university of groningen
faculty of science and engineering
biology biomolecular sciences

The impact of pesticide utilisation on the biodiversity and ecological stability

A call for drastic change

By: Ing. Reinier Blok
Date: 18 May 2022

The impact of pesticide utilisation on the biodiversity and ecological stability

A call for drastic change

Ing. Reinier Blok

By: Ing. Reinier Blok
Student number: 4995090
Submission date: 18 May 2022
Type: Bachelor Thesis
As part of: Pre-Master Biology
Major: Biomolecular Sciences
Faculty: Faculty of Science and Engineering
At: University of Groningen, The Netherlands

First assessor: Prof. Dr. J.T.M. Elzenga

Preface

As far as I can remember, I have been fascinated by the natural world. From a young age going ladybird ‘hunting’ in the front garden to joining 'mini expeditions' in lime quarries searching for remnants of the biodiversity of the past. It almost leads me to believe that my profound interest in the natural world and its biodiversity is, somehow, encoded in my epigenome. Therefore, it is deeply concerning to witness the effect of human activity on the decline of the natural world and its biodiversity first-hand. As such, I have made it my life's mission to explore and help improve the field of biodiversity research before the (by some speculated inevitable) point of total ecological collapse has been reached.

With this thesis on the topic of pesticide ecotoxicology, I would like to further educate myself and other novices on the subject to become more aware of the dire situation we have put ourselves, as a species, in. Change in the utilisation of xenobiotics is imminent for the survival of our biosphere.

Reinier
2022

Abstract

With the ongoing expansion and intensification of the world’s agriculture, the utilisation of pesticides is now greater than ever before. With the many adverse effects that active ingredients (AI’s) and co-formulants in pesticide formulations bring along, the demand for data about the ecotoxicological impact of these xenobiotics is increasing equally. Numerous independent studies and research groups around the globe have tried to tackle the issues surrounding these xenobiotics. But often these studies only assess the toxicology of a handful of AI's in only a small selection of taxa. The few meta-studies on this topic, although impressively elaborate by themselves, simply lack essential data/ parameters to be subsequently used in an all-encompassing ecotoxicological assessment (excluding crucial taxa or not accounting for synergistic effects). In this extensive review of the most up-to-date scientific findings and reviews on the compounds, molecular modes of action and dispersal of these pesticides, it is suggested that the situation is more ominous than ever possibly quantified so far. This phenomenon is mainly due to the endless number of parameters and ever-increasing complexity involved in such an assessment. However, although difficult to quantify, extrapolations to some degrees are feasible.

Abbreviations

AI	active ingredient
AMPA	aminomethylphosphonic acid
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
nAChR	nicotinic acetylcholine receptor
NEC	no effect concentration
POEA	polyethoxylated tallow amine
ROS	radical oxygen species
RS	risk score

Table of Contents

1. Introduction

Pesticides are used worldwide in agriculture in an endeavour to maintain better control regarding the adverse effects of many environmental factors, of which soil microorganisms and invertebrates are the predominant targets. As an example, the 2019 sales of pesticides in the European Union (EU) (including 'plant growth regulators' data) have indicated to have used 392,307 metric tonnes of pesticides, which is equivalent to 312.8 grams per EU capita [2]. This number naturally raises concerns regarding human exposure and long-term health effects, as long-term studies in Sweden and France indicated a frequent low concentration xenobiotics exposure to humans [3-5]. Despite the EU/ international pesticide phase-out initiatives, loopholes and other means of escape regarding the rules and regulations of pesticide usage allow countries to, even now, use otherwise banned compounds [6,7]. The use of restricted compounds and chemical analogues is a significant basis of concern, not only to human exposure and health but predominantly to the surrounding natural environment. The reasoning as to why many of the compounds are currently restricted or banned is that after reconsideration many have demonstrated to have a detrimental effect on the local biodiversity and ecology [8 - 13]. But by only regulating a handful of potent compounds, compared to the still regularly used synthetic alternatives, one can dispute that the environment will retain its exposure level and risk factor regarding the stability of the ecosystem and biodiversity.

The biodiversity of our world is, thus far, undoubtedly unequivocally to anything discovered in the observable universe. However, with the continuous systemic exploitation of the natural world, like intensive agriculture to sustain the ever-growing needs of the human population, the point of no return concerning the global ecological collapse is closer than ever [14]. Fortunately, many studies that were conducted over the past couple of decades have accumulated a better understanding of the impact of xenobiotics on the environment. However, one can quickly notice a trend amidst the hundreds of published research outcomes. Many studies only focus on select compounds and/ or taxa, or are significantly estimate-based on at that time limited pesticide data availability [11,15], and/ or frequently use arbitrary units of measurement, giving the cursory peer a biassed view of the situation. One example of this practice is the already impactful publication by Tang *et al.*, where they created a global map concerning pesticide pollution risks, taking water scarcity and biodiversity into account (**Figure 1**) [16]. Despite the accuracy and resolution claims on the generated environmental risk maps, one point of critique is that the biodiversity analysis is based only on data regarding Tetrapoda. As Tetrapoda only account for 2.77% of extant described species and $3.83 \cdot 10^{-6}$% of all predicted extant species [17,18], and the majority of the used pesticides have a predominant effect on plants, soil microorganisms and insects, it is to be disputed that the created environmental risk map concerning biodiversity is incomplete and/ or deceiving by design.

As mentioned before, soil microorganisms, plants and insects are beyond question the predominantly targeted organisms for the application of pesticides, which is directly evident in the global pesticide sales data. According to Maggi *et al.* [19], the global estimate of the most common annually used pesticides are the herbicides glyphosate and metam-potassium (±700,000 metric tonnes), metam and dichloropropene (±450,000 tonnes) and 2,4-

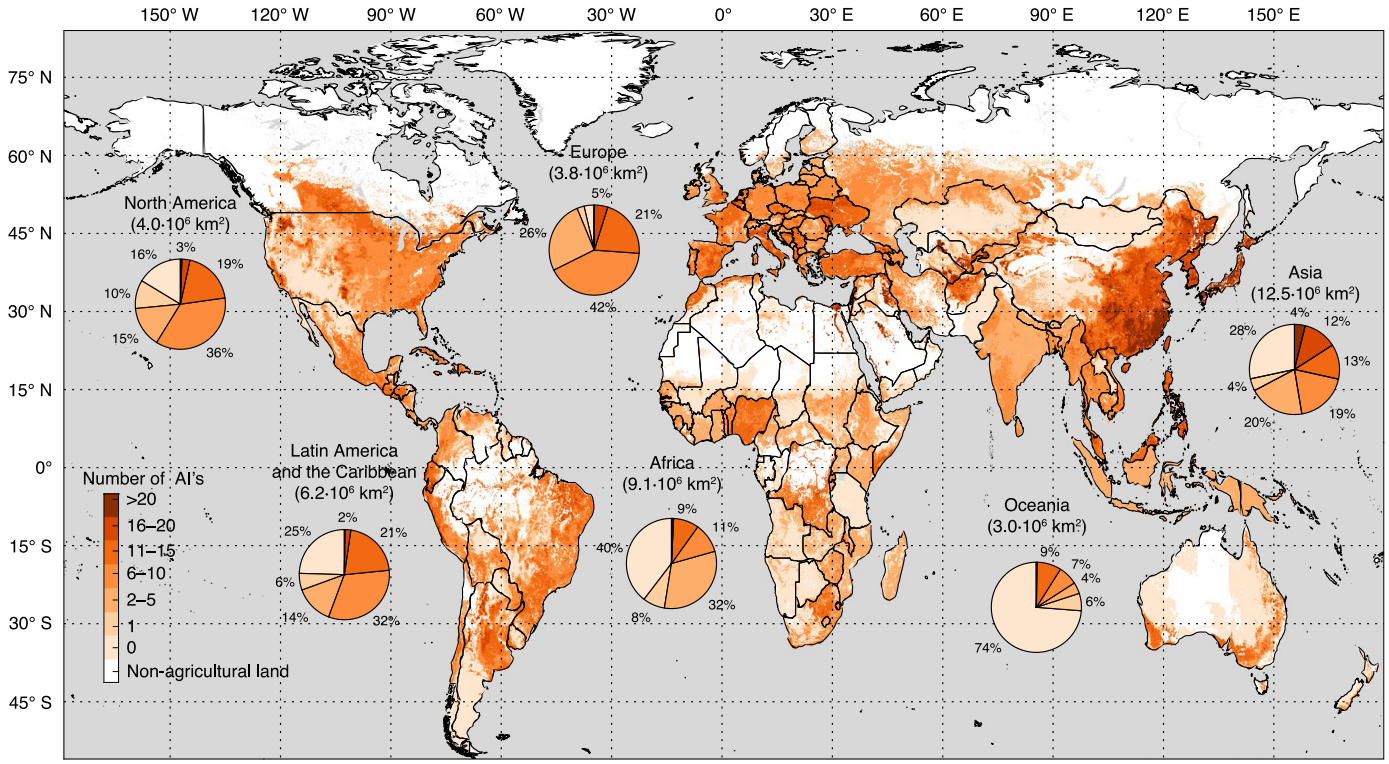

Figure 1 – Global map of the number of active ingredients (AI) posing a risk to the environment. Based on the 92 most used AI's (of which 59 are classified as herbicides, 21 as insecticides and 19 as fungicides). Synergism between AIs is excluded. The map has a spatial resolution of 5 arcmin (±10km^2 at the equator). The pie charts represent the fraction of agricultural land contaminated by different amounts of AI's in each continent. The values in parentheses above the pie charts denote the total agricultural land in that continent. Adapted from Tang *et al.* [**16**].

Dichlorophenoxyacetic Acid (±150,000 tonnes); the insecticides metam potassium, metam and calcium polysulfide (±50,000 tonnes) and chlorpyrifos (±20,000 tonnes); and the fungicides metam potassium and petroleum oil (±150,000 tonnes), and chlorothalonil (±120,000 tonnes). Many of these compound/ pesticide groups have demonstrated significant multitarget/ off-target toxicity, resulting in potentially serious ecotoxicological outcomes [20-29]. According to the global trends in annual pesticide usage, it is expected that the application of pesticides will begin to plateau after an expected 8 years, assuming no phase-out initiatives are active [30]. This prediction that agrochemicals are continued to be used for many years to come, therefore also continuing as a significant stressor on the environment, is by some hypothesised to be one of the most significant contributors to the current mass extinction and subsequent biodiversity loss [9,31,32] and is disputed to be frequently underestimated [33]. With this in mind, this report aims to explore, evaluate and clarify the current understandings of the effects of the utilisation of pesticides on the ecology and biodiversity and what constraints the field may have.

2. Pesticide (A-)Specificity

As mentioned before in the introduction, millions of metric tonnes of pesticides are utilised annually by the agricultural sector [19]. A large percentage of the agrochemicals are applied through 'crop-spraying' and/ or the, often disputed [34,35], practice of seed-coating. But, the most overlooked group of pesticides that also pose a significant environmental risk are the pesticides for the treatment of endoparasites in livestock. All these different types of pesticide application led to unwanted dispersal causing exposure to off-target organisms as a side effect. Due to the vast number of different agrochemical compounds being utilised throughout the world, only a handful of the most used AI's or classes of AI's will be explained in further detail, merely to explain and clarify the concept of pesticide specificity and mode of actions.

2.1 Active Ingredients

Besides just the inevitable exposure to taxonomically related off-target organisms, the toxicological effects the AI's induce in target organisms are also found in taxonomically non-related off-target organisms. The simplest example of this is found in the adverse effect of the utilisation of fungicides, bactericides and alike on beneficial soil microbes [36]. As with many synthetic pesticides, the toxicodynamics is not only limited to specific taxa and is more often than not effective among all of the related taxa the compound is designed for. The broadly used fungicides of the (tri)azole group for instance are effective against all yeasts and moulds and, due to the high similarities of the targeted enzymes and metabolic pathways, also to some Protozoa [37,38], and in some rare cases animals and plants [39]. This is due to the inhibition of the enzymes belonging to the cytochrome P450 monooxygenase (CYP) superfamily, among which the main targeted fungal sterol 14-demethylases (CYP51). In fungi and protozoa, CYP51 is responsible for the anabolism of ergosterol, which serves a similar function as cholesterol does in animal cells. Impairment of this biochemical pathway leads to significant membrane destabilisation and, eventually, cell lysis.

