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Reducing overall herbicide use may reduce risks to humans but increase toxic loads to honeybees, earthworms and birds

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Abstract

Background: Pesticide use has been associated with risks for human health and an overall decline in biodiversity. Although herbicides are the most commonly used pesticides worldwide, they have received less attention in this debate. We investigated the extent to which long-term trends in herbicide use in Austria influence potential toxic exposures to non-target organisms and potential risks to humans. We analyzed official sales data of 101 herbicide active ingredients (AIs) approved in Austria between 2010 and 2019 regarding their ecotoxicological properties based on lethal doses (LD_{50} and LC_{50}) weighed by their persistence in the environment (DT_{50}) for honeybees (*Apis mellifera*), earthworms (*Eisenia fetida*), and birds (*Serinus serinus*). Human health risks were qualitatively assessed based on official hazard statements for the AIs used.

Results: In Austria, herbicide amounts sold decreased significantly by 24% from 1480 to 1123 tonnes between 2010 and 2019. This also led to a considerable decrease in the amounts of AIs classified by H-statements of the EU Pesticides Database: − 71% acute inhalation toxicity, − 58% reproductive toxicity,− 47% specific target organ toxicity. Yet, 36% of herbicides used were still classified as highly hazardous pesticides according to the Pesticide Action Network. Surprisingly, over the same period, toxic loads to honeybees increased by 487% (oral exposure), while lethal toxic loads to earthworms increased by 498%, and to birds by 580%. This can be attributed to a shift toward the use of more acutely toxic and especially more persistent AIs. The most problematic AI for honeybees, earthworms, birds and humans was the highly persistent diquat. The further ranking of the most toxic herbicides varied considerably depending on the organism. It is important to note that this toxic load assessment, like official environmental risk assessments, evaluates the potential risk but not the actual fatalities or real-world exposure.

Conclusions: Our results show a trade-off between herbicide amounts and toxicological hazards to humans and other non-target organisms. These interdependencies need to be considered when implementing pesticide reduction targets to protect public health and biodiversity, such as the EU´s "farm-to-fork" strategy, which aims to reduce the amounts and risks of synthetic pesticides.

Keywords: Agrochemicals, Environmental pollution, Farmland biodiversity, Insect pollinators, Human health, Pesticide non-target effects, Pesticide reduction, Soil fauna, Farmland birds, Sustainable agriculture

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Background

Trends of declining diversity and biomass of insects [1, 2], earthworms [3] and birds [4] are observed in agroecosystems and non-crop ecosystems around the world [5–7]. The loss of biodiversity in agricultural landscapes is the result of the interaction of various stress factors, including intensified management with poorly structured landscapes, as well as the use of pesticides [8–11]. In addition, pesticide use has been linked to human health problems, including diabetes, reproductive disorders, neurological dysfunction, cancer, and respiratory disorders [12, 13]. Of the pesticides used worldwide, herbicides account for the largest share [14, 15]. Nevertheless, most research reporting non-target effects of pesticides has focused on insecticides [16], largely due to the widespread assumption that herbicides specifically kill weeds without having serious impacts on non-plant organisms [17].

Herbicides are widely used in conventional agriculture, horticulture, landscaping, by railway companies, and in home gardens [18], but are not allowed in organic farming, at least in Europe [19]. Glyphosate alone accounts for 33% of the herbicide amount used in Europe in 2017, with one-third of the acreage of annual cropping systems and half of the acreage of perennial tree crops treated annually with glyphosate [20]. Herbicide residues have been shown to contaminate ambient air throughout Germany [21], were found in insects from nature conservation areas in Germany [22], and in grass samples of public places in an intensively managed agricultural area in Northern Italy [23, 24]. Although herbicides target weeds, their active ingredients (AIs) can have many direct [25] and indirect [26] effects on non-target organisms. Both lethal and sublethal effects have been reported for (honey) bees [27–29], earthworms [30–32] and birds [33]. In addition, numerous human health effects (cancer, reproductive and developmental toxicity, acute toxicity) of herbicides have been documented [33–35], some of which, such as glyphosate, are highly controversial [36, 37].

Honeybees may ingest pesticides orally via contaminated guttation droplets [38], nectar and pollen from freshly treated flowering plants or weeds nearby [27, 39], or by ingesting contaminants on body parts during cleaning [40]. Additionally, contact exposure of bees can occur through foliar sprays, drift, particle-bound soil contaminants spread by wind erosion [27], or via contaminated plant surfaces and wild-bee nesting material [41, 42]. Indeed, herbicide residues (e.g., pendimethalin, linuron) have been detected in bee bread and dead honeybees [43] and wild bees (e.g., atrazine, metolachlor) [44]. For earthworms the major routes of exposure are dermal uptake of dissolved herbicides in pore water and ingestion of contaminated organic matter, plant residues and soil [45, 46]. Samples of earthworms from agricultural soils in France contained 31 pesticide residues of which 6 were herbicides (e.g., diflufenican, pendimethalin, pyroxsulam) [47]. Depending of habitat use, birds are exposed to herbicides as well [48] by ingesting granular pesticides [49] or contaminated seeds, earthworms, insects and water, by inhalation, preening [50] or dermal uptake [51]. Herbicides (e.g., atrazine, linuron, pendimethalin) have also been found in insect boluses fed to nestlings [52] or in carcasses of grey partridge (e.g., mesosulfuron-methyl, fluroxypyr, florasulam) [53]. Humans can take up herbicides through ingestion of drinking water and food (e.g., glyphosate, MCPA, metolachlor) [54–56]. Moreover, herbicides pose a risk to agricultural workers during application [57] but also to bystanders and residents by breathing herbicide-contaminated ambient air [21, 58] when drifted to public places from agricultural fields [23, 24].

Approaches to assessing the toxic load (TL) of pesticides to non-target organisms, including humans, link applied amounts to ecotoxicological measures [59–62]. More advanced assessments additionally incorporate the persistence of a substance [63]. The results of these studies show that despite decreasing amounts of pesticides applied, there is an upward-trend in the applied toxicity to non-target species such as plants and insects, largely due to an increased use of more efficient but more hazardous AIs [59–61]. However, results for vertebrates in general [61] and birds in particular [64] show a significant decrease in TL as pesticide amounts decrease. Similarly, toxic risks to humans can be assessed by considering globally harmonized hazard statements [65]. Such qualitative assessments (without thresholds) are also performed for the approval of active substances in Europe [66]. For endocrine disrupting pesticides, which interfere with the hormone system of organisms, the existence of safe thresholds is unlikely [67].

