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Ecotoxicity of Pesticides Approved for Use in European Conventional or Organic Agriculture for Honeybees, Birds, and Earthworms

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Abstract: Pesticides affect biota inside and outside agricultural fields due to their intrinsic mode of action. This study investigated whether pesticide active substances (AS) approved for conventional agriculture in Europe differ in their ecotoxicity from AS approved for organic agriculture. The evaluation was based on official ecotoxicological data for surrogate honeybee, bird, and earthworm species, which also serve as a reference for official environmental risk assessments in the pesticide authorization process. In October 2022, 268 chemical-synthetic AS approved for conventional and 179 nature-based AS approved for organic agriculture were listed in the EU Pesticide Database. Ecotoxicological data were only available for 254 AS approved for use in conventional agriculture and 110 AS approved for use in organic agriculture. The results showed a higher ecotoxicity of conventional AS: 79% (201 AS), 64% (163 AS) and 91% (230 AS) were moderately to acutely toxic to honeybees, birds, and earthworms, respectively, compared to 44% (48 AS), 14% (15 AS) and 36% (39 AS) of AS approved for organic agriculture. We have only considered the potential ecotoxicities of individual substances in this assessment; actual exposure in the field, where multiple AS formulations with other chemicals (including impurities) are applied, will be different. Nevertheless, these results emphasize that an increase in organic agriculture in Europe would reduce the ecotoxicological burden on biodiversity and associated ecosystem services.

Keywords: environmental effects; plant protection; European agriculture; biodiversity loss; non-target effects

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1. Introduction

The decline in biodiversity is caused by various factors such as the destruction of habitats, climate change, or the intensity of land use [1]. Pesticides, by their mode of action, influence the overall biodiversity in agroecosystems and beyond [2–4]. The European Union (EU) is aware of this fact and has proposed to reduce the use of hazardous pesticides and increase the proportion of organic farmland [5]. However, critics of this proposal argue that an increase in organic agriculture would lead to a higher overall hazard to non-target organisms in Europe, as the application rates of nature-derived pesticides used in organic farming are much higher than those of synthetic pesticides used in conventional farming [6]. In contrast, an assessment of hazards to humans and aquatic organisms has shown that the active substances (AS) used in pesticides of conventional agriculture are by orders of magnitude more toxic than the AS used in organic farming [7]. The extent to which other non-target organisms such as honeybees, birds, or earthworms would be affected is not known. The aim of this study is to assess this.

The AS approved for use in conventional agriculture in the EU are mainly carbon-based, synthetic chemicals produced with large amounts of fossil fuels [8], while the

AS approved for use in organic agriculture are of natural origin, such as plant extracts, pheromones, microorganisms, or inorganic elements [7]. Regardless of the origin of an AS, it can only be used in a pesticide in the EU if its safety has been officially approved in accordance with Regulation (EC) 1107/2009 and listed in Annex 1 of Directive 91/414/EEC [9]. In environmental risk assessments (ERA), the effects on non-target organisms are tested on a wide range of surrogate species representing the wider biodiversity. The endpoints of acute toxicity tests indicate, for example, the dose at which 50% of the population dies after short-term exposure (acute lethal dose LD_{50}) or the concentration (LC_{50}). In chronic toxicity tests, the highest concentration is determined at which no effects are observed after long-term exposure (NOEC; [10]).

Honeybees, birds, and earthworms are commonly used surrogate taxa in ERAs to evaluate the potential impact on non-target organisms. Honeybees are important insect pollinators that affect the yields of many crops such as apples, melons, cherries, pumpkins, or tomatoes [11], although wild bees are often more efficient pollinators [12]. Pesticides can be carried into hives through the storage of pollen and nectar, which can expose subsequent generations to pesticides without direct contact in the field [13]. Birds also play a very important role in agroecosystems as natural pest controllers that consume potential insect pests [14]. In addition, birds contribute to the diversity of wild plants by spreading plant seeds [15]. Pesticides have been shown to cause numerous lethal and sublethal effects in birds, affecting survival, reproduction, behavior, and orientation [16–20]. Pesticides also affect the abundance, biomass, richness, and diversity of soil fauna communities in a wide range of environmental conditions [21,22]. Earthworms are often tested as representatives of soil organisms as they convert organic matter into humus, which is very important for soil fertility and plant production [23]. Pesticides have been shown to affect the activity and reproduction [24,25] or the midgut bacteria of earthworms [26].

In the field, non-target organisms can be exposed to pesticides at different concentrations, with different frequencies and by different routes. Organisms can come into direct contact with the pesticide when it is applied in the field or be affected by drift to neighboring fields. In the case of systemic pesticides, the AS are distributed among all parts of the treated plant, including pollen, nectar, and roots, and can affect non-target organisms feeding on these plants above- and belowground [27].

The objective of this study was to compare the potential ecotoxicological impact of all pesticide active ingredients approved in Europe for conventional or organic agriculture. We focused on honeybees, birds, and earthworms as they inhabit European agroecosystems and because they are often used as surrogate species in ERAs. To our knowledge, this assessment is novel as no studies have yet been conducted comparing all pesticide AS approved in the EU between conventional and organic agriculture. The results could help to assess whether a further strengthening of organic agriculture would lead to a higher potential ecotoxicity and burden for biodiversity in agroecosystems in Europe.

2. Materials and Methods

2.1. Data Procurement

The AS authorized for conventional and organic agriculture were obtained from the official EU Pesticide Database published by the European Commission (https://ec.europa.eu/food/plants/pesticides/eu-pesticides-database_en; accessed on 23 June 2024). In summary, 447 AS were authorized by October 2022, of which 268 AS were for use in conventional agriculture (conAS) and 179 in organic farming (orgAS), which are regulated in Annex 1 of Regulation 2021/1165 [28]. Substances that are approved for organic farming can also be used in conventional agriculture, but not vice versa.

For the present study, the ecotoxicological endpoints of conAS were taken from the Pesticide Properties Database of the University of Hertfordshire (PPDB) [29]. The ecotoxicological endpoints of orgAS were derived from the Bio-Pesticide Database (BPDB), which is an addition to the PPDB. Typically, the tests from which these ecotoxicological endpoints were derived are conducted with surrogate species such as honeybees (*Apis mellifera*), birds

(*Colinus virginianus*, *Corturnix japonica*, and *Anas platyrhynchos*), and earthworms (*Eisenia foetida*). For the current evaluation, we took the acute oral and contact LD_{50} in µg bee^{-1} for honeybees, the acute LD_{50} in mg kg^{-1} and chronic NOEL in mg kg bw^{-1} d^{-1} for birds, and the LC_{50} in mg kg soil^{-1}, as well as the NOEC values in mg kg soil^{-1} for earthworms from the PPDB and BPDB. LD_{50} values with a "greater than" sign (>) were treated as equal to the value, which may overestimate the associated hazards.