Due to the ability to target many other taxa besides the ones that the application is aimed at, soil microorganism abundance and diversity are drastically affected. A practical study conducted by Onwona-Kwakye *et al.* [40] quantified this phenomenon in irrigated rice fields. Although it is debatable that deciding to use irrigated rice fields, a small niche of all of the arable land which is also accompanied by its characteristic biodiversity, as a study field, the general concept and findings of the study are expected and applicable to any form of arable land. Soil samples in a 4-hectare study area, known to be affected by pesticide contamination, were collected. Samples originated from a water source upstream (unexposed), a pesticide-contaminated rice field (exposed), and an area downstream of the irrigation line (residual). Bacteria were cultured under aerobic and anaerobic conditions from which the cultures were later used for 16S rRNA sequencing. Simpson's and Shannon's diversity index tests were conducted which both indicated a significant decline in the bacterial diversity in the exposed soil samples (**Figure 2**). Although not statistically significant, a decline in bacterial diversity from the residual exposure samples when compared to the unexposed can also be observed.

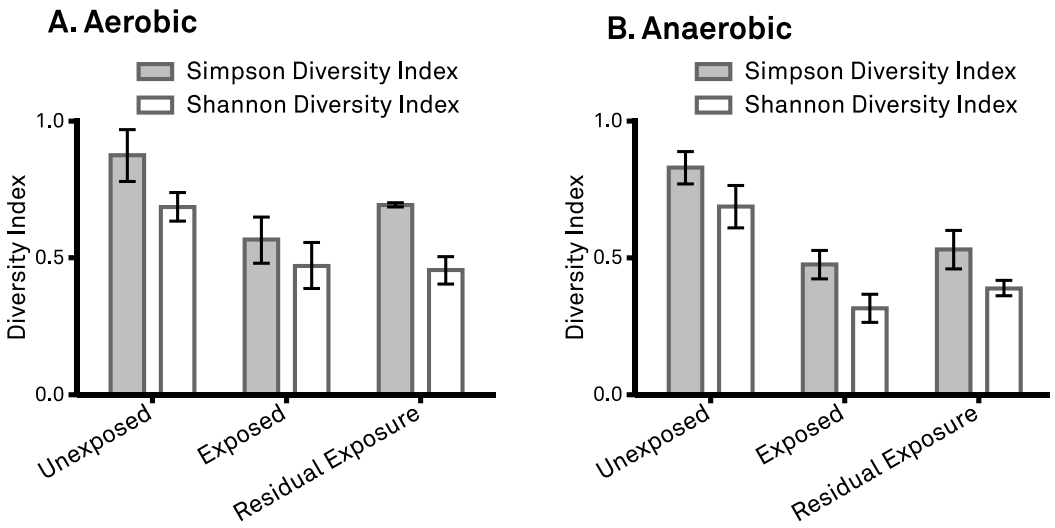

Figure 2 – The Simpson and Shannon bacterial diversity indices of pesticide-treated irrigated soil samples. Pesticides-treated irrigated soil samples were collected from the unexposed, pesticide-exposed, and residual exposure areas and incubated for 24 h under aerobic (**A**) and anaerobic (**B**) conditions. DNA was extracted and analysed by 16S rRNA sequencing. **B:** In the anaerobic samples, a two-sample t-test showed that the mean Simpson and Shannon diversity indices were significantly different between the pesticide-exposed and unexposed areas ($p = 3{\cdot}10^{-5}$ and $p = 5{\cdot}10^{-5}$, respectively). **A:** In the aerobic samples, the mean Simpson and Shannon diversity indices were also significantly different between the pesticide-exposed and unexposed areas ($p = 1{\cdot}10^{-3}$and $p = 3{\cdot}10^{-3}$, respectively). The error bars represent the upper and lower bounds of the indices of the replicate samples from each exposure group. Adapted from Onwona-Kwakye *et al.* [**40**].

As many microorganisms have similar (analogous), if not identical metabolic pathways, it is indeed expected that many AI's targeting those specific pathways are effective against these organisms as well. Another phenomenon is for a compound to affect unrelated taxa in their unique toxicological manner. The dithiocarbamate pesticide class, compounds analogous to carbamates, have demonstrated to do so. Dithiocarbamates are generally complex molecules harnessing the dithiocarbamate functional group. Although carbamates are chemically related, they have entirely different toxicological effects. Carbamates are mainly utilised as an insecticide and act, non-covalently, on (inhibiting) the enzyme acetylcholinesterase, leading to an over-stimulation at the neuronal synapse due to an excess of acetylcholine (cholinergic poisoning). Dithiocarbamate, on the other hand, is designed as a fungicide. Dithiocarbamates are hydrolysed in aqueous solutions which release ethylene bisisothiocyanate sulphide (EBIS), which in turn acts as a thiol inhibitor that inactivates sulfhydryl groups, leading to incorrectly folded proteins and membrane instability [41]. Even though the rate of decomposition of dithiocarbamates due to hydrolysation or photodegradation is rather quick, residues can, under certain conditions, still seep into the environment. In animals, dithiocarbamates interfere with the synthesis of the neurotransmitter catecholamine by inhibiting the conversion of dopamine to epinephrine and norepinephrine catalysed by the enzyme dopamine-β-hydroxylase (endocrine disruptor) [42]. Catecholamines are the group of hormones excreted in responses to stress, thus playing a big part in the 'fight-or-flight' response of an animal. Impairment in this biochemical process results in significantly abnormal behaviour and so the ability to survive [43]. The metabolite ethylene thiourea has also been recognised to be a cause of the impairment in fertility and reproduction and as a carcinogen [42,44,45].

In addition to impacting related off-target taxa, AI's meant for higher organisms generally pose a significantly greater risk to many more taxa, although more phylogenetically related. A perfect example of this is the AI in the most utilised herbicide Roundup, glyphosate. As a herbicide, glyphosates are designed as a structural analogue for the amino acid glycine and also have a competitive inhibiting effect on the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) by acting as a transition state analogue [46,47]. The incorporation of glyphosates in polypeptides leads to a stiffer protein backbone, resulting in incorrectly folded or completely denatured proteins. Glyphosate substitution for conserved glycines has been linked to many disease symptoms, among which adrenal insufficiency, ALS, Alzheimer's disease, COPD, glaucoma, hypothyroidism, lupus, mitochondrial disease, non-Hodgkin's lymphoma, Parkinson's disease, prion disease, and osteoporosis [48]. Although heavily debated in scientific literature, it must be noted that all the risk and toxicity assessments on glyphosates have been conducted in the context of humans [49-51].

The EPSP enzyme is essential for the creation of aromatic amino acids by being part of the shikimate pathway [52]. Due to the absence of the shikimate pathway in animal cells, it is considered a safe AI to use. However, several practical studies both agree and disagree with the claims of glyphosates to be toxic to animals, predominantly taxa of the family Apidae (bees and bumblebees) [10,53-57]. Aside from the direct toxicity of the glyphosates, the metabolite aminomethylphosphonic acid (AMPA) has also been proven to induce toxicological effects on animals [58-60], contradicting the claims of quick riddance of glyphosate toxicity when it is in contact with soil. Although, through practical experiments in *Daphnia magna*, AMPA indicated low chronic toxicity, while in the larval stage of the fish *Pimephales promelas* [60] and additionally in the embryonic stages of the toad *Bufo spinosus,* genotoxic effects have been observed at environmentally documented levels [59-62].

Another fiercely debated class of AI's are those acting on the central nervous system, which mainly are the carbamates, neonicotinoids, organochlorides, organophosphates and pyrethroids. As previously discussed, carbamates (and also organophosphates) act by non-covalent inhibition of the enzyme acetylcholinesterase, leading to cholinergic poisoning [63]. Neonicotinoids also lead to neuronal overstimulation, but through interference with a different pathway. They bind with high affinity and specificity to the nicotinic acetylcholine receptors (nAChRs), which are a type of ion-channel, leading to overstimulation of the neuron by the uncontrolled influx of cations [64]. Blockages of nAChRs are usually resolved by the enzyme acetylcholinesterase. But, as the binding of some neonicotinoids to the nAChRs is irreversible [65,66], acetylcholinesterase is unable to resolve the blockage, causing paralysis and death [64]. Therefore, neonicotinoids are exceptionally effective as an insecticide as nAChRs in an insect are only found in the central nervous system. Organochlorides, like neonicotinoids, also affect an ion-channel protein. Organochlorides can be categorised into two classes (DDT-type and chlorinated alicyclics) depending on the mode of action. DDT-type organochlorides, like the pyrethroids, act on the voltage-gated sodium channels by preventing gate closure after activation. This leads to sodium ions leaking through the channel protein creating a destabilising negative afterpotential with hyperexcitability of the nerve. This leakage causes repeated discharges in the neuron either spontaneously or after a single stimulus [67]. Chlorinated alicyclic-type organochlorides act at the GABA(A) chloride ionophore complex, inhibiting chloride flow into the nerve [68]. Exposure to chlorinated alicyclic-type

organochlorides may lead to a depressed activity of the central nervous system, followed by neuronal hyperexcitability, tremors, and seizures.

As of February 2020, the EU has banned the use of the four most used neonicotinoids clothianidin, imidacloprid, thiacloprid and thiamethoxam for all outdoor use. Ironically, many more neonicotinoid compounds are available for utilisation besides the 4 banned, among which are compounds with similar toxicological effects. A good example of this is the N-cyanoamidines neonicotinoid acetamiprid. which, together with thiacloprid, are proven to have a lower affinity to insect nAChRs. This is due to the cyano functional group, which induces a lower affinity when compared to the nitro functional group N-nitroguanidine neonicotinoids [69]. According to Article 53 regarding the ban, member states are temporarily authorised (up to 120 days) to use the otherwise banned compounds in case of an emergency derogation. As of writing, already more than 235 emergency derogations have been 'granted', with many of which a questionable reasoning [70]. This leads to the suspicion that, if genuinely desired, regulated compounds can be used at will. This comprehensively contradicts the proposition behind the restriction of these compounds. Besides that, the main argument for the restriction of those four neonicotinoids named repetitively throughout the literature is to halt the rapid decline in bee populations correlated with the use of neonicotinoids [71,72].

Even with the recent advancements in the understanding of the adverse effects of many of the neurotoxic AI's, they are still frequently widely used throughout the globe. Numerous studies conclude that the utilisation of many neurotoxic pesticides has detrimental effects on all animals, many of which influence behavioural aspects like circadian rhythm, memory disruption, locomotion, sleep, spatial orientation and more [71-74]. Although many of the neurotoxic AI's currently in use have a higher affinity for insect receptors [75], a multitude of studies has proven that environmental doses can already have detrimental effects on off-target organisms besides insects [63]. One well-known example is the eggshell thinning and reproductive impairments found in some bird species caused by the metabolites of the DDT-type organochloride neurotoxins (DDE and DDD) [68,76,77]. Another study by Molina *et al.* [78] demonstrated a lower leukocyte count in deer mice (*Peromyscus maniculatus*) and cotton rats (*Sigmodon hispidus*) chronically exposed to environmental levels of DDT and its metabolites by acting as a mediator of oxidative damage. The synergistic effects of these neurotoxic compounds are, however, poorly documented. Besides the few studies on the western honey bee *Apis* mellifera (or any other member outside of the family Apidae for that matter), or the standard fruit fly laboratory model organism *Drosophila melanogaster*, not much is known. Studies of synergistic effects may hold the potential to give new insights into the ecotoxicological effects of many of these neurotoxins.

2.2 Co-formulants

One significant aspect frequently overlooked in pesticide risk assessments/ research, besides AI's and the metabolites thereof, are the pesticide co-formulants. These co-formulants may include defoliants, emulsifiers, (heavy)metal ions and surfactants, and may all be classified as synergists when improving the AI's toxicity. As with section '2.1 Active Ingredients', only a handful of the most used/ well-studied compounds will be discussed since there are hundreds of compounds in active usage and a few dozens of classes.

Synergists may also be classified as AI by themselves, depending on the mechanism of effect. Although a synergist is usually defined as a compound used to enhance the toxicological effects of another toxin used within the pesticide formulations, synergists that are effective on their own may (also) be classified as an AI. Well-studied and widely used synergists include piperonyl butoxide (PBO) and N-Octyl bicycloheptene dicarboximide (MGK-264), used in combination with carbamate- and pyrethroid-class neurotoxic pesticides, and polyethoxylated tallow amine (POEA), commonly used as a wetting agent in combination with glyphosates. Numerous studies have demonstrated that the addition of a synergist can significantly reduce, if not completely remove, resistance in target organisms [79]. As previously mentioned, some synergists can exhibit toxicological effects by themselves, among which are the non-ionic surfactant POEA. A study by EFSA [80] and later also agreed upon by Mesnage *et al.* [81] has indicated POEA to have considerably higher toxicity in animals compared to glyphosates alone, making the combination of the two all the more effective. Due to the chemical nature of many of the synergists classified as 'wetting agents' (surfactants and emulsifiers), endocrine disrupting and genotoxic effects are commonly observed in higher organisms [82-86].

