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REVIEW

Wildlife Ecotoxicology of Pesticides: Can We Track Effects to the Population Level and Beyond?

Heinz-R. Köhler^{1*} and Rita Triebkorn^{1,2}

During the past 50 years, the human population has more than doubled and global agricultural production has similarly risen. However, the productive arable area has increased by just 10%; thus the increased use of pesticides has been a consequence of the demands of human population growth, and its impact has reached global significance. Although we often know a pesticide's mode of action in the target species, we still largely do not understand the full impact of unintended side effects on wildlife, particularly at higher levels of biological organization: populations, communities, and ecosystems. In these times of regional and global species declines, we are challenged with the task of causally linking knowledge about the molecular actions of pesticides to their possible interference with biological processes, in order to develop reliable predictions about the consequences of pesticide use, and misuse, in a rapidly changing world.

Wildlife ecotoxicology has its roots in acute poisoning events in the late 19th century; however, public concern over the undesirable environmental effects of chemicals arose in the early 1960s with the publication of Rachel Carson's *Silent Spring*, which publicly broached the issue of the environmental risks of pesticide use for the first time. Shortly thereafter, DDT and its metabolites were found to be responsible for population-level effects in raptorial birds and, with the realization of the global nature of organochlorine pesticide contamination, long-range studies on wildlife exposure, mainly on the basis of environmental analytical chemistry, were launched (1). At that time, in industrialized countries, attention was focused on acute mortality effects in wildlife after pesticide use, abuse, or misuse, mostly involving birds or fish. Currently, pesticide use is widespread in agriculture all over the world, but still only very few countries have established wildlife poisoning surveillance programs (2). As a result, many data on pesticides remain scattered and/or not publicly available (3). Even 15 years ago, incident registration was already considered an insufficient approach for understanding the side effects of pesticide use in agriculture (4). Further shortcomings that are inevitably associated with research on incidents are the difficulties in discriminating between poisoning and other causes of death and the limitations of the analytical detection of pesticides in carcasses (2).

Consequently, in the past 25 years, research interest has shifted from documenting incidents,

and exclusively quantifying chemical exposure, to effect studies aimed at linking laboratory, mesocosm, and field experiments. Since the early 1990s, the proportion of effect-related publications has continuously increased, even though a large number of mechanistically oriented studies have been conducted on laboratory or domestic species, particularly mammals. In terms of sheer numbers of publications, most research on wildlife ecotoxicology deals with fish, insects, and, to a lesser extent, birds, amphibians, and arachnids (Fig. 1A). Effect-related research, which has addressed insecticides, herbicides, and fungicides in a rather constant proportion of published papers for more than 20 years, does not reflect the actual proportions of active ingredients applied in the United States or Europe, but rather overemphasizes the effects of insecticides (Fig. 1, C and D). Within the literature on pesticide effects, increasing numbers of publications have been recorded for some distinct insecticide classes in recent years, which is indicative of the importance of these currently dominating active ingredients (Fig. 1B). In this context, the past 5 years have revealed a particular progression of interest in the effects of organophosphates, pyrethroids, and the rather "new" class of neonicotinoids. However, there remains ongoing interest in first-generation organochlorine pesticides, such as DDT, which is still in use in many developing countries (5). Even though the banning of highly persistent organochlorines in developed countries has shifted pesticide use toward a vast diversity of readily biodegradable ingredients, the explosiveness of organochlorines on a global scale cannot be ignored. The Food and Agriculture Organization of the United Nations estimates that half a million tons of "old" obsolete pesticides have been scattered throughout developing regions in Asia, Latin America, and Africa.

Regulatory programs have considerably changed the array of pesticides used in agriculture. Since 1993, both the United States and the European Union have implemented programs to update risk assessments for pesticides in use, which made manufacturers pull highly acutely toxic organophosphate and carbamate insecticides from these markets voluntarily. Current-use pesticides are mainly designed on the basis of their desired mode of action, which is aimed at displaying optimal efficiency in target, and minimum side effects in nontarget, organisms. Because of the frequently close phylogenetic relationships of beneficial and pest species, however, it is ambitious to both target and protect. One of the major challenges in wildlife ecotoxicology, therefore, is to trace the effects and side effects of chemicals, from their cellular targets through levels of increasing complexity to communities of species and the function of ecosystems. Here we provide an integrated view of the existing knowledge regarding pesticides of the past and present. This includes synthetic chemicals and biological compounds [such as spinosyns, azadirachtin, and *Bacillus thuringiensis* (Bt) δ -endotoxin] applied in agriculture but excludes nonagricultural biocides used as antifouling or fracking compounds, parasiticides, or antibiotics.

