synonyms	Additional identifiers of the chemical. Synonyms are only listed if they are used by at least one of the databases included in the MAGIC graph.
Chemical Class	The chemical class according to the PAN and WQP dataset. Only chemicals occurring in one of these datasets are classified and classifications are given here as is. Chemical classifications will be extended by considering further databases and harmonized among databases in the future.
Stereochemical	An "x" indicates stereo-chemical information is associated with the chemical.
Insecticide	An "x" indicates that the chemical is used as an insecticide according to the PAN database.
Herbicide	An "x" indicates that the chemical is used as an herbicide according to the PAN database.
Fungicide	An "x" indicates that the chemical is used as a fungicide according to the PAN database.
Microbiocide	An "x" indicates that the chemical is used as a microbiocide according to the PAN database.
Other Uses	A list of other uses of this chemical (excluding insecticide, herbicide, fungicide and microbiocide) according to the PAN and WQP databases. As with chemical classes, use type classification will be improved continuously over the next versions of the MAGIC graph.
WQP	Entries marked "x" indicate that the WQP database contains records of this chemical, using any of its identifiers.
ECOTOX	Entries marked "x" indicate that the ECOTOX database contains records of this chemical, using any of its identifiers.
FOODTOX	Entries marked "x" indicate that the FOODTOX database contains records of this chemical, using any of its identifiers.
USE	Entries marked "x" indicate that the USE database contains records of this chemical, using any of its identifiers.
PAN	Entries marked "x" indicate that the PAN database contains records of this chemical, using any of its identifiers.

2.3. Database Linkage and Pesticide Use Types

The databases listed in Table 1 were integrated into the MAGIC graph and subsequently analyzed individually regarding the chemical identifiers they contain (Table 4). These databases were selected because they are the most comprehensive resources for large-scale ecotoxicological core data from governmental and non-governmental sources. The MAGIC graph made it possible to evaluate how many of the identifiers used by each dataset actually identified chemicals, and how the identified chemicals were distributed among chemicals with stereo-chemical information and those without. Further, the number of synonymous identifiers within each dataset was identified.

Table 4. Characterization of ecotoxicologically-relevant datasets using the MAGIC graph.

Dataset	ID Type	Identifiers		Syno-nyms [4]	Chemicals	
		Chemical [1,2]	Other [2,3]		Stereo- [5]	Non-Stereo [5]
WQP	CAS RN, name	6384 (65%)	3374 (35%)	3133	385 (11%)	2987 (89%)
ECOTOX	CAS RN	11,550 (73%)	4214 (27%)	28	1454 (13%)	10,068 (87%)
USE	Name	451 (92%)	40 (8%)	2	58 (13%)	391 (87%)
PAN	CAS RN, name	10,399 (69%)	4640 (31%)	4453	707 (12%)	5388 (88%)
FOODTOX	CAS RN, name	4190 (75%)	1375 (25%)	752	636 (18%)	2802 (82%)

[1] Identifiers that were linked to specific structurally unique compounds using the CDDB. [2] Percentages refer to the entirety of chemical identifiers in the dataset. [3] Identifiers used by the respective dataset that could not be linked to a specific chemical using the CDDB. [4] Synonyms refer to the amount of additional chemical identifiers attributed to chemicals. [5] Percentages refer to the entirety of chemicals in the dataset.

The considered databases vary in the absolute number of chemicals they cover and the proportion of identifiers for chemicals (Table 4). For instance, 35% of WQP identifiers are not categorized as a "chemical", because they refer to mixtures, physical attributes (e.g., temperature and flow velocity), biological parameters (e.g., algal density and toxicity endpoints) or other, non-chemical information. Lower proportions of chemical identifiers may primarily indicate that the respective database is not only focused on PEICs but also on other entities, such as formulations, mixtures, etc. However, lower proportions may also be a result of low-quality data reporting, such as non-adherence to standardized nomenclature.

The characterization of databases further reveals that PEICs with isomeric information constitute 11–18% of chemicals in all analyzed databases (Table 4). Integration of hierarchical structuring is therefore a graphs' valuable feature that not only allows for a more detailed differentiation among chemicals but also enables transparent analyses over multiple levels of specificity. Synonym analysis shows that, for instance, in the ECOTOX database, synonymous relationships are rare ($n = 28$; <0.2%), which underlines the CAS RNs' suitability as identifiers. Nonetheless, while CAS RNs uniquely identify chemicals, there may be multiple CAS RNs (e.g., CAS RN vs. deprecated CAS RN) referring to the same chemical (e.g., cyfluthrin). This may produce spurious analysis results, if unaddressed. With the MAGIC graph, however, analyses are based on chemicals, instead of identifiers, and all data, related to a chemical, are considered equally, regardless of the chemical identifier used.

We also assessed to what extent the MAGIC graph allows linking more chemicals over the different datasets compared to a relational approach where only same-spelling identifiers were considered linkable (Figure 3). We found that linkage increased only marginally (1–2%) when using the graph in the case both merged databases used CAS RN (see Table 4). This increase, although only small, underlines that, even with CAS RN, nomenclature can be an issue for data linkage. Relational joins using same-spelling names were only successful for 0–63% compared to the graph approach (Figure 3), signifying that joins relying on names are substantially affected by differing nomenclatures. In contrast, the graph approach successfully linked 21–99% of entries. With relational joins, it was impossible to link CAS RN from one dataset and chemical names from another, whereas, with the graph approach, we successfully linked 98% of the data from ECOTOX and FOODTOX, a linkage that depends on using CAS RN and names simultaneously (Table 4). While this case may also be partially resolved using relational joins, prior manual harmonization of chemical identifiers would be required, which is time-intensive, yet unnecessary, when using the graph approach. Figure 3 provides further information, e.g., on fractions for individual database pairs or total linkage of chemical data. In large-scale ecotoxicological assessments, transcending national or continental boundaries, harmonization and subsequent linking of data may become unfeasible, while the MAGIC graph approach can provide better performance and coverage compared to traditional relational joins.
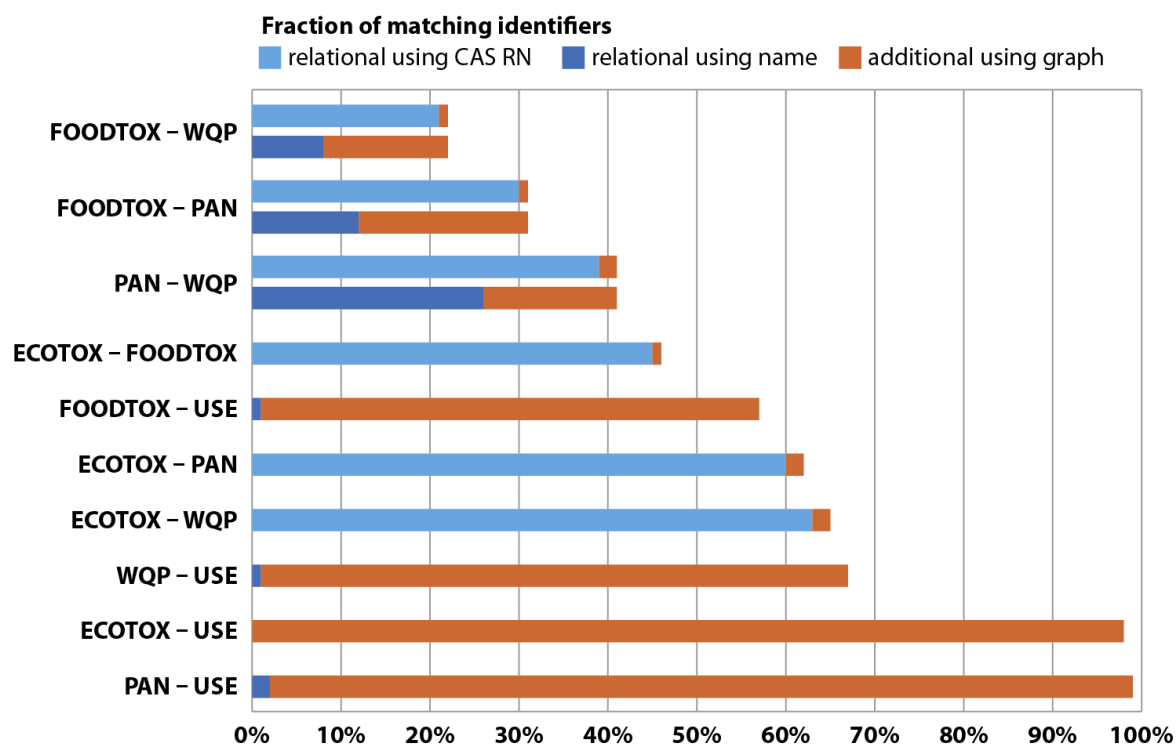
Figure 3. Linkage of chemical data relative to a theoretical maximum for different databases. Complete linkage presumes that all chemicals listed in the smaller database are contained in the larger one. Linkage with a relational approach, where only CAS RN (light blue) or same-spelling chemical identifiers match (dark blue), is compared to the additional gain with the graph approach (orange). See Table 4 for the types of identifiers that were available for each database.

The use types and chemical classes of the PAN database give an example of how data, included in the MAGIC graph, can be used for characterizing datasets: the ECOTOX, FOODTOX and PAN databases cover a broad range of chemicals, including similar proportions of insecticides, herbicides, fungicides and microbiocides (Figure 4). In contrast, the USE dataset shows a higher proportion of insecticides, herbicides and fungicides, and a lower proportion of microbiocides, reflecting its focus on agricultural pesticide applications. Similarly, the WQP contains relatively fewer data of insecticides, herbicides and fungicides, since the number of chemicals being breakdown products (classified as other use type) in this environmental concentration dataset is rather high. The integration of the PAN database use types into the MAGIC graph thus enables an unprecedentedly comprehensive overview of the kind of PEICs that are contained in individual datasets (Figure 4). In addition, the successful data harmonization further demonstrates that ancillary chemical data can be readily incorporated into the MAGIC graph. For instance, supplementing regulatory information (e.g., regulatory status, environmental quality criteria) may now be added with only little effort.
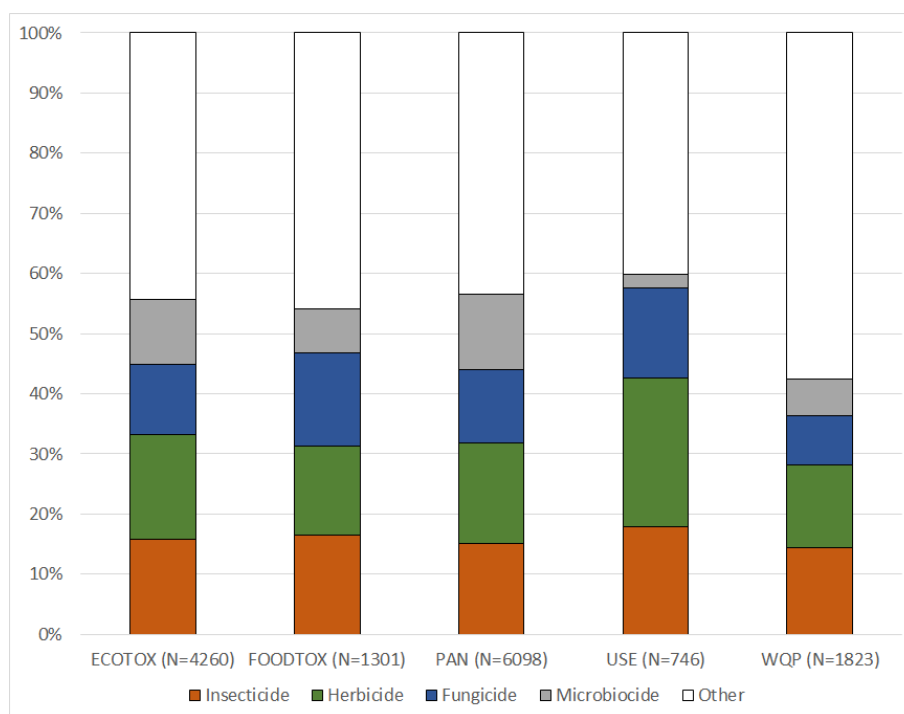
Figure 4. PAN use types of the chemicals in different datasets after linking them with the MAGIC graph. Chemicals may have multiple use types and are then included in several categories.

3. Methods

In contrast to relational database management systems, graph databases do not depend on predefined schemata. Briefly, nodes, relationships, labels, types and properties can be added, modified and removed ad hoc and as needed. While this tremendously facilitates the management of changing and growing heterogeneous datasets, it also complicates the usage of these data. Without a static and technically binding schema, the current semantics have to be discovered dynamically: it has to be found what kinds of nodes there are, what properties they have, how nodes are related to other nodes, etc. These concerns were addressed by specifying features of the data model informally outside the database and included semantics of node labels, relationships between nodes and restrictions of properties. To maintain consistency between this specification and the content of the database, as well as safeguard data integrity, 32 rules, checking specific aspects of the data model, were implemented (Appendix B, Table A1). Rules were iteratively formulated by expert judgment whenever new conceptual or technical requirements arose, while it was generally aimed at maintaining a small set of rules. Violations of the rules result in notifications that have to be resolved manually or semi-automatically (Figure 5). This rule-based approach provides a balanced tradeoff between benefits of an agreed schema and flexibility of a graph database. Turning the a priori schema known from relational databases into a posteriori applied consistency rules also resulted in work-flows that resemble those of test-driven developments [24]. For instance, extension of the domain of the graph database application, e.g., by additionally linking taxonomic data to effect data, is achieved in the two following steps. First, one specifies and implements a set of additional rules, e.g., "species and genus are allowed labels", "an effect must be linked to a species", "a species belongs to a genus", etc., and afterwards modifies the database by adding nodes and relationships until all rules are fulfilled. Fulfillment of all rules then marks a new version of the database application that provides additional information.
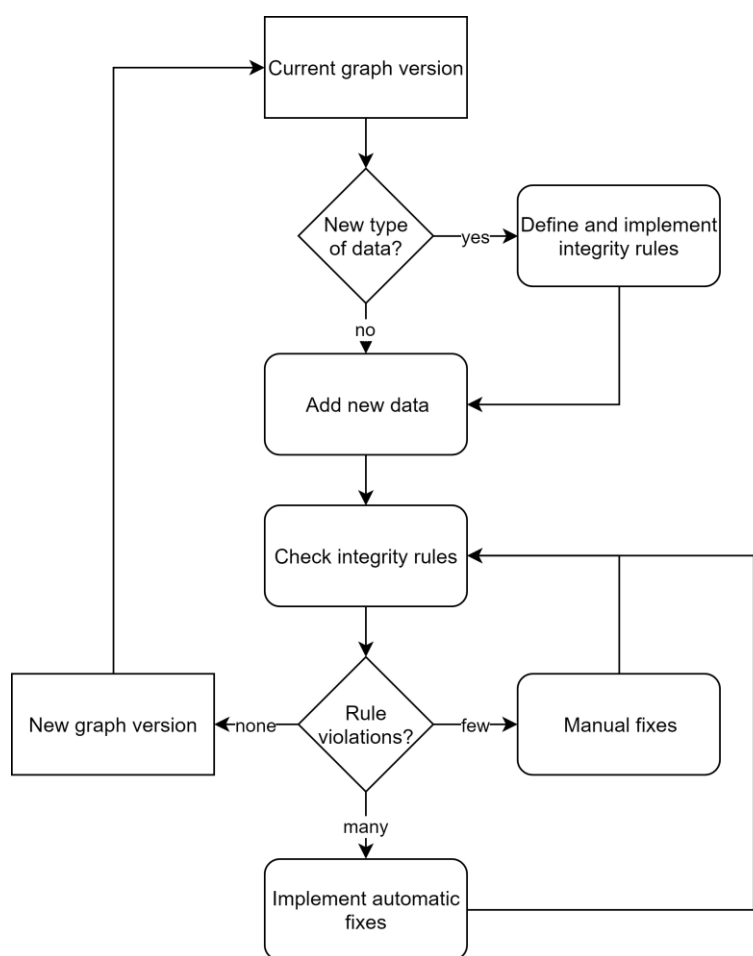
Figure 5. Workflow for adding data to the MAGIC graph while maintaining its integrity.

Initially, six publicly available chemical databases were identified and compared regarding quality of synonyms they provide for an array of organic pesticides (*n* = 655). After extensive quantity and quality assessments of the generated synonym links, the U.S. EPA Chemical Dashboard [11], containing approximately 765,000 chemical entries, was chosen as a synonym provider (see Appendix C).

In the first implementation, synonymous chemical identifiers (e.g., substance names) were interlinked directly (Figure 6a). However, following this concept, the number of steps necessary to collect all synonyms of a given identifier varied between queries, which resulted in complex queries. This concept also complicated the estimation of the quality of synonym relationships, as two distant identifiers could be linked over relationships of different certainty. A later refined representation distinguished between the chemical itself and its identifiers (Figure 6b), leading to a representation where the step sequence for collecting all synonyms of a chemical is well defined and only requires two steps. This adjustment improved the computational efficacy, at the same time allowing to add further chemical identifiers without increasing the maximum number of steps required.
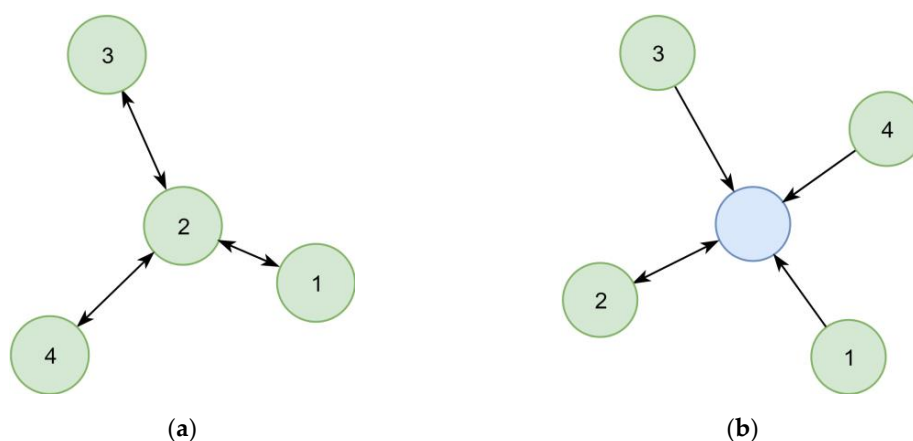
Figure 6. Two representations of four synonym identifiers (1–4) in a graph. (**a**) Links between identifiers indicate known synonym relationships. Synonyms of an identifier are all directly or indirectly connected other identifiers. (**b**) Identifiers point to the identified chemical (blue circle). All identifiers pointing to the same chemical are synonyms. The chemical has a preferred identifier (double arrow).

Consistency between chemical query results was achieved by attributing each chemical a preferred identifier (used by the CDDB), so that chemicals can be identified in a default way. A descriptive property was attributed to relationships between identifiers and chemicals to reflect the identification type, e.g., CAS RN. Data output for chemicals can, thus, be restricted to specific types of relationships for identifying chemicals.