In the PPDB and BPDB, there was no ecotoxicological assessment for 14 conAS and 69 orgAS for the surrogate species under consideration, so they were excluded from the current analyses. Therefore, 254 conAS and 110 orgAS were considered for the comparison of potential ecotoxicity. We considered all AS that were ecotoxicologically assessed, including those used for post-harvest applications or for storage gases such as ethylene and CO_2.

2.2. Comparison of Potential Ecotoxicity

In the PPDB, the acute toxicity of an AS for honeybees and birds was given as the LD_{50} value. For honeybees, the acute oral toxicity was mainly assessed using OECD tests no. 213 and no. 214 [30,31]. For birds, the acute toxicity in birds was assessed using OECD test no. 223 [32]; the acute LD_{50} and chronic NOEL for birds was determined using OECD test no. 205 [33]. Earthworm ecotoxicity was assessed using OECD test no. 207; the ecotoxicological endpoint of acute toxicity in earthworms is expressed as acute LC_{50} in mg kg soil^{-1} [34]. Chronic toxicity to earthworms is usually assessed using OECD test no. 222 [35] by determining the endpoint "no observed effect concentration" NOEC in mg kg^{-1} soil.

Based on these ecotoxicological data, we evaluated all AS using the PPDB classification system, which consists of three toxicity categories: low, moderately, and highly toxic (Table 1).

Table 1. Terminology used to classify toxicity adapted from PPDB [29].

Organism	Species	Exposure	Ecotoxicological Endpoint	Thresholds			Reference
				Low Toxic	Moderately Toxic	Highly Toxic	
Honeybees	Western honeybee (*Apis mellifera*)	Oral and contact, 48 h	Acute LD_{50} (µg bee^{-1})	>100	1–100	<1	OECD test no. 213: Honeybees, acute oral toxicity test; OECD test no. 214: Honeybees, acute contact toxicity test
Birds	Mallard duck (*Anas platyrhynchos*), bobwhite quail (*Colinus virginianus*), Japanese quail (*Coturnix japonica*)	Oral, 14 days	Acute LD_{50} (mg kg bw^{-1})	>2000	100–2000	<100	OECD test no. 223: Avian, acute oral toxicity test; OECD test no. 206: Avian, reproduction test
		Mixed into food before egg deposition, 21 days	Chronic NOEL (mg bw^{-1} d^{-1})	>200	10–200	<10	
Earthworms	Compost worm (*Eisenia foetida*)	Mixed into artificial soil, 14 days	Acute LD_{50} (mg kg soil^{-1})	>1000	10–1000	<10	OECD test no. 207: Earthworm, acute toxicity test;
		Mixed into artificial soil, 28 days	Chronic NOEC, reproduction (mg kg soil^{-1})	>100	0.1–100	<0.1	OECD test no. 222: Earthworm, reproduction test

Even if the LD_{50} records the lethality, the calculated number of LD_{50} doses per AS does not correspond to the actual number of non-target organisms killed in the field. Their effect may also be indirect [36] or, depending on the actual exposure, may have no effect at all. Furthermore, no interactions between different simultaneously applied AS were considered.

3. Results

3.1. Comparison of Active Substances

Of the 254 conAS, 91 AS were herbicides (36%), 81 AS were fungicides (32%), 42 AS were insecticides (17%), and 25 AS (10%) were plant growth regulators (Figure 1). The "Others" category included nematicides, rodenticides, molluscicides, and other AS.

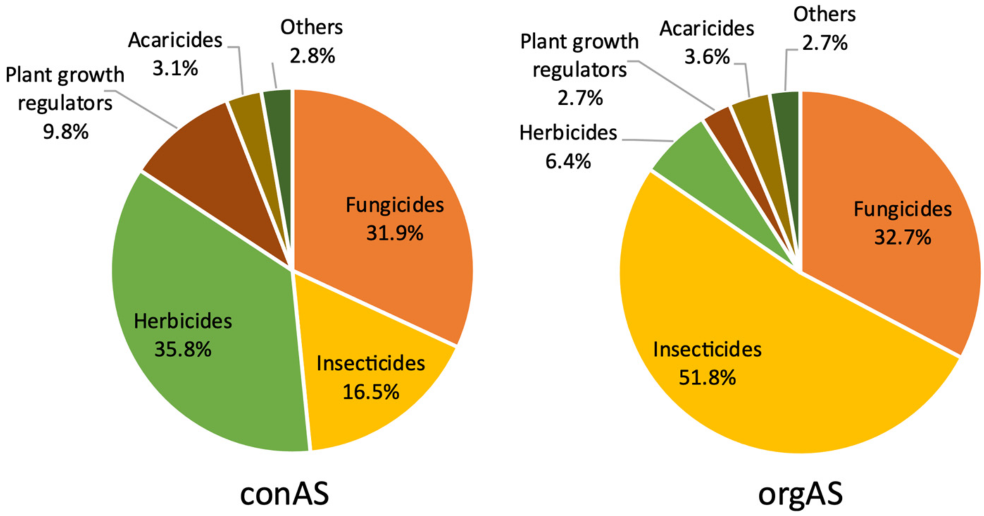

Figure 1. Pesticide types of active substances (AS) allowed in conventional farming (conAS, n = 254) and organic farming (orgAS, n = 110) in Europe according to the official EU Pesticide database.

Of the 110 orgAS, 57 AS were insecticides (52%), 35 AS were fungicides (32%), 7 AS were herbicides (6%), and 3 AS (2.7%) were plant growth regulators (Figure 1). All four fatty acids (methyl decanoate, lauric acid, methyl octanoate, oleic acid) and all pheromones were counted separately. Pelargonic acid, the four fatty acids, sodium chloride, and citronella oil were categorized as herbicides in the database but are used in the non-agricultural sector and are not permitted in organic farming [28]. If an AS fell into more than one category, it was only counted once. For example, azadirachtin was only counted as an insecticide, although it is also authorized as an acaricide in Europe [28]. Supplementary Table S1 lists all conAS and Supplementary Table S2 lists all orgAS used in this study.

3.2. Comparison of Pesticide Classes

Based on the information contained in the PPDB and BPDB, the pesticide classes were compared in terms of their toxicity to honeybees, birds, and earthworms.