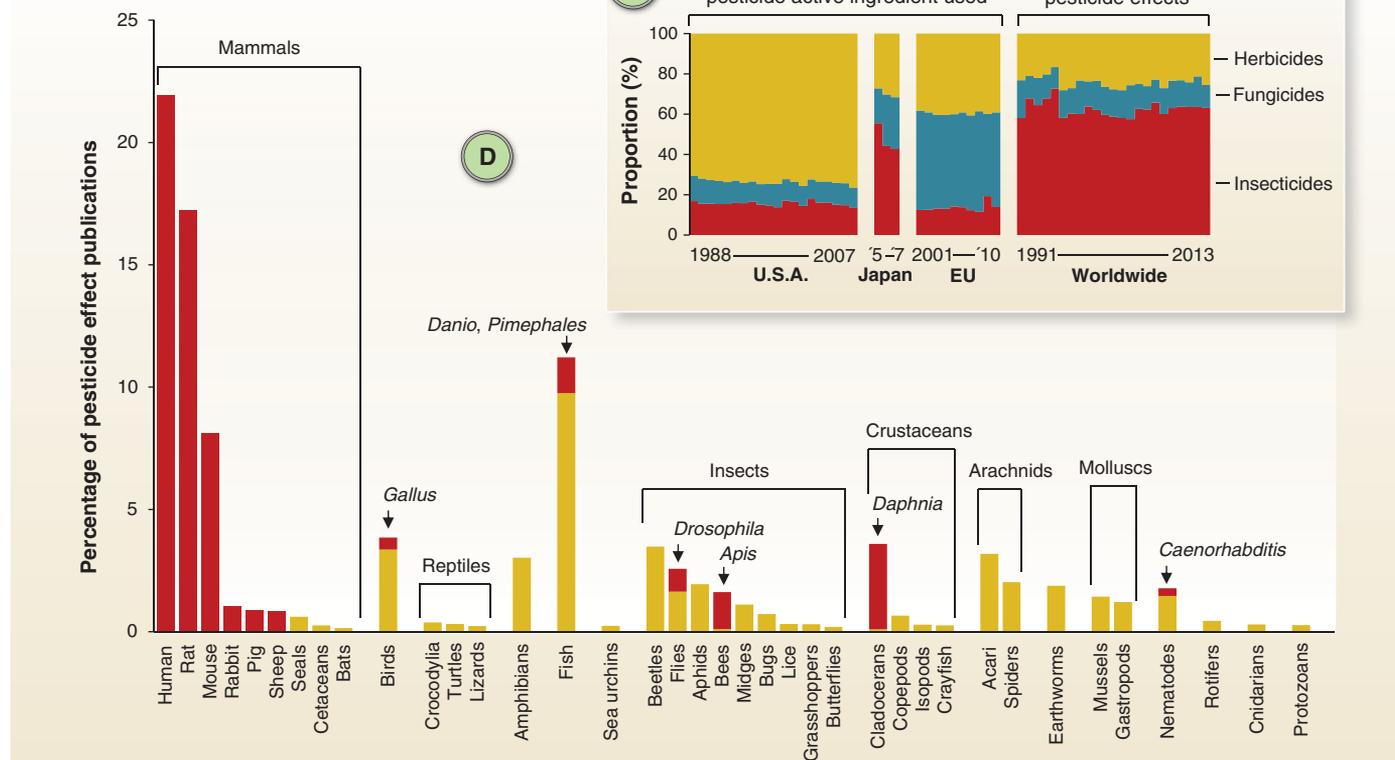
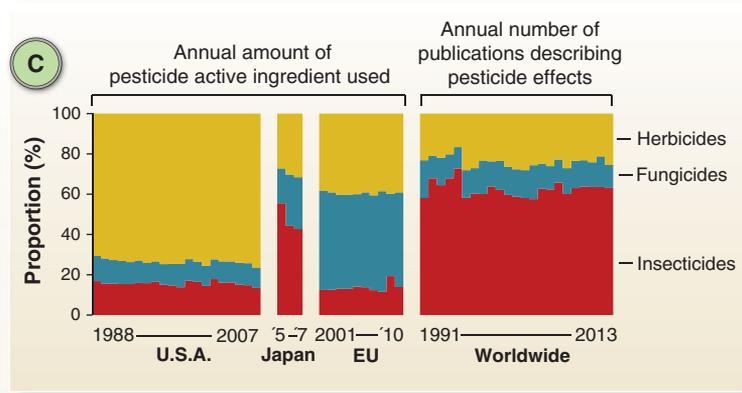
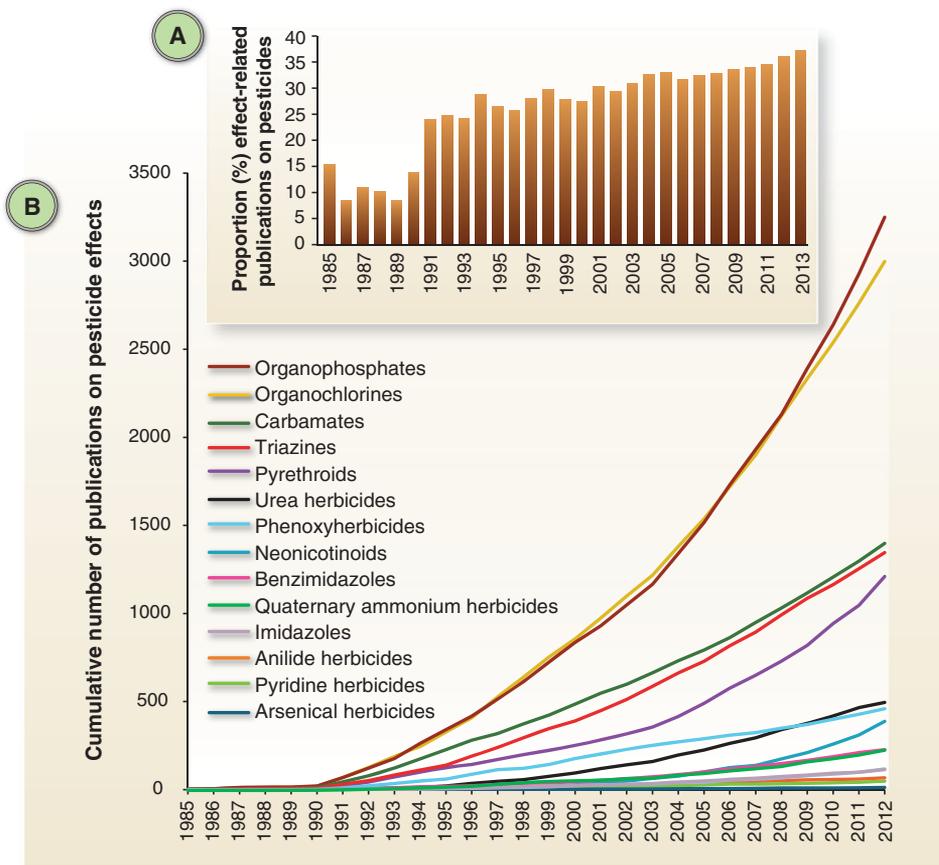
Individuals and Populations