The combination of pesticide AI's, other co-formulants, and heavy metals can result in a variety of effects depending on the metal and synergistic compound (**Table 1**) [87]. Of the heavy metals commonly found in pesticide formulations, arsenic, cadmium, mercury, and lead are the most significant due to their observed pathophysiological effects when bioaccumulated. When exposed to these heavy metals, molecular complexes are formed between the metal and cellular compounds containing sulphur, oxygen, and/ or nitrogen [88-90], which may result in tissue necrosis [91]. Heavy metals are known to affect reproductive systems, nervous systems, gastrointestinal tracts, and mucous tissues [91,92]. Although the mechanism of effect is not

Table 1 – The synergistic effects of heavy metals and their synergists in pesticide formulations. Adapted from Singh *et al.* [**87**].

Metal	Synergist	Effect	Reference
Cadmium	Ethanol	Elevation of norepinephrinein in the hypothalamus and midbrain	[93]
	Dimethoate	Affects relative body weight gain and relative liver weight	[94]
	Propoxur	Alters immuno- and neurotoxicological functions	[95]
	Diazinon	Notable loss of spermatogenic elements, disorganization and seminiferous epithelium and lacking maturation of germs cells	[96,96]
Lead	Dimethoate	Affects relative body weight gain, relative liver weight, relative thymus weight and the mean corpuscular volume value	[94]
	Arsenic/ Mercury	Alteration in central monoaminergic system neurotoxicity and cytotoxicity	[98]
Mercury	Dimethoate	Alteration in body weight gain, relative liver and kidney weights and in IgM-plaque-forming cells	[94]
Arsenic	Dimethoate	Change in relative liver weight mean corpuscular volume and IgM-plaque-forming cell content of the spleen	[94]

exactly known, it is speculated that heavy metals induce the formation of radical oxygen species (ROS) that, together with the pesticide AI as a synergist, lead to oxidative stress [91,99,100]. Besides that, it has also been reported that cadmium and lead can inhibit acetylcholinesterase in the blood [101] and brain [92], respectively. Therefore, the combination of heavy metal co-formulants in neurotoxic pesticide formulations can achieve remarkable toxicological efficiency.

In 'modern day' pesticides, meant for private use and aiming to reduce or eliminate the main AI('s) within the formulation, the concentration of co-formulants has been documented to frequently exceed the legal limits. This is probably to compensate for the lost effect of the AI attempting to 'replace'. A study by Seralini *et al*. [102] has reported that the concentration of heavy metals is often found to be within the mg/ml (several hundred to a thousand times the chronic toxicity level), and polycyclic aromatic hydrocarbons (often associated with their carcinogenic properties) in the tens to sometimes hundreds of µg/ml. Besides polycyclic aromatic hydrocarbons being well-established carcinogens, it is known that their decomposition product/ metabolites also pose significant toxicity [102].

2.3 Abiotic Effectors

Interestingly enough, specific abiotic factors also seem to play a role in the significance of toxicity in certain pesticide components. This significance is mainly contributed by the specific mechanism of effect being impaired by the AI's and/ or synergists. A review by Gomes and Juneau [103] explored this phenomenon in algae. Summarised; the effect of how temperature and certain herbicides affect growth/ the ability to survive tends to be species-specific. For example, the EC_{50} of the herbicide atrazine for the cyanobacteria *Oscillatoria limneltica* is 24.2µg/L at 13°C and 52.3µg/L at 20°C [104], while for the microalga *Raphidocelis subcapitata* it is 20.5µg/L at 15°C and 45.6µg/L at 20°C [105]. As the herbicide affects the Q_b-binding site on the D1 protein of photosystem II by inhibition, impairment results in the formation of ROS ultimately inducing oxidative bursts that damage lipids, pigments and proteins [106]. This implies that, when photosynthetic organisms are exposed to atrazine (or any other AI acting on a photoinduced electron transport chain), toxicity increases with light exposure [103].

Temperature, for that matter, can also play a role as an additional stressor, thus resulting in varying toxicity. It is known that organophosphates exhibit elevated levels of toxicity in invertebrates at a higher temperature, whereas pyrethroids have elevated levels of toxicity at lower temperatures [107-109]. Furthermore, Willming *et al.* [110] have demonstrated that the natural fluctuation in temperature on a day indeed does affect the toxicity of pesticides to aquatic invertebrates. Survival of the midge larvae *Chironomus dilutes* exposed to bifenthrin (a pyrethroid) was significantly reduced under temperature-fluctuating conditions than when compared to a constant temperature. The increase of toxicity under temperature-fluctuating conditions was also observed on a molecular level in *Daphnia magna* exposed to malathion (an organophosphate) by an increase of cholinesterase activity.

3. Environmental dispersal

The most significant aspect of an ecological risk assessment concerning the effects of pesticides on the ecology is, undoubtedly, the environmental dispersal and subsequent bioaccumulation. Considering the many associated toxicological effects, it is of great concern that only 1% of the utilised pesticides reach their target, while the remaining 99% are 'released' into the global environment [111,112]. Many different mechanisms of action are recognised and explored in literature, which can be separated into two classes; abiotic- and biotic factors. Abiotic factors include distribution through chemical properties and/ or through wind and/ or water erosion. This includes the commonly described 'primary and secondary pesticide movement', dispersal at the time of application, and dispersal through any other mechanism after the application, respectively [113]. Biotic factors may include distribution through, possibly already pesticide bioaccumulated, organisms themselves.

3.1 Abiotic Factors

Of all of the abiotic factors, the aspect most significant for the distribution of pesticides through the environment, besides over-spraying and wind distribution on pesticide aerosols during the application, is due to the chemical properties of the various compounds within the formulation. The volatility of the compound(s) and/ or the pesticide being classified as a surface pesticide (contrasting to systemic pesticides) are the main aspects identified [114]. As Damalas *et al.* [115] unveiled, nearly 80-90% of the pesticide formulation is volatilized into the environment within a few hours of the application. However, it is far from the truth to conclude that the 10-20% non-volatile partition consists of the AI's. Many of the compounds within the volatile partition have a naturally higher vapour pressure, like ethanol or many of the polycyclic aromatic hydrocarbons like the benzene or naphthalene derivatives [116]. Additionally, co-formulants and/ or spray adjuvants can also alter the volatility of the pesticide formulation, as demonstrated by Dan and Hageman [117], generally resulting in higher volatility with the addition of adjuvants. The volatilisation of these compounds leads to further dispersal through (predominantly) the atmospheric parts of the hydrological cycle, resulting in the potential dispersal over vast distances [113].

Airborne pesticide particle dispersal is dependent on the thickness of the surface boundary layer (the lowest region of the troposphere) [118], which can vary greatly throughout the day. A taller surface boundary layer, most common in the daytime often reaching a few kilometres in height, provides a greater opportunity for dispersion and subsequent dilution [113,119], thus reducing the dose and subsequent toxicity upon precipitation. Atmospheric turbulence, ground cover, topography, and nearby bodies of water all influence the height of the surface boundary layer. Conditions promoting the dispersal and precipitation of high doses of pesticides can induce noticeable ecotoxicological damage. As uncovered by Nelemans *et al*. [120], herbicide drift can significantly influence the production of biomass, plant coverage and seed germination in numerous plant species. They further presumed that this effect can lead to species composition shifts and vegetation capacity in adjacent off-target areas.

However, far from the volatilized 80-90% of the applied formulation remains stable from their initial dispersal through to the eventual precipitation. The stability of the compounds through this journey will be affected by photodegradation/ -bleaching, oxidation, hydrolysis, and half-life degradation [121,122]. These forms of degradation are considered to be the main form of 'natural' degradation [121], followed by metabolic degradation by microorganisms. The compounds which are resilient against these forms of degradation pose an undeniably greater risk to the environment [123]. One way to combat this issue is a direct ground injection and/ or using seed coatings, as many of these forms of degradation are enhanced through air- and/ or UV-exposure.

Direct ground injection of pesticide formulations and/ or using seed coatings has frequently been proven to be detrimental to soil microorganisms [36,40]. However, general erosion by rain and wind forces pesticide residues further and deeper into the ground, eventually leaching and contaminating groundwater, flowing water, and eventually estuaries and adjoining seas and oceans. Tang *et al.* [16] have computed global pesticide risk scores (RS) based on the 92 most utilised AI on 4 environmental 'compartments' (soil, surface water, groundwater and atmosphere), visualised in **Figure 3**. However, due to the current lack of (ecotoxicological) data on the synergism of many pesticide formulations (and individual compounds), synergism is not taken into account. As it is known that formulations are much

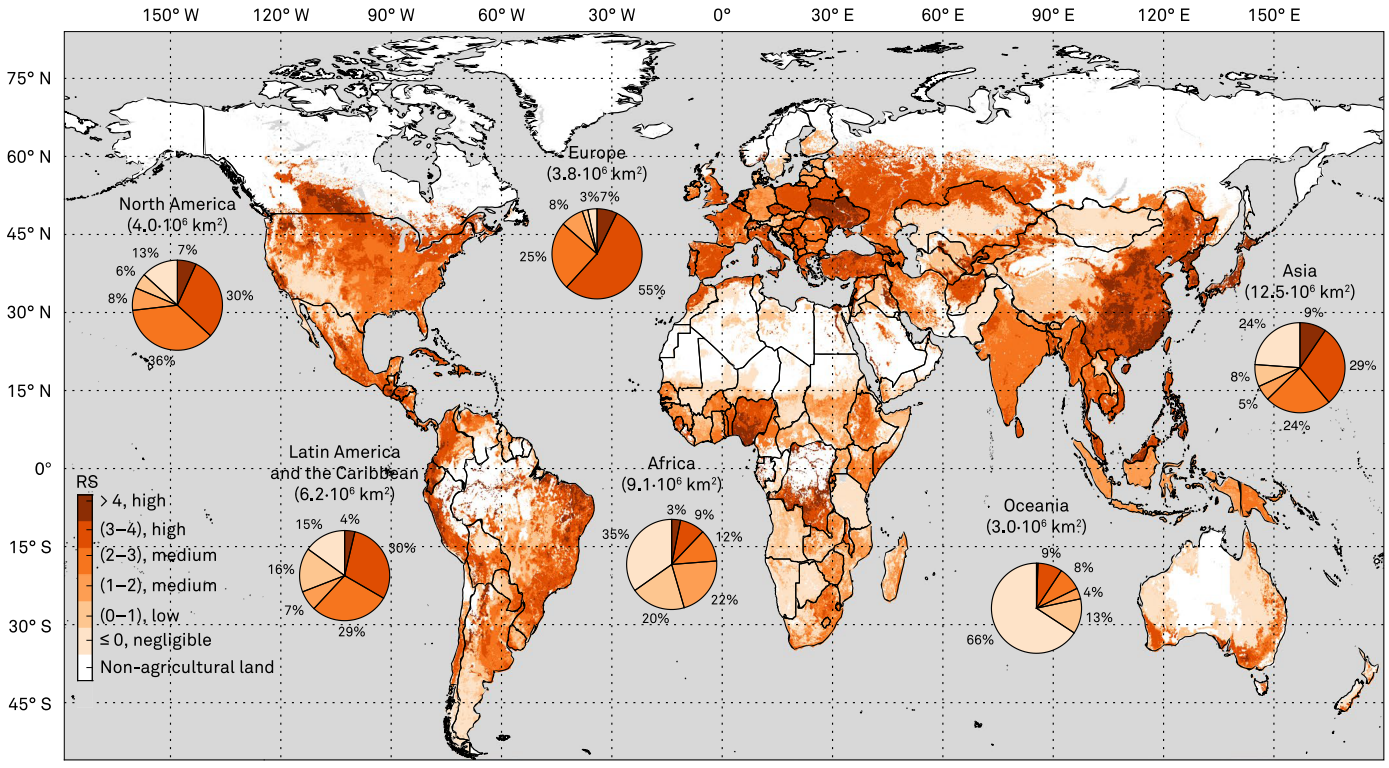

Figure 3 – Global map of pesticide risk score. Based on the 92 most used AI's (of which 59 are classified as herbicides, 21 as insecticides and 19 as fungicides). Synergism between AI's is excluded. The map has a spatial resolution of 5 arcmin (±10km² at the equator). The pie charts represent the fraction of agricultural land classed under different RS in each region, and the values in parentheses above the pie charts denote the total agricultural land in that region. RS was determined as the ratio between the predicted environmental concentration and the predicted no-effect concentration derived from each AI's ecotoxicities. The 'risk point' of each environmental compartment (soil, surface water, groundwater and atmosphere) was then evaluated as the log-transformed sum of all risk quotients. Finally, the overall RS in a grid cell was calculated as the maximum risk point across the four environmental compartments. Adapted from Tang *et al.* **[16]**.

more toxic than their declared active principles in many marine and terrestrial ecosystems [124,125], factual data can be expected to have a much greater RS [123].