Policy makers in Europe have already responded to the harmful effects of pesticides on humans and biodiversity by formulating the "Farm-to-Fork Strategy", which aims to reduce overall pesticide use and risks by 50% by 2030 [68]. Therefore, in the current study we wanted to know the extent to which herbicide amounts are associated with potential toxic loads to honeybees (*Apis mellifera)*, earthworms (*Eisenia fetida),* birds (*Serinus serinus*), as well as risks to human health. Using data on herbicide sales in Austria between 2010 and 2019, we expected that herbicide amounts would decrease in these years as organic farming area increased from 20% of agricultural area in Austria in 2010 to 26% in 2019 [69].

The assessment of potential herbicide effects on honeybees, earthworms and birds is important because they provide essential functions and services in agricultural landscapes and are also used as surrogate species in official environmental risk assessments of pesticides [25, 26, 70–72]. Moreover, honeybees (and wild bees) are increasingly facing health problems throughout Europe [73]. Earthworms make up the majority of soil faunal biomass in many temperate agroecosystems and promote plant productivity [74], but their activity, reproduction and survival have also shown to be impaired by herbicides [47, 75, 76]. Finally, populations of farmland birds have declined across Europe in recent decades, with populations of European serins (*Serinus serinus*) in Austria declining by 85% since 1998 [77].

Material and methods

Amounts of herbicidal AIs

Our analysis was based on official herbicide AI sales data between 2010–2019 kindly provided by the Austrian Federal Agency of Health and Food Safety (AGES, www.ages.at) upon request. In the absence of actual application data, we assumed that the AI sales correspond to the amounts used in Austria during the year the pesticides were sold. Stockpiling may result in higher sales than use per year but over the years a balance between sales and stock depletion is expected [78]. Therefore, the amounts of AIs sold per year are referred to as amounts applied in the same year.

A list of 106 AIs approved during the requested period was provided, while amounts of some AIs ($n=23$) were not provided due to trade secrecy. However, these sales data do not distinguish between the share for agricultural or non-agricultural purposes. If the total amounts of the chemical class were listed, the difference between this chemical class amount and the corresponding known AI amounts could be used for further calculations. If the chemical class amounts were not provided, they were assumed to be zero. Four unclassified AIs had to be omitted completely due to lacking data: acetic acid, flurochloridone, pelargonic acid, caprylic acid; however, they are also used only in small quantities. Chlorpropham was not included in our analyses because it is only used as a sprout inhibitor in potato storage, and we consider it unlikely to affect organisms in the field. Thus, the final analysis included 101 AIs, of which 80 AIs of 34 chemical classes had attributable amounts during 2010–2019. The excluded AIs represent on average 1.7% of the total amount of all AIs between 2010 and 2019, with a minimum contribution of 0.6% in 2014 and a maximum contribution of 5.8% in 2010. A complete list of all 106 AIs sold can be found in the supplemental information, but we cannot provide detailed amounts due to commercial confidentiality (Additional file 1: Table S1).

For the analysis we grouped the individual herbicides according to chemical classes. The chemical class "other herbicides" contained the following AIs: triazolinone, triazolone, triketone, phenylpyrazole, pyridazinone, pyridinecarboxamide, pyridine acetic acid, pyridinecarboxylic acid, quinolone, benzothiazinone, isoxazole, nitrile, imidazolinone, cyclohexanedione, diazine, dicarboximide, aryloxyphenoxypropionate, benzofurane, dicarboximide and for the unclassified group the AIs clomazone and quinoclamine. Additionally, for each of these AIs we assessed whether it was approved for arable farmland, orchards and vineyards, railway tracks, golf courses, nurseries, parks, lawns, private use and for other purposes. This was done using the Austrian plant protection products register (PSMR; psmregister.baes.gv.at) provided by the Austrian Authority for Food Safety [79] and other sources [80–82].

Calculating potential toxic loads

Ecotoxicological testing of acute toxicities of herbicides are conducted with surrogate species and are standardized by guidelines of the Organization for Economic Co-operation and Development (OECD) [83–86]. They aim to determine the median lethal dose (LD_{50}) or median lethal concentration (LC_{50} in artificial soil for earthworms), defined as the dose or concentration of a pesticide, that kills 50% of the tested population of organisms. Other tests assess chronic, sublethal effects over a longer period e.g., for earthworms [87] or birds [88] by calculating the no observed effect concentration (NOEC) or no observed effect level (NOEL in mg $kg_{bodyweight(bw)}{}^{-1}$ d^{-1}), the maximum concentration or dose at which no significant effects on reproduction or development are observed relative to control groups [89].

In assessing potential toxic loads (TL), we focused on European honeybees (*Apis mellifera*), compost worms (*Eisenia fetida*), and the European serin (*Serinus serinus*). These species were selected because they serve as surrogate species for official environmental risk assessments of herbicides. The TL depends on the acute substance toxicity (LD_{50}) and applied amount [59] and was additionally weighted by the persistence (DT_{50}) of the respective AI [63] (Fig. 1).

It is assumed that a higher persistence means a higher exposure risk to non-target organisms over a longer period of time, resulting in a higher TL. Formula 1 was used to include the persistence of an AI in the toxic load calculations [63].

$$\text{Toxic load} = \sum^{n_{AI}} \frac{\text{Amount}_{AI}}{n_{days}\,\text{per year}.LD_{50AI}} * \frac{DT_{50AI}}{\ln(2)}. \tag{1}$$

The TL of each AI was calculated by dividing the annual amount used by the corresponding LD_{50} value. This required converting the applied kg to µg for honeybees (LD_{50} in µg bee^{-1}) and to mg for earthworms (LC_{50} in mg kg^{-1} soil and LD_{50} in mg $earthworm^{-1}$) and birds (LD_{50} in mg kg^{-1} in mg $bird^{-1}$). The TL of each AI was then multiplied by the half-life (DT_{50}: 50% dissipation time until a pesticide is degraded to 50% of its