Substances relevant in ecotoxicological contexts can be described by varying detail of specificity (e.g., isomerism), which was addressed by creating hierarchical chemical sub-graphs. For example, permethrin (Figure 7), an insecticidal compound, represents a stereoisomeric mixture of cis- and trans-permethrin isomers. The respective relationships between chemicals were resolved considering the presence of stereo-layers in their standard InChI strings [25], creating a hierarchical sub-graph (Figure 7). Further distinction of hierarchical levels (e.g., enantiomers and diastereomers) currently is not technically possible, as standard InChI strings do not support this operation [25]. However, it is also rarely needed for ecotoxicological assessments using field concentrations.
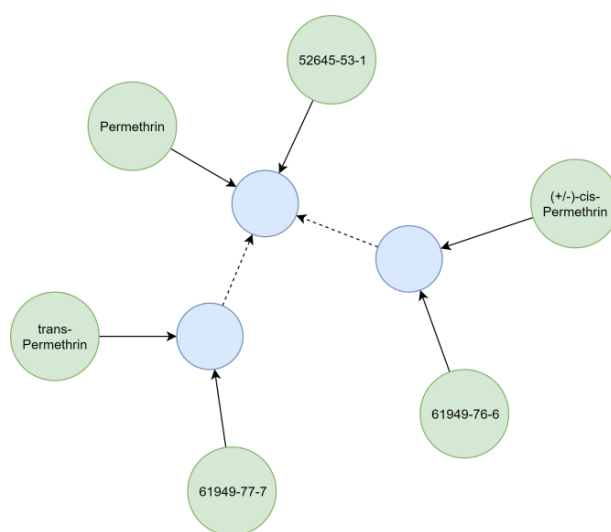

Figure 7. Representation of some synonyms and different levels of specificity for permethrin. Identifiers (green) refer (solid arrows) to chemicals (blue). Chemicals with stereo-information refer (dashed arrow) to a structural identical chemical without stereo-information.

After evaluation of different solutions, the MAGIC graph was hosted using the Neo4j native graph database, a mature, actively developed and widespread graph database product that is available as an Open Source Community Edition (GPLv3 license) and as an extended Enterprise Edition. The MAGIC graph was implemented using versions 3.4.5 to 3.5.0 of the Community Edition (updates were applied as soon as available). Validation rules (Appendix B), as well as tools for automatic rule violation fixes, were implemented using PHP and integrated into an Apache 2 web server. For conducting manual fixes, a set of graphical tools was evaluated (Appendix D). The web server hosts a publicly available website (https://magic.eco) that allows, among other functions, to access the data of the MAGIC graph (see user notes).

Currentness of data is accomplished by synchronization routines, which update the MAGIC graph when some external data sources, especially the synonym provider, change. To detect differences between the graph and external sources, we reapplied the rule-based approach by implementing a set of synchronization rules (Table A2). Violations of these rules indicated differences between databases, prompting a synchronization routine.

4. User Notes

The website https://magic.eco provides access to the most recent version of the MAGIC graph. It offers the possibility to visit individual chemical identifiers and to discover the synonyms and generalizations as well as the data that are currently connected to the chemical. The website also provides a user with an option to download an up-to-date version of the Microsoft® Excel worksheet published with this data descriptor.

Appendix A

A graph consists of vertices that are connected by edges. There is a variety of graph models that define the specifics of how vertices are connected by edges, but we adhere to the conceptually simple, yet powerful, labeled property graph model. A graph consists, according to this model, of vertices named nodes that are interconnected by directed edges named relationships [19]. Relationships always connect two nodes but nodes may have an arbitrary number of out- or ingoing relationships, including relationships to itself or multiple relationships to the same other node. Nodes have zero to many labels that allow differentiating them functionally. Similarly, relationships have types, but the number of types per relationship is limited to one. Nodes and relationships can be enriched by attaching an arbitrary number of properties to them, each containing additional information to the node or relationship. In essence, the labeled property graph model offers a high degree of flexibility while it provides constructs such as labels, types, and properties that help in structuring the data.

Appendix B

A rule-based approach has been chosen to maintain integrity of the MAGIC graph and to keep it synchronized with its synonym provider. This appendix sections lists the rules that have been defined and implemented in the MAGIC graph.

Table A1. Rules defining the MAGIC chemical graph.

ID	"Rule" and Rule Description
G1	"All nodes must have a single label": Ensuring that each node has exactly one label results in a graph that is easier to maintain, as other rules can refer to specific sets of nodes without having to deal with possible labeling overlaps. If the graph gets more complex in the future, it might however become advantageous to allow multiple labels per node.
G2	"Only a set of predefined labels is allowed for nodes": Restricting labels to a predefined set prevents nodes in the graph that are not targeted by rules. The MAGIC graph may currently contain nodes with labels "ChemicalIdentifier", "Chemical" and "Dataset".
G3	"Nodes should have a label with an associated view": Making sure that each label has a defined way that it is represented by the front-end website makes the MAGIC graph completely navigable.
G4	"Only a set of predefined types is allowed for relationships": Predefining types assures that all relationships are addressed by rules. Currently, the relationship types "identifies", "prefers_identifier", "uses_identifier" and "specifies" are allowed in the MAGIC graph.
G5	"Every item in the MAGIC graph must have a name": Naming items provides an endpoint for visiting the item using the website front-end and facilitates modifications and synchronization of the graph by allowing identification of individual nodes. For many types of items, such as chemical identifiers, the name is a natural part of the data.
G6	"Labels should have an associated edit view": Edit views allow smaller modifications of items using the website front-end and the provision of such edit views helps in maintaining the graph database.
G7	"MAGIC graph items should have at least one ingoing relationship": Items, having no ingoing relationship, lack in significance because they are not navigable along the graph relationships and should not be part of the graph. Some items are considered as entry points to the graph (e.g., datasets) and are marked as globally available. The rule does not apply to these items.
CI1	"All chemical identifiers nodes must have a timestamp": Registering the date of item creation assists in synchronization chemical identifiers with external data sources and helps to resolve rule conflicts by indicating which item is more recent.
CI2	"All chemical identifiers that actually identify a chemical should be linked to exactly one chemical": A chemical identifier should identify a chemical, otherwise it is irrelevant for the chemical graph. However, there are two typical occasions when a chemical identifier does not identify a chemical: (1) when a data source, from which data were imported into the graph listed an identifier as a chemical identifier, but further investigation revealed that the identifier did not refer to a chemical in a strict sense (e.g., it identifies a mixture of chemicals); and (2) when a chemical identifier was not found by the synonym provider. Violating this rule gives the user a chance to recognize and mitigate the second occasion, e.g., by adding manual synonym relationships. The user also has a chance to mark a chemical identifier in such a way that it does not trigger this rule anymore (by marking it as a chemical identifier that does not actually identify a chemical), which also signifies that the rule violation was recognized and managed manually.
CI3	"All chemical identification relationships should have a timestamp": Registering the date of relationship creation assists in synchronization with external data sources and helps to resolve rule conflicts by indicating which relationship is more recent.
CI4	"All chemical identifications should have a type": Specifying the mode in which a chemical identifier identifies a chemical helps in estimating the quality and uncertainty of the relationship. It also helps to output specific sets of identifiers, e.g., only CAS RN. The types used for specifying the relationship of identification are currently not restricted but may be a predefined set in the future.
CI5	"All chemical identifiers should be used by at least one dataset": The data sources where a chemical identifier is used should be given. If this is not the case, retracing the origin of chemical identifiers is not possible which decreases the overall quality of the MAGIC graph.
CI6	"All chemical identifiers should have an identifier from a predefined list showing what is actually identified": Other rules depend on the information that a chemical identifier actually identifies a chemical, that is, there applies a stricter meaning of chemical than in some other databases. To provide this information, a chemical identifier should describe what it actually identifies. Currently, the following possibilities are considered here: chemical, mixture, unmatched chemical (by no means a corresponding chemical could be identified), unspecific (is not specific enough to identify exactly one chemical) and ignored (for any reason).

Table A1. *Cont.*

ID	"Rule" and Rule Description
CI7	"A chemical identifier, that does not actually identify a chemical, should not be linked with a chemical": Specifying that a chemical identifier identifies a chemical only does make sense in case the chemical identifier is marked as actually identifying a chemical. If this is not the case, but an identifying relationship exists nonetheless, a manual examination of the case is advised.
C1	"All chemicals must have a timestamp": Registering the date of item creation assists in resolving conflicts involving chemicals by indicating which item is more recent.
C2	"All chemicals should have exactly one preferred name": According to the MAGIC graph data model, a chemical is considered having many names. Making sure that every chemical has exactly one designated preferred name still allows it to be referred to in outputs in a harmonized way.
C3	"All chemicals should be identified by at least one chemical identifier": Chemicals that have no identifier cannot be related to actual chemicals and should be removed from the graph.
C4	"All identifier preferences should have a timestamp": Name preferences of chemicals, especially when taken from external sources, may change over time. In these occasions, timestamps help to identify the more recent preference.
C5	"All chemicals should indicate whether they have bond stereo-chemical information": To understand which level of specificity regarding stereo-chemistry a chemical has, presence or absence of stereo-information at double bonds should be indicated.
C6	"All chemicals should indicate whether they have tetrahedral stereo-chemical information": To understand which level of specificity regarding stereo-chemistry a chemical has, presence or absence of stereo-information at tetrahedral stereo centers should be indicated.
C7	"Chemicals with stereo-information should specify other chemicals or indicate to not do so": The purpose of considering stereo-information is to distinguish two levels of specificity regarding stereo-chemistry: absence and presence of stereo-information. In the case of stereo-information presence, a chemical should specify a chemical without stereo-information but with the same chemical structure, so representing the two levels of specificity in the graph. For some chemicals, it is not reasonable to find a chemical with the same structure but without stereo-information. In this case, the more specific chemical should be marked such that this rule can be ignored.
C8	"Chemicals may not specify themselves": Violations of this rule may occur when stereo-information in external data sources changes.
D1	"All datasets must have a timestamp": Registering the date of item creation assists in resolving conflicts in datasets by indicating which item is more recent.
D2	"All datasets must have a title": A title provides a more extensive way for a short description of the dataset but is not, unlike its name, used as an identifier.
D3	"All datasets must have a description": A description is an even more extensive opportunity to characterize a dataset by text.
D4	"All datasets should haven an indicator of whether they are published": Distinction between published and non-published datasets allows to decide which datasets are accessible by the website front-end.
D5	"All datasets should be published": At least at later stages, after inserting a dataset into the graph and fixing possible rule violations, the dataset should be published to make its data available.
D6	"All datasets must have at least one author": Assigning authors to a dataset is an attribution to the persons who were responsible for inserting the dataset into the graph.
UTI1	"All Use Type Identifiers should be used by at least one dataset": Use type identifiers originate from datasets and attributions to these datasets should be given.
UTI2	"All Use Type Identifiers should be used by at least one chemical identifier": All use type identifiers should be linked to at least one chemical identifier, otherwise they are of limited use for assessments.
CCI1	"All Chem Class Identifiers should be used by at least one dataset": Chemical class identifiers originate from datasets and an attribution to this dataset should be given.
CCI2	"All Chem Class Identifiers should be used by at least one chemical identifier": All chemical class identifiers should be linked to at least one chemical identifier, otherwise they are of limited use for assessments.

Table A2. Synchronization rules.

ID	Rule and Description
SyncCDDB1	"All identifiers in the MAGIC graph that actually identify a chemical should have exactly one match in the CDDB": Identifiers that have no match in the CDDB have been removed from there and should also be removed from the MAGIC graph. In some (rare) cases, chemical identifiers have two or more matches in the CDDB. These cases should be resolved manually, e.g., by ignoring the chemical identifier.
SyncCDDB2	"All identifiers of a specific chemical in the MAGIC graph should have the same preferred name and DTXSID in the CDDB": Having different preferred names among the synonym identifiers of a chemical is a strong indicator that synonym relationships in the CDDB have changed. This should result in an update of synonym relationships in the MAGIC graph as well.
SyncCDDB3	"The preferred name of a chemical in the MAGIC graph should be the same as the preferred name in the CDDB": Preferred names of the CDDB may change. Making sure we use the same preferred name in the MAGIC graph as in the CDDB circumvents the need to establish a custom scheme for preferred names.
SyncCDDB4	"The stereo-information of a chemical in the MAGIC graph should be the same as the stereo-information of that chemical in the CDDB": This rule captures changes in the chemical structure stored in the CDDB. Again, these changes should be synchronized with the MAGIC graph to reflect the most recent specifying relationships.
SyncCDDB5	"The identifier type of the relationship between a chemical identifier and a chemical in the MAGIC graph should be the same as in the CDDB": Synchronizing the identifier type between CDDB and MAGIC graph eliminates the necessity to manage a custom set of identifier types while still allowing to use the benefits of typed identifiers.
SyncCDDB6	"All identifiers in the MAGIC graph that do not actually identify a chemical should have no match in the CDDB": On some occasions, new identifiers become recognized by the CDDB. This rule captures those instances where the newly recognized identifiers match identifiers in the MAGIC that previously have been marked as not actually identifying chemicals.

Appendix C

In total, 655 substance names—categorized as organic contaminants—were obtained from the Water Quality Portal (https://www.waterqualitydata.us/) and used for benchmarking six databases. First, successful synonym attribution was compared quantitatively (Table A3) between databases, and then the quality of synonym relationships was manually assessed by validating correctness of generated links using assigned standard InChI-Keys.

Table A3. Comparison of chemical synonym providers regarding automated attribution of InChI-Keys for 655 organic contaminants.

Database	Coverage (%)	Remarks
U.S. EPA Chemical Dashboard [1]	586 (89.5%)	correct links
PubChem [2]	645 (98.5%)	ambiguous response, incorrect links
PUG REST [3]	604 (92.2%)	ambiguous response, incorrect links
SRS [4]	601 (91.8%)	rarely incorrect links
ChemSpider [5]	346 (52.8%)	low coverage
Chemical Translation Service [6]	613 (93.6%)	ambiguous response, incorrect links

[1] https://comptox.epa.gov/dashboard/. [2] https://pubchem.ncbi.nlm.nih.gov/. [3] https://pubchemdocs.ncbi.nlm.nih.gov/pug-rest. [4] http://www.exchangenetwork.net/data-exchange/srs/. [5] http://www.chemspider.com/. [6] http://cts.fiehnlab.ucdavis.edu/.

Attribution of synonyms and InChI-Keys was high for all six databases with the exception of one (ChemSpider), which was removed from subsequent analyses due to its comparatively low coverage (Table A3). Following this, manual validation of assigned InChI-Keys revealed that attribution was frequently incorrect or query responses were ambiguous, except for the U.S. EPA Chemical Dashboard (CDDB). Although multiple factors leading to misattribution of InChI-Keys or synonyms were identified, automatic aggregation of synonym lists from online-sources lacking expert curation was found the most prevalent factor that adversely affected attribution quality. The CDDB, that, unlike

the other considered databases, curates records and gives quality indicators, was thus identified as the most reliable source for synonyms and corresponding InChI-Keys (see below for sample data retrieved from the CDDB). Synonym quality provided by the CDDB was further assessed to sustain the robustness of the method. Although automated linking of Chemical Identifiers to chemicals was high (89.5%, *n* = 586) when using the CDDB (Table A3), 69 WQP entries could not be automatically assigned to Chemical Identifiers. Missing entries were manually assigned CAS RN and InChI-Keys via cross-validation using, among other databases, PubChem, PAN and PPDB. Attributed CAS RN and InChI-Keys were then used to link missing entries with the corresponding CDDB entry, which was successful in 75.4% (*n* = 52) of remaining cases. No manual links could be established in 24.6% (*n* = 17) of cases, because no corresponding entry was found in the CDDB. Failure to automatically establish synonym relationships was mostly due to chemical names in WQP being abbreviated, using wrong or uncommon identifiers, or referring to entities that are not chemicals in a strict sense (i.e., mixtures). Most importantly, a manual check revealed that no false synonym relationships (i.e., incorrect links) were generated automatically using the CDDB as synonym provider, which was manually checked. Thus, overall correctness and reliability of generated links were without any noticeable concern.

Data were retrieved from the EPA Chemistry Dashboard (https://comptox.epa.gov/dashboard) using its batch search. The following is an excerpt of data retrieved:

1. Input: Lindane, Found by: Approved Name, DTXSID: DTXSID2020686, Preferred name: Lindane, InChI key: JLYXXMFPNIAWKQ-GNIYUCBRSA-N, InChI string: InChI=1/C6H6Cl6/c7-1-2(8)4(10)6(12)5(11)3(1)9/h1-6H/t1-,2-,3-,4+,5+,6+

2. Input: cis-Permethrin, Found by: Expert Validated Synonym, DTXSID: DTXSID0038338, Preferred name: (+/−)-cis-Permethrin, InChI key: RLLPVAHGXHCWKJ-HKUYNNGSSA-N, InChI string: InChI=1/C21H20Cl2O3/c1-21(2)17(12-18(22)23)19(21)20(24)25-13-14-7-6-10-16(11-14)26-15-8-4-3-5-9-15/h3-12,17,19H,13H2,1-2H3/t17-,19-/s2

3. Input: lambda-Cyhalothrin, Found by: Synonym from Valid Source, DTXSID: DTXSID7032559, Preferred name: λ-Cyhalothrin, InChI key: ZXQYGBMAQZUVMI-GCMPRSNUSA-N, InChI string: InChI=1/C23H19ClF3NO3/c1-22(2)17(12-19(24)23(25,26)27)20(22)21(29)31-18(13-28)14-7-6-10-16(11-14)30-15-8-4-3-5-9-15/h3-12,17-18,20H,1-2H3/b19-12-/t17-,18+,20-/s2

These data are sufficient: (1) to identify synonymous identifiers (Input, DTXSID, Preferred name, (InChI key, InChI string)); (2) to evaluate the quality of the synonym relationship (Found by); (3) to assess the presence of stereo-chemical information in a chemical and compare chemicals with the same structure (InChI string, InChI key); and (4) to assign a common preferred name to a chemical (Preferred name).

Appendix D

Various graphical user interfaces have been tested to identify a suitable tool for minor interactive modifications of the MAGIC graph, preferably without coding Cypher queries (Table A4).

Table A4. Comparison of graphical user interfaces for Neo4j databases.

Name	Version	License	Graph Edit	Last Update	Remarks
Bloom		commercial	+	recently	https://neo4j.com/bloom/
Cytoscape	3.7.0	GNU	−	10/2018	Does not support Neo4j natively, but possibly via (outdated) plug-in [1]; http://www.cytoscape.org/
Gephi	0.9.2	commercial (free edition)	−	9/2017	Does not support Neo4j natively, but possibly via plug-in[2]; https://gephi.org/
Graphexp		Apache	+	10/2018	Does not support Neo4j natively; https://github.com/bricaud/graphexp

Table A4. *Cont.*

Name	Version	License	Graph Edit	Last Update	Remarks
Graphileon	2.0.0-beta	GNU	+	8/2018	Graphs with datetime properties (introduced in Neo4j v3.4) cannot be visualized or edited; https://graphileon.com/graphileon-personal-edition/
Keylines	5.0	commercial	−	11/2018	https://cambridge-intelligence.com/keylines
Linkurious	2.5.4	commercial	+	7/2018	Offers trial version, without price information; https://linkurio.us/solution/neo4j/
Neo4j Browser	3.2.5	GNU	−	11/2018	Shipped with Neo4j database; https://neo4j.com/developer/guide-neo4j-browser/
Neo4j Browser (forked)	3.2.7	GNU	+	11/2018	Extends Neo4j Browser by editing functionality; https://github.com/phdd/neo4j-browser
Neo4js	2	open source	+	5/2018	https://github.com/adadgio/neo4j-js-ng2
Neoclipse	1.9.5	open source	+	9/2014	Does not support current Neo4j version; https://github.com/neo4j-contrib/neoclipse
Structr	3.0.3	commercial	+	9/2018	https://structr.com/
Tom Sawyer	8.2.2	commercial	−	11/2018	https://www.tomsawyer.com/graph-database-browser/

[1.] https://apps.cytoscape.org/apps/cyneo4j. [2] https://tbgraph.wordpress.com/2017/04/01/neo4j-to-gephi.