3.2 Biotic Factors

Biotic pesticide distribution, often associated with the concepts of bioaccumulation, -magnification and -concentration (**Figure 5**), can confront organisms otherwise less affected with much higher doses than solely through abiotic pesticide distribution factors. The majority of organisms in the lower trophic levels are frequently (in some cases chronically) exposed to no effect concentration (NEC) doses, while the upper trophic levels (primarily consisting of predatory heterotrophs) are exposed to the more severe/ lethal doses. However, through the different methods of utilisation, direct biomagnification in higher trophic levels is possible, as demonstrated in **Figure 4**. Bioaccumulation, -magnification and -concentration is highly dependent on the stability of the compound, with many synergists and DDT and derivatives being more stable and thus having enhanced accumulation, unlike lesser stable molecules like carbamates and glyphosates.

Figure 4 – Example of atypical trophic bioaccumulation. Metaldehyde, a since the 30th of June 2019 in the UK sale-banned molluscicide, consumed by a red-legged partridge (*Alectoris rufa*). Image © Rob, 2021 **[156]**.

Great examples of pesticide bioaccumulation and -magnification in literature are in regards to the countless marine studies. An estimate has put the total amount of leached pesticide (based on ametryn, atrazine, diuron, hexazinone, simazine and tebuthiuron) through erosion into the great barrier reef world heritage site alone at 34 metric tonnes annually [124,125], which has a significant detrimental effect on all aspects of the reef/ marine ecology. When compared to the general RS of North-East Australia in **Figure 3** (taking the different erosion mechanisms into account), much greater values are expected in, for example, South-East Asia and Europe. Even though the (bio)concentration of many compounds is detected in sub-lethal/ NEC concentrations, due to the chemical nature of many of these compounds (many of which are lipophilic), chronic exposure and bioaccumulation are inevitable. Monitoring these low-concentration compounds is crucial for understanding the environmental RS. As Bayo *et al.* [126] have stated (in the context of neonicotinoids); if initial assessments consider the concentrations to be harmless to (aquatic) organisms, it may lead to a relaxation of monitoring efforts, resulting in the worldwide contamination of many (aquatic) ecosystems, including the deepest and most isolated parts of the ocean [127]. To combat this problem, two studies by Villegas *et al.* [128] and Righi *et al.* [129] used fiddler crabs *Leptuca festae* and *Minuca ecuadoriensis*, and swimming crabs *Callinectes ornatus* and *C. bocourti* as pesticide contaminants bioindicators, respectively. Both demonstrated that biomarkers stemming from pollutant accumulation within the hepatopancreas were indicated to be a reliable method for pollutant/ pesticide monitoring. Another vast group of organisms frequently exposed to chronic

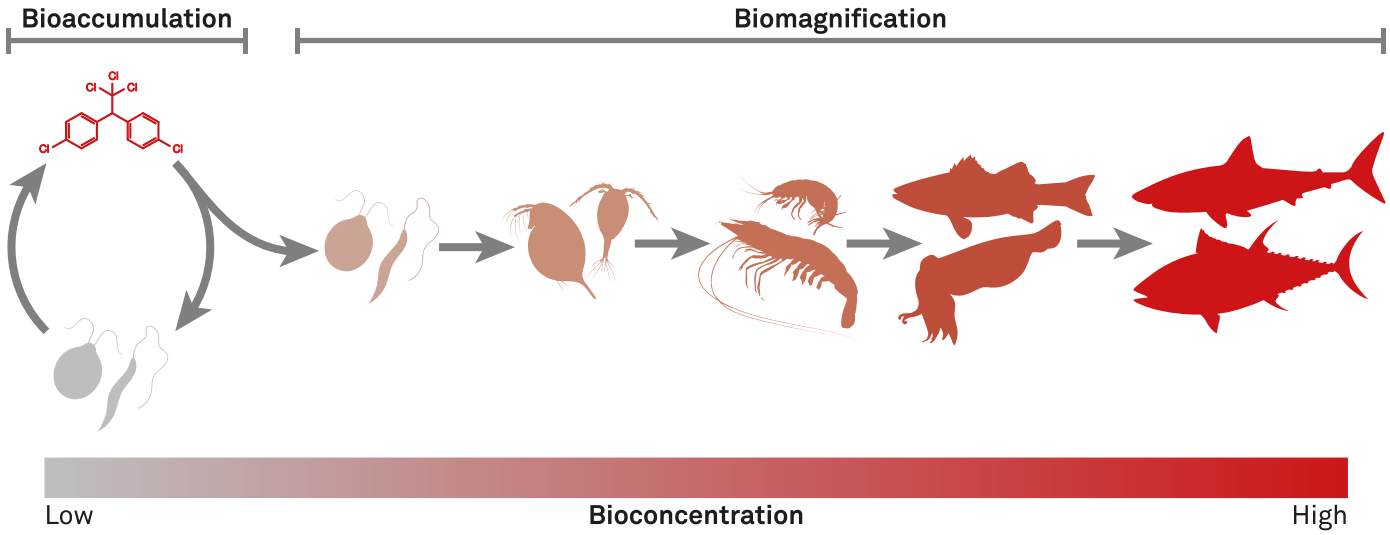

Figure 5 – Graphical visualisation of the concept of bioaccumulation, -magnification, and -concentration. Trophic accumulation is graphically visualised with DDT and the marine ecosystem as example. It must be noted that bioaccumulation and the start of -magnification are possible through any of the trophic levels and that the general concept is possible in any ecosystem.

NEC doses are the corals and aquatic vegetation. The corals in particular are most at risk, as the rising sea temperature and increase in pollutant concentration put tremendous stress on these organisms [130,131]. Up to a point, both the host organism and photosynthesising symbiont cannot compensate for the induced stressors, resulting in excessive production of ROS, oxidative stress, and eventually coral bleaching [132,133]. As corals are considered key habitat-forming species, the decline of these species and subsequent habitat have depreciated returns throughout the entire oceanic ecosystem.

Terrestrial bioaccumulation, -magnification, and -concentration experience similar mechanisms to that of the aquatic counterpart, with the first typical trophic levels being the soil microorganisms, closely followed by detritivores like earthworms, woodlice and slugs/snails. However, as in any ecosystem, any trophic level can receive the primary exposure, although bioaccumulation is primarily induced in the first typical trophic levels and smaller organisms in subsequent trophic levels. Therefore, these lower-level organisms make for suitable candidates in the biomonitoring for pesticide residues, as frequently demonstrated with earthworm tissue [58,134,135]. The fat bodies in arthropods can also be utilised for biomonitoring, as fat body mass is dependent on both environmental and physiological conditions [136-138]. Additionally, insect fat bodies can be directly correlated to the immunocompetence of the animal [139], and due to the nature of the tissue, bioindicators or direct accumulation can also assist in biomonitoring [140].

Although favourable in biomonitoring, bioaccumulation within the fat bodies of arthropods can certainly exceed non-lethal bioconcentrations, as observed in *A. mellifera* and the use of neonicotinoids. This phenomenon can lead to enhanced biomagnification and possible lethality in organisms of higher trophic levels. Besides only functioning as an energy storage tissue, adipose tissue in many animals is responsible for the storage of a variety of (lipophilic) molecules. Biomagnification will increase the toxicant accumulation within the adipose tissue resulting in all the consequences this entails [141]. As demonstrated in wild Nile crocodiles (*Crocodylus niloticus*) by Humphries *et al*. [142], toxicant accumulation in adipose tissue is offloaded after egg creation and oviposition. Not only does this result in direct

contamination of the new generation, but all adverse effects of (chronic) exposure to the toxins during the development and subsequent further biomagnification also need to be taken into account.

Another biotic mechanism of pesticide accumulation and distribution, much overlooked in the literature, is through general excretion (mainly defecation). Besides the accumulation of the predominantly beforehand mentioned agrochemicals through, for example, consumption, antiparasitic drugs administered to livestock and pets can be as detrimental to the environment. As faeces are an invaluable nutrient source to many detritivorous arthropods, long-term chronic contamination can significantly impact this niche of essential arthropods. Faeces contaminated with pesticides have been demonstrated to slow down or, in specific species, completely halt the development of many insect larvae [143]. As a consequence of the improper digestion and burial of the faeces by the insects, 'life reduced' toxin-hotspots are consequently created, promoting an increase in the production of greenhouse gasses like methane and nitrous oxide [144].

Although widely recognised in aquatic ecosystems, direct toxin absorption and accumulation through the skin in terrestrial animals is frequently overlooked. Thin-skinned animals, or with a relatively high ratio of surface area to body mass, migrating through pesticide-contaminated areas can receive as great a dose as through typical bioaccumulation and -magnification. Soft-bodied invertebrates and amphibians are the major groups at risk. As an example, glyphosates penetrated the skin of the edible frog (*Pelophylax kl. esculentus*) 26 times faster when compared to pigskin [145]. As briefly mentioned in the prior chapter, pesticide residue exposure to these hypersensitive animals can result in the formation of deformities during any part of the development, underdevelopment of embryonic stages and genotoxicity [59,61,62]. A field study conducted by Berger *et al.* [146] assessed the regional migration patterns of the Great crested newt (*Triturus cristatus*) and Fire-bellied toad (*Bombina bombina*) in correlation to the application of glyphosates over 20 years. Their results reviled a high temporal coincidence in the change of migration patterns and the application of glyphosates.

4. Discussion

The research progress on the infamous topic of pesticide ecotoxicology is currently being outpaced by the (excessive) utilisation of pesticides accompanied by the many adverse effects on the ecology and biodiversity [33]. Bioaccumulation and -magnification, together with the persistence of habitat destruction/fragmentation, will result in enhanced genetic-erosion of species' population, with 16.5% of vertebrate pollinator species (30% for island species) now threatened with extinction [147]. Although a lot has been uncovered in the last couple of years, not nearly enough is yet known to fully grasp and understand the ever-worsening situation and the subsequent future effects it may unfold [11,15,148]. Most practical research findings of last years on this subject have focussed on the effects of only a handful of compounds (in most cases around 1-5), usually not considering synergism, on a handful of taxa. Furthermore, most studies are short-term, thus creating knowledge gaps concerning long-term effects on species populations and ecosystems [126]. This is due to limiting the scale of the research down to a realistic level. However, although the collective accumulation of findings and data seems impressive at first glance, problems can be found rather quickly.

The lack of taxa diversity is an apparent problem, which is obviously of unquestionable importance. As many of the toxicological research findings concerning individual species will be uploaded to the comprehensive knowledge base ECOTOX, thus representing literature, a quick browse will quickly indicate signs of this issue. As an example, although important in modern western agriculture, the insect biodiversity of the natural world consists of more than *A. mellifera*. Therefore, despite hypothesised similarities in the target enzymes of related organisms, toxicological data of significantly more organisms need to be acquired to sketch a greater image concerning the different induced effects on specific organisms.

Another great issue with pesticide data availability is consistency and standardisation (or lack thereof). This is regarding both determinations of taxa-specific toxicological concentrations and monitoring of environmental distribution. As numerously discussed within the literature, controlled laboratory tests are significantly different to that of field studies, almost to a point that some suggest a direct comparison of the two is considered a bold move. A probable solution to this controversy is the design of practical methods in controlled environments closely simulating field studies. However, two problems will arise with the first one being the over- or under-complication of the designed protocol. Introducing too many parameters will lead to a significant reduction in reproducibility. This in turn leads to the second problem, being the acquisition of 'unreliable' toxicological data. Besides over- or under-complicating, utilisation of (arbitrary) measurements and units can further complicate/ unintentionally deceive research outcomes, making the incorporation in downstream applications harder, if not impossible. Widespread adoption of proposed standardised protocols can be a viable solution. Examples of these are the use of rodent faeces [134] and worms [149,150] in pesticide residue monitoring.