As events of acute poisoning in wildlife have decreased in number during recent decades, at least in developed countries, the problem of chronic pesticide toxicity has moved into the focus of scientific interest. Wherever pesticide application is spatially restricted and buffer zones (such as riparian buffers) are respected, wildlife vertebrates currently are considered unlikely to be exposed to pesticide levels that are acutely toxic, with the exception of some examples of exceedances of acute toxicity values in aquatic systems (6, 7) and anticholinesterase poisoning of birds (8). Chronic toxicity, however, has to be taken into account for all pesticides that are applied at regular intervals, particularly those that are highly persistent, such as organochlorines. In addition to their acute toxicity, which has occasionally led to mass deaths in the past, this group of insecticides (including DDT and its metabolite DDE, an androgen receptor antagonist) is known to chronically act as endocrine disruptors (9), exerting estrogenic and/or androgenic effects in rats, birds, and fish (10). DDT itself is carcinogenic (9). To date, more than 120 endocrine-disruptive pesticides are known, covering numerous chemical classes (11). Organochlorines, organophosphates, carbamates, pyrethroids, thiocarbamates, triazines, and triazoles furthermore exhibit thyroid disruption properties in rodents, birds, amphibians, and fish (10). Immunotoxicity, which is primarily caused by the inhibition of serine hydrolases or esterases, oxidative damage, and the modulation of signal transduction pathways

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Fig. 1. Trends in research on pesticide effects and pesticide use. (A) Steadily increasing proportion of effect-related research among publications on pesticides in the past 28 years. (B) Journal publication numbers on effects related to pesticide classes. During the most recent years, the most substantial increase in the rate of publication was recorded for organophosphates, pyrethroids, and neonicotinoids. (C) The proportions of effect-related publications on herbicides, fungicides, and insecticides remained rather constant throughout the past 23 years but did not reflect the proportions of these pesticide classes used in the United States and Europe. (D) Effect-related research shows a bias toward domestic and lab model species (in red; including human cell lines) in relation to wildlife animals (in yellow) [calculated from data obtained from the Web of Science (March 2013), the U.S. Environmental Protection Agency, the European Crop Protection Association, and (88)].



has been reported for organophosphates (12). The organochlorine chlordane, carbamates, the phenoxy herbicide 2,4-D, and atrazine were found to interact with the immune system of vertebrates (13). Organophosphates and carbamates impair metabolic functions such as thermoregulation, water and/or food intake, and behavior (activity, foraging time, learning ability) in vertebrates. Further consequences are weight loss, impaired development, and reduced reproduction and hatching success (14). Particularly in aquatic biota, a plethora of studies have revealed a broad range of pesticides representing a variety of chemical classes to induce embryotoxicity and teratogenic-

ity in nontarget fish, amphibia, and invertebrates, which result in organ malformations, delayed hatching, growth suppression, and embryonic mortality (15). Some of these pesticide effects at the sub-individual or individual levels have been causally or plausibly linked to their consequences in populations (Fig. 2).