Moderate acute toxicity to honeybees was found for 79.1% of the evaluated conAS and 43.6% of the evaluated orgAS (Table 2). Supplementary Table S3 provides an overview of the ecotoxicological evaluation of all AS considered in this study.

Moderate or high acute toxicity to birds was observed in 64.2% (163 AS) of conAS and in 13.6% (15 AS) of orgAS (Table 2). Moderate chronic toxicity to birds was found in 48.0% (122 AS) of conAS, but only in 1.8% of orgAS. Of the conAS, 9.1% (23 AS) were highly chronically toxic to birds, but no orgAS fell into this category (Table 2).

Most of the conAS, 90.6% but only 35.5% of orgAS were moderately or highly acutely toxic to earthworms (Table 2). In terms of chronic toxicity to earthworms, 58.7% of conAS were moderately chronically toxic, but only 7.3% of orgAS were. Of the conAS, 0.3% (1 AS) were highly chronically toxic to earthworms but no orgAS fell into this category.

Table 2. Overview of the ecotoxicological assessment of all pesticide active substances (AS) approved in the European Union (n = 447). Some AS could not be assessed because no ecotoxicological profile was available. Because AS can be toxic to different organisms, the addition of the counts can be higher than the total number of AS considered. See Supplementary Table S4 for more details on the specific toxicity categories among test organisms.

Ecotoxicological Classification	Approved in Conventional Agriculture		Approved in Organic Agriculture	
	%	n	%	n
Total number of EU-approved AIs	60	268	40	179
Of these, those that were ecotoxicologically assessed	94.8	254	61.5	110
Honeybees—moderate or high acute toxicity	79.1	201	43.6	48
Birds—moderate or high acute toxicity	64.2	163	13.6	15
Birds—moderate or high chronic toxicity	57.1	145	1.8	2
Earthworms—moderate or high acute toxicity	90.6	230	35.5	39
Earthworms—moderate or high chronic toxicity	58.7	149	7.3	8

3.3. Potential Hazards to Honeybees

Honeybee acute contact toxicity was caused by 18 highly toxic and 164 moderately toxic conAS, while these categories were caused by 2 highly toxic and 34 moderately toxic orgAS (Figure 2). The acute oral toxicity to honeybees was contributed by 16 highly toxic conAS and 116 moderately toxic conAS; for orgAS, acute oral toxicity was contributed by 3 highly toxic AS and 26 moderately toxic orgAS (Figure 2).

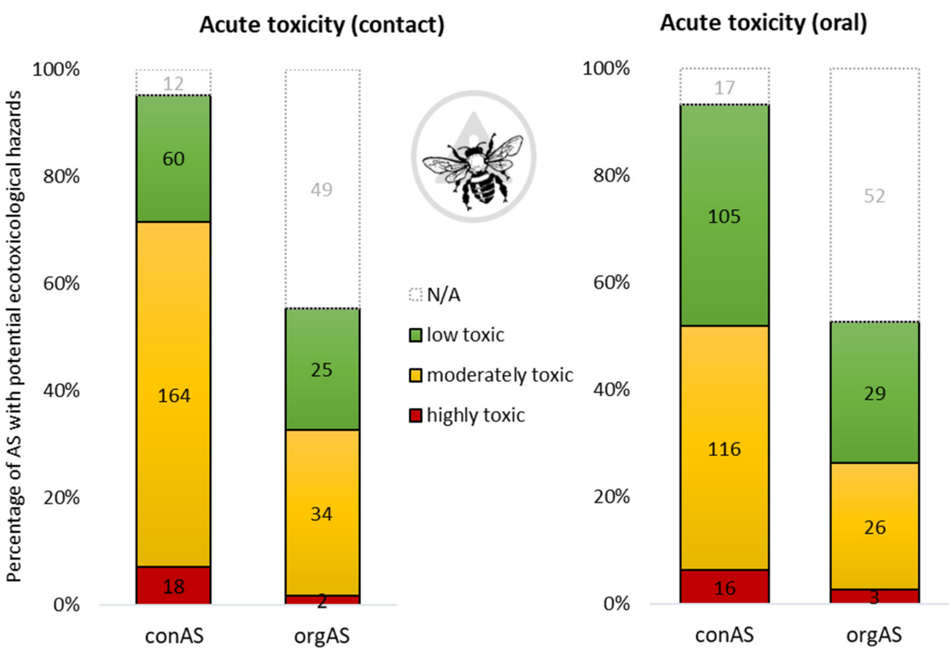

Figure 2. Ecotoxicological risk assessment of active substances approved in Europe in conventional agriculture (conAS; n = 254) and organic agriculture (orgAS; n = 110) for honeybees based on acute oral LD_{50} (µg bee^{-1}) and acute contact LD_{50} (µg bee^{-1}).

The ranking of the most toxic AS in terms of contact and oral toxicity to honeybees is dominated by insecticides. The most toxic conAS regarding contact toxicity to honeybees were abamectin, deltamethrin, and gamma-cyhalothrin, with LD_{50} of 0.001, 0.002, and 0.005 µg bee^{-1}, respectively (Table 3). The most toxic orgAS regarding honeybee contact toxicity were spinosad, pyrethrins, and azadirachtin, with LD_{50} of 0.004, 0.013, and 11.81 μg bee^{-1}, respectively (Table 3). The most toxic conAS regarding honeybee oral toxicity were abamectin, milbemectin, and deltamethrin, with LD_{50} of 0.004, 0.026, and 0.070 μg bee^{-1}, respectively (Table 3). The most toxic orgAS regarding honeybee oral toxicity were spinosad, (Z)-8-Dodecen-1-ol, and (Z)-8-Dode-cen-1-yl acetate, with LD_{50} of 0.06, 0.85, and 0.85 μg bee^{-1}, respectively (Table 3).

Table 3. Honeybee top-10 active substances (AS) approved in Europe in conventional agriculture (conAS; n = 254) and organic agriculture (orgAS; n = 110) based on acute oral and acute contact LD_{50}. F = fungicide, H = herbicide, I = insecticide.