Although a significant proportion of (taxa-specific) toxicological data is 'unavailable', extrapolation of currently available data and estimations will give, to some degree, an indication of the ecotoxicological trend. However, caution must be taken when doing so as these significantly under-supported predictions/ hypotheses will most likely have a bias

towards specific taxa and only consider a handful of AI's without synergy. Even if the predictions are intended to be utilised on taxa other than those used in the (meta)analyses. The PEST-CHEMGRIDS v1.01 by Maggi *et al.* [19] (further applied by Tang *et al.* [16]) is, so far, the only global comprehensive (RS-based) pesticide contamination meta-analysis. Apart from that, the risk assessments are constructed from limited data, and in the subsequent analysis by Tang *et al.* [16] only considering Tetrapoda as representative of biodiversity. When correlating with other (local) risk assessments in literature, together with a healthy knowledge of the (synergistic) effects of compounds found in pesticide formulations, one can quickly deduce that the situation is more ominous than ever quantified so far. RS-quantification based on all these aspects and parameters, although not impossible, will be reaching the limits of ecological meta-analyses.

Besides solely monitoring directly for pesticide residues and subsequent distribution, using next-generation biodiversity monitoring and correlating it to the limited available pesticide distribution data, it is possible to get a more direct answer regarding ecotoxicology and its effect on biodiversity. Next-generation biodiversity monitoring may include the mass deployment of remote operated artificial intelligence-driven (visual) recognition devices (DIOPSIS camera, for example) or making frequent use of DNA-metabarcoding to monitor the change in species composition within an ecosystem [28]. Although the methodologies are officially still considered to be in their infancy, both have demonstrated to be a valuable asset in both biodiversity monitoring and the correlation of the decline due to agricultural stressors and xenobiotics [28].

With many of the AI's and co-formulants being in non-lethal environmental concentrations, together with that numerous AI's exploit similar molecular mechanisms, forms of pesticide resistance are expected to eventually emerge within the lower trophic levels. Both genomic- [151,152] and transcriptomic adaptations [153] have already been discovered. However, even with increased levels of detoxification within the adapted (lower trophic) populations, increased bioconcentration due to biomagnification will always outpace, as higher trophic organisms generally have a significantly lower generation time and thus take longer to adapt.

So far, two solutions can be proposed to the pesticide ecotoxicology problem, both of which can be closely associated with one another. The first one being simply to reduce and more strictly regulate the utilisation of agrochemicals. Although simply said on paper; as said by Zaller [154], "many agronomists, and agricultural lobbyists are promoting the myth that pesticides are an essential part of modern agriculture and that their benefits will definitely outweigh any effects on the environment or human health." This influence is of such significant effect that the farmer's perception and awareness of pesticide ecotoxicology are overruled. The second (obvious) proposed solution is to work more with nature, instead of against it. This involves expanded utilisation of sustainable agricultural methods like biodynamic-, ecological-, organic-, permaculture-, and regenerative-agriculture [154]. The main focus of these types of sustainable agriculture is limited, to no use of agrochemicals and exploiting nature's mechanisms for increased yields. As concluded by Janssen and van Rijn [155], biological control through natural predation can be/ is as effective in reducing arthropod pest densities as the use of pesticides. This again demonstrates the importance of a balanced and healthy system. But with decades-long systemic overexploitation of arable fields, years of intensive management and soil acclimatisation are required to slowly return this ecological balance to

the otherwise barren arable fields. This is most predominantly due to the persistence of agrochemical residues, maintaining the disruptive effect on the microbial balance in the soil. Only after a sufficient quantity is eliminated by either microbial decomposition, chemical decomposition or physical erosion, more delicate/ sensitive microbial structures can settle. Mycorrhizal networks and natural vertical soil microorganismal gradients are an example of this, which are also frequently disturbed by the act of tilling. Having a variation in the vegetation can help with the microbiome diversity and the formation of these structures [147], emphasising the importance of mixed agricultural systems.

5. Conclusion

Taking all research outcomes, from physiology to ecosystem functioning, concerning the issue of pesticide ecotoxicology collectively; the independent conclusion of this report will not differ greatly. Considering the vast amount of pesticides being applied annually throughout the globe, without a great understanding of the adverse effects and synergism to off-targets, it becomes apparent that the ecology is under immense pressure from these xenobiotics. This should be a wake-up call to drastically remodel current risk assessments, as they have been demonstrated to be inadequate for the protection of biodiversity and ecology.

The major point of attention is the (still) limited quantity and quality of freely accessible data. Better knowledge and education concerning these chemicals, even within the scientific community, is of great necessity. The ecotoxicology of agrochemicals needs to be tested on a vastly greater variety of organisms, covering as much taxonomic diversity as possible. Preferably in field test settings and using whole formulations to explore the synergic effects. The utilisation of standardised practices and units of measure are of great value.

The second major point of attention is the inclusion of as much taxonomic diversity within pesticide risk assessment meta-analyses. Although considered a computational challenge, it is far from impossible to execute as basing a biodiversity risk assessment solely on Tetrapoda is simply inadequate and unacceptable.

The third point is the necessity of next-generation biodiversity monitoring. As the ecotoxicological effects of pesticide residues increase and expand at an unprecedented rate, the need for biodiversity data, to be subsequently correlated to pesticide residue distribution, is now more important than ever.

However, the direct solution to the issue of pesticide ecotoxicity is not just to spread awareness, test toxicodynamics and monitor species trends, but to directly reduce the vast utilisation of agrochemicals as a whole.

References

[1] D. Kostic, *Tractor spraying pesticides on vegetable field with sprayer at spring,* Djurdjevo, Bačka: Shutterstock, 2018.

[2] Eurostat, "Pesticide sales," Eurostat, 22 12 2021. [Online]. Available: https://ec.europa.eu/eurostat/databrowser/view/aei_fm_salpest09/default/table?lang=en. [Accessed 5 January 2022].

[3] E. Norén, C. Lindh, L. Rylander, A. Glynn, J. Axelsson, M. Littorin, M. Faniband, E. Larsson and C. Nielsen, "Concentrations and temporal trends in pesticide biomarkers in urine of Swedish adolescents, 2000–2017," *Journal of Exposure Science and Environmental Epidemiology,* vol. 30, no. 4, p. 756–767, 24 February 2020.

[4] E. Norén, C. Lindh, A. Glynn, L. Rylander, D. Pineda and C. Nielsen, "Temporal trends, 2000–2017, of perfluoroalkyl acid (PFAA) concentrations in serum of Swedish adolescents," *Environment International,* vol. 155, no. 106716, 1 October 2021.

[5] J. Baudry, L. Debrauwer, G. Durand, G. Limon, A. Delcambre, R. Vidal, B. Taupier-Letage, N. Druesne-Pecollo, P. Galan, S. Hercberg, D. Lairon, J.-P. Cravedi and E. Kesse-Guyot, "Urinary pesticide concentrations in French adults with low and high organic food consumption: results from the general population-based NutriNet-Santé," *Journal of Exposure Science and Environmental Epidemiology,* vol. 29, no. 3, p. 366–378, 5 September 2018.

[6] B. A. Woodcock, L. Ridding, S. N. Freeman, M. G. Pereira, D. Sleep, J. Redhead, D. Aston, N. L. Carreck, R. F. Shore, J. M. Bullock, M. S. Heard and R. F. Pywell, "Neonicotinoid residues in UK honey despite European Union moratorium," *PLOS One,* vol. 13, no. 1, p. e0189681, 3 January 2018.

[7] D. Wintermantel, J.-F. Odoux, A. Decourtye, M. Henry, F. Allier and V. Bretagnolle, "Neonicotinoid-induced mortality risk for bees foraging on oilseed rape nectar persists despite EU moratorium," *Science of The Total Environment,* vol. 704, 20 February 2020.

[8] E. A. Straw and M. J. F. Brown, "Co-formulant in a commercial fungicide product causes lethal and sub-lethal effects in bumble bees," *Scientific Reports,* vol. 11, 5 November 2021.

[9] C. A. Brühl, N. Bakanov, S. Köthe, L. Eichler, M. Sorg, T. Hörren, R. Mühlethaler, G. Meinel and G. U. C. Lehmann, "Direct pesticide exposure of insects in nature conservation areas in Germany," *Scientific Reports,* vol. 11, 16 December 2021.

[10] E. A. Straw, E. N. Carpentier and M. J. F. Brown, "Roundup causes high levels of mortality following contact exposure in bumble bees," *Journal of Applied Ecology,* vol. 58, no. 6, pp. 1167-1176, 6 April 2021.

[11] N. Donley, "The USA lags behind other agricultural nations in banning harmful pesticides," *Environmental Health,* vol. 18, no. 44, 7 June 2019.

[12] European Food Safety Authority, "Assessment of the scientific information from the Italian project "APENET" investigating effects on honeybees of coated maize seeds with some neonicotinoids and fipronil," *EFSA Journal,* vol. 10, no. 6, 27 June 2012.

[13] European Food Safety Authority, "Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin," *EFSA Journal,* vol. 11, no. 1, 16 January 2013.

[14] WWF, Living Planet Report 2020 - Bending the curve of biodiversity loss, R. E. A. Almond, M. Grooten and T. Petersen, Eds., Gland, Vaud: World Wide Fund for Nature, 2020.

[15] R. Mesnage, E. A. Straw, M. N. Antoniou, C. Benbrook, M. J. F. Brown, M.-P. Chauzat, R. Finger, D. Goulson, E. Leadbeater, A. López-Ballesteros, N. Möhring, P. Neumann, D. Stanley and J. C. Stout, "Improving pesticide-use data for the EU," *Nature Ecology & Evolution,* vol. 5, p. 1560, 14 October 2021.

[16] F. H. M. Tang, M. Lenzen, A. McBratney and F. Maggi, "Risk of pesticide pollution at the global scale," *Nature Geoscience,* vol. 14, no. 4, p. 206–210, 29 March 2021.

[17] C. Mora, D. P. Tittensor, S. Adl, A. G. B. Simpson and B. Worm, "How Many Species Are There on Earth and in the Ocean?," *PLOS Biology,* vol. 9, no. 8, p. e1001127, 23 August 2011.

[18] IUCN, "Summary Statistics," 13 November 2014. [Online]. Available: https://www.iucnredlist.org/resources/summary-statistics#Reasons%20for%20change. [Accessed 7 January 2022].

[19] F. Maggi, F. H. M. Tang, D. la Cecilia and A. McBratney, "PEST-CHEMGRIDS, global gridded maps of the top 20 crop-specific pesticide application rates from 2015 to 2025," *Scientific Data,* vol. 6, no. 1, 12 September 2019.

[20] D. Goulson, "Pesticides linked to bird declines," *Nature,* vol. 511, pp. 295-296, 17 July 2014.

[21] B. Ndakidemi, K. Mtei and P. A. Ndakidemi, "Impacts of Synthetic and Botanical Pesticides on Beneficial Insects," *Agricultural Sciences,* vol. 7, no. 6, pp. 364-372, 1 June 2016.

[22] C. A. Hallmann, M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson and H. de Kroon, "More than 75 percent decline over 27 years in total flying insect biomass in protected areas," *PLOS ONE,* vol. 12, no. 10, p. e0185809, 18 October 2017.

[23] M. Hvězdová, P. Kosubová, M. Košíková, K. E. Scherr, Z. Šimek, L. Brodský, M. Šudoma, L. Škulcová, M. Sáňka, M. Svobodová, L. Krkošková, J. Vašíčková, N. Neuwirthov, L. Bielská and J. Hofman, "Currently and recently used pesticides in Central European arable soils," *Science of the Total Environment,* Vols. 613-614, pp. 361-370, 1 February 2018.

[24] P. Uhl and C. A. Brühl, "The Impact of Pesticides on Flower-Visiting Insects: A Review with Regard to European Risk Assessment," *Environmental Toxicology and Chemistry,* vol. 38, no. 11, p. 2355–2370, 8 August 2019.

[25] C. A. Brühl and J. G. Zaller, "Biodiversity Decline as a Consequence of an Inappropriate Environmental Risk Assessment of Pesticides," *Frontiers in Environmental Science,* vol. 7, no. 177, 31 October 2019.

[26] H. C. Ito, H. Shiraishi, M. Nakagawa and N. Takamura, "Combined impact of pesticides and other environmental stressors on animal diversity in irrigation ponds," *PLOS ONE,* vol. 15, no. 7, p. e0229052, 2 July 2020.