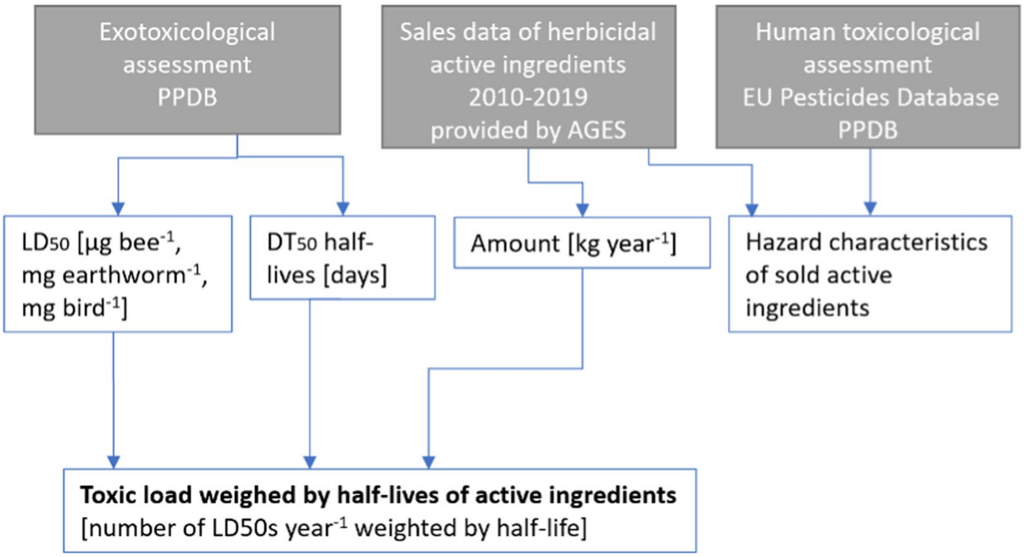

Fig. 1 Data sources and parameters for toxic load calculations and human toxicological assessments. *AGES* Austrian agency for health and food safety, provided data upon request, *PPDB* pesticide property database lists ecotoxicological properties (LD_{50}) and half-lives (DT_{50})

original amount) divided by ln (2), assuming first order degradation kinetics [90]. By inserting the number of days per year (n_{days}) into the formula, the unit "day" (of DT_{50} and number of days year^{-1}) cancels out, giving the persistence-weighed unit number of LD_{50} doses released to the environment. The total TL per year is obtained by summing the TL of all AIs (n_{AI}).

It is important to note that the calculated number of LD_{50} doses cannot be interpreted as actual kills, as actual exposure may in fact be much lower [59]. However, the results can be interpreted as the potential risk of poisoning per year. The method allows assessing trends of used amounts in relation to ecotoxicity and to clarify which AIs are most persistent and pose the greatest threat. Our calculations did not consider the degradation products of AIs, their toxicity, or interactions between different AIs or other co-formulants.

The LD_{50}, LC_{50} values and DT_{50} (soil) were primarily collected from the Pesticide Property Database (PPDB) established by the University of Hertfordshire [91]. To include half-lives, DT_{50}-field values were preferentially chosen. If these were not available, the arithmetic mean was calculated for the range of DT_{50} values from EU dossier laboratory studies reported in the PPDB (e.g., for dichlorprop-P, MCPB, amidosulfuron, foramsulfuron and fenoxaprop-P-ethyl). DT_{50}-typical (often mean of field and laboratory studies) was used for the AIs mecoprop, triflusulfuron and iron sulphate. No DT_{50} values were provided for chlorotoluron, so the value was taken from Pub-Chem [92].

For honeybees, the acute oral LD_{50} (worst case from 24, 48 and 72 h values), for earthworms (*Eisenia fetida)* the acute 14 day LC_{50}, and for birds acute oral LD_{50} was used. If the LD_{50}/LC_{50} values were not available in the PPDB, other sources were chosen in the following descending order of priority: PubChem for dimethenamid [93], OPP Pesticide Ecotoxicity Database [94], European Food Safety Authority (EFSA) Journals and Conclusions on pesticide peer reviews for metosulam [95] and the Australian Pesticides and Veterinary Medicines Authority for tepraloxydim [96].

Whenever there was a "greater than" sign preceded the LD_{50}/LC_{50} in the databases, the value was adopted assuming an "equal sign" potentially overestimating TLs. In cases where LD_{50} values of AIs were missing, LD_{50} values of closely related substances were assumed as substitutes (e.g., ioxynil octanoate instead of ioxynil) or available LD_{50} values listed in the section "unknown" mode of exposure in the PPDB were taken (e.g., iron sulphate for honeybees). Earthworm NOECs were available for 60 AIs and bird NOELs (21 days, mg $kg_{bw}{}^{-1}$ d^{-1}) were available for 76 AIs.

Since all toxicity values for earthworms are LC_{50} values [mg $kg_{soil}{}^{-1}$] we developed a formula to convert them into LD_{50} values. By dividing the LC_{50} value by the mean number of earthworms [n_{EW}] per mass [kg] agricultural soil in Austria, the LD_{50} value can be derived (Formula 2):

$$LD_{50EW} = \frac{LC_{50EW}}{n_{EW}\ per\ mass_{soil}}. \tag{2}$$

Earthworm densities per square meter were taken from two studies, in which earthworms were assessed in 68 subplots in arable farmland soils in Austria [97, 98] and averaged to 208 ± 90 earthworms (n_{EW}) per m^2. The area of one m^2 was multiplied with an extraction depth of 0.28 m to get the extracted soil volume. This soil volume was then multiplied with an assumed soil density (bulk density for generic soil type) of 1.52 g cm^{-3} resulting in the extracted mass of soil (containing the stated n_{EW}). Finally, the n_{EW} was divided by the total mass of soil extracted to obtain the average number of 0.5 earthworms per kg soil (n_{EW} per $mass_{soil}$ in Formula 2).

Bird TL was calculated for the granivorous European serin (*Serinus serinus*) because its population trends are strongly declining in Austria according to the farmland bird index and because of its low bodyweight compared to other listed species [77]. Bird LD_{50} values were only available for three surrogate species: *Colinus virginianus, Coturnix japonica* or *Anas platyrhynchos* and were expressed in mg $kg_{bw}{}^{-1}$ [91]. For calculations, these LD_{50} values were converted into mg per bird by multiplying them with the body weight of each bird species. Then, the LD_{50} in mg per surrogate bird species was extrapolated to *S. serinus* using Formula 3 [64]:

$$\log(LD50_2) = \log(LD50_1) + (\log(W_2) - \log(W_1)) * 1.239. \quad (3)$$

The variable $LD50_1$ indicates the LD_{50} in mg $bird^{-1}$ and W_1 the mean bodyweight that was available for the surrogate bird species. $LD50_2$ as well as W_2 indicate the values and bodyweight for the selected *S. serinus*. The mean bodyweight of *S. serinus* (W_2) was assumed to be 13 g (range 11–15 g) [99]. As W_1 the mean bodyweight of either *C. virginianus* 189 g (167–214 g) [100], of *C. japonica* 95 g (90–100 g) [101], or *A. platyrhynchos* 1115.5 g (967–1264 g) [102] was chosen. If the LD_{50} reported in the PPDB was not attributable to a surrogate species, it was assumed to belong to *C. virginianus*. The value 1.239 in the formula is the average slope by calculating scaling factors for 130 pesticides [103].

In case of missing AI amounts within a herbicide chemical class, the total "rest-amount" (total amount minus known AI-amounts) of this chemical class was used in combination with the arithmetic mean of LD_{50} and DT_{50} values of all missing AIs. Unclassified AIs with missing amount and ecotoxicological values such as acetic acid, flurochloridone, pelargonic acid and caprylic acid were omitted entirely.