In general, information on the hazards of pesticides to wildlife is based on the knowledge of their environmental fate, persistence, application rate, and toxicity (14); the latter have been largely gained from laboratory experiments predominantly conducted on vertebrates, including mammalian model organisms. Although modern

insecticides such as neonicotinoids previously were expected to exert only low toxicity on mammals, birds, and fish, because these compounds have a low affinity for vertebrates relative to insect nicotinic receptors (16), current research has provided evidence for respiratory, cardiovascular, neurological, and immunological toxicity in rats and humans (17, 18). However, information about many endangered mammalian species, particularly arctic marine biota, is scarce and is limited to measurements of compounds and a few selected biomarkers, such as CyPIA1 activity (19). Effects indicative of endocrine disruption were reported for river otters, bears, seals, sea lions,

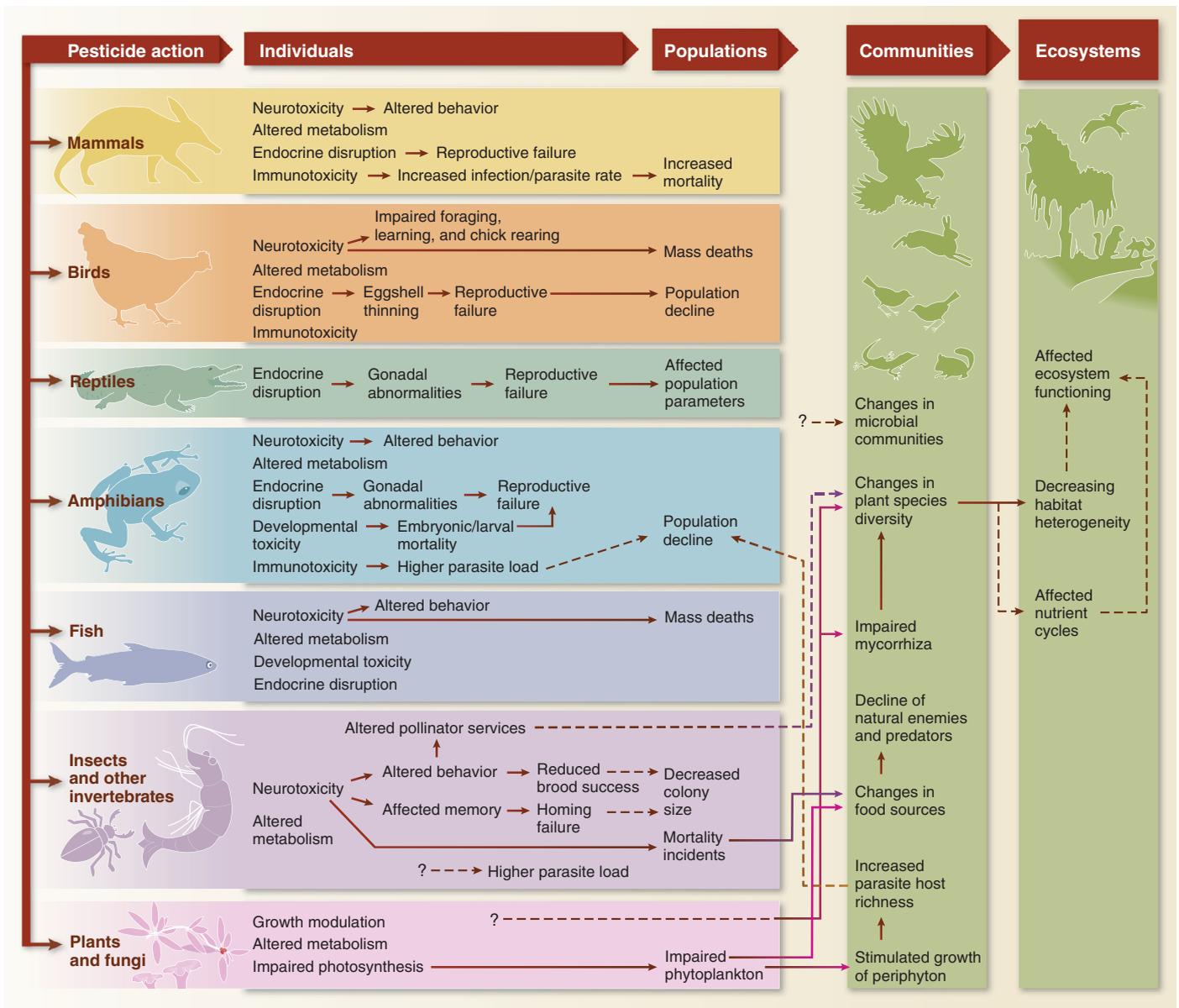


Fig. 2. Documented pesticide effects on wildlife at different levels of biological organization and known (solid arrows) or evidence-supported, anticipated (dashed arrows) interrelations among them. Research remains

to be conducted wherever plausibly interrelated effects are not connected by arrows. Most of the sub-individual data for mammals are derived from non-wildlife studies.

and beluga whales from organochlorine-polluted environments, but it was impossible to separate the effects of DDT from those of sympatrically present nonpesticide organochlorines (20).