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PESTICIDES

Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops

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Pesticide impacts are usually discussed in the context of applied amounts while disregarding the large but environmentally relevant variations in substance-specific toxicity. Here, we systemically interpret changes in the use of 381 pesticides over 25 years by considering 1591 substance-specific acute toxicity threshold values for eight nontarget species groups. We find that the toxicity of applied insecticides to aquatic invertebrates and pollinators has increased considerably—in sharp contrast to the applied amount—and that this increase has been driven by highly toxic pyrethroids and neonicotinoids, respectively. We also report increasing applied toxicity to aquatic invertebrates and pollinators in genetically modified (GM) corn and to terrestrial plants in herbicide-tolerant soybeans since approximately 2010. Our results challenge the claims of a decrease in the environmental impacts of pesticide use.

R ecent debates about the potential side effects of pesticides on humans (1) and the environment (2, 3) have been dominated by the comparison of use rates (e.g., kilograms per hectare) or applied amounts (e.g., kilograms per year) (4–9). These weight-based measures are not necessarily informative from an environmental perspective because toxicity among pesticides varies over several orders of magnitude (tables S1 to S3). This suggests that environmental effects strongly depend on the shares of individual pesticides in the total applied amount (1).

In this study, we extended a weight-based assessment of 381 pesticides for the years 1992 to 2016 (figs. S1 to S3) by 1591 regulatory threshold levels (RTLs) [as officially derived thresholds indicative of potential biodiversity impacts (3)] for eight different groups of nontarget species (10). We multiplied the annually applied amount (i.e., mass) of individual pesticides [data from the US Geological Survey (USGS)] with the reciprocal of the pesticide- and species group–specific RTLs (10) [data mainly from the US Environmental Protection Agency (EPA), see tables S1 and S2; species groups were unequally represented, see table S5] to derive the total applied toxicity (TAT) per substance, species group, and year (for TAT sensitivity, see fig. S4). The TAT is predictive of the potential pesticide impact (fig. S5). The annual TAT values were aggregated over different sets of substances (e.g., pesticide use types, chemical classes, and modes of action) to derive relative measures of temporal trends in agriculture both overall and for genetically modified (GM)–dominated crops specifically.

A comparison of the applied pesticide amount and the TAT reveals different temporal phases

for the different species groups. Regarding vertebrate toxicity (Fig. 1A), great reductions in acute toxicity have been achieved over the past few decades, driven almost entirely by insecticides (fig. S6A), whose TATs decreased by approximately a factor of 9 for mammals (Fig. 1C) and birds (Fig. 1D) through the replacement of organophosphorus and carbamate insecticides by pyrethroids and neonicotinoids (figs. S2 and S7, A and B). This development, which coincided with a proportional decrease in the applied amount (Fig. 1A, phase a) and an increase in corn acreage (fig. S10A), occurred in response to the high toxicity found in vertebrates (9). The fish TAT (TAT_{fish}) (Fig. 1B) remained constant overall since 2004 because of pyrethroid toxicity, which is relevant for this group (fig. S7C).

In sharp contrast, the invertebrate TAT has markedly increased since approximately 2005 (Fig. 1E, phase c). Both aquatic invertebrate TAT ($TAT_{aqua-inverts}$) and $TAT_{pollinators}$ more than doubled, with an increase of ~8% per year between 2005 and 2015 (Fig. 1, F and G; fig. S6B; and fig. S8, A and C), whereas the terrestrial arthropod TAT ($TAT_{terr-arthropods}$) (referring here to nonpollinating species) increased less (Fig. 1H and figs. S6B and S8B; note, there is lower data availability for terrestrial arthropods, table S5). The TAT was driven solely by insecticides in all invertebrate groups (fig. S6B), coinciding with a proportional increase in cultivated area in relevant crops (figs. S10, B and C, and S11). Simultaneously, the applied insecticide amount decreased by ~40% (fig. S1B).

Although pollinators and aquatic invertebrates show similar temporal patterns regarding the applied amount of pesticides and TAT (Fig. 1E, phases b and c), the toxicities are driven by distinct classes of insecticides. For pollinators—e.g., bees or bumble bees—neonicotinoids are increasingly responsible for the TAT (Fig. 1G and fig. S8A). Neonicotinoids have been documented as being highly

toxic to bees (11, 12), and some of them have therefore been banned in the European Union (EU). Although seed treatments constitute >80% of all neonicotinoid use in the US (4) and restrictions on postbloom applications in perennial (tree) crops receiving spray applications have reduced pollinator risks (13), neonicotinoid use remains problematic (12), for example because of oral-based bee toxic load, which has increased particularly in heartland corn and soybeans (13). For aquatic invertebrates (e.g., crustaceans, mayflies, caddisflies, and dragonflies), pyrethroid insecticides have dominated the TAT since 1992, and they have also become increasingly relevant for terrestrial arthropods (nonpollinators such as mites, flies, and beetles; Fig. 1, F and H, and fig. S8, B and C). Figure 1E highlights a regime shift in the evolution of pesticide use in the mid-2000s (phase bc), when a phase of prevailing structural change in insecticide use (b) turned into a phase of TAT growth that is completely decoupled from trends in the total applied amount (c). Increases in applied pyrethroid toxicity have previously been implied only for fish (14). In the case of $TAT_{aqua-inverts}$, just four pyrethroids explained >80% of the increase since 2006. Because the detection limits of these four compounds in water are more than two orders of magnitude higher than their respective RTLs (table S3), it appears virtually impossible to track them at the entire range of ecologically relevant concentrations through scientific monitoring efforts (3, 15, 16). The highly effective, low–use rate insecticides (tables S3 and S4), often associated with an environmentally benign character (6, 9), increase in toxicity and use (fig. S12) and have the potential to be a considerable but widely unrecognized threat to both terrestrial and aquatic invertebrates (2, 3, 16).

The TAT for nontarget plants, which has been driven solely by herbicide use, showed an upward trend since approximately 2006 (Fig. 1I, phases a' and f, and figs. S6C and S9), likely related to resistance in crops (17). Although no single mode of action dominates plant toxicity, growth regulators (e.g., acetochlor) contribute mainly to the terrestrial and aquatic plant TAT, and amino acid synthesis inhibitors, such as the increasingly used glyphosate (fig. S3B) and cell membrane disruptors (e.g., oxyfluorfen), contribute to the $TAT_{terr-plants}$ (Fig. 1, J and K, and fig. S9). The increases in plant TAT may have major impacts on terrestrial food webs, for example through reduced plant seed production (18) or plant species decline (19), requiring a systemic evaluation of previously unrecognized aspects of pesticide use.

Toxicity-weighted use is the strongest predictor of the potential impact of a pesticide on the environment (20). Its application in the

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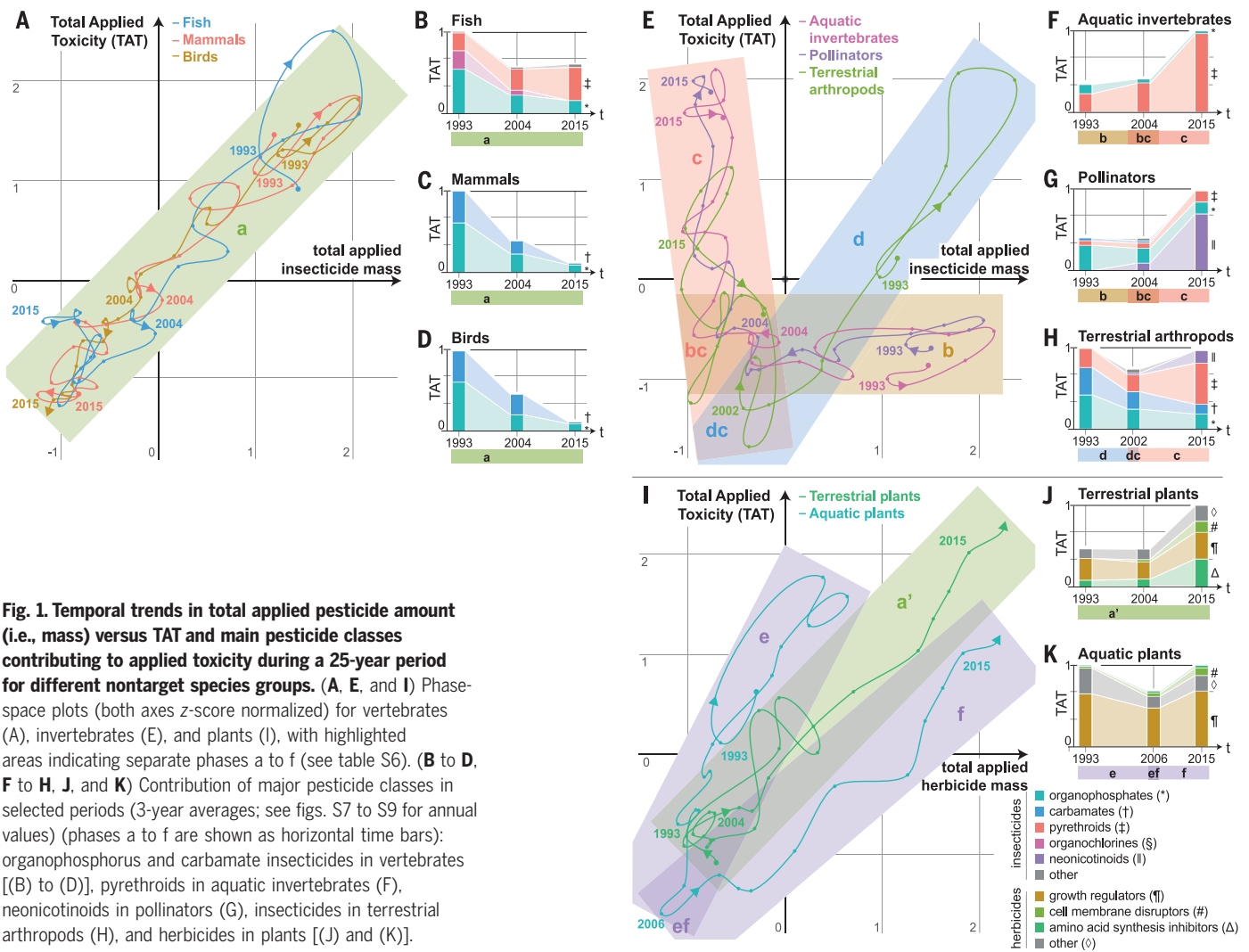*Corresponding author. Email: schulz@uni-landau.de

Fig. 1. Temporal trends in total applied pesticide amount (i.e., mass) versus TAT and main pesticide classes contributing to applied toxicity during a 25-year period for different nontarget species groups. (**A**, **E**, and **I**) Phase-space plots (both axes z-score normalized) for vertebrates (A), invertebrates (E), and plants (I), with highlighted areas indicating separate phases a to f (see table S6). (**B** to **D**, **F** to **H**, **J**, and **K**) Contribution of major pesticide classes in selected periods (3-year averages; see figs. S7 to S9 for annual values) (phases a to f are shown as horizontal time bars): organophosphorus and carbamate insecticides in vertebrates [(B) to (D)], pyrethroids in aquatic invertebrates (F), neonicotinoids in pollinators (G), insecticides in terrestrial arthropods (H), and herbicides in plants [(J) and (K)].

present study relies on the assumption that pesticide use and its effects on organisms are robustly connected to each other at large scales, even though there is tremendous variability in substance properties (table S4), application patterns, and local exposure situations. This assumption is, however, supported by multiple lines of evidence, even in the crucial case of pyrethroid risk to aquatic invertebrates. Monitoring data from a total of 89 available peer-reviewed studies [1977 insecticide concentrations from 231 different surface waters across the US (20, 21)] show that the rate at which measured insecticide and pyrethroid concentrations exceed the RTL$_{aqua\text{-}inverts}$ is significantly correlated with the applied toxicity to aquatic invertebrates (fig. S5). RTL exceedance in surface waters is indicative of negative effects on aquatic biodiversity (3), and pyrethroids exhibit the highest RTL exceedance rates (3, 21). Pyrethroids show adverse effects in midwestern streams (15), occur regularly in stream biofilms (22), and even cause resistance in nontarget freshwater am-

phipods (23). Taken together, multiple lines of evidence provide a clear link between the use of, exposure to, and effects of pyrethroid insecticides in aquatic systems. This link likely also applies to other pesticide and species groups, although further investigation in this field is needed.

From a broader perspective, decreases in vertebrate TAT were achieved at the cost of increased invertebrate TAT (Fig. 1, A and E). Additionally, ecologically linked pollinators and terrestrial plants (11, 24) are among those with the largest TAT increases (Fig. 1, E and I). The cumulative direct impact of modern insecticides on invertebrates and the indirect impact of herbicides on invertebrates through the food chain thus likely contribute to the currently debated decline in arthropods (2, 3, 5, 25–27). This decline may ultimately lead to indirect effects on vertebrate predators (2).

The TAT increased even in GM crops (Fig. 2, B, D, and F). Herbicide use has undergone substantial changes with the implementation of herbicide-tolerant GM crops (Fig. 2A),

which has led to a strong increase in the use of glyphosate (8, 28) (Fig. 2A and fig. S3B). TAT$_{terr\text{-}plants}$ has increased steadily since approximately 2008 for herbicides in herbicide-tolerant soybeans (Fig. 2B and fig. S14, A and B), likely in response to glyphosate resistance (17). However, downward trends have been reported for GM soybean herbicide toxicity to humans (1).

In the most widely grown GM crop that produces a *Bacillus thuringiensis* (*Bt*) toxin, corn, the insecticide TAT increased. Considering only data for corn, of which 79% in 2016 was *Bt* hybrids (Fig. 2C), TAT increased for both aquatic invertebrates (mainly because of pyrethroids; Fig. 2D) and terrestrial pollinators (mainly because of neonicotinoids; Fig. 2E) at the same rate observed for US agriculture as a whole (fig. S8, A and C). We verified that the toxicity per hectare of insecticides applied to *Bt* corn is equal to that for non-*Bt* corn (Fig. 2D, fig. S13, and fig. S14, C to F). The increasing insecticide TAT may be a result of preemptive, possibly unnecessary applications (4) or resistance (17). Our analysis suggests that

Fig. 2. Annual applied amount (i.e., mass) and nontarget species toxicity of the main classes of pesticides used in two of the most widely grown GM crops—soybeans and corn—in the US between 1992 and 2016.
(**A**) Amount of herbicides applied in soybeans (Mann-Kendall tests for monotonic trend: $\tau = 0.873$, $P < 0.001$). (**B**) $TAT_{terr\text{-}plants}$ of 78 herbicides applied in soybeans ($\tau = 0.387$, $P = 0.129$). (**C**) Amount of insecticides applied in corn ($\tau = -0.553$, $P = 0.019$). (**D**) $TAT_{aqua\text{-}inverts}$ of 72 insecticides applied in corn ($\tau = 0.5$, $P = 0.017$). (**E**) $TAT_{pollinators}$ of 63 insecticides applied in corn ($\tau = 0.653$, $P = 0.009$). Lines in (A) and (C) were fitted using generalized additive models.
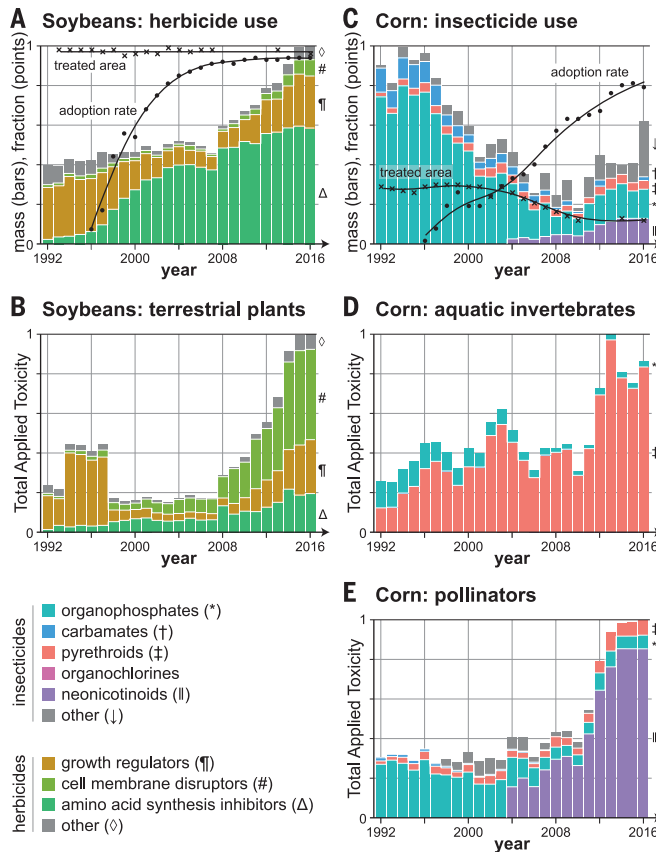
claims of reduced chemical insecticide use in US *Bt* crops (*8, 9, 28*) simply reflect the considerably lower application rates required for more recently developed, more toxic insecticide classes, whereas the $TAT_{pollinators}$ and $TAT_{aqua\text{-}inverts}$ both continue to increase.

TAT values may increase even more in global agriculture than they do in the US as important TAT drivers increase simultaneously. Such global developments include increased pesticide sales in Asia, Latin America, and Europe (*9*); expanded global cropland area (www.fao.org/faostat); and increased global pesticide use (*29*). Widespread resistance development (*17*), increasing field size (*7*), decreasing crop diversification (*7*), international market connectedness (*30*), and increasing temperatures (*5*) are key drivers of these developments. Although insecticide effects have been repeatedly documented (*2, 15, 22*), invertebrate biodiversity trends are generally debated (*25, 26*), and large-scale studies often do not focus on pesticides (*25, 26, 31, 32*). The unavailability of open-access pesticide use data in many regions, such as Latin America, the EU, China, and Russia, also prevents analyses such as those presented here, which potentially masks a crucial driver of the global biodiversity decline.

Despite being based on high-quality, curated data, our analysis carries some uncertainties.

The link between pesticide use and potential ecological impacts is formed by complex processes at different scales, and the methods applied here can only be indicative of the role pesticide use plays in the degradation of ecosystems, even at large scales. In light of the multiple emergent risks and resistance problems (*6, 17*), pesticide risks should be more integrated into policy strategies (*33*) to develop resilient global production systems (*30, 31*). Advancements in precision agriculture, mixed and organic farming, and nano-scale delivery platforms provide examples of how agriculture can develop productively while reducing environmental impacts (*34, 35*). Because 61% of US drinking water originates from surface waters, according to the EPA, TAT-based pesticide evaluations may also benefit human health evaluations. Overall, a system-centric view is conducive to understanding the dynamics of pesticide use on medium to large scales (*36*), and this is even more important given that ecosystem-wide pesticide impacts are becoming increasingly evident (*2, 11, 12, 16*).

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and have been supplemented for some species groups with data from www.efsa.europa.eu/en/data/chemical-hazards-data. The RTLs, the data for additional analysis regarding GM corn, and the code for this analysis can be found at https://static.magic.eco/TAT and are archived on Zenodo (37).

Science

Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops

Ralf Schulz, Sascha Bub, Lara L. Petschick, Sebastian Stehle and Jakob Wolfram

A shifting burden

In 1962, Rachel Carson's *Silent Spring* was published, and the world was forced to take notice of the unintended impacts of pesticides on wildlife. Since then, there has been a perceived reduction in the amount of pesticides used and a shift in the kinds of pesticides available. Schulz *et al.* looked at the type, amount, and toxicity of pesticides applied over the last 25 years. They found that despite decreasing total amounts applied and decreased impacts on vertebrates, toxicity––in particular to insects and aquatic invertebrates––has increased substantially.