		Honeybee Oral Toxicity						Honeybee Contact Toxicity				
Rank	**Type**	**LD_{50} (μg Bee^{-1})**	**conAS**	**Type**	**LD_{50} (μg Bee^{-1})**	**orgAS**	**Type**	**LD_{50} (μg Bee^{-1})**	**conAS**	**Type**	**LD_{50} (μg Bee^{-1})**	**orgAS**
1	I	0.004	Abamectin	I	0.06	Spinosad	I	0.001	Abamectin	I	0.004	Spinosad
2	I	0.026	Milbemectin	I	0.85	(Z)-8-Dodecen-1-ol	I	0.002	Deltamethrin	I	0.013	Pyrethrins
3	I	0.07	Deltamethrin	I	0.85	(Z)-8-Dodecen-1-yl acetate	I	0.005	Gamma-cyhalothrin	I	11.81	Azadirachtin
4	I	0.14	Spinetoram	I	8.1	Azarirachtin	I	0.023	Cypermethrin	F	12.5	COS-OGA
5	I	0.146	Sulfoxaflor	F	10	COS-OGA	I	0.024	Pyridaben	I	20	Isaria fumosorosea
6	I	0.16	Formetanate	F	12.1	Copper oxychloride	I	0.024	Spinetoram	F	22	Copper oxide
7	I	0.172	Cypermethrin	F	23.3	Bordeaux mixture	I	0.025	Milbemectin	F	23.5	Tribasic copper sulfate
8	I	0.21	Esfenvalerate	F	24	Potassium hydrogen carbonate	I	0.036	Emamectin	H	25	Methyl decanoate
9	I	0.22	Pirimiphos-methyl	F	40	Tribasic copper sulfate	I	0.038	Etofenprox	H	25	Lauric acid
10	I	0.24	Aluminum phosphide	F	48	Streptomyces K61	I	0.038	Lambda-cyhalothrin	H	25	Methyl octanoate

The most toxic orgAS fungicide to honeybees was COS-OGA, with an LD_{50} for contact toxicity of 12.5 μg bee^{-1} and an LD_{50} for oral toxicity of 10.0 μg bee^{-1} (Table 4). The most toxic orgAS herbicide for honeybees was methyl decanoate, with an LD_{50} for contact toxicity of 25.0 μg bee^{-1} (Table 3)

Table 4. Bird top-10 pesticide active substances (AS) approved in Europe in conventional agriculture (conAS; n = 254) and organic agriculture (orgAS; n = 110) for birds based on acute LD_{50} (mg kg bw^{-1}) and chronic NOEL (mg kg bw^{-1} d^{-1}). F = fungicide, H = herbicide, I = insecticide, P = plant growth regulator, R = rodenticide.

		Bird Chronic Toxicity						Bird Acute Toxicity				
Rank	**Type**	**LD_{50} (mg kg bw^{-1} d^{-1})**	**conAS**	**Type**	**LD_{50} (mg kg bw^{-1} d^{-1})**	**orgAS**	**Type**	**LD_{50} (mg kg bw^{-1})**	**conAS**	**Type**	**LD_{50} (mg kg bw^{-1})**	**orgAS**
1	I	0.7	Abamectin	I	68.4	Spinosad	I	3.2	Oxamyl	F	72.4	Tribasic copper sulfate
2	I	1.5	Oxamyl	I	82	Pyrethrins	I	10	Fosthiazate	F	173	Copper oxychloride
3	I	2	Tefluthrin	F	2222	Trichoderma asperellum strain T25	I	11.5	Formetante	F	223	Copper hydroxide
4	I	2.1	Bifenazate		1405	Orange oil	R	12.9	Zinc phosphide	F	616	Bordeaux mixture
5	I	2.5	Fosthiazate				I	20.9	Pirimicarb	I	1000	Azadirachtin
6	H	2.95	Prosulfuron				I	26	Abamectin	F	1000	*Bacillus amyloliq-uefaciens*
7	I	3.3	Lambda-cyhalothrin				I	49	Magnesium phosphide	F	1183	Copper oxide
8	A	3.65	Fenpyroximate				I	49	Aluminum phosphide	F	1667	*Ampelomyces quisqualis*
9	I	4.29	Cypermethrin				I	76	Emamectin	F	1700	Laminarin
10	F	4.3	Ipconazole				I	81	1-methylcyclo propene	I	2000	Spinosad

3.4. Potential Hazards to Birds

Acute bird toxicity was contributed by 12 highly toxic and 151 moderately toxic conAS, while these categories were contributed by 1 highly toxic and 15 moderately toxic orgAS (Figure 3). The chronic toxicity to birds was contributed by 23 highly toxic and 122 moderately toxic conAS, while these categories were contributed by 0 highly toxic and 2 moderately toxic orgAS (Figure 3).

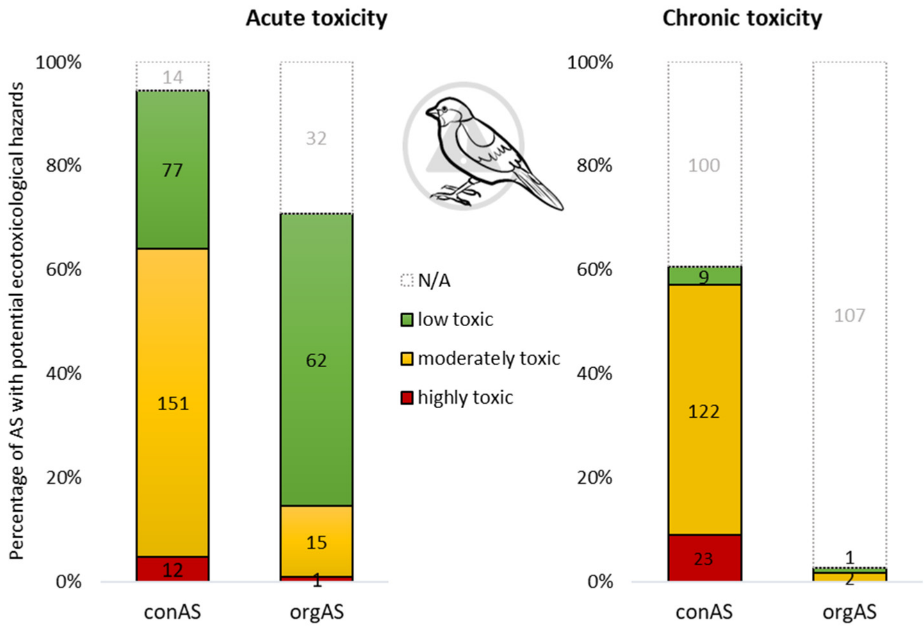

Figure 3. Ecotoxicological risk assessment of active substances approved in Europe in conventional agriculture (conAS; n = 254) and organic agriculture (orgAS; n = 110) for birds based on acute LD_{50} (mg kg bw^{-1}) and chronic NOEL (mg kg bw^{-1} d^{-1}).