In birds, population effects of pesticides have been linked to neurotoxicity and endocrine disruption. Although acute mortality could be attributed to inhibition of acetylcholine esterase activity exerted by organochlorines, organophosphates, and carbamates (8, 21), chronic exposure via oral uptake to organochlorines and organophosphates in particular, but also to carbamates and a variety of herbicides and fungicides, resulted in disturbances of the endocrine and reproductive system. DDT and its metabolite DDE had a devastating effect on many Laurentian Great Lakes bird species due to a reduction of eggshell thickness of up to 90% and, consequently, cracking, and even have affected migrating eagles that had consumed fish from the Great Lakes 2 years previously (5, 22). Similar effects of organochlorines were detected in ducks and herons from the Ebro Delta in Spain (21). It is commonly accepted that these endocrine effects have caused the observed population declines. However, behavioral effects, including impaired incubation and chick-rearing behavior (23), which have been detected in captive birds after chronic exposure to all neurotoxic pesticide classes, have as yet not been linked to population declines (24).

The spill of highly persistent organochlorines (DDT and its metabolites, diclofol, dieldrin, and toxaphene) in Lake Apopka, Florida, in 1980 is well known as the only example linking the endocrine effects of pesticides to juvenile population densities and unexpected adult mortality in wildlife reptiles (25). The population parameters of American alligators were impaired by disrupted steroidogenesis, reduced testosterone levels and penis lengths in males, and elevated 17 β -estradiol levels in juvenile females (5). Worldwide, amphibians have also been suffering alarming population declines. Signs of endocrine disruption, such as gonadal abnormalities and the feminization of males (5, 26, 27), interference with metamorphosis (28), changing behavior (5, 28), and retarded development (26), have been frequently found in wildlife frogs and toads, but it has been difficult to relate these pesticide effects directly to population parameters, gene frequencies, or sex ratios (28). A recent meta-analysis revealed overall environmental pollution to have large effects on abnormality frequencies but only medium effects on survival and no effects on time of development (29). A key to mechanistically link pesticide impact and population declines in amphibians may lie in an impaired immune function and, consequently, in increased infection rates (28). Whether high acute mortality recently observed in European common frogs after direct dermal application of recommended rates of four fungicides, two herbicides, and the insecticide dimethoate (30) is field-relevant remains to be investigated.

Fish ecotoxicology faces similar challenges. Although literature on laboratory studies provides rich detail for sub-individual pesticide effects, attempts to link these to fish populations are rare. Apart from obvious relations in cases where pesticide runoff from orchards reached streams and caused fish kills (31), the difficulty in separating pesticide action from potentially interacting parameters in freshwater ecosystems in industrialized regions has hampered causality analysis. There is compelling global evidence that exposure to endocrine-disruptive chemicals is compromising the physiology and sexual behavior of fish, including effecting permanent alterations of sexual differentiation and impairment of fertility; however, it is thus far impossible to quantify the specific contribution of pesticides to these impairments (20). Whereas pesticide-induced neurological, endocrine, and olfactory dysfunction after cholinesterase inhibition have been correlated with fish behavior (32), effects at the population level associated with exposure to mixtures of pesticides and other chemicals have at most been plausibly linked to sub-individual effects by the application of Bradford-Hill's criteria of causation (33, 34). Generally, single-chemical risk assessment will probably underestimate the actual risks of pesticide mixtures to fish, as combinations of organophosphates and carbamates were shown to exert synergistic neurotoxicity and unpredicted mortality in Pacific salmon (35).

Research on interrelations between individual and population effects of pesticides on invertebrates is dominated by studies on insects, particularly bees. Honey bee poisoning incidents in developed countries such as the United Kingdom or Germany declined from the mid 1990s onward, in parallel to a decline in organophosphate incidents (36). The phenomenon of colony collapse disorder (CCD) and the suspicion that neonicotinoids and formamidines could be involved (37), however, has stimulated much recent research. There is evidence that neonicotinoid pesticides disrupt biogenic amine signaling and cause subsequent olfactory dysfunction, as well as affecting foraging behavior, learning, and memory abilities (3, 37, 38), but it is still unclear whether bee societies can buffer individual effects at field-realistic dosages (3, 39). Two recent studies found that bumblebees exposed to field-realistic concentrations of imidacloprid suffer from impaired foraging, brood development, and colony success in terms of growth rate and new queen production, particularly in combinatorial exposure to the pyrethroid λ -cyhalothrin (39, 40). In honey bees, thiamethoxam caused high worker mortality due to homing failure (41), but possible risks for colony collapse remain controversial (41, 42). Alternative approaches designed to reduce impact on beneficial insects, such as bees, favor compounds of microbial origin such as spinosyns or the Bt δ -endotoxin *Cry*. Spinosyns,

however, affect various physiological and behavioral traits of beneficial arthropods, particularly hymenopterans (43), whereas transgenic crops expressing *Cry* were shown to cause negative effects on the abundance of some insect taxa, predominantly on susceptible lepidopteran herbivores as well as their predators and parasitoids (44–46). So, despite all efforts to increase the specificity of insecticides, there is as yet no compound that both targets insect pests and leaves nontarget insects unaffected.