Science, this issue p. 81

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Trends of Total Applied Pesticide Toxicity in German Agriculture

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ABSTRACT: Expressing temporal changes in the use of pesticides, based not only on amounts (masses) but also on their toxicity for different species groups, was proposed as a sensible approach for evaluating potential environmental risks. Here, we calculated the total applied toxicity (TAT) between 1995 and 2019 for Germany, mapped it, and compared it to the US TAT and other risk indicators. Results show that the German TAT for terrestrial vertebrates decreased over time by about 20%. The TAT increased by a factor of three for fishes, largely due to insecticides, by a factor of two for soil organisms, largely due to fungicides and insecticides, and, to a lower extent, for terrestrial plants, solely due to herbicides. Other species groups showed no trends in TAT, which for pollinators likely results from neonicotinoid use restrictions. Many TAT trends from Germany and the US differ, partly due to different insecticide and fungicide uses. TAT, SYNOPS risk indicators, and the EU Harmonized Risk Indicators, currently being used to assess the German National Action Plan's goal to reduce risks by 30% by 2023, lead to clearly different risk perceptions. Validated approaches are needed for evaluation of risk quantifications at the national scale.

KEYWORDS: EU, Farm to Fork Germany, NAP, pesticides, risk analysis, SYNOPS risk indicators, USA
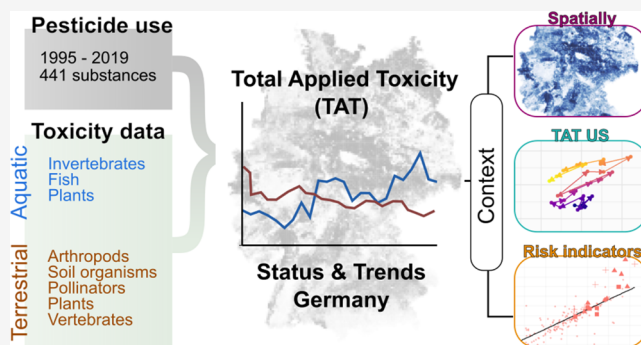
INTRODUCTION

Approximately 30 000 tons of pesticides are applied in German agriculture yearly (2019).[1] Herbicides contribute the most to these applications (59% of mass), followed by fungicides (38%) and insecticides (3%). Potential ecological side effects of this pesticide use for aquatic and terrestrial biodiversity in Germany[2−5] and the European Union[6−9] have been critically debated. Previously, trends in the ecological impact of pesticide use on human health and the environment were discussed mostly by use rates and applied masses.[10−12] However, trends based on these mass-based quantities ignore the important impact of the changing toxicity of the applied substance spectrum.[13,14]

Recently, total applied toxicity (TAT) has been used as an environmental risk indicator for pesticide use in US agriculture.[14] TAT integrates the total amount of applied pesticides, weighted by their individual toxicities. It thereby avoids the shortcomings of solely mass-based evaluations and has been shown indicative of potential biodiversity effects on aquatic organisms.[14] Similar approaches had already been applied to specific pesticide or species groups;[13,15−20] however, so far, TAT provides the most comprehensive large-scale approach for the identification of long-term temporal trends of potential pesticide risks for different nontarget species groups. Calculation of TAT is straightforward but depends on the availability of two data sets: high-quality ecotoxicity data (endpoints) for a large range of pesticides and species, and use

data for these pesticides over a sufficiently long time period. The European Food Safety Authority's (EFSA) OpenFoodTox database[21] provides ecotoxicity data used during the registration of pesticides in the European Union. Here, substance-specific toxicity thresholds were derived by applying the following principles of the official EFSA regulatory risk assessment:[8,22] quality criteria for the consideration of individual ecotoxicity endpoints, application of assessment factors, and usage of both acute and chronic endpoints. With regard to pesticide use, only censored data (broad mass classes per substance and year) had long been published for Germany, but recently, more detailed data for 1995−2019 have been made available by the German Federal Office of Consumer Protection and Food Safety (BVL).[1]

Here, we calculate TAT trends for three aquatic (invertebrates, fish, and plants) and five terrestrial (arthropods, pollinators, vertebrates, soil organisms, and plants) species groups. This study extends the species groups of the US study[14] by calculating TAT for soil organisms, which, on the one hand, are particularly important for ecosystem services

(e.g., nutrient cycling or carbon storage), soil health, and agricultural sustainability,[23] and, on the other hand, are exposed to risks from pesticide use.[19,24] Compared to aquatic and terrestrial off-field ecosystems, in-field soil ecosystems are more directly exposed to applied pesticides.[25,26] We also present maps of the spatial TAT distribution for the eight species groups in Germany based on the available use data, the information on the use of individual pesticides in different crops,[27] and a high-resolution crop map for Germany.[28]

Agriculture in Germany and the US differs substantially in extent, crop composition, field sizes, farming practices, and landscape context.[12,29] These factors, plus different pest pressures and different regulatory contexts, lead to a dissimilar pesticide use in both countries. In US agriculture, fungicides contribute to the applied pesticide amount by only 12% (compared to 38% in Germany), and insecticides by 7% (with only 3% in Germany).[14] The comparison of TAT trends in both countries, as conducted here, shows how the trends for various species groups differ between the countries and helps to understand the large-scale impact of pesticide use globally.[14] Reduction of pesticide risks is a declared goal of the European Union, and in 2009, the European Commission issued a directive on the sustainable use of pesticides (2009/128/EC). From each member state, this directive requests to set up a National Action Plan (NAP) to "reduce risks and impacts of pesticide use on human health and the environment". The Federal Government of Germany enacted its NAP in 2013 with the major goal to reduce the environmental risk potentials for aquatic and terrestrial nontarget organisms by 2023 by 30%, compared to the mean risk potentials of the period from 1996 to 2005.[30] Recently, the EU Farm to Fork Strategy has even promised a reduction of both use and risks of chemical pesticides by 50% by 2030.[31] The NAP initially stipulated the use of a complex of risk indicators called SYNOPS (Synoptische Bewertung von Pflanzenschutzmitteln; synoptic assessment of plant protection products) to assess these target quotas.[27,30,32] SYNOPS compares modeled exposure estimates with effect endpoints to calculate risk estimates. With the EU directive 2019/782, the use of common Harmonized Risk Indicators (HRI) was stipulated for all member states. HRI are calculated as the sum of used amounts per substance multiplied by substance-specific weighting factors that are defined by hazard groups (group 1: low risk; group 2: approved; group 3: candidates for substitution; group 4: not approved), regardless of substance-specific toxicities.

In the present study, we calculate TAT trends for Germany, compare them to mass trends, identify the main pesticides contributing to these trends, and map the TAT for different nontarget species groups. We then compare TAT trends between 1995 and 2019 with existing data from the US[14] to identify differences in pesticide risk trends between the two countries. Also, we compare the TAT to the SYNOPS risk indicators[33] and to the EU HRI to identify advantages and disadvantages of the three approaches for a large-scale pesticide risk indication in Germany as required by the National Action Plan.

■ MATERIALS AND METHODS

Pesticide Use Data. From the BVL,[1] data on pesticide sales in Germany are available for 562 active ingredients, of which 441 outdoor-use pesticides (145 fungicides, 163 herbicides, and 133 insecticides) were selected for the analysis in this study. Overall sales remained constant over time; only

fungicide sales increased significantly and monotonously between 1995−1999 and 2015−2019 (Figure S1 and Table S1). For some individual pesticide classes (e.g., azoles, triazines, pyrethroids), sales trends were significant (Figure S2). Although sold pesticides are not necessarily used in the year of purchase, we assume that the potential lag between sales and applications is negligible for a large-scale analysis, and that pesticide sales are a robust proxy for pesticide use.

Toxicity Data. Here, we used ecotoxicity endpoints from the EFSA OpenFoodTox database.[34] This database compiles endpoints that were considered during the official risk assessments of pesticides in Europe. In a few cases, data for relevant species groups (Table S2) were added from sources like the Pesticides Properties DataBase (PPDB)[35] or the US EPA Office of Pesticide Programs Pesticide Ecotoxicity Database.[36] For 292 out of the 441 pesticides considered in this study, ecotoxicity data were retrieved for the following species groups: aquatic invertebrates (e.g., crustaceans, insect larvae; nine species in total), fish (e.g., trout, carp; 10 species in total), aquatic plants (e.g., algae, macrophytes; 13 species in total), arthropods (e.g., predatory mites, parasitoid wasps; two species in total), pollinators (bees; one species), vertebrates (e.g., birds, mammals; five species in total), soil organisms (e.g., earthworms, springtails, mites; five species in total), and terrestrial plants (e.g., onion, ryegrass; 13 species in total). These ecotoxicity data represent 92.0−98.0% (mean = 96.0%) of the applied mass of relevant pesticide types per species group (Table S2 and Figure S3). Following the tier-1 risk assessment principles for pesticide registration in the EU[22] and previous studies,[8] all endpoints were divided by an assessment factor and also by an adjustment factor for vertebrate endpoints based on concentrations in feed (Table S3). The most sensitive, and therefore most protective, endpoint per substance and species group was finally used for TAT calculation.

TAT Trend Calculation. The TAT for Germany was calculated according to Schulz et al.[14] TAT serves as a tool to display large-scale temporal trends of how changes in pesticide use are reflected in different species groups.[14] An increasing TAT does not necessarily translate into an equally higher toxicity that is actually faced by the respective species group, yet it indicates an overall increase of mass and/or toxicity characteristics of the pesticides applied in agriculture. A positive correlation between applied insecticide amounts and ecological threshold exceedances has been shown using 1977 field measurements in 231 surface waters in the US.[14] For other pesticide types and terrestrial ecosystems, similar links need to be proved, but the required data are largely missing. For better readability, TAT values were displayed 0-max scaled and without physical units throughout this study (see also ref 14). Differences in TAT trends were analyzed by both comparing absolute differences between the 5-year periods of 1996−2000 and 2015−2019 and by assessing the monotony of trends over the entire time period. Significance of differences was assessed using nonparametric Wilcoxon rank-sum tests (α-level of 0.05 for all statistics). Monotony was assessed with a variance-corrected Mann−Kendall Test, which accounts for serial autocorrelation.[37]

TAT Mapping. To visualize its spatial distribution, we calculated the TAT for 1 km × 1 km grid cells in Germany for each species group. The individual TAT calculation of each grid cell was based on the estimated amount of active ingredients used in this cell and their toxicity thresholds.
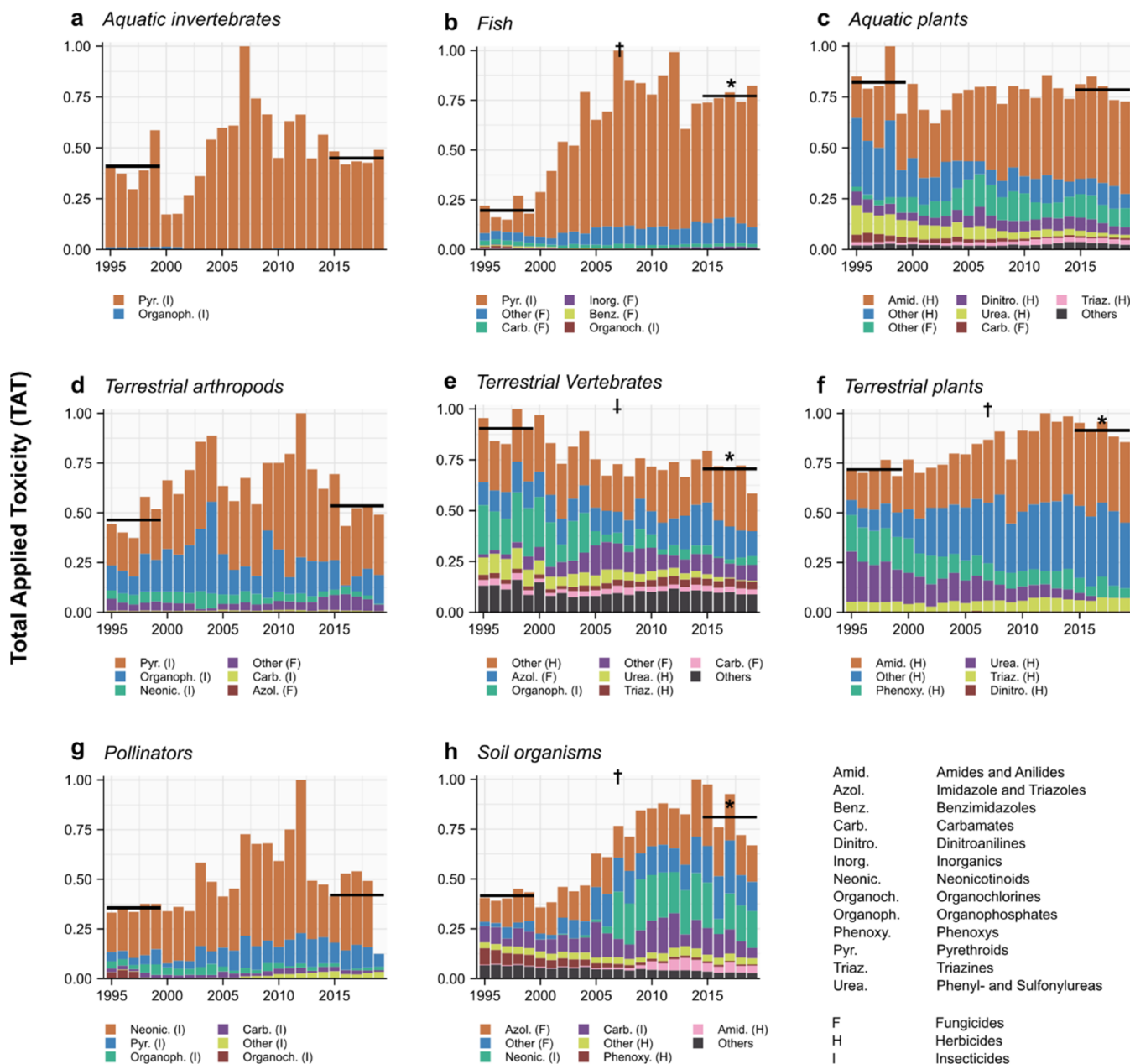
Figure 1. Total applied toxicity (TAT) of relevant pesticide types (Table S2) in German agriculture by pesticide classes for (a) aquatic invertebrates, (b) fish, (c) aquatic plants, (d) terrestrial arthropods, (e) vertebrates, (f) terrestrial plants, (g) pollinators, and (h) soil organisms. Horizontal lines show the average TAT for 1995−1999 and 2015−2019. Significant differences between average TAT for these two 5-year intervals are marked by an asterisk. If the underlying trends are monotonic, they are additionally marked by a dagger (Table S1).

Amounts were estimated by first calculating nationwide fractions of use, with data from Strassemeyer et al.,[27] for each pesticide in 27 crop classes in 2015 (latest year for which these data are available) and then by linking these fractions to the 18 crop types depicted in the 10-m-resolution crop map of Germany for 2017 (first year for which these data are available), provided by Blickensdörfer et al.[28] The linking was done by combining various pome fruits and vegetables into broader classes. By integrating the 100 m² crop cells, for which species group-specific TATs were calculated by dividing the total TAT per crop class by the number of 100 m² cells of this class, an estimate for 1 km² grid cells was made under consideration of their crop composition. It should be noted, though, that spatial homogeneity of use and TAT rates in

entire Germany is a generalizing assumption. Use data with higher resolution would be necessary to depict regional differences more accurately.

Comparison of TAT Germany with TAT US and SYNOPS. The TAT approach was applied for Germany in the same way as reported for the US,[14] yet country-specific pesticide use and ecotoxicity endpoints were considered. Bird and mammal TAT were combined into a vertebrate TAT for Germany, as the ecotoxicity data for birds and mammals were insufficient to represent both groups separately. Soil organisms were considered in the TAT Germany, yet not in the TAT US. For the comparison with SYNOPS, TAT trends were calculated individually for acute and chronic risks in aquatic and terrestrial ecosystems, reflecting the same statistical
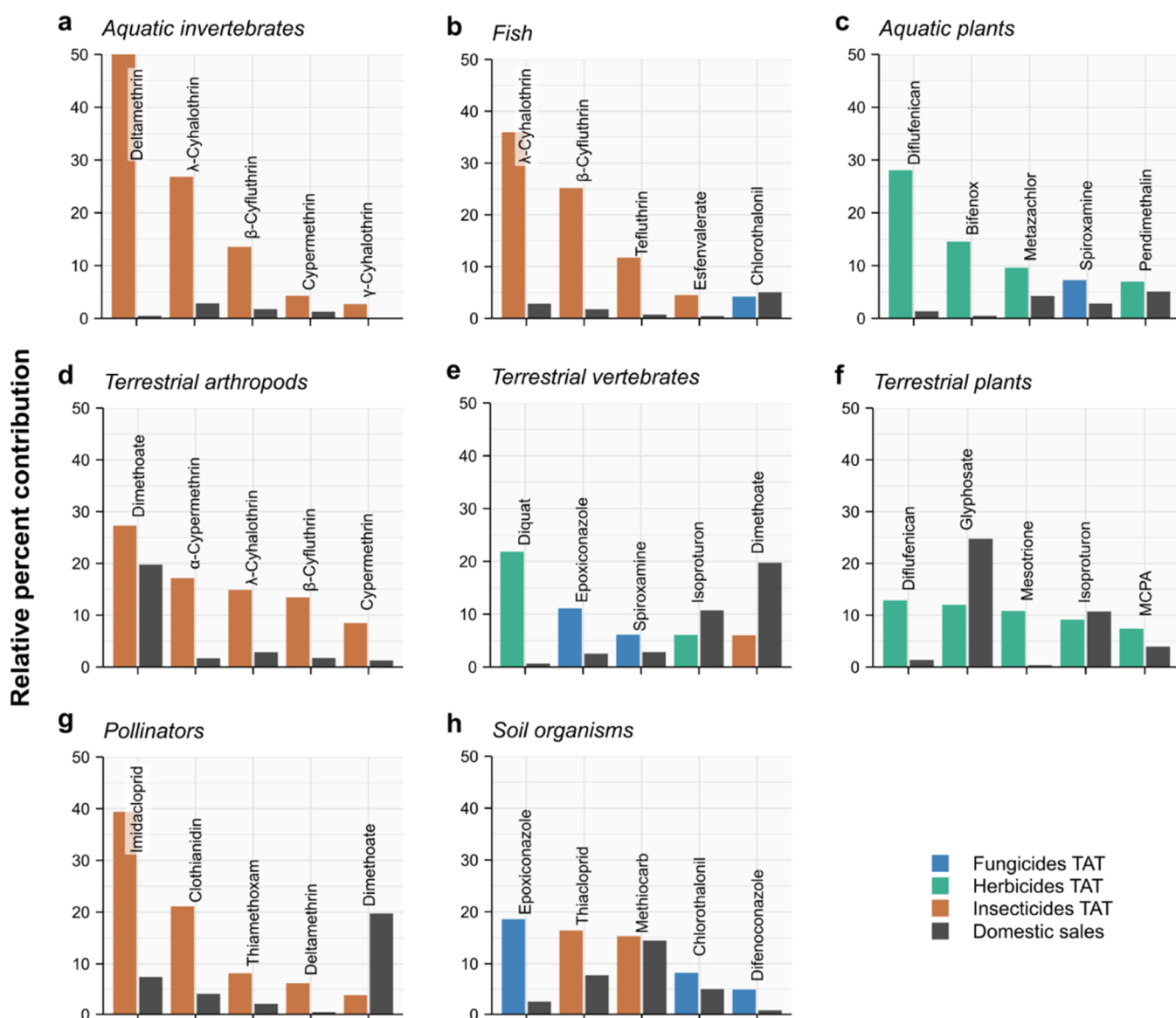
Figure 2. Relative contribution of individual pesticides to the TAT of (a) aquatic invertebrates, (b) fish, (c) aquatic plants, (d) arthropods, (e) vertebrates, (f) terrestrial plants, (g) pollinators, and (h) soil organisms. Domestic sales refer to the sold mass of the pesticide relative to the total sold mass of its pesticide type. For display, the top five pesticides per species group regarding TAT contribution were selected. Percentages refer to the total of the years 1995−2019.

populations as found in the SYNOPS risk indicator reports.[4] The acute aquatic TAT was calculated using the minimum acute endpoint for aquatic invertebrates, fish, and plants; the chronic aquatic TAT using the minimum chronic endpoint for aquatic invertebrates and fish. The pollinator TAT corresponds with the SYNOPS's acute terrestrial risk, and the soil organism TAT with the SYNOPS's chronic terrestrial risk.