The ranking of the most toxic AS in terms of acute toxicity to birds was dominated by insecticides and fungicides. The most toxic conAS regarding bird acute toxicity were the insecticides oxamyl, fosthiazate, and formetante, with LD_{50} of 3.2, 10.0, and 11.5 mg bw^{-1}, respectively (Table 4). The most toxic orgAS regarding bird acute toxicity were the fungicides tribasic copper sulfate, copper oxychloride, and copper hydroxide, with LD_{50} of 72.4, 173.0, and 223.0 mg bw^{-1}, respectively (Table 4). The most toxic conAS regarding bird chronic toxicity were the insecticides abamectin, oxamyl, and tefluthrin, with NOEL of 0.7, 1.5, and 2.0 mg kg bw^{-1} d^{-1}, respectively (Table 4). The most toxic orgAS regarding bird chronic toxicity were the insecticides spinosad, pyrethrin, and the fungicide *Trichoderma asperellum* strain T25, with NOEL of 68.4, 82, and 2222 mg kg bw^{-1} d^{-1}, respectively (Table 4).

The most toxic conAS herbicide for birds was metribuzin with an LD_{50} of 164.0 mg bw^{-1}; the most toxic rodenticide of conAS was zincphosphide with an LD_{50} of 12.9 mg bw^{-1} (Table 4).

3.5. Potential Hazards to Earthworms

Acute toxicity to earthworms was contributed by 6 highly toxic and 224 moderately toxic conAS, while these categories were contributed by 39 moderately toxic orgAS; there was no highly earthworm-toxic orgAS (Figure 4). Earthworm chronic toxicity was contributed by 1 highly toxic and 148 moderately toxic conAS and 8 moderately toxic orgAS, while no orgAS was considered highly chronically toxic to earthworms (Figure 4).

The ranking of the most toxic AS regarding earthworm acute and chronic toxicity is dominated by various pesticide classes including molluscicides and plant growth regulators. The most toxic conAS for earthworm acute toxicity were the insecticides sulfoxaflor and tefluthrin and the fungicide mefentrifluconazole, with LD_{50} of 0.9, 1.0, and 2.7 mg kg^{-1} soil, respectively (Table 5). The most toxic orgAS regarding earthworm acute toxicity were the molluscicide ferric phosphate, the insecticide pyrethrins, and the plant growth regulator ethylene, with LD_{50} of 10.0, 23.7, and 60.0 mg kg^{-1} soil, respectively (Table 5). The most toxic conAS in terms of earthworm chronic toxicity were the fungicide dimoxystrobin, the insecticide sulfoxaflor, and the herbicide nicosulfuron, with LD_{50} of 0.09, 0.1, and 0.1 mg kg^{-1} soil, respectively (Table 5). The most toxic orgAS to earthworm chronic toxicity were the insecticides pyrethrins and spinosad and the molluscicide ferric phosphate, with LD_{50} of 0.3, 1.8, and 6.7 mg kg^{-1} soil, respectively (Table 5).

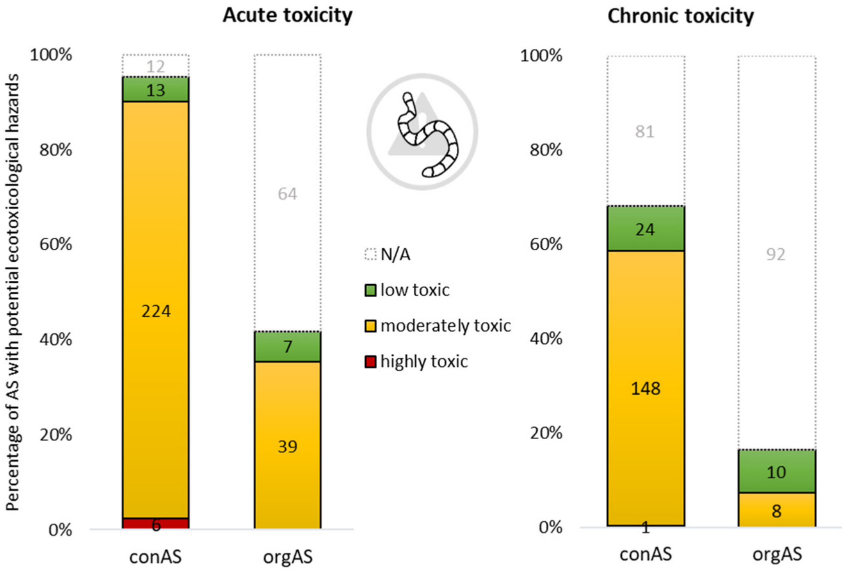

Figure 4. Earthworm acute and chronic toxicity of active substances (AS) approved in Europe in conventional agriculture (conAS; n = 254) and organic agriculture (orgAS; n = 110) based on acute LC_{50} (mg kg soil^{-1}) and chronic NOEC (mg kg soil^{-1}).

Table 5. Ecotoxicological risk assessment of active substances approved in Europe in conventional agriculture (conAS; n = 254) and organic agriculture (orgAS; n = 110) for earthworms based on acute LC_{50} (mg kg soil^{-1}) and chronic NOEC (mg kg soil^{-1}). F = fungicide, H = herbicide, I = insecticide, M = molluscicide, P = plant growth regulator.

	Earthworm Chronic Toxicity						Earthworm Acute Toxicity					
Rank	**Type**	**NOEC (mg kg soil^{-1})**	**conAS**	**Type**	**NOEC (mg kg soil^{-1})**	**orgAS**	**Type**	**LC_{50} (mg kg soil^{-1})**	**conAS**	**Type**	**LC_{50} (mg kg soil^{-1})**	**orgAS**
1	F	0.09	Dimoxystrobin	I	0.3	Pyrethrins	I	0.9	Sulfoxaflor	M	10	Ferric phosphate
2	I	0.1	Sulfoxaflor	I	1.8	Spinosad	I	1	Tefluthrin	I	23.7	Pyrethrins
3	H	0.1	Nicosulfuron	M	6.7	Ferric phosphate	F	2.7	Mefentrifluconazole	P	60	Ethylene
4	I	0.109	Milbemectin	F	15	Tribasic copper sulfate	P	5	1-methylcyclopropene	H	105	Methyl decanoate
5	H	0.13	Sulfosulfuron	F	15	Copper hydroxide	I	6.5	Dazomet	H	105	Lauric acid
6	I	0.16	Tefluthrin	F	15	Copper oxide	I	9	Acetamiprid	H	105	Methyl octanoate
7	I	0.165	Deltamethrin	F	40.5	Copper oxychloride	I	10.6	Esfenvalerate	H	105	Oleic acid
8	H	0.167	Picloram	A	55.35	Paraffin oil	P	11.8	Sodium 5-nitroguaiacolate	H	105	Pelargonic acid
9	H	0.17	Beflubutamid	H	250	Pelargonic acid	A	13.3	Fenazaquin	F	155	Tribasic copper sulfate
10	F	0.175	Fluazinam	I	1000	Cydia pomonella Granulovirus	H	15.7	Flazasulfuron	F	195.5	Bordeaux mixture