Across the Levels of Biological Organization

For the most part, pesticide research remains a scattered assemblage of data recorded at the molecular, cellular, physiological, or individual levels for different species on the one hand, and records of population declines or altered community structure in areas with high pesticide input or persistence on the other hand. Evidence for causal links across the levels is still scarce and restricted to the mentioned examples. At present, two strategies are favored to move from one level of biological organization to the next, more complex one. First, a multi-tiered approach combining controlled lab experiments, mesocosms, and field studies is needed to provide the basis for the application of Hill's criteria of causation (33, 47). Second, computational methods either relating observed population effects to underlying parameters [a top-down strategy (20)] or translating toxicity data derived from individuals to the level of wild populations and beyond (a bottom-up strategy) are increasingly being developed and refined (48). Refinement includes criteria quantifying the "best" model selection (49) and the adoption of population dynamics and food web modeling from ecology, accepting that a sophisticated understanding of species interactions is essential to detect and explain indirect pesticide effects (50). New approaches in population modeling include population-level measures of toxicant effects (such as those on population growth rate or age structure) and different sensitivities of life-history traits, and aim to determine the probability of extirpation or recovery of populations after pesticide exposure (51–53). Despite recent promising achievements (54, 55), however, population modeling is still considered a relatively new subdiscipline in ecotoxicology (48) and is not yet developed well enough to fully assess pesticide impacts on endangered species (56).

Biotic Interactions and Communities: Indirect Effects

The current scarcity of incidents in developed countries, the shift from long-lived to mostly less-persistent compounds (except for sulfonylurea herbicides and neonicotinoids), and the awareness of long-term sublethal effects of pesticides have turned the attention of scientists and administrators toward the indirect consequences of

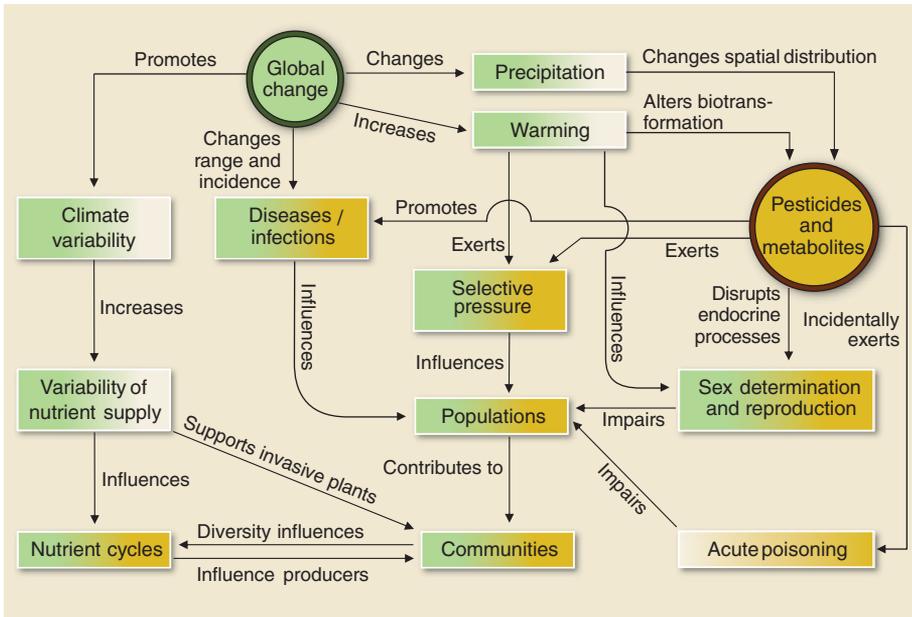


Fig. 3. Anticipated interactions of global change and pesticide effects on the physiology and ecology of wildlife species. The presumed impact of pesticides is depicted in yellow, and the presumed global climate change impact is in green.

pesticide use, which address changes in biotic interactions. Here, three main aspects have moved into focus: parasite-host interactions, predator-prey relationships, and pollination.