[27] S. Ali, M. I. Ullah, A. Sajjad, Q. Shakeel and A. Hussain, "Environmental and Health Effects of Pesticide Residues," in *Sustainable Agriculture Reviews 48 Pesticide Occurrence, Analysis and Remediation Vol. 2 Analysis*, vol. 48, Inamuddin, M. I. Ahamed and E. Lichtfouse, Eds., Cham, Springer International Publishing, 2021, pp. 311-336.

[28] K. K. Beentjes, S. H. Barmentlo, E. Cieraad, M. Schilthuizen, B. B. van der Hoorn, A. G. C. L. Speksnijder and K. B. Trimbos, "Environmental DNA metabarcoding reveals comparable responses to agricultural stressors on different trophic levels of a freshwater community," *Molecular Ecology,* p. 1–14, 15 December 2021.

[29] F. Sánchez-Bayo, "Indirect Effect of Pesticides on Insects and Other Arthropods," *Toxics,* vol. 9, no. 8, 30 July 2021.

[30] FAOSTAT, "Pesticides indicators," Food and Agriculture Organization of the United Nations, 10 December 2021. [Online]. Available: https://www.fao.org/faostat/en/#data/EP/visualize. [Accessed 18 January 2022].

[31] R. Isenring, "Pesticides and the loss of biodiversity," Pesticide Action Network Europe, 2010.

[32] T. Tscharntke, Y. Clough, T. C. Wanger, L. Jackson, I. Motzke, I. Perfecto, J. Vandermeer and A. Whitbread, "Global food security, biodiversity conservation and the future of agricultural intensification," *Biological Conservation,* vol. 151, no. 1, pp. 53-59, 1 July 2012.

[33] N. Dudley, S. J. Attwood, D. Goulson, D. Jarvis, Z. P. Bharucha and J. Pretty, "How should conservationists respond to pesticides as a driver of biodiversity loss in agroecosystems?," *Biological Conservation,* vol. 209, pp. 449-453, 1 May 2017.

[34] C. Accinelli, H. K. Abbas, W. T. Shier, A. Vicari, N. S. Little, M. R. Aloise and S. Giacomini, "Degradation of microplastic seed film-coating fragments in soil," *Chemosphere,* vol. 226, pp. 645-650, July 2019.

[35] J. G. Zaller, N. König, A. Tiefenbacher, Y. Muraoka, P. Querner, A. Ratzenböck, M. Bonkowski and R. Koller, "Pesticide seed dressings can affect the activity of various soil organisms and reduce decomposition of plant material," *BMC Ecology,* vol. 16, no. 37, 17 August 2016.

[36] X.-M. Lu and P.-Z. Lu, "Response of microbial communities to pesticide residues in soil restored with Azolla imbricata," *Applied Microbiology and Biotechnology,* vol. 102, no. 1, p. 475–484, 8 November 2017.

[37] P. A. Cano, A. Islas-Jácome, Á. Rangel-Serrano, F. Anaya-Velázquez, F. Padilla-Vaca, E. Trujillo-Esquivel, P. Ponce-Noyola, A. Martínez-Richa and R. Gámez-Montaño, "In Vitro Studies of Chromone-Tetrazoles against Pathogenic Protozoa, Bacteria, and Fungi," *Molecules,* vol. 20, no. 7, pp. 12436-12449, 8 July 2015.

[38] V. W. Rabelo, T. F. Santos, L. Terra, M. V. Santana, H. C. Castro, C. R. Rodrigues and P. A. Abreu, "Targeting CYP51 for drug design by the contributions of molecular modeling," *Fundamental & Clinical Pharmacology,* vol. 31, no. 1, pp. 37-53, 3 August 2016.

[39] B. Holečková, K. Šiviková, J. Dianovský and M. Galdíková, "Effect of triazole pesticide formulation on bovine culture cells," *Journal of Environmental Science and Health, Part B,* vol. 48, no. 12, pp. 1080-1088, 5 September 2013.

[40] M. Onwona-Kwakye, K. Plants-Paris, K. Keita, J. Lee, P. J. van den Brink, J. N. Hogarh and C. Darkoh, "Pesticides Decrease Bacterial Diversity and Abundance of Irrigated Rice Fields," *Microorganisms,* vol. 8, no. 3, p. 318, 25 February 2020.

[41] T. S. Thind and D. W. Hollomon, "Thiocarbamate fungicides: reliable tools in resistance management and future outlook," *Pest Management Science,* vol. 74, no. 7, pp. 1547-1551, July 2018.

[42] S. Cecconi, R. Paro, G. Rossi and G. Macchiarelli, "The Effects of the Endocrine Disruptors Dithiocarbamates on the Mammalian Ovary with Particular Regard to Mancozeb," *Current Pharmaceutical Design,* vol. 13, no. 29, pp. 2989-3004, February 2007.

[43] J. Motiejunaite, L. Amar and E. Vidal-Petiot, "Adrenergic receptors and cardiovascular effects of catecholamines," *Annales d'Endocrinologie,* vol. 82, no. 3-4, pp. 193-197, June 2021.

[44] P. Houeto, G. Bindoula and J. R. Hoffman, "Ethylenebisdithiocarbamates and ethylenethiourea: possible human health hazards.," *Environmental Health Perspectives,* vol. 103, no. 6, p. 568–573, June 1995.

[45] F. Belpoggi, M. Soffritti, M. Guarino, L. Lambertini, D. Cevolani and C. Maltoni, "Results of long-term experimental studies on the carcinogenicity of ethylene-bis-dithiocarbamate (Mancozeb) in rats," *Annals of the New York Academy of Sciences,* vol. 982, no. 1, pp. 123-136, December 2002.

[46] E. Schönbrunn, S. Eschenburg, W. A. Shuttleworth, J. V. Schloss, N. Amrhein, J. N. S. Evans and W. Kabsch, "Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail," *Proceedings of the National Academy of Sciences,* vol. 98, no. 4, pp. 1376-1380, 13 February 2001.

[47] L. Pollegioni, E. Schonbrunn and D. Siehl, "Molecular basis of glyphosate resistance - different approaches through protein engineering," *The FEBS Journal,* vol. 278, no. 16, pp. 2753-2766, 1 August 2011.

[48] A. Samsel and S. Seneff, "Glyphosate pathways to modern diseases V: Amino acid analogue of glycine in diverse proteins," *Journal of Biological Physics and Chemistry,* vol. 16, no. 1, pp. 9-46, 31 March 2016.

[49] IARC, "IARC Monograph on the Evaluation of Carcinogenic Risks to Humans," in *Some Organophosphates Insecticides and Herbicides: Diazinon, Glyphosate, Malathion, Parathion and Tetrachorvinphos; International Agency for Research on Cancer (IARC)*, vol. 112, Lyon, 2015.

[50] K. Z. Guyton, D. Loomis, Y. Grosse, F. El Ghissassi, L. Benbrahim-Tallaa, N. Guha, C. Scoccianti, H. Mattock and K. Straif, "Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate," *The Lancet Oncology,* vol. 16, no. 5, pp. 490-491, 1 May 2015.

[51] EFSA (European Food Safety Authority), "Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate.," *EFSA Journal,* vol. 13, no. 11, p. 4302, 2015.

[52] L. Aristilde, M. L. Reed, R. A. Wilkes, T. Youngster, M. A. Kukurugya, V. Katz and C. R. S. Sasaki, "Glyphosate-Induced Specific and Widespread Perturbations in the Metabolome of Soil Pseudomonas Species," *Frontiers in Environmental Science,* vol. 5, no. 34, 20 June 2017.

[53] L. Battisti, M. Potrich, A. R. Sampaio, N. d. C. Ghisi, F. M. Costa-Maia, R. Abati, C. B. d. R. Martinez and S. H. Sofia, "Is glyphosate toxic to bees? A meta-analytical review," *Science of The Total Environment,* vol. 767, no. 145397, 1 May 2021.

[54] E. A. Straw, "Letter to the editors regarding Battisti et al (2021): is glyphosate toxic to bees? A meta-analytical review," *Science of The Total Environment,* vol. 790, no. 147556, 10 October 2021.

[55] L. Battisti, M. Potrich, A. R. Sampaio, N. d. C. Ghisi, F. M. Costa-Maia, R. Abati, C. B. d. R. Martinez and S. H. Sofia, "Response to Letter to the Editor "Is glyphosate toxic to bees? A meta-analytical review"," *Science of The Total Environment,* vol. 790, no. 147517, 10 October 2021.

[56] E. A. Straw and M. J. F. Brown, "No evidence of effects or interaction between the widely used herbicide, glyphosate, and a common parasite in bumble bees," *PeerJ,* vol. 9, no. e12486, 17 November 2021.

[57] N. Blot, L. Veillat, R. Rouzé and H. Delatte, “Glyphosate, but not its metabolite AMPA, alters the honeybee gut microbiota,” *PLOS ONE,* vol. 14, no. 4, p. e0215466, 16 April 2019.

[58] A. Domínguez, G. G. Brown, K. D. Sautter, C. M. R. de Oliveira, E. C. de Vasconcelos, C. C. Niva, M. L. C. Bartz and J. C. Bedano, “Toxicity of AMPA to the earthworm Eisenia andrei Bouché, 1972 in tropical artificial soil,” *Scientific Reports,* vol. 6, no. 1, p. 19731, 21 January 2016.

[59] M. Cheron and F. Brischoux, “Aminomethylphosphonic acid alters amphibian embryonic development at environmental concentrations,” *Environmental Research,* vol. 190, p. 109944, 1 November 2020.

[60] S. L. Levine, G. von Mérey, T. Minderhout, P. Manson and P. Sutton, “Aminomethylphosphonic acid has low chronic toxicity to Daphnia magna and Pimephales promelas,” *Environmental Toxicology and Chemistry,* vol. 34, no. 6, pp. 1382-1389, 1 June 2015.

[61] F. Baier, M. Jedinger, E. Gruber and J. G. Zaller, “Temperature-Dependence of Glyphosate-Based Herbicide's Effects on Egg and Tadpole Growth of Common Toads,” *Frontiers in Environmental Science,* vol. 4, no. 51, 24 August 2016.

[62] E. Adams, C. Leeb and C. A. Brühl, “Pesticide exposure affects reproductive capacity of common toads (Bufo bufo) in a viticultural landscape,” *Ecotoxicology,* vol. 30, no. 2, p. 213–223, 20 January 2021.

[63] J. V. Peter, T. I. Sudarsan and J. L. Moran, “Clinical features of organophosphate poisoning: A review of different classification systems and approaches,” *Indian Journal of Critical Care Medicine,* vol. 18, no. 11, pp. 735-745, November 2014.

[64] I. Yamamoto, “Nicotine to Nicotinoids: 1962 to 1997,” in *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*, 1 ed., vol. I, Tokyo, Springer-Verlag, 1999, pp. 3-27.

[65] J. Abbink, “The biochemistry of imidacloprid.,” *Pflanzenschutz-Nachrichten Bayer,* vol. 42, no. 2, pp. 183-195, 1991.

[66] H. Mehlhorn, N. Mencke and O. Hansen, “Effects of imidacloprid on adult and larval stages of the flea Ctenocephalides felis after in vivo and in vitro application: a light- and electron-microscopy study,” *Parasitology Research,* vol. 85, no. 8, p. 625–637, June 1999.

[67] J. R. Coats, “Mechanisms of toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecticides.,” *Environmental Health Perspectives,* vol. 87, pp. 255-262, 1 July 1990.

[68] C. W. Tubbs, “California condors and DDT: Examining the effects of endocrine disrupting chemicals in a critically endangered species,” *Endocrine Disruptors,* vol. 4, no. 1, 27 April 2016.

[69] T. Blacquière, G. Smagghe, C. A. M. van Gestel and V. Mommaerts, “Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment,” *Ecotoxicology,* vol. 21, no. 4, p. 973–992, 18 February 2012.

[70] European Commission, “Search for Emergency Authorisations,” European Commission, 31 January 2022. [Online]. Available: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/ppp/pppeas/screen/home. [Accessed 31 January 2022].

[71] S. Tosi, G. Burgio and J. C. Nieh, “A common neonicotinoid pesticide, thiamethoxam, impairs honey bee flight ability,” *Scientific Reports,* vol. 7, 26 April 2017.

[72] D. B. Smith, A. N. Arce, A. R. Rodrigues, P. H. Bischoff, D. Burris, F. Ahmed and R. J. Gill, “Insecticide exposure during brood or early-adult development reduces brain growth and impairs adult learning in bumblebees,” *Proceedings of the Royal Society B,* vol. 287, no. 1922, p. 20192442, 4 March 2020.