4. Discussion

In this comprehensive evaluation of the ecotoxicity of all AS approved for use in agriculture in the EU, we found that the AS approved for use in conventional agriculture generally have a higher inherent ecotoxicity to honeybees, birds, and earthworms than the AS approved for use in organic agriculture. Most synthetic AS used in conventional agriculture act through selective interaction with biochemical processes that are important for the survival of populations of pests and disease organisms [37]. In contrast to these pesticidal AS, microorganisms and pheromones, as well as the vast majority of plant extracts and mineral AS approved in organic agriculture, exert their effects via a non-specific chemical, physicochemical, or physical mode of action, by deterring or confusing pests or the strengthening of the plant's defense [38]. The strengthening of plant defenses is also the main reason why the development of resistance is rarely observed in orgAS in contrast to conAS [39]. However, it is important to note that our evaluation was only based on the intrinsic toxicity of the approved AS to the non-target organisms considered, based on the available LD_{50}/LC_{50} values for individual AS, and that the actual exposure in the field will be different with multiple applications of pesticides.

4.1. Honeybee Ecotoxicity

Honeybees and other pollinators play an important role in providing pollination services, maintaining biodiversity, and supporting crop production. Given the overall decline in insect biodiversity, assessments of potential threats must be taken seriously.

Overall, 79% of conAS (201 conAS) but only 44% of orgAS (48 orgAS) were moderately or highly toxic to honeybees, both by contact and oral exposure. For conAS, this was caused by herbicides (72 conAS), fungicides (65 conAS), and, to a lesser extent, insecticides (37 conAS). For orgAS, this toxicity was mainly contributed by insecticides (22 orgAS), fungicides (19 orgAS), and, to a lesser extent, herbicides (4 orgAS).

The top-three of moderately or highly honeybee toxic conAS (abamectin, deltamethrin, gamma-cyhalothrin) were 791 times more toxic than the top-three orgAS (spinosad, pyrethrins, azadirachtin).

The most toxic conAS for honeybees was the insecticide abamectin and the most toxic orgAS was the insecticide spinosad. However, abamectin (LD_{50} = 0.001 μg bee^{-1}) was four times more toxic to honeybees than spinosad (LD_{50} = 0.004 μg bee^{-1}). Both abamectin and spinosad are bacterial fermentation products of soil bacteria: abamectin of the actinomycete *Streptomyces avermitilis*, while spinosad is derived from the actinomycete *Saccharopolyspora spinosa*. Usually, there are restrictions on the use of pesticides only when honeybees are not actively foraging in the crop (e.g., for spinosad, deltamethrin, and others); however, this does not take into account the possible drift onto flowering weeds in the vicinity of the field [40]. The approval for the AS abamectin was renewed on 1 April 2023, but only for use in greenhouses, as a high risk was identified for several groups of non-target organisms [41]. The second highly toxic orgAS for honeybees was pyrethrins, with an LD_{50} of 0.013 μg^{-1}. While spinosad and pyrethrins were the only two orgAS with high bee toxicity after contact exposure, a total of 18 conAS fell into this category.

We found that two orgAS insecticidal pheromones ((Z)-8-Dodecen-1-ol, (Z)-8-Dodecen-1-yl acetate) were classified as highly toxic to honeybees after oral exposure. The highly toxic risk exists for honeybee larvae after exposure to pheromone residues, whereas the risk for adult honeybees is considered negligible [42,43]. In addition to synthetic pesticides, biopesticides such as microbial, biochemical, and plant-incorporated protectant-based products can also have lethal and sublethal effects on honeybees and native pollinators [44].

In general, both lethal and sublethal effects have been documented for conAS [13,45]. Pesticides have been shown to affect the functioning of the nervous system [46], the respiratory system [47], the digestive system [48], and the reproductive system [49], as well as eating behavior [13], orientation [50], and mobility [51]. Even low doses of AS can impair the immune system of pollinators, making them less resistant to infections and parasites such as the Varroa mite [52]. Neurotoxic insecticides inhibit normal nerve system functions by different mechanisms such as chlorpyrifos, which inhibits the enzyme acetylcholinesterase [53], imidacloprid, which blocks nicotinic acetylcholine receptors [54], and lambda-cyhalothrin, which interferes with sodium channels [46]. These effects cause nerval overstimulation in non-target organisms, leading to paralysis and eventual death [55,56]. However, spinosad [57] and lambda-cyhalothrin [46] have also been shown to impair midgut functions and functions associated with nutrient absorption following oral exposure. Other conAS can also lead to irritation in orientation and communication [58].

4.2. Bird Ecotoxicity

Declines in European bird populations have been reported for decades, and agricultural intensification, particularly the heavy use of pesticides and fertilizers, has been identified as the main cause of declines in most bird populations, especially invertebrate feeders [59].

Overall, 64% of conAS (163 conAS) but only 14% of orgAS (15 orgAS) were moderately or highly acutely toxic to birds. Almost 57.1% of conAS (145 conAS) showed moderate or high chronic toxicity to birds, while 2 orgAS fell into this category. The moderate or high acute bird toxicity of conAS was mainly caused by herbicides (60 conAS) and fungicides (55 conAS) and, to a lesser extent, insecticides (19 conAS). Among orgAS, moderate or high acute bird toxicity was mainly attributable to fungicides (11 orgAS) and insecticides (3 orgAS), while no herbicidal orgAS contributed to this category. Moderate or high chronic toxicity to birds was mainly contributed by herbicides (60 conAS) and fungicides (55 conAS). The only 2 orgAS classified as moderately toxic were insecticides.

The top-three moderately or highly acute toxic AS to birds among the conAS (oxamyl, fosthiazate, formetante) were, on average, approximately 20 times more toxic than the top-three orgAS (tribasic copper sulfate, coper oxychloride, copper hydroxide).

While 12 conAS were highly toxic to birds, only 1 orgAS, tribasic copper sulfate, belonged to this category. Other copper-based substances were categorized as moderately toxic, such as copper oxychloride, copper hydroxide, Bordeaux mixture, and copper oxide. In general, copper substances are considered "candidates for substitution" in the European Union [60]. However, it should also be noted that copper-based AS are also used in conventional agriculture [61].