A number of pesticide compounds have been proven to affect immune parameters, and some cases of immunosuppression (exerted by organochlorine pesticides, organophosphates, carbamates, atrazine, and 2,4-D) were correlated to higher susceptibility of organisms to infection and parasite-caused diseases. For example, oysters exposed to DDT, toxaphene, and parathion were shown to be susceptible to fungal infection, and earthworms from triazine-treated orchards became infected with monocystid gregarines (13, 57). In mammals, the use of anticholinesterase agents in agriculture can pose a threat of infections, disease outbreaks, and higher mortality, such as by tularemia in hares (58). Work on seals showed that organochlorine pollutants, including pesticides, have immunotoxic properties, impairing resistance to phocine distemper virus (59). Particularly in view of the global loss of amphibian populations, which has resulted in nearly one-third of the world's species being threatened, this subject seems to be crucial. Laboratory exposure experiments and field studies have shown an association between atrazine, malathion, esfenvalerate, or glyphosate exposure and increased infection of tadpoles with trematodes (60, 61). A field survey of the northern leopard frog, *Rana pipiens*, revealed that atrazine pollution and inorganic phosphate accounted for 74% of the variation in the abundance of trematodes (62). Further mesocosm studies in ponds

showed that atrazine killed the phytoplankton, thus allowing light to penetrate the water column and periphyton to assimilate the nutrients, including inorganic phosphate, released from the plankton. Presumably, periphyton growth provided more food to grazers and thus increased the richness of snails, which act as trematode intermediate hosts (62).

Other prominent indirect pesticide effects act on food webs and species competition through the removal of prey or competing species. Herbicides, which reduce the plant cover of soil and change plant species diversity, were found to be responsible for reduced food availability and thus adverse secondary effects on soil invertebrates and butterflies (63). The fungicide benomyl, which suppresses arbuscular mycorrhizal fungi, altered the patch-level floral display and resulted, after 3 years of fungal repression, in a two-thirds reduction of the total number of floral visits and in a shift in the community of floral visitors from large-bodied bees to small-bodied bees and flies (64). Indirect herbicide effects have also been reported for many vertebrate species, because weed and many noncrop plants are important components of their diet. Pesticide-induced diet shifts decreased species abundance and diversity in small mammals (63), reduced survival and reproductive rates in seed-eating or carnivorous birds (65, 66), and resulted in declines of bird populations and species diversity (63). However, declining bird species are not found to be associated with particular plants but rather with reductions in overall diversity and the abundance of food plants in intensely managed arable land. Con-

comitantly, in these areas, a loss of insects and spiders, important sources of food for chicks of a wide range of bird species, was observed (67). Accompanying the trend toward monoculture on a large scale in the United States and parts of the developing world, herbicide use—particularly in combination with the cultivation of herbicide-tolerant crops—has frequently contributed to an overall reduction in habitat heterogeneity in agricultural landscapes and degraded their suitability as habitat for wildlife, including pollinators (63). Also, the biological pesticide spinosad has a wide variety of sublethal effects on natural enemies of pests and can drastically affect demographic traits in parasitoids and predators (43). Bt-transgenic crops, as an alternative to conventional insecticide use, did not impair the function and abundance of natural pest enemies in a 6-year study, but secondary effects by sublethally poisoned prey and diminished food quality for predators cannot be excluded for this kind of pest control (45). In aquatic systems, the most detrimental effects of herbicides address the reduction of the complexity and structure of the plankton and the submerged vegetation, including periphyton, all acting as food sources and refuges for phytophagous species such as waterbirds and amphibian tadpoles (21, 68). In this regard, structural alterations in the planktonic community can result from direct herbicide effects on microalgae, from indirect consequences of pesticides on filter feeders (69), or from changes in competitive interactions [for example, small zooplankton (rotifers) were found to increase after larger zooplankton (cladocerans) were selectively decimated (70)]. Species of higher trophic levels, such as salmon, are most likely to be affected in population growth and productivity by indirect pesticide effects (71). Fleeger and co-workers list 56 cases of indirect pesticide effects on competition or predation in aquatic biota, identified in studies across trophic levels (50). It has to be concluded that, at least in aquatic systems, pesticides exert strong selection on invertebrates. Freshwater habitats are best-studied in this respect, whereas marine and estuarine systems are underrepresented. Furthermore, it is noteworthy that not only modulations in the population structure of prey or predator species, but also pesticide effects on interspecific behavior, may change predator-prey interactions, as shown for glyphosate in tiger salamanders (72) or imidacloprid in zebrafish (73) and their respective prey.

Probably the most meaningful example of indirect pesticide effects, however, does not address the aquatic environment but insect pollination. In bumblebee (*Bombus terrestris*) workers chronically exposed to realistic concentrations of imidacloprid and λ -cyhalothrin, pesticide-altered behavior was found to be associated with a declined pollen-collecting efficiency (39). For these insecticides, as well as for spinosad impact on

bees, it is likely that diminished foraging efficiency affects overall pollination services (43).

Can Microevolution Catch Up?