In addition, all AIs were qualitatively classified according to their ecotoxicological properties using the PPDB three category classification system (Table 1).

The four-part classification according to PPDB was also used for persistence. AIs with a $DT_{50} > 365$ days are classified as "very persistent", with 100–365 days as "persistent", with 30–99 days as "moderately persistent", and with < 30 days as "non-persistent" [91].

HHP and human toxicological assessment

For each AI, a classification according to the International List of Highly Hazardous Pesticides (HHP) compiled by the Pesticide Action Network (PAN) was made. An AI is classified as a HHP if it either impairs human health or environmental integrity or if it is listed in the annex of certain environmental conventions (e.g., Rotterdam, Stockholm, Montreal) [104].

Additionally, human health hazard statements (H-statements) were evaluated for each AI based on the official EU Pesticides Database [105]; Table 2; complete list in Additional file 1: Table S6). Of 101 evaluated AIs, 54 had at least one H-statement. To assess changes in human toxicology we compared the number and amounts of used AIs classified with H-statements in 2010 with those used in 2019. Not all AIs could be included because the authorities did not provide information for 23 AIs.

Table 1 Classification of active ingredients in toxicity categories regarding honeybees, earthworms and birds [91]

Species	Toxicity category		
	Highly toxic	Moderately toxic	Non-toxic
Honeybee LD_{50} [µg bee^{-1}] (oral and contact exposure)Honeybee LD50 [µg bee^{-1}] (oral and contact exposure)	< 1	1–100	> 100
Earthworm LC_{50} [mg $kg_{soil}{}^{-1}$]	< 10	10–1000	> 1000
Earthworm NOEC [mg $kg_{soil}{}^{-1}$]	< 0.1	0.1–100	> 100
Bird LD_{50} [mg $kg_{bw}{}^{-1}$]	< 100	100–2000	> 2000
Bird NOEL [mg $kg_{bw}{}^{-1}$ d^{-1}]	< 10	10–200	> 200

Table 2 Assessed GHS hazard statements for human health of herbicidal AIs ($n=54$) approved in Austria 2000–2019, classified by EU regulation (EC) 1272/2008

Code	Hazard class and category	Hazard statement
H301	Acute toxicity (oral), Cat 3	Toxic if swallowed
H302	Acute toxicity (oral), Cat 4	Harmful if swallowed
H312	Acute toxicity (dermal), Cat 4	Harmful in contact with skin
H315	Skin corrosion/irritation, Cat 2	Causes skin irritation
H317	Sensitization—skin, Cat 1	May cause an allergic skin reaction
H318	Serious eye damage/eye irritation, Cat 1	Causes serious eye damage
H319	Serious eye damage/eye irritation, Cat 2	Causes serious eye irritation
H330	Acute toxicity (inhalation), Cat 1,2	Fatal if inhaled
H331	Acute toxicity (inhalation), Cat 3	Toxic if inhaled
H332	Acute toxicity (inhalation), Cat 4	Harmful if inhaled
H335	Specific target organ toxicity, single exposure, Cat 3, respiratory tract irritation	May cause respiratory irritation
H351	Carcinogenicity, Cat 2	Suspected of causing cancer
H360D	Reproductive toxicity Cat 1A, 1B	May damage the unborn child
H360Df	Reproductive toxicity Cat 1A, 1B	May damage the unborn child. Suspected of damaging fertility
H360FD	Reproductive toxicity Cat 1A, 1B	May damage fertility; may damage the unborn child
H361d	Reproductive toxicity Cat 2	Suspected of damaging the unborn child
H361fd	Reproductive toxicity Cat 2	Suspected of damaging fertility and of damaging the unborn child
H372	Specific target organ toxicity, repeated exposure, Cat 1	Causes damage to organs through prolonged or repeated exposure
H373	Specific target organ toxicity, repeated exposure, Cat 2	May cause damage to organs through prolonged or repeated exposure

GHS globally harmonized system of classification and labeling of chemicals [106], *Cat* category

Statistical analysis

We used the software R-Studio (R 3.0.1 + and R-Studio Desktop 1.4.1717; The R Foundation for Statistical Computing; http://www.R-project.org) to run simple linear regression models (R-packages: ggplot2, dplyr, broom, ggpubr) to assess whether the amounts or TLs showed significant trends across years. The significance level was set to 0.05. Additionally, linear models were run for 13 herbicide classes and for the top ten AIs contributing most to the total TL in 2019. In the models the years represented the independent variable of the linear model, while the amounts and TLs were the dependent variable. For each linear model, the homogeneity of variances of residuals was assessed using the residuals vs. the fitted-plots, and normal distribution of the residuals was checked using qq-plots.

Results

Amounts of used herbicides

Sales data were provided for the period 2010–2019 for 80 herbicidal AIs grouped into 13 chemical classes. Most of these AIs, 93% (74 AIs), were approved for arable crops, 23% (18 AIs) for orchards and 11% (9 AIs) for viticulture; 9% (seven AIs) were simultaneously approved for arable crops, orchards and viticulture. Three AIs (iron sulphate, triclopyr, quinoclamine) were approved for use in turf, grassland, ornamental and sports lawns, pot plants, and cultivating trees and shrubs.

The total amount of herbicides decreased by 24% from 1480 t in 2010 to 1123 t in 2019 (Fig. 2, Table 3). In 2019, organophosphates, containing glyphosate and glufosinate-ammonium accounted for 22% of herbicides used, followed by amides and anilides (21%) and triazine and triazinones (14%); all other classes were used at proportions < 8%. While the amounts of most classes decreased between 2010 and 2019, amounts of the classes bipyridylium (AI: diquat) increased by 646%, inorganics by 187% (AI: iron sulphate) and thiocarbamate (AIs: prosulfocarb and tri-allate) by 119% during this period. But while those three classes increased proportionally, their total contribution is minor when compared to all others (Fig. 2). Some classes such as phenoxy-phytohormones and benzoic acid showed a marginally significant decrease ($P<0.10$), while amides and anilides and dinitroaniline fluctuated from year to year and showed no overall trend (Table 3).