[73] M. Calvo-Agudo, J. González-Cabrera, Y. Picó, P. Calatayud-Vernich, A. Urbaneja, M. Dicke and A. Tena, “Neonicotinoids in excretion product of phloem-feeding insects kill beneficial insects,” *Proceedings of the National Academy of Sciences,* vol. 116, no. 34, pp. 16817-16822, August 2019.

[74] K. Tasman, S. Hidalgo, B. Zhu, S. A. Rands and J. J. L. Hodge, “Neonicotinoids disrupt memory, circadian behaviour and sleep,” *Scientific Reports,* vol. 11, no. 1, p. 2061, 21 January 2021.

[75] M. Tomizawa and J. E. Casida, “Selective Toxicity of Neonicotinoids Attributable to Specificity of Insect and Mammalian Nicotinic Receptors,” *Annual Review of Entomology,* vol. 48, no. 1, pp. 339-364, 1 January 2003.

[76] R. Kamata, F. Shiraishi and K. Nakamura, “Avian eggshell thinning caused by transovarian exposure to o, p ′-DDT: changes in histology and calcium-binding protein production in the oviduct uterus,” *The Journal of Toxicological Sciences,* vol. 45, no. 3, p. The Journal of Toxicological Sciences, 2020.

[77] R. Kesic, J. E. Elliott, K. M. Fremlin, L. Gauthier, K. G. Drouillard and C. A. Bishop, “Continuing Persistence and Biomagnification of DDT and Metabolites in Northern Temperate Fruit Orchard Avian Food Chains,” *Environmental Toxicology,* vol. 40, no. 12, pp. 3379-3391, 24 September 2021.

[78] E. M. Molina, A. N. Kavazis and M. T. Mendonça, “Chronic Exposure to Environmental DDT/DDE in 2 Species of Small Rodents: Measures of Contaminant Load, Immune Dysfunction, and Oxidative Stress,” *Environmental Toxicology,* vol. 40, no. 6, pp. 1619-1629, 4 February 2021.

[79] J. Chanda, K. Saili, F. Phiri, J. C. Stevenson, M. Mwenda, S. Chishimba, C. Mulube, B. Mambwe, C. Lungu, D. Earle, A. Bennett, T. P. Eisele, M. Kamuliwo and R. W. Steketee, “Pyrethroid and Carbamate Resistance in Anopheles funestus Giles along Lake Kariba in Southern Zambia,” *The American Journal of Tropical Medicine and Hygiene,* vol. 103, no. 2, p. 90–97, 2 July 2020.

[80] EFSA, “Request for the evaluation of the toxicological assessment of the co-formulant POE-tallowamine,” *EFSA Journal,* vol. 13, no. 1, p. 4303, 12 November 2015.

[81] R. Mesnage, C. Benbrook and M. N. Antoniou, “Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides,” *Food and Chemical Toxicology,* vol. 128, pp. 137-137, 1 June 2019.

[82] P. A. Marchand, C. Dimier-Vallet and R. Vidal, “Biorational substitution of piperonyl butoxide in organic production: effectiveness of vegetable oils as synergists for pyrethrums,” *Chemistry, Activity and Impact of Plant Biocontrol products,* vol. 25, no. 30, p. 29936–29942, October 2018.

[83] M. K. Horton, A. Rundle, D. E. Camann, D. B. Barr, V. A. Rauh and R. M. Whyatt, “Impact of Prenatal Exposure to Piperonyl Butoxide and Permethrin on 36-Month Neurodevelopment,” *Pediatrics,* vol. 127, no. 3, p. 699–706, 1 March 2011.

[84] R. N. Singh, P. Kumar, N. Kumar and D. K. Singh, “Efficacy of Binary Combination of Deltamethrin+Mgk-264 on Levels of Biochemical Changes in The Snail Lymnaea Acuminata,” *International Journal of Pharmacy and Pharmaceutical Sciences,* vol. 12, no. 8, pp. 111-116, August 2020.

[85] H. L. Wong, D. G. Garthwaite, C. T. Ramwell and C. D. Brown, “Assessment of occupational exposure to pesticide mixtures with endocrine-disrupting activity,” *Environmental Science and Pollution Research,* vol. 26, no. 2, p. pages1642–1653, 21 January 2019.

[86] L. d. B. Rodrigues, G. G. Costa, E. L. Thá, L. R. Silva, R. de Oliveira, D. M. Leme, M. M. Cestari, C. K. Grisolia, M. C. Valadares and G. A. R. de Oliveira, "Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms," *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* vol. 842, pp. 94-101, 1 June 2019.

[87] N. Singh, V. K. Gupta, A. Kumar and B. Sharma, "Synergistic Effects of Heavy Metals and Pesticides in Living Systems," *Frontiers in Chemistry,* vol. 5, no. 70, 11 October 2017.

[88] G. Aguilera, A. L. Colín-González, E. Rangel-López, A. Chavarría and A. Santamaría, "Redox Signaling, Neuroinflammation, and Neurodegeneration," *Antioxidants & Redox Signaling,* vol. 28, no. 18, pp. 1626-1651, 20 June 2018.

[89] A. Kumar, B. Yegla and T. C. Foster, "Redox Signaling in Neurotransmission and Cognition During Aging," *Antioxidants & Redox Signaling,* vol. 28, no. 18, pp. 1724-1745, 20 June 2018.

[90] B. M. Trost and J. S. Tracy, "Carbon–Nitrogen Bond Formation via the Vanadium Oxo Catalyzed Sigmatropic Functionalization of Allenols," *Organic Letters,* vol. 19, no. 10, p. 2630–2633, 3 May 2017.

[91] B. Sharma, S. Singh and N. J. Siddiqi, "Biomedical Implications of Heavy Metals Induced Imbalances in Redox Systems," *BioMed Research International,* vol. 2014, p. 640754, 12 August 2014.

[92] V. K. Gupta, A. Kumar, N. J. Siddiqi and B. Sharma, "Rat Brain Acetyl Cholinesterase as a Biomarker of Cadmium Induced Neurotoxicity," *Open Access Journal of Toxicology,* vol. 1, no. 1, pp. 1-7, 8 January 2016.

[93] S. J. Flora and S. K. Tandon, "Effect of combined exposure to cadmium and ethanol on regional brain biogenic amine levels in the rat," *The International Journal of Biochemistry & Cell Biology,* vol. 15, no. 4, pp. 863-871, October 1987.

[94] L. Institóris, O. Siroki, U. Undeger, N. Basaran and I. Dési, "Immunotoxicological investigations on rats treated subacutely with dimethoate, As3+and Hg2+ in combination," *Human & Experimental Toxicology,* vol. 20, no. 7, pp. 329-336, July 2001.

[95] L. Institóris, A. Papp, O. Siroki, B. D. Banerjee and I. Dési, "Immuno- and neurotoxicological investigation of combined subacute exposure with the carbamate pesticide propoxur and cadmium in rats," *Toxicology,* vol. 178, no. 2, pp. 161-173, 2 September 2002.

[96] D. M. Creasy, "Pathogenesis of Male Reproductive Toxicity," *Toxicologic Pathology,* vol. 29, no. 1, pp. 64-76, 1 January 2001.

[97] M. Adamkovicova, R. Toman, M. Cabaj, P. Massanyi, M. Martiniakova, R. Omelka, V. Krajcovicova and H. Duranova, "Effects of Subchronic Exposure to Cadmium and Diazinon on Testis and Epididymis in Rats," *The Scientific World Journal,* vol. 2014, p. 632581, 7 December 2014.

[98] J. J. Mejía, F. Díaz-Barriga, J. Calderón, C. Ríos and M. E. Jiménez-Capdeville, "Effects of lead-arsenic combined exposure on central monoaminergic systems," *Neurotoxicology and Teratology,* vol. 19, no. 6, pp. 489-497, November-December 1997.

[99] V. K. Gupta, S. Singh, A. Agrawal, N. J. Siddiqi and B. Sharma, "Phytochemicals Mediated Remediation of Neurotoxicity Induced by Heavy Metals," *Biochemistry Research International,* vol. 2015, p. 534769, 11 November 2015.

[100] A. Ullah, A. Khan and I. Khan, "Diabetes mellitus and oxidative stress—A concise review," *Saudi Pharmaceutical Journal,* vol. 24, no. 5, pp. 547-553, 5 September 2016.

[101] V. K. Gupta, R. Pal, N. J. Siddiqi and B. Sharma, "Acetylcholinesterase from Human Erythrocytes as a Surrogate Biomarker of Lead Induced Neurotoxicity," *Enzyme Research,* vol. 2015, p. 370705, 22 October 2015.

[102] G.-E. Seralini and G. Jungers, "Toxic compounds in herbicides without glyphosate," *Food and Chemical Toxicology,* vol. 146, p. 111770, 1 December 2020.

[103] M. P. Gomes and P. Juneau, "Temperature and Light Modulation of Herbicide Toxicity on Algal and Cyanobacterial Physiology," *Frontiers in Environmental Science,* vol. 5, no. 50, 14 August 2017.

[104] A. Bérard, C. Leboulanger and T. Pelte, "Tolerance of Oscillatoria limnetica Lemmermann to Atrazine in Natural Phytoplankton Populations and in Pure Culture: Influence of Season and Temperature," *Archives of Environmental Contamination and Toxicology,* vol. 37, no. 4, p. 472–479, 1 November 1999.

[105] L. Baxter, R. A. Brain, L. Lissemore, K. R. Solomon, M. L. Hanson and R. S. Prosser, "Influence of light, nutrients, and temperature on the toxicity of atrazine to the algal species Raphidocelis subcapitata: Implications for the risk assessment of herbicides," *Ecotoxicology and Environmental Safety,* vol. 132, pp. 250-259, 1 October 2016.

[106] Ó. González-Barreiro, C. Rioboo and C. Herrero, "Atrazine-Induced Chlorosis in Synechococcus elongatus Cells," *Archives of Environmental Contamination and Toxicology,* vol. 46, no. 3, p. 301–307, 1 April 2004.

[107] M. J. Lydy, J. B. Belden and M. A. Ternes, "Effects of temperature on the toxicity of m-parathion, chlorpyrifos, and pentachlorobenzene to Chironomus tentans," *Archives of Environmental Contamination and Toxicology,* vol. 37, no. 4, pp. 542-547, November 1999.

[108] A. D. Harwood, J. You and M. J. Lydy, "Temperature as a toxicity identification evaluation tool for pyrethroid insecticides: Toxicokinetic confirmation," *Environmental Toxicology and Chemistry,* vol. 28, no. 5, pp. 1051-1058, May 2009.

[109] J. R. Coats, D. M. Symonik, S. P. Bradbury, S. D. Dyer, L. K. Timson and G. J. Atchison, "Toxicology of synthetic pyrethroids in aquatic organisms: An overview," *Environmental Toxicology and Chemistry,* vol. 8, no. 8, pp. 671-679, August 1989.

[110] M. M. Willming, G. Qin and J. D. Maul, "Effects of environmentally realistic daily temperature variation on pesticide toxicity to aquatic invertebrates," *Environmental Toxicology and Chemistry,* vol. 32, no. 12, pp. 2738-2745, 17 August 2013.

[111] F. Wania and D. Mackay, "Global Fractionation and Cold Condensation of Low Volatility Organochlorine Compounds in Polar Regions," *Ambio,* vol. 22, no. 1, pp. 10-18, February 1993.

[112] W. Zhang, F. Jiang and J. Ou, "Global pesticide consumption and pollution: with China as a focus," *Proceedings of the International Academy of Ecology and Environmental Sciences,* vol. 1, no. 2, pp. 125-144, 28 August 2011.

[113] M. Bish, E. Oseland and K. Bradley, "Off-target pesticide movement: a review of our current understanding of drift due to inversions and secondary movement," *Weed Technology,* vol. 35, no. 3, pp. 345 - 356, June 2021.

[114] M. E. Balmer, D. Janser, U. Schaller, J. Krauss, H. C. Geiser and T. Poiger, "Magnitude and decline of pesticide co-formulant residues in vegetables and fruits: results from field trials compared to estimated values," *Pest Management Science,* vol. 77, no. 3, pp. 1187-1196, March 2021.

[115] C. A. Damalas and I. G. Eleftherohorinos, "Pesticide Exposure, Safety Issues, and Risk Assessment Indicators," *International Journal of Environmental Research and Public Health,* vol. 8, no. 5, pp. 1402-1419, 6 May 2011.