The selection of resistant phenotypes after multi-generation exposure can be a problem in pest control and, perhaps, a chance for nontarget species with high reproductive output and short generation time. As a matter of principle, the elucidation of long-term pesticide effects in communities of animals and plants is often hampered by the long generation times of the species involved and thus the inevitable inertia of these systems. In contrast, microbial communities display microevolutionary responses within a rather short time period. Transient effects of herbicides, including diuron and simazine, the dithiocarbamate fungicide mancozeb, and DDT, on microbial populations and communities and their function in ecosystems (as, for example, their role in nitrification) are regularly measurable, but studies have congruently revealed their high capacity to recover and to develop tolerance to these pesticides (68). Quite often, these tolerant bacteria benefit from pesticide application and use the compound itself as a carbon source (74). Similarly, insect and pathogen pests were found to benefit from elevated protein levels in 2,4-D-treated corn plants (75), whose yields on a per-hectare basis may thus equal those from organically managed corn (76). Studies also report at least partial restoration of community functions despite structural changes after pesticide treatment in communities of freshwater microalgae (77). It is, however, unclear to what extent the selection of resistance traits and/or a functional resilience of the community can be generalized throughout ecosystems, because studies on metazoans are rare. Recent field experiments revealed λ -cyhalodrin treatment to select 10-fold higher resistance against this pyrethroid in lady beetles (*Eriopsis connexa*) after 55 generations (78). Furthermore, the selection of resistance against deltamethrin was reported for the common green lacewing *Chrysoperla carnea* (79) but, up to now, there has been no indication regarding pesticide-tolerant bees, probably because the queens are not directly exposed to the toxin (38). However, the scarcity of information about nontarget species does not allow the degree to which resistance contributes to the regeneration of populations to be judged. Independent from evolutionary processes, however, ecological networks often allow for restoration by means of recruitment from the filial generation or immigrating individuals. Microcrustacean populations in stagnant waters, for example, usually recover from pesticide effects within a few weeks, provided that the compound is not persistent, the physicochemical environment remains intact, generation times are short, and immigration from the residual population is possible (80). There is also evidence that the reversal of intense pesticide use in arable systems can

result in the rapid recovery of food sources for birds (67). In a review of the ecological consequences of insecticide use, Devine and Furlong listed a variety of cases in which terrestrial and aquatic insect, crustacean, lumbricid, and fish populations recovered within months when the pesticide treatment stopped (81). In this context, multilevel modeling allows situations in which reduced pesticide application will have the most benefit on restoring biodiversity to be detected (54).

Future Challenges in a Changing World

It is to be assumed that the global changes we are going to experience during the coming decades pose larger questions regarding pesticide impact on wildlife than we have been accustomed to. We cannot predict the consequences of a possible release of the bulk of obsolete pesticides that remain in developing countries. Shifts from the use of "old" and highly persistent pesticides to modern compounds may surely improve the situation in many countries of the world but, as outlined, they are also far from being unproblematic. As far as we know, even the latest generation of biopesticides poses problems for wildlife; perhaps not directly by receptor interaction in nontarget species, but at least indirectly via the impairment of species interactions.

Climate change will surely interact with the spatial distribution and effects of pesticides in nature (Fig. 3). Currently, it is possible to identify reasonable points of expected interactions, even though the magnitude of interference remains unclear. Elevated water temperatures may change the metabolite pattern of pesticides via alterations in biotransformation processes, and changes in precipitation may result in changes in volatilization and deposition (82). Global warming is decisively expected to affect the ecotoxicological potency of pesticides, because 83% of ecotoxicological studies on the combined effects of elevated temperature and pesticide exposure have revealed the synergistic action of these factors (83). Experimental evidence for this expectation has been provided by a study on the fungicide pyrimethanil, applied under thermally realistic global change summer conditions simulated for central Europe. In comparison to current temperatures, responses to the conditions in this study predict increased mortality, a declining population growth rate, and considerably reduced genetic diversity in the midge *Chironomus riparius* (84). Pesticide interactions with global warming will probably influence the direction in which selection acts upon biota, a factor that will be particularly problematic for populations or species living at the edge of their physiological tolerance (82). Further problems in a warming world may result from temperature interactions with the metabolic rates of heterothermic organisms and, with respect to endocrine-disruptive compounds, with physiological processes involved

in temperature-dependent sex determination, as is known for reptile species (25). In addition, changes in the geographic range and incidence of many infectious diseases that may be fostered by pesticide-exerted immunotoxicity have been predicted (60). Higher-level pesticide effects, such as changes in plant communities, will probably interfere with the effects of global change on biodiversity and thus affect ecosystem function. Increased heterogeneity of nutrient supply associated with global change was shown to strongly promote plant invasion and thus to alter plant communities (85). In turn, plant diversity is known to influence biomass production (86) and nitrogen cycling (87).

In the coming years, there will be a paramount need to causally link both direct and indirect pesticide effects across levels of increasing biological complexity. Specifically, it will be essential to detect and quantify confounding factors that act synergistically with pesticide exposure, and to identify processes of particular vulnerability to interactions of pesticide impact and climate change.

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