Toxic loads for honeybees, earthworms and birds

Of the 80 AIs used from 2010 and 2019, the proportion of highly toxic AIs to the evaluated species ranged from 0 to 2.5% for acute toxicity and accounted for 2.0% of 50 AIs with available chronic earthworm toxicity measures (NOEC) and about 6.6% of 61 AIs with chronic bird

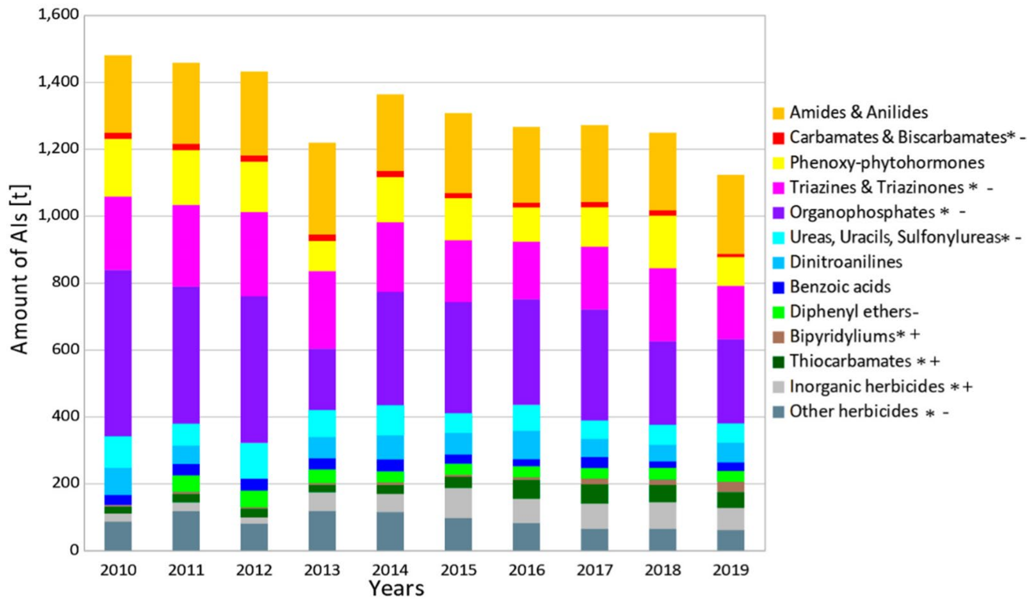

Fig. 2 Amounts of herbicidal AIs sold in Austria between 2010 and 2019 split into 13 chemical classes. *indicates significant increases (+) or decrease (–) across years. See Table 3 for statistical results

Table 3 Herbicide chemical classes market share based on amounts sold, and change between 2010 vs 2019 in Austria

Chemical class	Market share 2019 (%)	Change 2010–19 (%)	R^2	*F*-value	β1	β0	*P*-value	
Amides and anilides	21.0	2	0.13	1.14	− 2E+03	4E+06	0.316	
Carbamates and biscarbamates	0.9	− 45	0.62	12.95	− 7E+02	1E+06	**0.007**	↓
Phenoxy-phytohormones	7.6	− 51	0.34	4.04	− 6E+03	1E+07	0.079	
Triazines and triazinones	14.2	− 27	0.53	9.10	− 7E+03	2E+07	**0.017**	↓
Organophosphates	22.4	− 49	0.45	6.65	− 2E+04	4E+07	**0.033**	↓
Ureas, uracils and sulfonylureas	5.1	− 39	0.47	7.00	− 4E+03	8E+06	**0.029**	↓
Dinitroanilines	5.3	− 27	0.15	1.23	− 2E+03	3E+06	0.304	
Benzoic acids	2.3	− 16	0.39	5.17	− 1E+03	2E+06	0.053	
Diphenyl ethers	2.9	[a]− 33	0.65	13.08	− 2E+03	4E+06	**0.009**	↓
Bipyridyliums	2.8	646	0.65	15.10	2E+03	− 5E+06	**0.005**	↑
Thiocarbamates	4.2	119	0.72	20.29	4E+03	− 8E+06	**0.002**	↑
Inorganic herbicides	5.9	187	0.67	16.55	7E+03	− 1E+07	**0.004**	↑
Other herbicides	5.4	− 30	0.43	6.16	− 5E+03	1E+07	**0.038**	↓
Total amounts	100	− 24	0.74	23.26	− 3E+04	7E+07	**0.001**	↓

P-values and parameters from linear models testing changes over the years. Significant *P*-values in bold, arrows indicate the direction of the change

[a] % Change from 2011–2019, because in 2010 amounts of diphenyl ether were missing

toxicity values (NOEL). Highly acutely toxic to honeybees by contact exposure was quinoclamine and to birds were ioxynil and diquat. Flupyrsulfuron-methyl exhibited high chronic toxicity to earthworms, and flufenacet, diquat, flurtamone and prosulfuron were highly chronically toxic to birds.

The majority of used AIs were moderately acutely toxic to all three species, ranging from 57.5 to 90.0%, while chronically moderately toxic AIs accounted for 90.0% for earthworms and 88.5% for birds (Table 4). Non-toxic AIs ranged from 10.0 to 42.5% for acute toxicity and accounted for only 4.9% of chronic earthworm toxicity and 8% of chronic bird toxicity.

Table 4 Assessment of herbicidal AIs sold in Austria from 2010 to 2019 in terms of acute toxicity for honeybees and earthworms ($n=80$) and available chronic toxicity for earthworms ($n=50$) and birds ($n=61$)

Species	Number of AI in toxicity category					
	Highly toxic		Moderately toxic		Non-toxic	
	No. AIs	%	No. AIs	%	No. AIs	%
Honeybee LD_{50} oral [µg bee^{-1}] ($n=80$)	0	0.0	46	57.5	34	42.5
Honeybee LD_{50} contact [µg bee^{-1}] ($n=80$)	1	1.25	55	68.8	24	30.0
Earthworm LC_{50} [mg $kg_{soil}{}^{-1}$] ($n=80$)	0	0.0	72	90.0	8	10.0
Bird LD_{50} [mg $kg_{bw}{}^{-1}$] ($n=80$)	2	2.5	58	72.5	20	25.0
Earthworm NOEC [mg $kg_{soil}{}^{-1}$] ($n=50$)	1	2.0	45	90.0	4	8.00
Bird NOEL [mg $kg_{bw}{}^{-1}$ d^{-1}] ($n=61$)	4	6.6	54	88.5	3	4.9

Persistence data were available for all 80 AIs used. Of these AIs, 2.5% (two AIs) were very persistent (> 365 days), 3.6% (three AIs) persistent, 21.3% (17 AIs) moderately and 72.5% (58 AIs) non-persistent (< 30 days). A detailed list of the ecotoxicological assessments of all AIs, including available acute LD_{50} (oral, contact) for honeybees and LC_{50}/LD_{50}, NOEC values for earthworms, LD_{50} and NOEL values for birds and DT_{50} (Additional file 1: Table S1) and ranking (Additional file 1: Table S2) can be found in the Additional file 1.