■ RESULTS AND DISCUSSION

TAT Trends in Germany. Out of the TAT trends calculated for the eight species groups, three show a significant and monotonic overall increase (fish, terrestrial plants, soil organisms) and one a significant and monotonic overall decrease (vertebrates; Figures 1, S4 and Table S1). The remaining four species groups (aquatic invertebrates, aquatic plants, terrestrial arthropods, pollinators) show no overall trend. TATs that are largely determined by insecticides (e.g., aquatic invertebrates and fish) show a high relative interannual

variability with changes up to 20%. This is likely a result of the flexible insecticide applications to control invertebrates that show high temporal variabilities in their population dynamics.

The strongest increase in TAT, up to a factor of more than three, is observed for fishes, with insecticides driving almost the entire increase (Figure 1b). Pyrethroid insecticides account for more than 82% of the TAT_{fish} in the last 5 years (Figure 1b) with lambda-cyhalothrin, tefluthrin, and β-cyfluthrin contributing the most (Figure 2b). Pyrethroid use in agriculture has been linked to decreases in California Bay-Delta fish species[38] and to potentially detrimental effects on fish, including endocrine disruption, growth, and development alterations.[39] Recently, pesticides have been debated as potential contributors to the observed fish declines in Switzerland.[40] It is curious that the TAT_{fish} in Germany is increasing despite all efforts taken in the past decades to reduce the vertebrate toxicity of pesticides.[41] Although being applied in low and even decreasing amounts, insecticides may be

critical to the decline of freshwater fish species,[42−44] and even to repeatedly observed fish kills.[45,46]

The TAT for soil organisms significantly increased by a factor of two between 1995 and 2019 (Figure 1h). This trend reflects an increasing use of fungicides and changes in the use of neonicotinoid insecticides (Figures 1h and S2a,c). The latter changes are also responsible for the decreasing contribution of insecticides to the TAT$_{soil-organisms}$ since 2013. For agriculture, biological soil health is of utmost importance;[23,47] so an increasing TAT should receive appropriate attention to understand whether and to what extent soil communities are disrupted. Increased applied toxicity faced by soil organisms may have long-term effects on the productivity of agricultural soils, such as lower soil productivity and soil matter turnover rates, and may so induce secondary responses, e.g., an increased use of fertilizers, causing potentially adverse environmental effects.[23] Recent studies have reported the widespread presence of herbicides, fungicides, and neonicotinoids at high concentrations in European topsoils.[25,26] The fungicide epoxiconazole, the largest contributor to the soil organism TAT (Figure 2h), was found in ~25% of soil samples,[25,26] and it was assumed that epoxiconazole and some other pesticides in field soils degrade much slower than expected after laboratory tests.[47] Chronic risks for soil organisms may, thus, be underestimated. Also, both fungicide and insecticide seed dressings were shown to negatively affect the surface activity of earthworms, with herbicides amplifying these effects.[48]

The data for both fish and soil organisms highlight that the TAT increases can be associated with some pesticides that do not contribute considerably to domestic sales (Figure 2b,h). In both species groups, the five pesticides that had a combined TAT contribution of ≥60% only accounted for ≤5% of the total mass applied (Table S4). A pure mass-based evaluation approach might, thus, overlook these pesticides that are highly relevant from an ecotoxicological point of view. Figure 2 clearly points out that often a very few pesticides in combination constitute a large share of the overall risk. This means that the selection of ecotoxicity endpoints, used for deriving the TAT, is of utmost importance, as further discussed below in the section on the comparison of risk indicators relevant for Germany.

The TAT for terrestrial plants shows an upward trend and a significant increase over time, which both can be attributed to herbicides (Figure 1f). The trend results mainly from increased uses of diflufenican, glyphosate, and mesotrione (Figures 1f and S2b), which, together, contribute to the TAT$_{terr-plants}$ ~36% (Figure 2f). Of these three herbicides, glyphosate alone makes a considerable percentage of domestic sales (Figure 2f). Urea-derived herbicides, after playing an important role for the plant TAT in the 1990s, started on a continuous decline and finally ceased to contribute to the TAT in the most recent years. Only very few large-scale experimental or field studies on herbicide effects on nontarget plants are available.[5] A large field-scale experiment in Germany found sublethal effects on the common buttercup (Ranunculus acris), e.g., an 85% reduced flower intensity, following exposure to sulfonylurea herbicides at concentrations typically occurring in field margins.[49] In the same study, this plant species was also documented to be absent in field margins along cereal fields, although it frequently occurred next to nonsprayed meadows. Also, declines in plant species may lead to indirect effects on herbivorous insect species. Modeling studies suggest that a

herbicide-induced landscape-level reduction in milkweed (Asclepias spp.) caused observed declines in monarch butterfly larvae (Danaus plexippus).[50] On a larger scale, nontarget plant species richness in Germany was found continuously declining by 1.9% per decade between 1960 and 2017,[51] and the increasing TAT for terrestrial plants shown here may have contributed to this decline.

A number of species groups show no significant overall TAT trends for Germany. The TAT for aquatic invertebrates is almost entirely determined by a few pyrethroids (Figures 1a and 2a and Table S4) and shows high interannual variability. Based on the fact that the total pyrethroid use in German agriculture increased (Figure S2c), these TAT variations (Figure 1a) are presumably a consequence of use shifts among individual pyrethroids. Continental or global studies[7,52,53] as well as studies from the US[54−60] or Germany[4,61] repeatedly highlighted pesticide and, more specifically, insecticide risks to aquatic invertebrates. The TAT for aquatic plants similarly lacks a temporal trend (Figure 1c). A prevalent and continuous increase up until 2012 in the use of amide-derived and aniline-derived herbicides (Figure S2b), as the main contributors to TAT$_{aqua-plants}$ (Figure 1c; especially diflufenican, Figure 2c), was compensated by a decrease in the combined use of other herbicides.

The terrestrial arthropod TAT shows high variation over time, yet no trend (Figure 1d), with various pyrethroids and organophosphates (especially dimethoate, Figure 2d) being the most important contributors (Figure 1d). The general shift from organophosphate to pyrethroid use (Figure S2c) does not level out the differences in TAT for this group in every year. It must be noted, though, that, due to the limited availability of ecotoxicological endpoints, only two species (Aphidius rhopalosiphi and Typhlodromus pyri) represent the enormous diversity of terrestrial arthropods,[62] which increases the uncertainty for assessment of this species group. Potential negative effects of insecticide and fungicide use have been concluded for carabid beetles in a large-scale study of nine areas across the EU,[2] and also for terrestrial invertebrates in a 21-year crop rotation experiment, comparing different conventional and organic farming approaches in Switzerland.[23]

The pollinator TAT increased until it peaked in 2012 and then decreased considerably (Figure 1g), showing no overall trend. As the pollinator TAT is primarily determined by neonicotinoids (Figure 1; see also ref 14), especially imidacloprid (Figure 2g), this decrease can be mainly explained by restrictions on neonicotinoid use enacted by the EU in 2013[63] that in fact consequently led to a decreasing use in Germany (Figure S2c). While data from France showed that use restrictions for neonicotinoids led to increased applications of pyrethroids,[64] this shift is not found in the German use data, where both neonicotinoid and pyrethroid use decreased since 2012. The German Ministry of Food and Agriculture has recently issued a number of exceptions to the neonicotinoid use restrictions (e.g., ref 65), which may have an impact on future use and TAT trends for pollinators.

Terrestrial vertebrates are the only species group in the present study that encounter a significant, also monotonic, TAT decrease (Figure 1e). Fungicides, herbicides, and insecticides are all relevant to the vertebrate TAT, but the decreasing TAT$_{terr-vertebrates}$ trend is, similar to the US TAT$_{terr-vertebrates}$ trend,[14] mainly based on the decreasing use of organophosphates (Figures S2c and 1e), whereas even larger

combined TAT contributions from fungicides and herbicides (especially diquat, Figure 2e) remain stable over time.

Spatial Distribution of TAT. Our unprecedented spatial mapping of the TAT reveals regional differences that can help to identify target regions for subnational risk reduction actions (Figure S5). Although the spatial TAT patterns for the eight considered species groups are generally determined by the areal intensity of agriculture, and so are overall similar (Figure S5), there are some evident differences. For the three species groups with increasing TAT trends, different crops and regions are important (Figure S5). For fish, high TAT values are observed in western North-Rhine-Westphalia (Figure S5b), likely due to strong pyrethroid use in vegetable crops; for soil organisms in the southeast of Rhineland-Palatinate (Figure S5h), likely due to fungicide use in vineyards; and for terrestrial plants in the southwest of Lower-Saxony (Figure S5f), likely due to high herbicide use. The described regional differences emerge from very broad assumptions, like homogeneous spatial distribution of pesticide usage within each crop class. Publication of higher resolved use data for Germany, preferably crop-specific and, e.g., at the level of the 19 Regierungsbezirke or 400 districts in Germany, would allow depicting spatial patterns and their changes over time much more accurately.

Comparison to US TAT Trends. The main finding of a previous study for the US[14] was postulating distinct phases and regime shifts in the applied mass and TAT phase space. The vertebrate and plant TAT responded proportionally to changes in the applied mass of insecticides (vertebrates) and herbicides (terrestrial and aquatic plants). The invertebrate TAT showed a regime shift around 2003, evolving from a phase of decreasing applied insecticide mass and a stable TAT into a phase of increasing TAT decoupled from mass changes, induced by pyrethroids in the case of aquatic invertebrates and by neonicotinoids in the case of pollinators. In Germany, unlike in the much larger US agriculture (~400 000 tons annual pesticide application compared to ~30 000 tons in Germany), no clear phases in pesticide use are discernible (Figure S6). Here, applied herbicide and insecticide masses, the sole drivers for the US TATs, fluctuate around an average value with high relative interannual variance. This is likely a scale effect that would also be seen in the US agriculture if individual states or counties were considered. The composition of the applied pesticide aggregate also differs considerably between the two countries. In Germany, 38% of the applied pesticide mass refers to fungicides, 59% to herbicides, and 3% to insecticides,[1] whereas in the US, it was 12% for fungicides, 81% for herbicides, and 7% for insecticides.[66] The higher share of fungicides in Germany is likely caused by climatic differences and a higher proportion of cultivated crops that receive high fungicide treatments. For instance, the percentage of vineyard acreage is ~4 times higher in Germany than in the US.[67]

There are, however, some interesting similarities and differences in the use trends and TAT trends of both countries for individual species groups. In both cases, the $TAT_{aqua\text{-}inverts}$ is nearly exclusively driven by pyrethroids and correlates with the applied amounts of this insecticide class. However, two of the most important TAT contributors at the substance level in the US, bifenthrin and esfenvalerate, are not important for the TAT in Germany, whereas β-cyfluthrin and cypermethrin are not important in the US. Nonetheless, the $TAT_{aqua\text{-}inverts}$ in the US strongly increased, yet remained overall stable in Germany.

In the US, the TAT_{fish} decreased between 1999 and 2006 because of a reduced use of organophosphorus and organochlorine insecticides.[41] In Germany, these insecticide classes contribute to the TAT_{fish} only very little (Figure 1b). An important finding of the present study is the fact that the TAT_{fish} in Germany is driven by the increasing use of pyrethroids. Organophosphates and organochlorines combined contribute 14 times the mass of pyrethroids to all US applications (mean between 1995 and 2019[66]), whereas in Germany this factor is only four.[1] Further, aquatic plants in Germany face no change in TAT (Figure 1c), whereas the US aquatic plant TAT has increased since 2006, along with the increased use of shoot-growth inhibitors, e.g., acetochlor, a herbicide that is not registered for use in Germany.[1]

The arthropod TAT is mainly affected by pyrethroids and organophosphates, both in Germany (Figure 1d) and in the US.[14] A considerable TAT reduction for mammals and birds in the US was caused by diminishing use of organophosphates and carbamates.[41] Organophosphate insecticides caused a decrease in the vertebrate TAT in Germany (Figure 1e), but the TAT nearly plateaued around 2005 at a level determined by fungicides and herbicides, both pesticide types that did not affect the US TAT for vertebrates. Vertebrates are, therefore, a prominent example of how the choice of ecotoxicity thresholds, as well as the composition of applied pesticides, both impact the reception of applied toxicity. The TAT trend for terrestrial plants is more similar between Germany and the US. In both countries, amide-derived and aniline-derived herbicides (although with differences at the substance level) and glyphosate contribute to an increase in the TAT (Figure 1f). The $TAT_{pollinator}$ in the US saw a stark and continuous increase caused by an increased use of neonicotinoids. In Germany, neonicotinoids are also the main contributor to the $TAT_{pollinator}$ (Figure 1g), but their use is reduced since 2012 due to regulatory restrictions. As a result of this comparison, it becomes evident that differences in pesticide use patterns are an important driver for dissimilarities in TATs between Germany and the US. Moreover, the regulatory system, the availability, and the choice of ecotoxicity data[21,36] are decisive. The increases in TATs for a number of species groups are, however, concerning in both Germany and the US.

Discussion of Risk Indicators Relevant for Germany. Given the fact that the NAP stipulates a quantitative reduction of pesticide risks in Germany by 2023,[30] the quantification of pesticide risks at a national scale is of special importance. Evaluating large-scale pesticide risks is not trivial and generally hindered by data availability. However, various indicators of differing complexity and underlying assumptions have been formulated by various institutions. Here, we discuss TAT and two other types of indicators that are important in the context of the NAP: SYNOPS risk indicators and European HRI.

TAT is conceptually based on the principle that pesticide risks at large scales decrease if smaller amounts of pesticides are used or if pesticides are (partly) replaced by the same amount of less toxic pesticides (considering a specific species group). Likewise, risks increase with increasing amounts or higher toxicity. This principle is expressed by the TAT and can be evaluated with a basic set of data that is normally available at the national level (applied amounts per pesticide and toxicity thresholds per pesticide). Although it is not necessarily true that each change in TAT equally translates into changes in risk (due to many factors, like differences in application, different environmental fates of chemicals, etc.), it was shown

that TAT is indicative of pesticide risks in the US,[14] and we argue here, that it is also suited for assessment of general pesticide risk trends in Germany.

SYNOPS risk indicators, which were used in previous NAP reports to evaluate risk trends,[33,68] show some methodological differences to TAT (Table S5) but are eventually largely based on the same input data, applied amounts, and pesticide toxicities. The main methodological difference is that SYNOPS risk indicators strive to include exposure and environmental fate of applied pesticides by predicting environmental concentrations through scenario-based simulations of registered usages for different exposure pathways. Scenarios are formulated for indications, i.e., combinations of one pesticide, one target pest, and one target crop, and weighted during consolidation of risk indicators, on a yearly basis, by the calculated area to which they are applied. This area is derived by distributing the sold pesticide amount for a year among individual indications. Although interannual changes in registered usages and crop composition could, thus, alter the overall relative contribution that individual pesticides have to the risk over time, this was the case only for very few pesticides within the time period from 1996 to 2020 (Figure S7). In essence, SYNOPS risk indicators can be generally described, like TAT, as a model of applying (mostly) constant proportionality factors to the applied amounts of a pesticide to estimate its overall risks. Determination of these factors, however, is not trivial due to the complexity of the involved exposure and environmental fate modeling. And with currently limited documentation of methodology (e.g., by source code) and input parameters (e.g., applied ecotoxicity endpoints), recalculation and evaluation of SYNOPS risk indicators are generally hindered. Additionally, quantification of the explanatory power of SYNOPS risk indicators at large scales, which would require comparing them to empirical monitoring data, remains open.

Despite their conceptual similarities, TAT and SYNOPS risk indicators show mostly different risk trends for Germany if TAT is calculated for the combinations of risk groups and pesticide types used by SYNOPS risk indicators (Figure S8). Compared to the average of 1996−2005, the SYNOPS risk indicators show lower risks for 2015−2019 in six of 10 considered combinations, unchanged risks in two combinations, and higher risks in the other two. The TAT is lower for 2015−2019, compared to 1996−2005, in four combinations, unchanged in two, and higher in four. Herbicide-based risk trends, except for soil organisms, and the fungicide-based risk trend for soil organisms show high concordance between the SYNOPS and the TAT approach, but all other combinations of risk groups and species groups show no common temporal trends (Figure S9). It could be assumed that the differences in trends result from the additional exposure and environmental fate modeling employed by SYNOPS risk indicators, but it is more likely that the selection of threshold values causes the discrepancies. Considered thresholds for the TAT span up to 10 orders of magnitude (for aquatic risks), reflecting the span of underlying ecotoxicity values. The proportionality factors for the SYNOPS risk indicators also span up to 9.4 orders of magnitude (for terrestrial nontarget arthropod risk), with toxicity presumably contributing to these factors in a very large proportion. Differing physicochemical properties and other factors, determining the outcome of the fate simulations, are less likely to impact individual risk contributions a lot.[69,70]

Differences in the choice of threshold levels can have significant effects on risk calculations. For instance, the acute aquatic invertebrate TAT is driven by deltamethrin, for which a threshold of 0.0017 ng/L[71] was used during TAT calculation, resulting in a 31 times higher relative risk contribution per applied unit than that of cypermethrin's (threshold of 0.053 ng/L[72]), the most contributing pesticide to the SYNOPS acute aquatic risk. If, instead of the EU tier-1 principle thresholds, EC_{50} *Daphnia magna* values from the PPDB were used, cypermethrin ($EC_{50} = 0.21$ μg/L) would contribute to the risk about 2.7 times more per unit applied mass than deltamethrin ($EC_{50} = 0.56$ μg/L), and it would decrease deltamethrin's absolute contribution to the TAT by five orders of magnitude. Although it is unknown to us as to which ecotoxicity endpoints are actually used for the SYNOPS risk indicators, we assume that differences in the choice of endpoints explain both most of the different weighting of individual pesticides regarding their risk contribution (Figure S10) and, eventually, most of the divergences in risk trends (Figure S8). In conclusion to the comparison of the TAT and the SYNOPS risk trends, we find that often only a few pesticides determine trends, and also that the identity of these trend-defining pesticides is highly dependent on *a priori* assumptions, e.g., the thresholds considered. We also think that large-scale risk quantifications can benefit from the consideration of differing exposures and environmental fates among pesticides, although further validation at large scales is required. Overall, the formal comparison of TAT with SYNOPS is hindered by a lack of transparency regarding how the SYNOPS indicator is exactly derived.