Oxamyl, as the most toxic conAS to birds (LD_{50} = 3.2 mg kg bw^{-1}), was 22.6 times more toxic than the most toxic orgAS, tribasic copper sulfate (LD_{50} = 72.4 mg kg bw^{-1}). The second-most toxic conAS, fosthiazate (LD_{50} = 10 mg kg bw^{-1}), was 17 times more toxic than the second-most toxic orgAS, copper oxychloride (LD_{50} = 173 mg kg bw^{-1}). Authorization for the AS oxamyl was not renewed in May 2023 due to the risks in relation to acceptable user exposure [62].

In terms of chronic toxicity, 57.1% of conAS were moderately to highly toxic after chronic exposure, while 1.8% of orgAS fell into this category. Abamectin (NOEL 0.7 mg kg bw^{-1} day^{-1}), the conAS with the highest chronic toxicity, was 97.7 times more toxic than Spinosad (NOEL = 68.4 mg kg bw^{-1} day^{-1}). In general, smaller birds are more sensitive to pesticides than larger birds, and as the toxicity tests were carried out on large species, the LD_{50} values reported are probably not representative of smaller songbirds and the risk of poisoning may have been underestimated [63,64]. In addition to direct skin contact with pesticides, there is also a risk of dietary ingestion of pesticides via food, as the offspring of birds are fed with pesticide-treated crop seeds or wild plant seeds and small insects, which may also be contaminated by spray drift [16]. Male house sparrows exposed to the neonicotinoid insecticide acetamiprid had a lower sperm density [17], the eggs were smaller after exposure, and the thickness of the eggshell was also reduced [18]. In the yellowhammer, the number of eggs laid per nest was reduced after exposure to various pesticides [65], as was the number of surviving offspring of northern bobwhite quail after exposure to imidacloprid [19]. In addition, a reduction in the size and weight of embryos and testicular malformations were observed in Japanese quail after exposure to the insecticide clothianidin [66]. As is already known from pollinators, neonicotinoid insecticides also affect the locomotor system [67] and orientation in birds [20].

4.3. Earthworm Ecotoxicity

The use of pesticides has been shown to have detrimental effects on earthworms and overall soil biodiversity [21,22]. As AS residues are frequently found in soils across Europe [68–71], the potential threat to soil organisms is obvious.

Overall, 91% of conAS (230 conAS) were moderately or highly acutely toxic to earthworms. Of the orgAS, only 36% (39 orgAS) were moderately toxic to earthworms, but no orgAS was highly toxic to earthworms. Of the conAS, 59% (149 conAS) showed moderate-to-high chronic toxicity to earthworms, while 7% of orgAS (8 orgAS) were moderately chronically toxic to earthworms, with no orgAS highly chronically toxic. For conAS, moderate or high acute earthworm toxicity was mainly due to herbicides (83 conAS) and fungicides (75 conAS) and, to a lesser extent, insecticides (39 conAS). For orgAS, moderately chronically toxic to earthworms was mainly contributed by insecticides (13 orgAS) and fungicides (12 orgAS), while 6 herbicidal orgAS contributed to this category. Herbicides are not allowed in organic agriculture and the listed herbicide orgAS is approved for non-agricultural purposes. Moderate or high chronic earthworm toxicity was mainly caused by fungicides (60 conAS) and herbicides (55 conAS) and, to a lesser extent, insecticides (23 conAS). Of the orgAS, fungicides (4 AS) and insecticides (2 orgAS), but no herbicides, contributed to this category.

The top-three moderately or highly acute toxic conAS to earthworms (sulfoxaflor, tefluthrin, mefentrifluconazole) were approximately 19 times more toxic than the top-three orgAS (ferric phosphate, pyretrhins, ethylene).

The four insecticides—sulfoxaflor, tefluthrin, acetamiprid, and dazomet—as well as the fungicide mefentrifluconazole and plant growth regulator 1-methylcyclopropene showed highly acute earthworm toxicity. The only highly chronically toxic AS was the fungicidal conAS dimoxystrobin. Insecticides have also been shown to be the most toxic AS to earthworms in other studies [72]. After insecticides, fungicides are placed second and herbicides are considered of lower ecotoxicological concern to earthworms [73]. However, if the toxic load is considered by multiplying the quantity applied by the toxicity of the herbicidal AS for earthworms, decreasing quantities could lead to higher toxicities if more toxic AS are applied, as has been the case in Austria over the last 10 years [74].

The conAS with the highest earthworm toxicity approved in the EU is currently sulfoxaflor (LD_{50} = 0.9 μg kg $soil^{-1}$), which was seen as a replacement for neonicotinoids after they were banned in Europe [75].

The orgAS with the highest toxicity to earthworms was the moderately toxic molluscicide ferric phosphate. However, in an experimental study, no adverse effects on earthworms were observed in the control of an invasive slug species [76]. However, it is still less chronically toxic to earthworms than the chemical-synthetic substance molluscicide metaldehyde. Ferric phosphate, ferric phosphonates, and metaldehyde showed the same acute toxicity (LD_{50} = 1000 mg kg^{-1}), but ferric phosphate and phosphonates were chronically low toxic (LD_{50} = 1000 mg kg $soil^{-1}$), while metaldehyde was 31 times more toxic (LD_{50} = 32 mg kg $soil^{-1}$).

The second-most acutely toxic substances to earthworms in conventional and organic farming both belong to the chemical group of pyrethrins. However, the orgAS pyrethrin (LD_{50} = 23.7 mg kg $soil^{-1}$) was 24 times less toxic than the conAS tefluthrin (LD_{50} = 1 mg kg $soil^{-1}$). In addition, a second approved chemical-synthetic pyrethroid called esfenvalerate is also more toxic to earthworms than natural pyrethrin, as the LD_{50} is 10.6 mg kg $soil^{-1}$ (natural pyrethrin LD_{50} = 23.7 mg kg $soil^{-1}$). Altogether, eight chemical-synthetic pyrethroids were approved as insecticides: tefluthrin, lambda-cyhalothrin, gamma-cyhalothrin, etofenprox, esfenvalerate, tau-fluvalinate, deltamethrin, and cypermethrin.