[116] A. J. Maldonado-Reina, R. López-Ruiz, A. G. Frenich, F. J. Arrebola and R. Romero-González, " Co-formulants in plant protection products: An analytical approach to their determination by gas chromatography–high resolution mass accuracy spectrometry," *Talanta,* vol. 234, p. 122641, 1 November 2021.

[117] S. Das and K. J. Hageman, "Influence of Adjuvants on Pesticide Soil–Air Partition Coefficients: Laboratory Measurements and Predicted Effects on Volatilization," *Environmental Science & Technology,* vol. 54, no. 12, p. 7302–7308, 21 May 2020.

[118] M. S. Majewski and P. D. Capel, "Pesticides in the atmosphere; distribution, trends, and governing factors," U.S. Geological Survey, Sacramento, 1995.

[119] X. M. Hu, "Boundary layer (atmospheric) and air pollution | air pollution meteorology," in *Encyclopedia of Atmospheric Sciences*, 2 ed., Oxford, Oxfordshire: Academic Press, 2015, pp. 227-236.

[120] J. B. Nelemans, R. P. A. van Wijngaarden, I. Roessink and G. H. P. Arts, "Effects of the Herbicide Metsulfuron-Methyl on a Plant Community, Including Seed Germination Success in the F1 Generation," *Frontiers in Environmental Science,* vol. 5, no. 10, 28 March 2017.

[121] S. Rani and D. Sud, "Degradation of Dimethoate Pesticide in Soil: Impact of Soil Moisture and Enhanced Sunlight Intensity," *Water, Air, & Soil Pollution,* vol. 233, no. 24, 10 January 2022.

[122] J. Chen, Q. Yu, E. Patterson, C. Sayer and S. Powles, "Dinitroaniline Herbicide Resistance and Mechanisms in Weeds," *Frontiers in Plant Science,* vol. 12, p. 634018, 25 March 2021.

[123] A. Sharma, V. Kumar, B. Shahzad, M. Tanveer, G. P. S. Sidhu, N. Handa, S. K. Kohli, P. Yadav, A. S. Bali, R. D. Parihar, O. I. Dar, K. Singh, S. Jasrotia, P. Bakshi and Ramakrish, "Worldwide pesticide usage and its impacts on ecosystem," *SN Applied Sciences,* vol. 1, no. 1446, 21 October 2019.

[124] F. J. Kroon, P. M. Kuhnert, B. L. Henderson, S. N. Wilkinson, A. Kinsey-Henderson, B. Abbott, J. E. Brodie and R. D. R. Turnerg, "River loads of suspended solids, nitrogen, phosphorus and herbicides delivered to the Great Barrier Reef lagoon," *Marine Pollution Bulletin,* vol. 65, no. 4-9, pp. 167-181, 2012.

[125] J. Waterhouse, J. Brodie and A. Mitchell, "Quantifying the sources of pollutants in the Great Barrier Reef catchments and the relative risk to reef ecosystems," *Marine Pollution Bulletin,* vol. 65, no. 4-9, pp. 394-406, 2012.

[126] F. Sánchez-Bayo, K. Goka and D. Hayasaka, "Contamination of the Aquatic Environment with Neonicotinoids and its Implication for Ecosystems," *Frontiers in Environmental Science,* vol. 4, no. 71, 2 November 2016.

[127] A. J. Jamieson, T. Malkocs, S. B. Piertney, T. Fujii and Z. Zhang, "Bioaccumulation of persistent organic pollutants in the deepest ocean fauna," *Nature Ecology and Evolution,* vol. 1, no. 3, p. 0051, 13 February 2017.

[128] L. Villegas, M. Cabrera and M. V. Capparelli, "Assessment of Microplastic and Organophosphate Pesticides Contamination in Fiddler Crabs from a Ramsar Site in the Estuary of Guayas River, Ecuador," *Bulletin of Environmental Contamination and Toxicology,* vol. 107, no. 1, p. 20–28, 23 April 2021.

[129] B. D. P. Righi, L. D. Abujamara, I. F. Barcarolli, M. B. Jorge, Y. D. Zebrala, P. G. Costa, C. B. R. Martinez and A. Bianchini, "Response of biomarkers to metals, hydrocarbons and organochlorine pesticides contamination in crabs (Callinectes ornatus and C. bocourti) from two tropical estuaries (São José and São Marcos bays) of the Maranhão State (northeastern Brazil)," *Chemosphere,* vol. 288, no. 3, p. 132649, February 2022.

[130] F. Flores, S. Kaserzon, G. Elisei, G. Ricardo and A. P. Negri, "Toxicity thresholds of three insecticides and two fungicides to larvae of the coral Acropora tenuis," *PeerJ,* vol. 8, p. e9615, 28 July 2020.

[131] Y. Kang, R. Zhang, K. Yu, M. Han, Y. Wang, X. Huang, R. Wang and F. Liu, "First report of organochlorine pesticides (OCPs) in coral tissues and the surrounding air-seawater system from the South China Sea: Distribution, source, and environmental fate," *Chemosphere,* vol. 286, no. 2, p. 131711, January 2022.

[132] R. Jones, "The ecotoxicological effects of Photosystem II herbicides on corals," *Marine Pollution Bulletin,* vol. 51, no. 5, pp. 495-506, 1 January 2005.

[133] A. P. Negri, F. Flores, T. Röthig and S. Uthicke, "Herbicides increase the vulnerability of corals to rising sea surface temperature," *Limnology and Oceanography,* vol. 56, no. 2, pp. 471-485, March 2011.

[134] R. Mesnage, F. Mazzacuva, A. Caldwell, J. Halket and M. N. Antoniou, "Urinary excretion of herbicide co-formulants after oral exposure to roundup MON 52276 in rats," *Environmental Research,* vol. 197, p. 111103, June 2021.

[135] W. van Hoesel, A. Tiefenbacher, N. König, V. M. Dorn, J. F. Hagenguth, U. Prah, T. Widhalm, V. Wiklicky, R. Koller, M. Bonkowski, J. Lagerlöf, A. Ratzenböck and J. G. Zaller, "Single and Combined Effects of Pesticide Seed Dressings and Herbicides on Earthworms, Soil Microorganisms, and Litter Decomposition," *Frontiers in Environmental Science,* vol. 8, no. 215, 21 February 2017.

[136] L. L. Keeley, "6 - Physiology and Biochemistry of the Fat Body," in *Integument, Respiration and Circulation*, G. A. Kerkut and L. I. Gilbert, Eds., Oxford, Oxfordshire: Pergamon, 1985, pp. 211-248.

[137] C. Alaux, F. Ducloz, D. Crauser and Y. Le Conte, "Diet effects on honeybee immunocompetence," *Biology Letters,* vol. 6, no. 4, p. 562–565, 23 August 2010.

[138] M. van der Planck, D. Michez, M. Albrecht, E. Attridge, A. Babin, I. Bottero, T. Breeze, B. Brown, M.-P. Chauzat, E. Cini, C. Costa, P. De la Rua, J. R. de Miranda, G. Di Prisco and Domi, "Monitoring bee health in European agroecosystems using wing morphology and fat bodies," *One Ecosystem,* vol. 6, no. e63653, 17 May 2021.

[139] M. Vanderplanck, S. Decleves, N. Roger, C. Decroo, G. Caulier, G. Glauser, P. Gerbaux, G. Lognay, A. Richel, N. Escaravage and D. Michez, "Is non-host pollen suitable for generalist bumblebees?," *Insect Science,* vol. 25, no. 2, pp. 259-272, 1 April 2018.

[140] E. L. Arrese and J. L. Soulages, "Insect Fat Body: Energy, Metabolism, and Regulation," *Annual Review of Entomology,* vol. 55, no. 1, pp. 207-225, 1 January 2010.

[141] E. Jackson, R. Shoemaker, N. Larian and L. Cassis, "Adipose Tissue as a Site of Toxin Accumulation," *Comprehensive Physiology,* vol. 7, no. 4, pp. 1085-1135, 12 October 2017.

[142] M. S. Humphries, J. G. Myburgh, R. Campbell, A. Buah-Kwofie and X. Combrink, "Organochlorine pesticide bioaccumulation in wild Nile crocodile (Crocodylus niloticus) fat tissues: Environmental influences on changing residue levels and contaminant profiles," *Science of The Total Environment,* vol. 753, p. 142068, 20 January 2021.

[143] F. Sánchez-Bayo, "Impacts of Agricultural Pesticides on Terrestrial Ecosystems," in *Ecological Impacts of Toxic Chemicals*, vol. 1, Bussum, North Holland: Bentham Science Publishers, 2011, pp. 63-87.

[144] E. M. Slade, T. Riutta, T. Roslin and H. L. Tuomisto, "The role of dung beetles in reducing greenhouse gas emissions from cattle farming," *Scientific Reports,* vol. 6, no. 1, p. 18140, 5 January 2016.

[145] A. Quaranta, V. Bellantuono, G. Cassano and C. Lippe, "Why Amphibians Are More Sensitive than Mammals to Xenobiotics," *PLOS ONE,* vol. 4, no. 11, p. e7699, 4 November 2009.

[146] G. Berger, F. Graef, B. Pallut, J. Hoffmann, C. A. Brühl and N. Wagner, "How Does Changing Pesticide Usage Over Time Affect Migrating Amphibians: A Case Study on the Use of Glyphosate-Based Herbicides in German Agriculture Over 20 Years," *Frontiers in Environmental Science,* vol. 6, no. 6, 14 February 2018.

[147] FAO, The State of the World's Biodiversity for Food and Agriculture, J. Bélanger and D. Pilling , Eds., Rome: FAO Commission on Genetic Resources for Food and Agriculture Assessments, 2019, p. 572.

[148] E. L. Murphy, S. Eikenberry, G. Iacona, G. Watson and L. R. Gerber, "The value of increased spatial resolution of pesticide usage data for assessing risk to endangered species," *Conservation Science and Practice,* vol. 3, no. 12, p. e551, 1 December 2021.

[149] C. Pelosi, C. Bertrand, G. Daniele, M. Coeurdassier, P. Benoit, S. Nélieu, F. Lafay, V. Bretagnolle, S. Gaba, E. Vulliet and C. Fritsch, "Residues of currently used pesticides in soils and earthworms: A silent threat?," *Agriculture, Ecosystems & Environment,* vol. 305, p. 107167, 1 January 2021.

[150] J. Römbke, R. M. Schmelz and C. Pélosi, "Effects of Organic Pesticides on Enchytraeids (Oligochaeta) in Agroecosystems: Laboratory and Higher-Tier Tests," *Frontiers in Environmental Science,* vol. 5, no. 20, 15 May 2017.

[151] S.-K. Guo, L.-J. Cao, W. Song, P. Shi, Y.-F. Gao, Y.-J. Gong, J.-C. Chen, A. A. Hoffmann and S.-J. Wei, "Chromosome-level assembly of the melon thrips genome yields insights into evolution of a sap-sucking lifestyle and pesticide resistance," *Molecular Ecology Resources,* vol. 20, no. 4, pp. 1110-1125, July 2020.

[152] K. I. Brans, R. A. Almeida and M. Fajgenblat, "Genetic differentiation in pesticide resistance between urban and rural populations of a nontarget freshwater keystone interactor, Daphnia magna," *Evolutionary Applications,* vol. 14, no. 10, pp. 2541-2552, October 2021.

[153] F. Gui, T. Lan, Y. Zhao, W. Guo, Y. Dong, D. Fang, H. Liu, H. Li, H. Wang, R. Hao, X. Cheng, Y. Li, P. Yang, S. K. Sahu, Y. Chen, L. Cheng, S. He, P. Liu, G. Fan and L, "Genomic and transcriptomic analysis unveils population evolution and development of pesticide resistance in fall armyworm Spodoptera frugiperda," *Protein & Cell,* 27 October 2020.

[154] J. G. Zaller, "Where Are the Solutions to the Pesticide Problem?," in *Daily Poison: Pesticides - an Underestimated Danger*, 1 ed., vol. 1, Cham, Canton of Zug: Springer, 2020, pp. 223-295.

[155] A. Janssen and P. C. J. van Rijn, "Pesticides do not significantly reduce arthropod pest densities in the presence of natural enemies," *Ecology Letters,* vol. 24, no. 9, pp. 2010-2024, September 2021.

[156] Rob, "Twitter," 8 November 2021. [Online]. Available: https://twitter.com/r_joutdoors/status/1458068690705002505. [Accessed 8 November 2021].