Like TAT and SYNOPS risk indicators, HRI adjust applied pesticide masses by proportionality factors to quantify risks at the national scale. According to EC 2019/718, one of four fixed factors is assigned to each pesticide depending on its regulatory status. Low-risk substances gain a factor of 1, approved substances a factor of 8, candidates for substitution a factor of 16, and nonapproved substances a factor of 64. The annex of EU 540/2011 thereby lists the category of each pesticide. This declarative approach has a series of implications. First, the assertion of an arbitrary fixed factor for all pesticides within a regulatory category, e.g., for all approved substances, makes risk trends within this category solely mass-based, ignoring the (eco)toxicological principle that substances are toxic at very dissimilar concentrations. Risk trends within categories are, therefore, determined by pesticides with large used amounts (e.g., inorganics), potentially masking risks of highly toxic but less used pesticides. Second, risk trends change if the categorization of a pesticide changes. There are several reasons why the classification of a pesticide is altered, e.g., why an approved pesticide becomes a candidate for substitution. These reasons include, for instance, evidence of a pesticide's carcinogenicity or reproductive toxicity, which are important properties for human risk, but which do not necessarily justify why ecological risk also should be considered with a doubled proportionality factor (16 instead of 8). Intermingling human and ecological toxicity, thus, limits the application of HRI for the quantification of environmental risks, which is necessary to monitor the environmental risk reduction goals of the NAP. Additionally, reclassification of pesticides alters the risk trends in retrospect. As the most conceivable sequence of a pesticide's classification is in the order *approved, candidate for substitution, nonapproved*, risk factors tend to increase over the lifecycles of

pesticides' regulations (from 8 to 16 to 64), leading to HRI suggesting stronger decreasing risks. This is because pesticide use normally drops with the decision to substitute or disapprove of a pesticide, but amounts used in the past are now multiplied by an increased factor. With these characteristics, it is less surprising that the HRI in Germany decreased by 11% for 2020[73] (17% for the entire EU[74]) compared to its baseline period 2011−2013 and by even 32% if carbon dioxide is excluded from index calculation.[73] Overall, the comparison of TAT with HRI is difficult since HRI are not real risk indicators: they rather represent trends in pesticide use weighted by legislative categories.

In summary, the present study provides initial evidence that for certain species groups (fish, terrestrial plants, and soil organisms), potential risks arising from changes in pesticide use in German agriculture have increased over the past 25 years. This contrasts the aim to reduce environmental risks of pesticides as stipulated by the EU directives and the German NAP. Currently, the progress of the German NAP is being evaluated using HRI, indicators that can be easily applied to the available data but that also show striking conceptual shortcomings for the quantification of environmental risks compared to the previously used SYNOPS risk indicators, which are rather untransparent. The TAT approach applied here illustrates, though, that it is also possible to describe both the development of risks over time and their spatial distribution by an easily calculable index that incorporates the most important risk determinant—the individual toxicity of a pesticide for different species groups. The TAT has been partly validated for insecticides through a comparison with 1977 insecticide monitoring results in 231 US surface waters.[14] Thorough validations of risk indicators are, however, generally nonexistent. The TAT approach can be easily used in many countries, at continental or even at the global scale. We argue that beyond any indicative index, thorough evaluation of large-scale pesticide risks requires approaches that are thoroughly validated, e.g., through extensive monitoring. This is needed to ensure that risks are estimated in a protective manner and to support both policy setting and a transparent environmental risk assessment for protection authorities, science, and the general public. To better fulfill these requirements, a much better availability of pesticide use data with a high spatial resolution is urgently needed. The available data highlight that strongly improved measures for the reduction of pesticide risks are needed.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c07251.

> Tables S1−S5 and Figures S1−S10 with additional information on the data used in the study, as well as on the methods and results (PDF)

> Toxicity thresholds used in the study (XLSX)

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Notes

The authors declare the following competing financial interest(s): S.B. and R.S. are employees of the University Koblenz-Landau and also work as part-time consultants in the field of ecotoxicology and environmental risk assessment. The authors declare no other competing interests.

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Supporting Information for

Trends of total applied pesticide toxicity in German agriculture

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Pages S1 – S21

Tables S1 – S5

Figures S1 – S10

Table S1. Results of Wilcoxon-Rank-Sum tests and variance-corrected Mann-Kendall[1] for the data shown in Figures 1 and S1. Wilcoxon-Rank-Sum tests refer to the comparison of the time intervals of 1995–1999 and 2015–2019 and Mann-Kendall tests to the analysis of monotonic temporal trends between 1995 and 2019. Significant differences and monotonic trends (p < 0.05) are printed in bold.

Figure	Category	Wilcoxon test	Mann-Kendall test	
		p-value	tau	p-value
1	Aquatic plants	0.841	0.027	0.870
	Terrestrial arthropods	0.310	0.120	0.614
	Fish	0.008	0.553	0.019
	Aquatic invertebrates	0.151	0.213	0.291
	Pollinators	0.151	0.36	0.043
	Soil organisms	0.008	0.627	<0.001
	Terrestrial plants	0.008	0.647	<0.001
	Terrestrial vertebrates	0.008	-0.547	<0.001
S1	All pesticides	0.840	0.213	0.141
	Fungicides	0.032	0.433	<0.001
	Herbicides	0.151	0.007	0.986
	Insecticides	0.095	-0.113	0.441

25   **Table S2.** Relevance of pesticide types for the TAT of different species groups (marked X). Relevance has been determined by calculating the TAT using the available toxicity data for all pesticide types from the EFSA OpenFoodTox database[2] and checking whether the pesticide type contributes >5% on average to the TAT of the respective species group in the years between 1995 and 2019, or at least 10% in a single year.

Species group	Fungicides	Herbicides	Insecticides
Aquatic invertebrates	--	--	X
Fish	X	--	X
Aquatic plants	X	X	--
Terrestrial arthropods	X	--	X
Terrestrial vertebrates	X	X	X
Terrestrial plants	--	X	--
Pollinators	--	--	X
Soil organisms	X	X	X

30

Table S3. Assessment and feed adjustment factors applied to ecotoxicological endpoints of different species groups according to the tier-1 EU risk assessment of pesticides. Assessment factors are a means of covering uncertainty in the representativeness of considered endpoints. Adjustment factors are used to compare vertebrate endpoints based on feed to those based on body weight.

Species group	Taxonomic entities	Duration	Assessment factor	Adjustment factor (feed)
Aquatic invertebrates		acute	100	-
		chronic	10	-
Fish		acute	100	-
		chronic	10	-
Aquatic plants		acute	10	-
		chronic	10	-
Terrestrial arthropods		acute/chronic	2	-
Terrestrial vertebrates	*Mus musculus*	acute	10	5
		chronic	5	5
	Rattus norvegicus	acute	10	10
		chronic	5	10
	Avian species	acute	10	10
		chronic	5	10
Terrestrial plants		acute/chronic	5	-
Pollinators		acute	50	-
Soil organisms	*Folsomia candida*	acute/chronic	5	-
	Hypoaspis acule	acute/chronic	5	-
	Eisenia spp.	acute	10	-
		chronic	5	-

Table S4. Relative contribution of pesticides to the TAT and applied mass for different species groups. Contribution is expressed as percentage of the TAT, and mass (USE) as percentage of the total from pesticide types relevant for the species group (see Table S2). For each species group, the top-10 contributors regarding the TAT are shown. Percentages refer to the total TAT and mass contributions for the years 1995-2019.

Species group	Chemical	Pesticide class	Pesticide type	%TAT	%USE
Aquatic invertebrates *acute*	Deltamethrin	Pyrethroids	Insecticides	50.51	0.49
	lambda-Cyhalothrin	Pyrethroids	Insecticides	26.83	2.94
	beta-Cyfluthrin	Pyrethroids	Insecticides	13.54	1.79
	Cypermethrin	Pyrethroids	Insecticides	4.30	1.31
	gamma-Cyhalothrin	Pyrethroids	Insecticides	2.70	0.07
	tau-Fluvalinat	Pyrethroids	Insecticides	0.58	0.70
	Parathion	Organophosphates	Insecticides	0.47	2.98
	alpha-Cypermethrin	Pyrethroids	Insecticides	0.28	1.74
	Tefluthrin	Pyrethroids	Insecticides	0.20	0.74
	Parathion-methyl	Organophosphates	Insecticides	0.17	1.25
Aquatic invertebrates *chronic*	lambda-Cyhalothrin	Pyrethroids	Insecticides	69.29	3.07
	beta-Cyfluthrin	Pyrethroids	Insecticides	22.72	1.87
	Fenoxycarb	Carbamates	Insecticides	1.51	0.49
	Bifenthrin	Pyrethroids	Insecticides	1.00	0.19
	alpha-Cypermethrin	Pyrethroids	Insecticides	1.00	1.82
	Methiocarb	Carbamates	Insecticides	0.77	15.57
	Chlorpyrifos-methyl	Organophosphates	Insecticides	0.74	1.49
	Esfenvalerate	Pyrethroids	Insecticides	0.64	0.49
	Deltamethrin	Pyrethroids	Insecticides	0.63	0.52
	Tefluthrin	Pyrethroids	Insecticides	0.48	0.77
Fish *acute*	lambda-Cyhalothrin	Pyrethroids	Insecticides	35.92	0.24
	beta-Cyfluthrin	Pyrethroids	Insecticides	25.18	0.15
	Tefluthrin	Pyrethroids	Insecticides	11.75	0.06
	Esfenvalerate	Pyrethroids	Insecticides	4.47	0.04
	Chlorothalonil	Other	Fungicides	4.22	6.22
	Mancozeb	Carbamates	Fungicides	2.49	16.00
	Captan	Other	Fungicides	2.46	3.13
	gamma-Cyhalothrin	Pyrethroids	Insecticides	1.87	0.01
	Bifenthrin	Pyrethroids	Insecticides	1.75	0.02
	Pyraclostrobin	Other	Fungicides	1.48	0.77
Fish *chronic*	Esfenvalerate	Pyrethroids	Insecticides	29.64	0.04
	beta-Cyfluthrin	Pyrethroids	Insecticides	27.03	0.14
	Tefluthrin	Pyrethroids	Insecticides	11.78	0.06
	Mancozeb	Carbamates	Fungicides	9.06	15.6
	lambda-Cyhalothrin	Pyrethroids	Insecticides	5.99	0.24
	alpha-Cypermethrin	Pyrethroids	Insecticides	3.67	0.14
	Chlorothalonil	Other	Fungicides	3.40	6.06
	Dithianon	Other	Fungicides	2.16	1.27

	Cypermethrin	Pyrethroids	Insecticides	1.08	0.11
	Bifenthrin	Pyrethroids	Insecticides	0.97	0.01
Aquatic plants *acute*	Diflufenican	Amides	Herbicides	28.08	0.93
	Bifenox	Other	Herbicides	14.53	0.34
	Metazachlor	Amides	Herbicides	9.59	2.91
	Spiroxamine	Other	Fungicides	7.25	1.24
	Pendimethalin	Dinitroanilides	Herbicides	6.98	3.50
	Flufenacet	Amides	Herbicides	4.78	1.26
	Isoproturon	Ureas	Herbicides	4.28	7.32
	Dimethachlor	Amides	Herbicides	2.12	0.61
	Epoxiconazol	Azoles	Fungicides	1.97	1.12
	Diuron	Ureas	Herbicides	1.82	0.24
Terrestrial arthropods *acute*	Dimethoat	Organophosphates	Insecticides	27.28	1.68
	alpha-Cypermethrin	Pyrethroids	Insecticides	17.17	0.14
	lambda-Cyhalothrin	Pyrethroids	Insecticides	14.88	0.24
	beta-Cyfluthrin	Pyrethroids	Insecticides	13.45	0.15
	Cypermethrin	Pyrethroids	Insecticides	8.48	0.11
	Imidacloprid	Neonicotinoids	Insecticides	6.58	0.64
	Prochloraz	Other	Fungicides	6.57	2.85
	gamma-Cyhalothrin	Pyrethroids	Insecticides	2.16	0.01
	Esfenvalerate	Pyrethroids	Insecticides	0.69	0.04
	Methiocarb	Carbamates	Insecticides	0.59	1.23
Terrestrial vertebrates *acute*	Mesotrione	Other	Herbicides	49.54	0.27
	Ioxynil	Other	Herbicides	18.42	0.42
	Carbofuran	Carbamates	Insecticides	7.23	0.02
	Methiocarb	Carbamates	Insecticides	4.40	0.50
	Dimethoat	Organophosphates	Insecticides	2.86	0.68
	Metconazol	Azoles	Fungicides	2.29	0.21
	Spiroxamine	Other	Fungicides	2.06	1.40
	Folpet	Other	Fungicides	1.53	1.54
	Carbosulfan	Carbamates	Insecticides	0.86	0.02
	Dimoxystrobin	Other	Fungicides	0.77	0.09
Terrestrial vertebrates *chronic*	Diquat	Other	Herbicides	21.84	0.46
	Epoxiconazole	Azoles	Fungicides	11.09	1.17
	Spiroxamine	Other	Fungicides	6.11	1.30
	Isoproturon	Ureas	Herbicides	6.06	7.69
	Dimethoate	Organophosphates	Insecticides	5.98	0.63
	Oxydemeton-methyl	Organophosphates	Insecticides	5.19	0.08
	Tebuconazole	Azoles	Fungicides	3.81	2.33
	Mancozeb	Carbamates	Fungicides	3.02	6.00
	Terbuthylazin	Triazines	Herbicides	2.05	3.00
	Pendimethalin	Dinitroanilides	Herbicides	1.99	3.67
Terrestrial plants *acute*	Diflufenican	Amides	Herbicides	12.86	1.49
	Glyphosat	Other	Herbicides	12.02	27.17
	Mesotrione	Other	Herbicides	10.81	0.38

	Isoproturon	Ureas	Herbicides	9.16	11.78
	MCPA	Phenoxy.	Herbicides	7.4	4.33
	Metazachlor	Amides	Herbicides	6.63	4.68
	S-Metolachlor	Amides	Herbicides	4.63	3.34
	Florasulam	Amides	Herbicides	3.91	0.03
	Flufenacet	Amides	Herbicides	3.21	2.03
	Metamitron	Triazines	Herbicides	3.09	6.81
Pollinators	Imidacloprid	Neonicotinoids	Insecticides	39.42	8.07
acute	Clothianidin	Neonicotinoids	Insecticides	21.13	4.43
	Thiamethoxam	Neonicotinoids	Insecticides	8.17	2.26
	Deltamethrin	Pyrethroids	Insecticides	6.23	0.52
	Dimethoat	Organophosphates	Insecticides	3.85	21.29
	Methiocarb	Carbamates	Insecticides	3.52	15.59
	beta-Cyfluthrin	Pyrethroids	Insecticides	2.83	1.88
	Etofenprox	Pyrethroids	Insecticides	2.75	2.28
	zeta-Cypermethrin	Pyrethroids	Insecticides	2.58	0.28
	Spinosad	Other	Insecticides	1.91	0.38
Soil organisms	Epoxiconazole	Azoles	Fungicides	18.58	1.14
chronic	Thiacloprid	Neonicotinoids	Insecticides	16.42	0.24
	Methiocarb	Carbamates	Insecticides	15.31	0.45
	Chlorothalonil	Other	Fungicides	8.23	2.28
	Difenoconazol	Azoles	Fungicides	4.95	0.37
	Fluazinam	Other	Fungicides	3.89	0.50
	Dichlorprop-P	Phenoxys	Herbicides	3.12	1.44
	Prothioconazol	Azoles	Fungicides	2.53	1.24
	Quinmerac	Other	Herbicides	2.52	0.72
	Pethoxamid	Amides	Herbicides	1.75	0.52

45

Table S5. Main methodological differences between TAT and SYNOPS risk indicators for deriving trends in pesticide risks of aquatic and terrestrial species groups.