Acute toxic loads increased fivefold for honeybees, earthworms and birds from 2010 to 2019 (Table 5). This was due to a general shift in the composition of AIs used, with a relative increase of more toxic and/or persistent AIs over the years (Fig. 3).

The increase in TL was slight from 2010 to 2016 but then increased steeply until 2019. This was mainly due to a strong increase in the amount of the class bipyridylium herbicides (Fig. 3, Table 6).

Of the 13 chemical classes evaluated, two (carbamates and biscarbamates, other herbicides) showed significantly and one (benzoic acid) marginally significantly reduced TLs for honeybees, earthworms and birds (Table 6). Three classes (bipyridyliums, thiocarbamates, inorganics) showed increased TLs for the three species and one class with declining amounts (diphenyl ether) showed increased toxic loads for honeybees and earthworms but reduced TLs for birds. Individual changes in TLs were observed due to the different, species-specific toxic relevance of used AIs per chemical class; individual reductions in TLs for honeybees (phenoxy-phytohormones), earthworms (organophosphates); individual increases in TLs for honeybees and birds were seen for the ureas, uracils and sulfonylureas with declining amounts. For three herbicide classes (amides and anilides, triazines and triazinones, dinitroanilines), there was no significant change in toxic loads between 2010 and 2019.

The ranking of AIs contributing most to the respective TLs in 2019 was topped by diquat for honeybees (oral), earthworms and birds with > 92% TL share for the considered organisms (Table 7). The second rank of iron sulphate was common to the three species, although the TL share was < 5%. Both diquat and iron sulphate increased significantly between 2010 and 2019, by 646% and 187%, respectively. Toxic load ranks 3–10 had species-specific AIs depending on their toxic relevance but their individual TL-shares were < 1% (honeybee contact TL in supplemental information Additional file 1: Table S3 and S4). Persistence (expressed in half-lives, DT_{50}) varied considerably among the listed AIs, ranging from 5500 days for the most persistent AI diquat to 10 days for the non-persistent prosulfocarb (Table 7).

Table 5 Toxic loads (measured as number of released LD_{50}s weighed by half-life, DT_{50}) for honeybees (*A. mellifera*, oral), earthworms (*E. fetida*) and birds (*S. serinus*) of herbicides used in Austria between 2010 and 2019, change between 2010 and 2019 and parameters from linear models testing changes of total toxic loads

Species	Toxic loads in 2019 [no. LD_{50} weighed by DT_{50}]	Change 2010–19 (%)	R^2	*F*-value	Regression line	*P*-value	
Honeybees	5.59E+13	487	0.70	18.97	Y=4E+12*X–8E+15	**0.002**	↑
Earthworms	3.67E+09	498	0.67	16.43	Y=3E+08*X–5E+11	**0.004**	↑
Birds	1.86E+12	580	0.67	16.36	Y=1E+11*X–3E+14	**0.004**	↑

P-values in bold if significant, arrows indicate the direction of the change

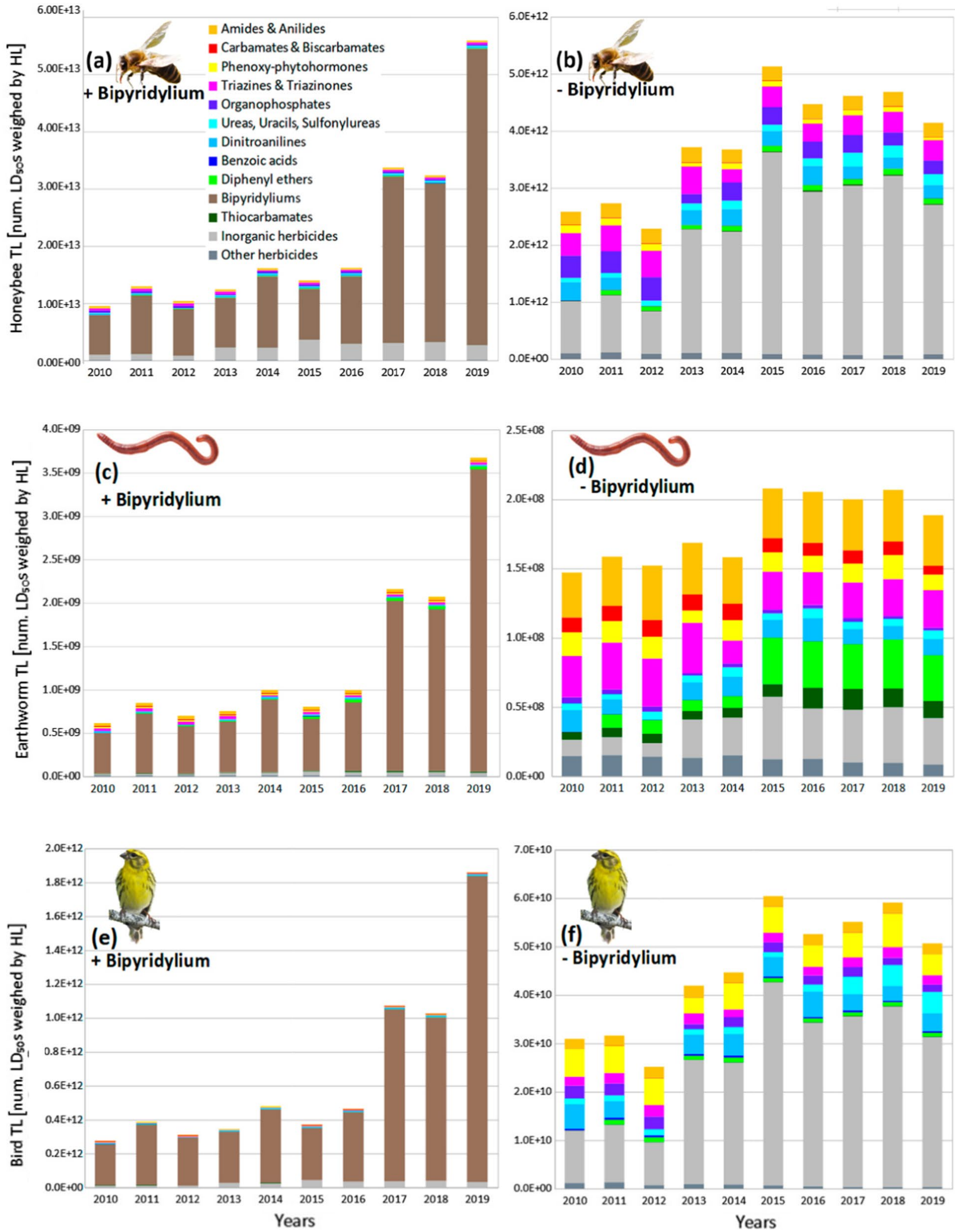

Fig. 3 Acute toxic loads (TL) of herbicidal chemical classes used from 2010 and 2019 in Austria for (**a**, **b**) honeybees (*A. mellifera*), (**c**, **d**) earthworms (*E. fetida*), and (**e**, **f**) birds (*S. serinus*). Calculations based on LD_{50} values weighted by soil half-lives (DT_{50}). Left column (**a**, **c**, **e**) showing all herbicide classes, right column (**b**, **d**, **f**) without the most problematic bipyridyliums. Note different y-axes scales. See Table 6 for statistical results