Copper compounds are very often discussed with regard to their impact on soil organisms. However, based on our evaluation, not a single copper compound was classified as acutely and chronically highly toxic, but as moderately toxic to earthworms (Bordeaux mixture, copper hydroxide, copper oxychloride, copper oxide, tribasic copper sulfate). According to the EU regulation on the use of copper, application is limited to a maximum of 4 kg Cu ha^{-1} $year^{-1}$ [77]. This restriction applies to the entire agricultural sector, not just organic farming, as conventional farmers also use copper-based products. A meta-analysis has shown that an application of 4 kg Cu ha^{-1} $year^{-1}$ has no effect on the biological quality and functions of the soil [78]. Copper-based fungicides used in vineyards reduced biomass and caused DNA damage to earthworms (*Eisenia foetida*) at the highest dose (150 mg kg^{-1}); however, after 28 days, the earthworms had fully recovered from the negative effects of pesticide exposure [79].

Neonicotinoid insecticides such as imidacloprid showed high toxicity to earthworms, as the midgut bacteria of the earthworms (*Lumbricus terrestris*) were affected after the recommended pesticide application [26].

Contamination of agricultural soils with pesticides is widespread and was found on 98% of arable land, vineyards, orchards, forests, grasslands, and brownfields, and even on organic fields, forests, grasslands, and fallow land [80]. The risk assessment identified a moderate-to-high risk for earthworms in arable soils, mainly due to insecticides and/or acaricides that were present much longer than their degradation would suggest.

Our results indicate that soil in conventional fields, which contain more pesticide residues than soils from organic fields, poses a high risk to soil invertebrates and may jeopardize soil functionality, especially as additive or synergistic "cocktail effects" may occur [81,82].

In general, the mode of action of pesticides is responsible for their higher or lower toxicity to non-target organisms [83]. The lower toxicity of orgAS to non-target organisms may be due to the fact that (i) their mode of action on the target receptor is usually agonistic, whereas that of conAS is more often antagonistic, and (ii) non-target organisms may have evolved in environments where poisonous plants grow and have developed neurophysiological mechanisms to cope with the potential threat of poisoning [83]. Therefore, most organisms have not been able to develop the appropriate physiological characteristics for synthetic conAS that could counteract their effect.

4.4. Study Limitations

By using LD_{50} and LC_{50} characteristics, our risk assessment focused only on lethal effects but did not consider sublethal and indirect effects [84]. In addition, our evaluation does not take into account that organisms are exposed to multiple pesticides during the growing season, legacy effects from previous applications [85,86], or interactions with other agrochemicals or heavy metals [87–89]. The assumption underlying ERAs that the organisms affected have sufficient time to recover from one pesticide application to the next does not correspond to reality [3].

Approaches have been developed that consider the ecotoxicological risks for biodiversity and ecosystems at environmental concentrations of an AS, taking into account persistence, bioaccumulation, and probability of exposure in different environmental compartments (water, sediment, soil, vegetation, air) [90]. We found no ecotoxicity data for 14 conAS and 69 orgAS that are authorized in European agriculture. While the lack of data for orgAS is understandable due to their origin as basic substances also used in the food industry, the lack of data for conAS is difficult to understand given the requirements for the authorization of pesticides.

It was also found that for 30% of the monitored pesticides currently in use, no data on chronic toxicity to soil invertebrates at different trophic levels were available, although the ERAs showed that pesticide residues in soil pose a high risk [91].

Similarly, to the official ERAs, our evaluation was based on a few surrogate species. However, it should be clear that not all species are equally exposed to pesticides due to different habitat use. For instance, the typical habitat of the earthworm species *Eisenia foetida* that is considered in ERAs lives in litter layers and very rarely in arable fields. In addition, a meta-analysis concluded that *E. foetida* is actually less susceptible to AS than earthworm species native to agroecosystems [92,93]. The honeybee has been used as an indicator species for standard ecotoxicological tests, but it has been pointed out that it is not always a good proxy for other species of eusocial and solitary bees, as species differ in autecology and sensitivity to various stressors [94]. For instance, stingless bees have been shown to be more sensitive to pesticides than honeybees [95]. The current ERA results for birds are also difficult to extrapolate from surrogate bird species to a field situation [96,97].

5. Conclusions

Our evaluation clearly showed that the AS approved in conventional agriculture are more toxic to honeybees, birds, and earthworms than the AS approved for organic agriculture. The differences can partly be explained by the fact that many orgAS are basic substances that are also used in the food industry or are obtained from foodstuffs (plants, plant by-products, plant-derived products, substances, and derived substances from animal origin) [98]. A much higher toxicity of conAS than orgAS was also found when the hazards to humans and aquatic organisms were assessed [7]. Although we did not consider application rates, the differences could be even more severe, as orgAS are estimated to be applied on only approximately 5–10% of organic farmland [99,100]. However, to accurately assess the adverse effects of pesticides on biodiversity, access to data on pesticide use [101] and monitoring of non-target species would be essential.

As pesticides have been found in various environmental matrices, including soil [70,71,102], water bodies [103], ambient air [4,104], and even at large distances from their application site in nature conservation areas or mountain peaks [2,105,106], a general reduction in pesticide use seems imperative. Furthermore, a reduction in pesticide use is possible [107], e.g., by introducing conservation biological control with more flowering plants and ground covers in apple orchards [108] or vineyards [109], by reducing fertilization and tillage, and by growing less disease-susceptible varieties [110]. Based on our analysis, we can conclude that an increase in organic agriculture in Europe would reduce the ecotoxicological burden on biodiversity and the associated ecosystem services for humans [101].

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/environments11070137/s1, Supplementary Table S1: Overview of all authorized AS in EU for conventional farming, their pesticide type and their ecotoxicological endpoints (for birds: acute LD50 and chronic NOEL, for honeybees: contact and oral acute LD50, for earthworms: acute LC50 and chronic NOEC). In the columns "classified as" to the right of each ecotoxicological endpoint, l.t. stands for "low toxic", m.t. for "moderately toxic", h.t. for "highly-toxic"; Supplementary Table S2: Overview of all authorized AS in EU for organic farming, their pesticide type and their ecotoxicological endpoints (for birds: acute LD50 and chronic NOEL, for honeybees: contact and oral acute LD50, for earthworms: acute LC50 and chronic NOEC). In the columns "classified as" to the right of each ecotoxicological endpoint, l.t. stands for "low toxic", m.t. for "moderately toxic", h.t. for "highly-toxic"; Supplementary Table S3: Overview of the ecotoxicological assessment of all pesticide active substances (ASs) approved in the European Union (n = 447). Some ASs could not be assessed because no ecotoxicological profile was available. Because AS can be toxic to different organisms the addition of the counts can be higher than the total number of AS considered; Supplementary Table S4: Comparison of highly and moderately pesticide classes approved in Europe for conventional (conAS) and organic agriculture (orgAS) towards their ecotoxicity against earthworms, honeybees, and birds.

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