| Characteristic	TAT	SYNOPS
Mass input	Sum of all outdoor pesticide applications per pesticide and year	Sum of all outdoor pesticide applications per pesticide and year distributed among individual application scenarios by target crop and target pest
Toxicity endpoints	Derived from endpoints according to European tier-1 risk assessment	LC_{50}/EC_{50}/NOEC/LOEC values of test species
Risk index accounting	Pesticide applications account in sum	Application scenarios account weighted by cropped area and application frequency
Pesticide class accounting	Pesticide types are evaluated together	Pesticide types are evaluated separately
Species group accounting	Species groups are evaluated separately	Risk indexes for aquatic and terrestrial ecosystems are aggregated from individual test species risks
Modeling	No further modeling	Exposure and environmental fate modeling based on physico-chemical properties and exposure scenarios
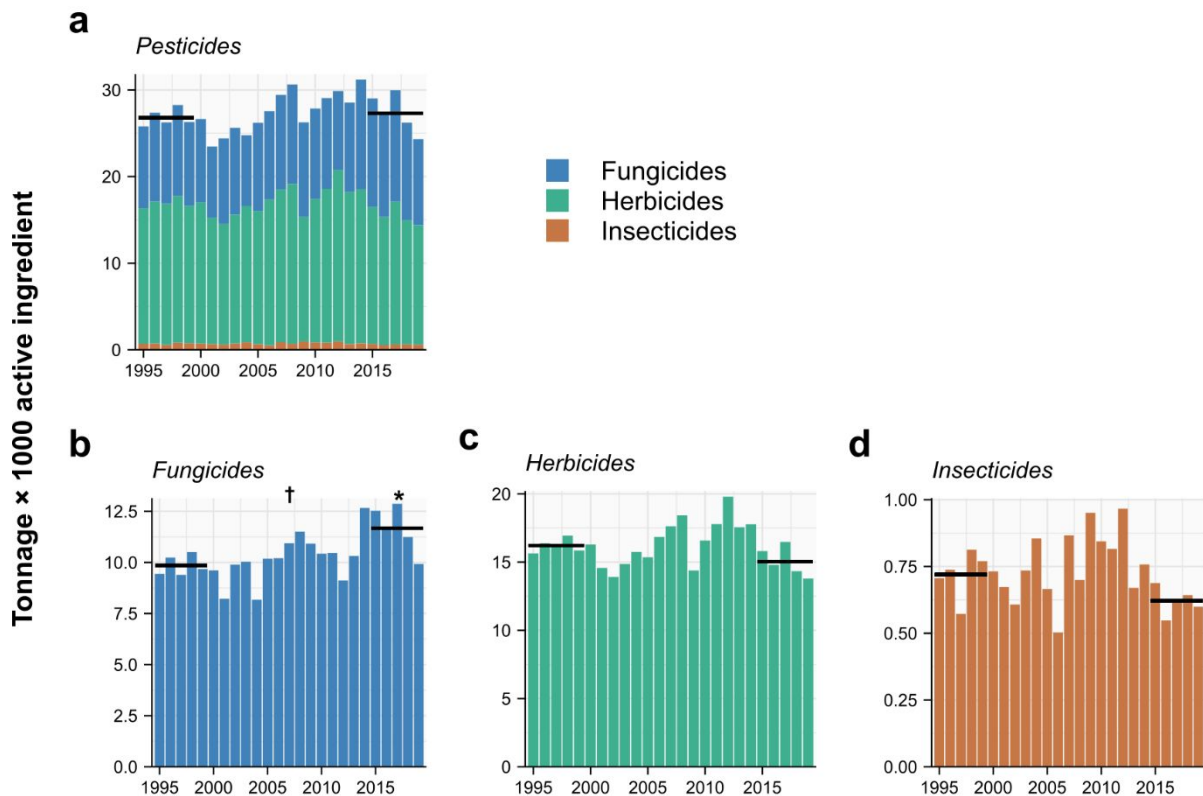
Figure S1. Domestic sales of active ingredients in Germany between 1995 and 2019 for (a) the total of 441 pesticides considered in this study, (b) the 145 fungicides, (c) the 163 herbicides and (d) the 133 insecticides contained therein. Annual domestic sales (mass in tons) are provided by the BVL[3] for 562 active ingredients, which were reported to federal authorities as required under §64 Pflanzenschutzgesetz (German Plant Protection Law). In concordance with the US TAT approach, we constrained the German dataset to chemical pesticides that are primarily used in outdoor-agriculture. 45 ingredients were not further considered because they were biological or physical agents or because they did not primarily act as toxicants, e.g., oils, suffocating or repelling pest organisms. Fourteen were not included in the analysis because they were found to be primarily used indoors. The remaining 503 active ingredients were classified into major use types: fungicides, herbicides, insecticides, molluscicides, plant growth regulators and other pesticides, according to the harmonized substance classification system used by EU regulation (2009/1185/EC). Initial analysis showed that molluscicides, plant growth regulators and other pesticides had virtually no contributions to the TAT for the assessed species groups (<0.5% each), thus, results for these groups (containing 62 pesticides) were not further considered. Significant differences between the first and last five-year averages (horizontal lines) are indicated in the figure by an asterisk and, if the underlying trend over the entire range of years is monotonic (variance-corrected Mann-Kendall-Test; Table S1), also by a dagger.
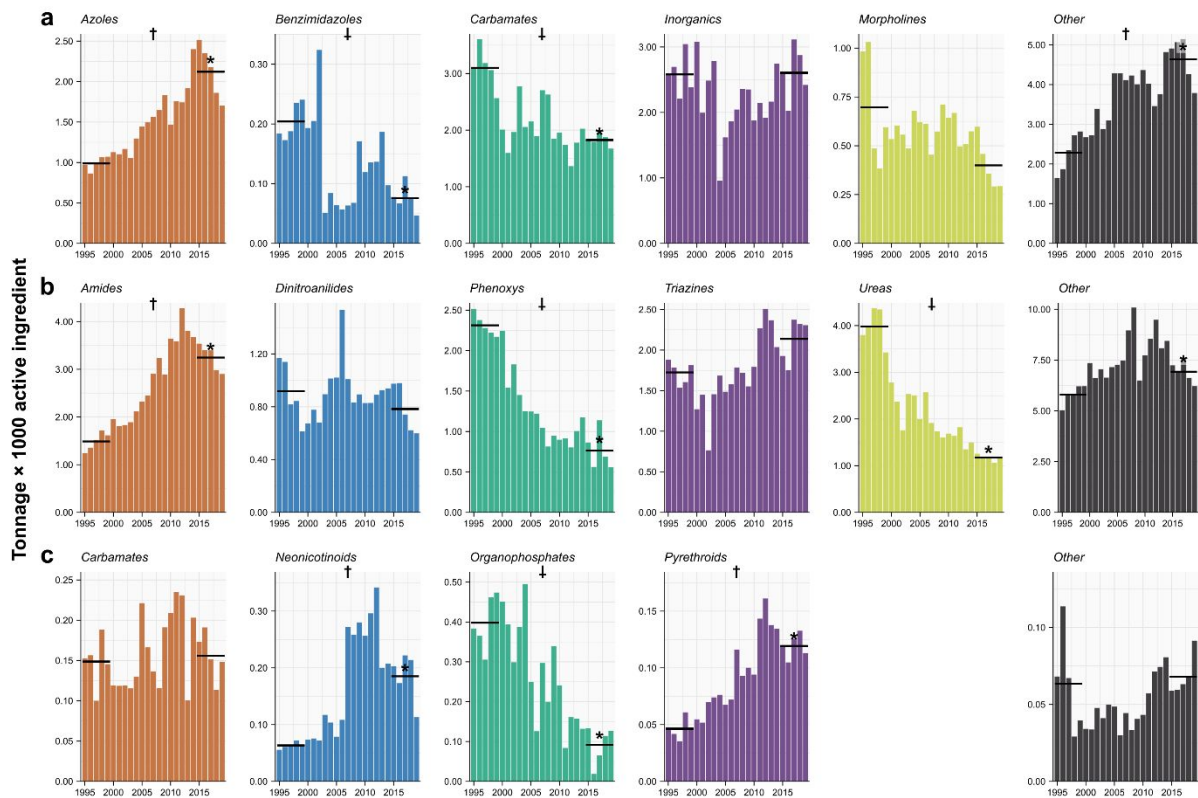
Figure S2. Domestic active ingredient sales in Germany between 1995 and 2019 for different classes of (a) fungicides, (b) herbicides and (c) insecticides. Significant differences between the first and last five-year averages (horizontal lines) are indicated in the figure by an asterisk and, if the underlying trend over the entire range of years is monotonic (variance-corrected Mann-Kendall-Test; Table S1), also by a dagger in case of an increasing trend or by an inverted dagger in case of a decreasing trend.
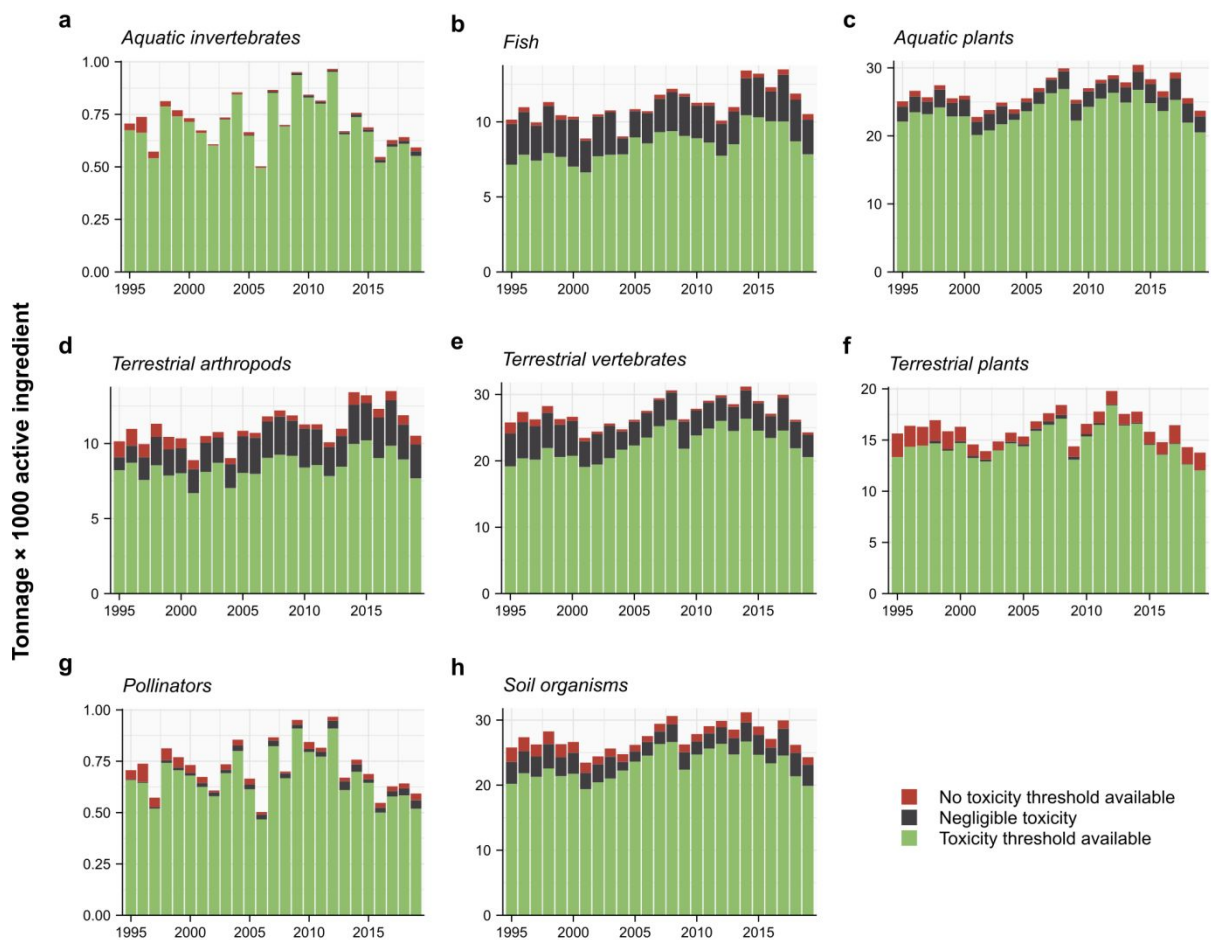
Figure S3. Tonnage of pesticide sales in Germany differentiated by the availability of toxicity data (thresholds) for the different species groups: (a) aquatic invertebrates, (b) fish, (c) aquatic plants, (d) terrestrial arthropods, (e) terrestrial vertebrates, (f) terrestrial plants, (g) pollinators and (h) soil organisms. Only the sales of relevant pesticide types (Table S2) were considered per species group.
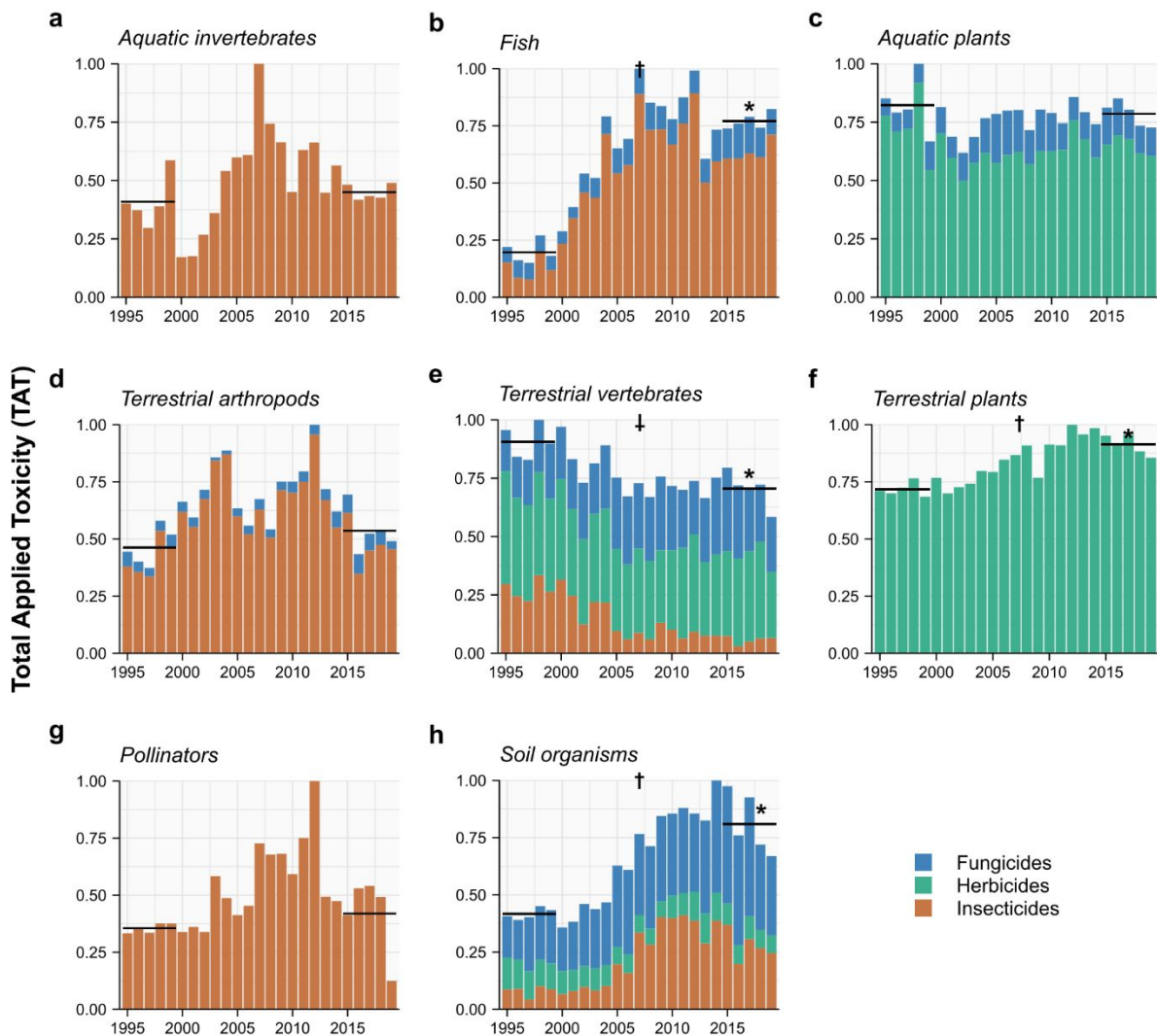
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Figure S4. The TAT in German agriculture for relevant pesticide types (Table S2) for (a) aquatic invertebrates, (b) fish), (c) aquatic plants, (d) terrestrial arthropods, (e) terrestrial vertebrates, (f) terrestrial plants, (g) pollinators and (h) soil organisms. Horizontal lines show the average TAT for 1995-1999 and 2015-2019. Significant differences between the first and last five-year averages (horizontal lines) are indicated in the figure by an asterisk and, if the underlying trend over the entire range of years is monotonic (variance-corrected Mann-Kendall-Test; Table S1), also by a dagger in case of an increasing trend or by an inverted dagger in case of a decreasing trend.
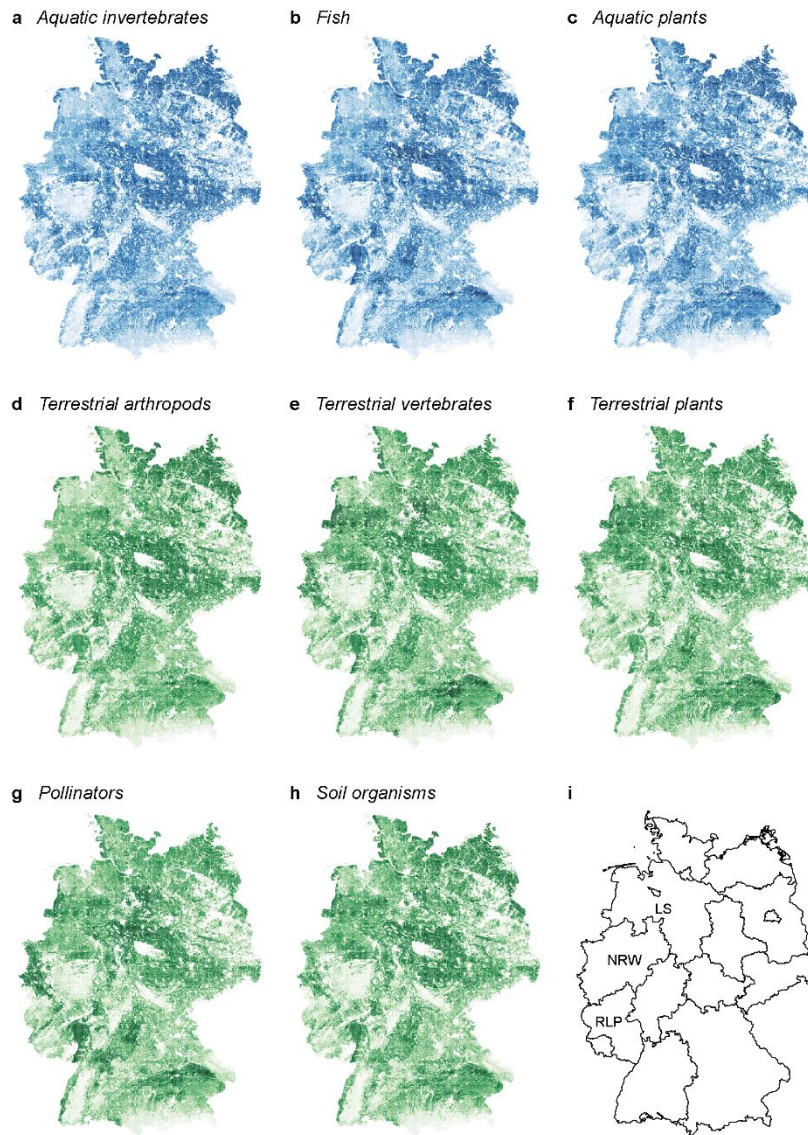
95