Table 6 Toxic load (TL) assessment of herbicide classes for honeybees (*A. mellifera*, oral), earthworms (*E. fetida*) and birds (*S. serinus*), TL share of herbicide class for each organism, change 2010 vs 2019 and parameters from linear models testing changes of toxic loads, *P*-values in bold if significant, arrows indicate the direction of the change

Chemical class	Species	TL share 2019 (%)	Change 2010–19 (%)	R^2	*F*-value	*P*-value	
Amides and anilides	Honeybee	0.4	11	0.06	0.47	0.510	
	Earthworms	1.0	13	0.14	1.33	0.283	
	Birds	0.1	15	0.12	1.10	0.326	
Carbamates and biscarbamates	Honeybee	< 0.1	−43	0.58	10.98	**0.011**	↓
	Earthworms	0.2	−41	0.56	10.09	**0.013**	↓
	Birds	< 0.1	−43	0.62	13.27	**0.007**	↓
Phenoxy-phytohormones	Honeybee	0.1	−64	0.62	12.96	**0.007**	↓
	Earthworms	0.3	−35	0.08	0.71	0.425	
	Birds	0.2	−26	0.00	0.02	0.883	
Triazines and triazinones	Honeybee	0.6	−10	0.24	2.51	0.152	
	Earthworms	0.7	−8	0.21	2.08	0.188	
	Birds	0.1	3	0.03	0.25	0.629	
Organophosphates	Honeybee	0.4	−39	0.36	4.56	0.065	
	Earthworms	0.1	−55	0.48	7.44	**0.026**	↓
	Birds	0.1	−42	0.39	5.15	0.053	
Ureas, uracils and sulfonylureas	Honeybee	0.3	149	0.74	22.87	**0.001**	↑
	Earthworms	0.2	30	0.18	1.74	0.223	
	Birds	0.2	280	0.67	16.57	**0.004**	↑
Dinitroanilines	Honeybee	0.4	−27	0.15	1.25	0.300	
	Earthworms	0.3	−27	0.15	1.25	0.300	
	Birds	0.2	−27	0.15	1.25	0.300	
Benzoic acids	Honeybee	< 0.1	−16	0.39	5.15	0.053	
	Earthworms	< 0.1	−16	0.39	5.15	0.053	
	Birds	< 0.1	−16	0.39	5.15	0.053	
Diphenyl ethers	Honeybee	0.2	[a]18	0.49	6.62	**0.037**	↑
	Earthworms	0.9	[a]240	0.73	19.00	**0.003**	↑
	Birds	< 0.1	[a]−23	0.60	10.70	**0.014**	↓
Bipyridyliums	Honeybee	92.6	646	0.65	15.08	**0.005**	↑
	Earthworms	94.9	646	0.65	15.08	**0.005**	↑
	Birds	97.3	646	0.65	15.08	**0.005**	↑
Thiocarbamates	Honeybee	< 0.1	109	0.71	19.65	**0.002**	↑
	Earthworms	0.3	118	0.72	20.39	**0.002**	↑
	Birds	0.0	102	0.70	19.06	**0.002**	↑
Inorganic herbicides	Honeybee	4.7	187	0.67	16.54	**0.004**	↑
	Earthworms	0.9	187	0.67	16.54	**0.004**	↑
	Birds	1.7	187	0.67	16.54	**0.004**	↑
Other herbicides	Honeybee	0.2	−20	0.63	13.51	**0.006**	↓
	Earthworms	0.2	−41	0.83	40.05	**< 0.001**	↓
	Birds	< 0.1	−75	0.87	51.24	**< 0.001**	↓

[a] % change from 2011 to 2019, because in 2010 amounts of diphenyl ether were missing

Human toxicology

Of all 80 AIs with documented usage during 2010–2019, 19% (15 AIs) were classified as highly hazardous pesticides (HHP), while for five HHP AIs (mecoprop, quizalofop-P-tefuryl, tepraloxydim, flumioxazin, pyraflufen-ethyl) the authorities did not provide amounts. In our quantitative analysis we excluded chlorpropham because it is used mainly indoors and thus assessed only 14 HHP AIs. Of all 14 used HHP-AIs 78.6% (11 AIs) were classified as

Table 7 Toxic loads (TL) rankings of herbicide AIs used in 2019 in Austria for honeybees (*A. mellifera*), earthworms (*E. fetida*) and birds (*S. serinus*), share on total TL for each organism, change 2010 vs 2019 and parameters from linear models testing changes of toxic loads, *P*-values in bold if significant, arrows indicate the direction of the change

Rank	Active ingredient	LD_{50}	Half-life [days]	Share on TL 2019 (%)	Change 2010–19 (%)	R^2	*F*-value	*P*-value	
Honeybees		[µg bee^{-1}]							
1	Diquat	13.0	5500	92.6	646	0.65	15.08	**0.005**	↑
2	Iron sulphate	100.0	1000	4.7	187	0.67	16.54	**0.004**	↑
3	Terbuthylazin	22.6	22	0.6	− 6	0.17	1.61	0.240	
4	Glyphosate	100.0	24	0.4	− 38	0.36	4.43	0.069	
5	Pendimethalin	101.2	101	0.4	− 27	0.15	1.25	0.300	
6	Chlorotoluron	88.7	70	0.2	4946	0.99	200.70	**0.001**	↑
7	S-metolachlor	85.0	23	0.2	4	0.02	0.13	0.728	
8	Aclonifen	107.0	80	0.2	[a]− 2	0.00	0.01	0.925	
9	Flufenacet	100.0	39	0.1	19	0.02	1.21	0.304	
10	Nicosulfuron	5.2	19	0.1	198	0.46	6.74	**0.032**	↑
Earthworms		[mg earthworms^{-1}]							
1	Diquat	193.0	5500	94.9	646	0.65	15.08	**0.005**	↑
2	Iron sulphate	7838.0	1000	0.9	187	0.67	16.54	**0.004**	↑
3	Aclonifen	307.1	80	0.9	[a]− 2	0.00	0.01	0.925	
4	Terbuthylazin	290.1	22	0.7	− 6	0.17	1.61	0.240	
5	Flufenacet	448.3	39	0.4	19	0.13	1.21	0.304	
6	Prosulfocarb	147.0	10	0.3	122	0.72	20.68	**0.002**	↑
7	Pendimethalin	2047.0	101	0.3	− 27	0.15	1.25	0.300	
8	MCPA	665.3	25	0.3	42	0.49	7.70	**0.024**	↑
9	S-metolachlor	1166.8	23	0.2	4	0.02	0.13	0.728	
10	Dimethenamid-P	602.6	16	0.2	77	0.18	1.77	0.221	
Birds		[mg bird^{-1}]							
1	Diquat	0.4	5500	97.3	646	0.65	15.08	**0.005**	↑
2	Iron sulphate	8.4	1000	1.7	187	0.67	16.54	**0.004**	↑
3	Chlorotoluron	2.2	70	0.2	4946	0.99	200.70	**0.001**	↑
4	MCPA	1.6	25	0.2	42	0.49	7.70	**0.024**	↑
5	Pendimethalin	6.4	101	0.2	− 27	0.15	1.25	0.300	
6	Glyphosate	15.4	24	0.1	− 38	0.36	4.43	0.069	
7	Terbuthylazin	8.5	22	0.1	− 6	0.17	1.61	0.240	
8	Metribuzin	1.1	19	< 0.1	76	0.68	16.97	**0.003**	↑
9	Aclonifen	13.7	80	< 0.1	[a]− 2	0.00	0.01	0.925	
10	S-metolachlor	11.3	23	< 0.1	4	0.02	0.13	0.728	