Figure S5. Spatial distribution of the estimated TAT in German agriculture for relevant pesticide types (Table S2) in 2015-2017 on a 1 km x 1 km grid for (a) aquatic invertebrates, (b) fish), (c) aquatic plants, (d) terrestrial arthropods, (e) terrestrial vertebrates, (f) terrestrial plants, (g) pollinators and (h) soil organisms. (i) shows the borders of the federal states and identifies those mentioned in the main text (NRW = North-Rhine-Westphalia; RLP = Rhineland-Palatinate; LS = Lower Saxony). Grid cells are colored by their percentile in the respective TAT distribution over all cells (darker areas indicate higher percentiles). Aquatic (blue) TAT values refer to a theoretical exposure of water bodies in a grid cell and are not projected onto actual water bodies.
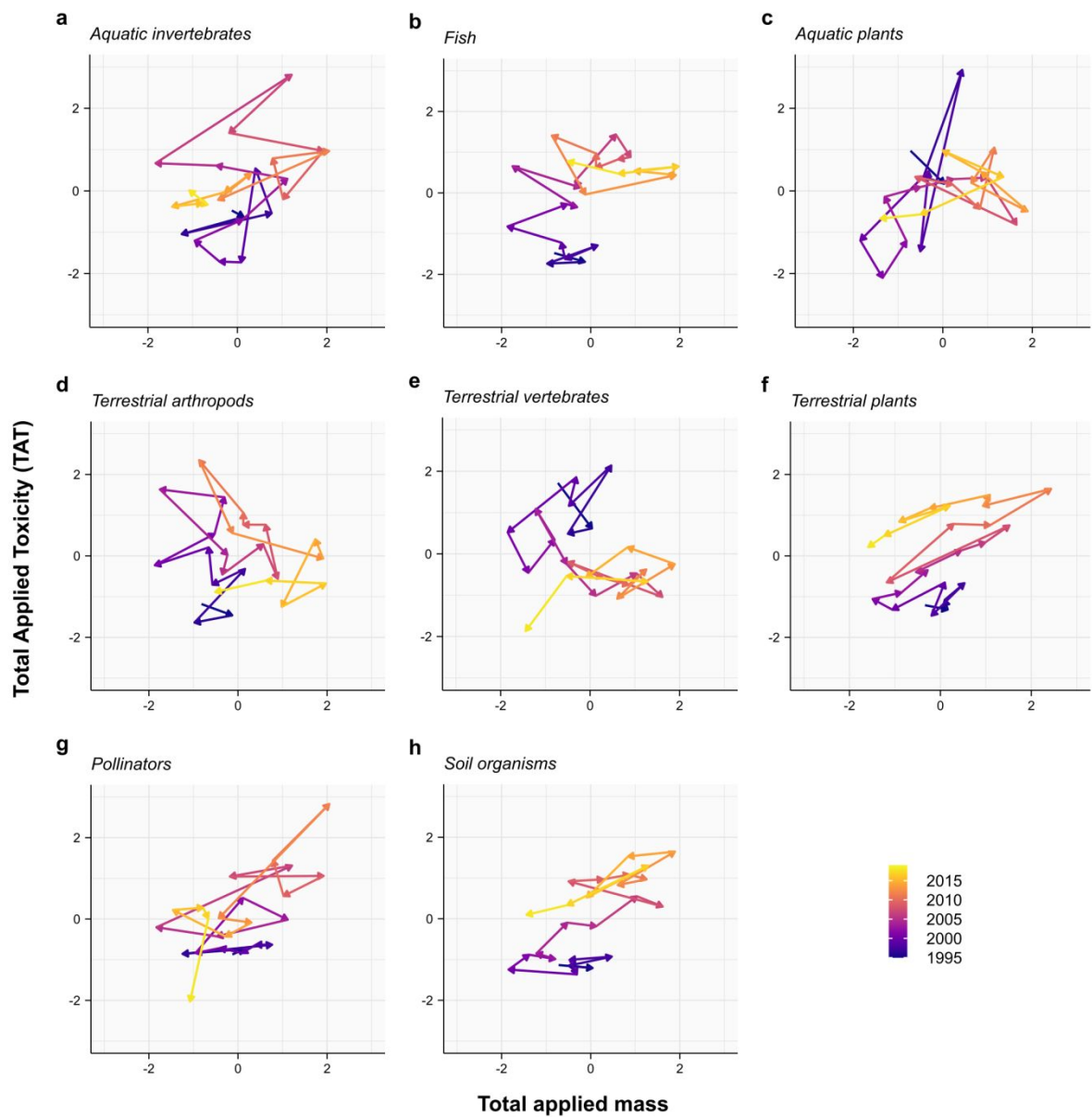
Figure S6. Phase space-plots (both axes z-score normalized) for (a) aquatic invertebrates, (b) fish, (c) aquatic plants, (d) terrestrial arthropods, (e) terrestrial vertebrates, (f) terrestrial plants, (g) pollinators and (h) soil organisms, showing temporal trends of applied pesticide mass (only relevant pesticide types according to Table S2) and the TAT in Germany.
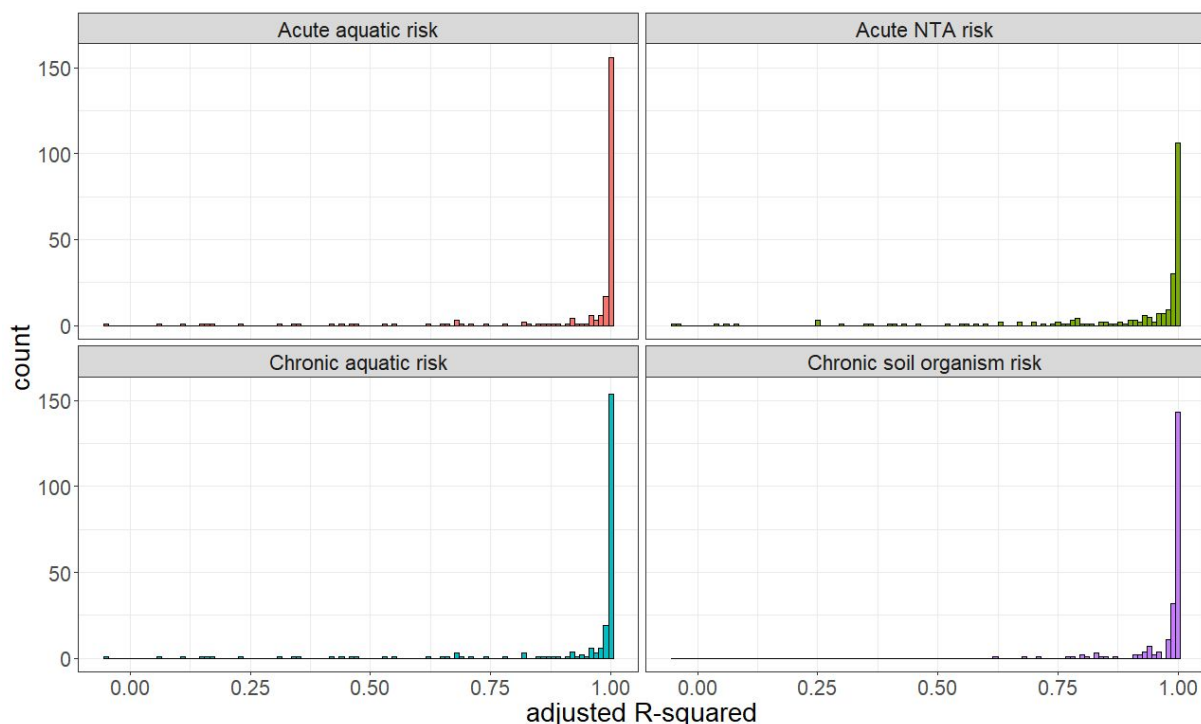
Figure S7. Adjusted R-square for linear models relating the annual SYNOPS trend risk indicator contributions of individual pesticides to their applied amounts in the same year.

High adjusted R-square (≥0.9) values indicate proportionality between the SYNOPS trend risk indicator and applied masses, i.e., applied amounts contribute by an approximately time-constant factor to the risk indicator. This is the case for most of the pesticides. For only few pesticides, factors vary over time (adjusted R-square <0.9) due to changes in registered usages or crop composition. Most of the combinations of pesticides with an adjusted R-square <0.9 and one of the four risk indicators (in total n=135, 59 pesticides) do, however, indicate comparably little contribution to the respective risk indicator (≤0.1 in any year), with only a few exceptions (n=14, 12 pesticides) that potentially impact the shape of the SYNOPS risk indicator trends: copper hydroxide (adjusted R-square 0.74; maximum yearly SYNOPS trend contribution: 0.25) for acute aquatic risk; abamectin (0.70; 0.36), clothianidin (0.56; 0.34), dimethoate (0.76; 0.81), dithianon (0.25; 0.16), imidacloprid (0.06; 1.35), copper oxychloride (0.77; 0.71), mancozeb (0.08; 0.19) and sulfur (0.85; 0.14) for acute non-target arthropod risk; fenoxycarb (0.66; 0.95), copper oxychloride (0.78; 0.25), mancozeb (0.15; 0.43) for chronic aquatic risk; prothioconazole (0.62; 0.27) for chronic soil organism risk. Linear models were fitted only for pesticides that contributed at least five years to a risk indicator.
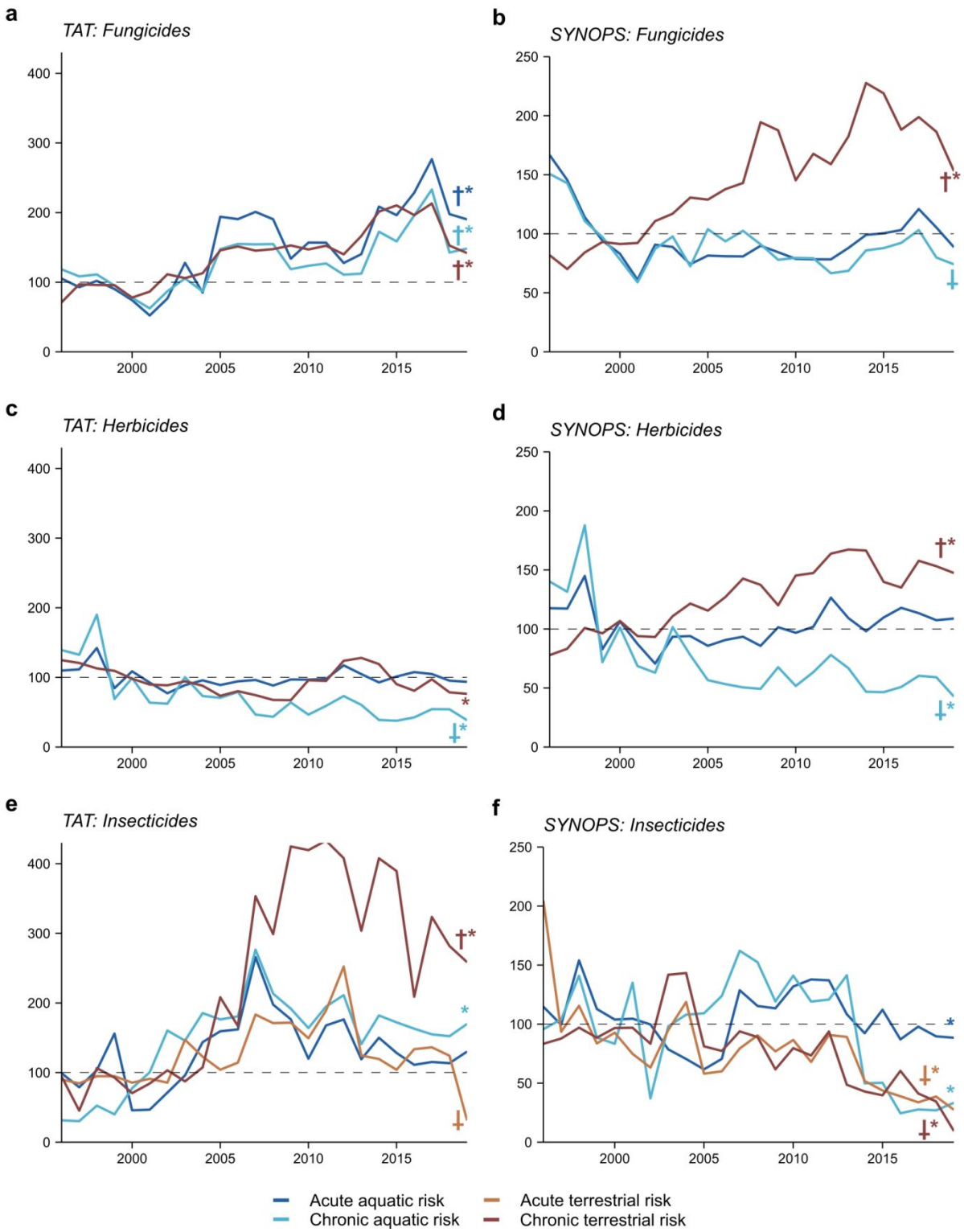
a *TAT: Fungicides*

b *SYNOPS: Fungicides*

c *TAT: Herbicides*

d *SYNOPS: Herbicides*

e *TAT: Insecticides*

f *SYNOPS: Insecticides*

Acute aquatic risk

Chronic aquatic risk

Acute terrestrial risk

Chronic terrestrial risk

Figure S8. Trends in fungicide, herbicide and insecticide risks in Germany according to the TAT approach and the SYNOPS-trend approach.[4] Results are represented as acute and chronic risks in aquatic and terrestrial ecosystems per pesticide type using the reporting units of the SYNOPS risk indicators. The acute aquatic risk TAT was calculated by the minimum acute endpoint of aquatic invertebrates, fish and aquatic plants per pesticide, the chronic aquatic risk TAT by the minimum chronic endpoint for aquatic invertebrates and fish per pesticide. The acute terrestrial risk is represented by the TAT for pollinators and the chronic terrestrial risk by the TAT for soil organisms, both fitting the species groups considered by the SYNOPS risk indicators for the respective risks. The reference line (=100) on the y-axis is the average value for the years 1996 to 2005 used as the baseline in the German NAP. The TAT values were normalized accordingly. Acute terrestrial risks are provided only for insecticides, since this is the only pesticide type relevant for the TAT of pollinators (Table S2). Asterisks mark trends where the risk in 2015-2019 significantly differs from that in 1996-2000. Daggers indicate statistically significant monotonic increasing trends, inverted daggers monotonic decreasing trends.[1] The yearly SYNOPS-trend indicators for 1996 to 2019 were retrieved from the Julius Kühn-Institute's Pesticide-Trends Database Explorer.[5] Monotony of potentially underlying trends were examined using a variance-corrected Mann-Kendall test[1], whereas differences between the two five-year time periods 1996 to 2000 and 2015 to 2019 were assessed using non-parametric Wilcoxon tests.
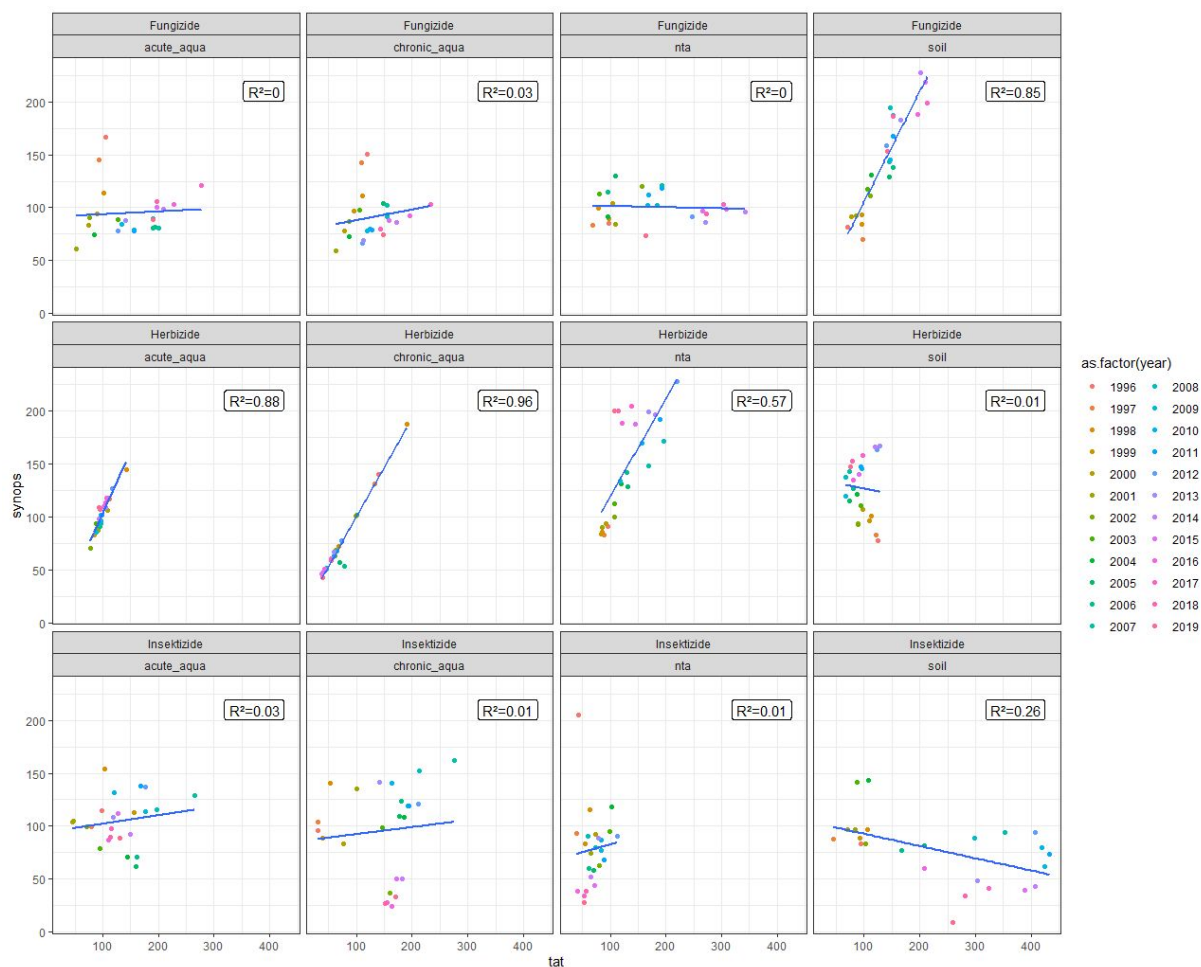
Figure S9. Comparison of the yearly SYNOPS and TAT indexes for different groups of chemical use types and species groups. Similarity of the SYNOPS and TAT trends was determined by a linear regression model relating the yearly normalized SYNOPS and TAT values for each considered group of chemical use type and risk, and expressed by the non-adjusted coefficient of determination of the fit. Higher values indicate higher similarity between the indicators.
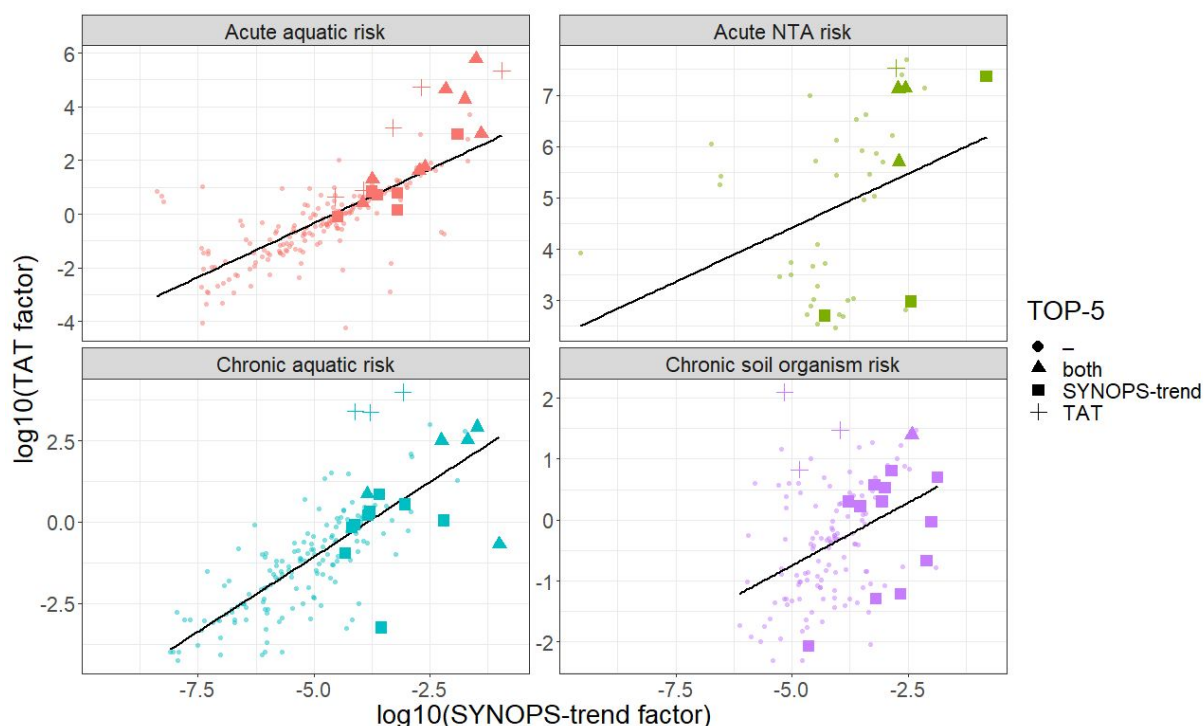
160

Figure S10. Comparison of proportionality factors applied to pesticide amounts for weighting the risk contribution of individual pesticides. For the SYNOPS risk indicators, proportionality factors were determined by fitting a linear regression model for applied pesticide amounts and individual pesticide risk contributions retrieved from [3]. For the TAT, factors are the

165   reciprocal of the pesticide's threshold. The nearer to the black regression line in the plot, the more similar is the relative weighting of a pesticide by both indicators. Pesticides that are among the top-5 contributors to an indicator are marked by symbols. For the SYNOPS risk indicators, top-5 contributors were considered individually per pesticide class (fungicide, herbicide, insecticide), i.e., up to 15 pesticides are marked. For the TAT, top-5 pesticides for

170   all species groups contributing to a risk group were considered (fish, aquatic invertebrates and aquatic plants for acute aquatic risk, fish and aquatic invertebrates for chronic aquatic risk, pollinators for acute NTA risk and soil organisms for chronic soil organism risk). Pesticides that are among the top-5 following the aforementioned method are marked by a triangle. Not all pesticides have quantified contributions to a risk group for both indicators,

175   e.g., due to missing thresholds. These pesticides are not shown in the figure, resulting in varying numbers of pesticides shown in the individual panels.

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