LD_{50} and half-life date from [91]

[a] %-change from 2015 to 2019 only, because data for aclonifen were missing in 2010

hazardous to human health, 21.4% (3 AIs) were listed because of high environmental concern (Additional file 1: Table S5). These 14 HHP-AIs accounted for 44% (646 t) of the total amounts used in 2010 and 36% in 2019 (399 t)). Amounts of 14 HHPs showed no significant trend from 2010 to 2019 ($R^2 = 0.33$, $F = 4.03$, $P = 0.08$), which could be due to large variations between years. The HHPs diquat, chlorotoluron and metribuzin increased.

Analysis of hazard statements of AIs approved between 2000 and 2019 showed that 64.8% (35 of 54 AIs with H-statements) had more than one H-statement regarding human health. Diquat was the most toxic AI with seven H-statements, ioxynil had six H-statements (Additional file 1: Table S6). The total number of H-statements of the herbicides used decreased from 78 H-statements in 2010 to 61 in 2019 (Fig. 4). Total amounts of AIs with at least one H-statement

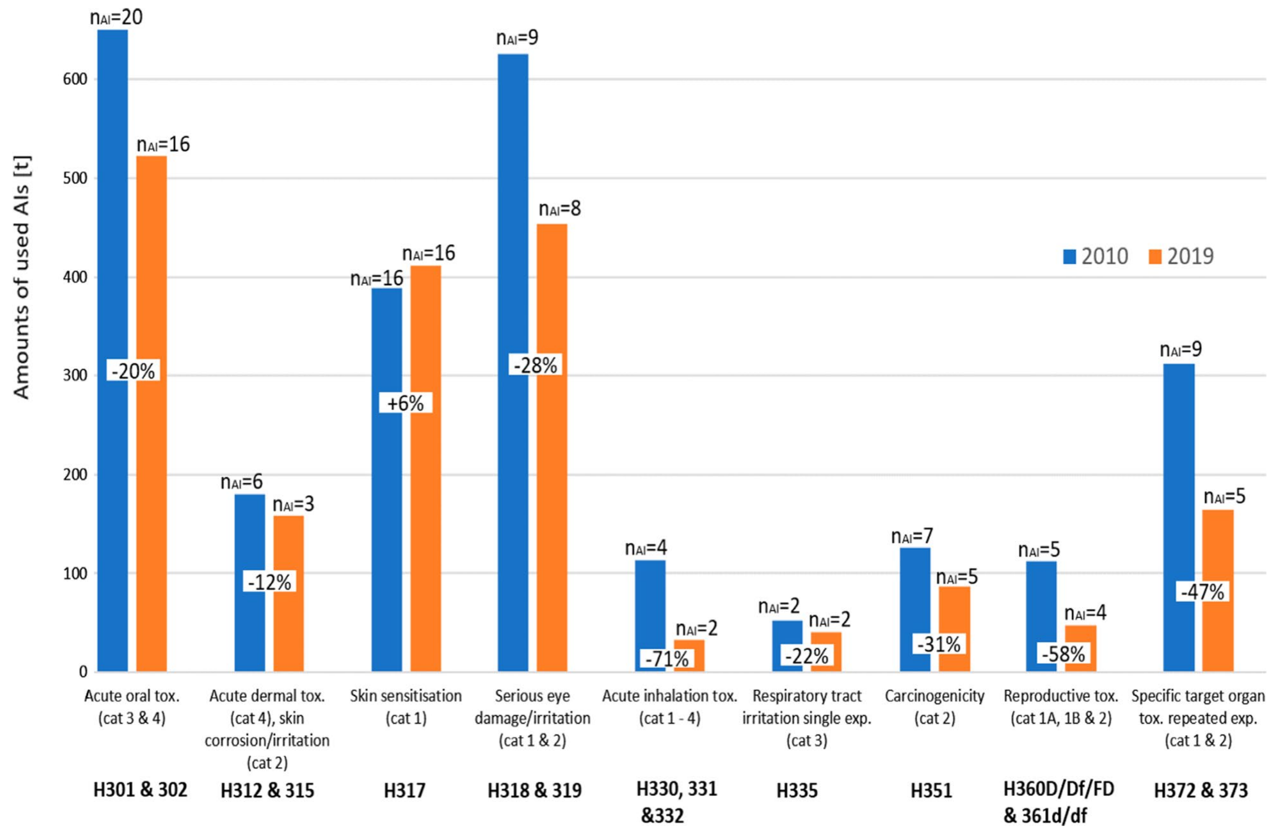

Fig. 4 Amounts (t) of AIs per H-statement used in 2010 and 2019, %-change of amounts 2010 vs 2019, number of AIs (n_{AI}) for each aggregated category. Only AIs with clearly attributable amounts were considered. *Cat* category, *exp* exposure, *tox* toxicity [107]. Codes of H-statements in bold

decreased from 1319.0 t in 2010 to 998.6 t in 2019 by 24%, amounts of AIs with at least two H-statements by 19%. Amounts of particularly hazardous AIs with at least four H-statements decreased by 75% from 168.5 t in 2010 to 42.2 t.

In 2019, there were 16 AIs with acute oral toxicity (H301 & H302) and 16 AIs with skin sensitization (H317). All other H-categories included between 2 and 8 AIs (Fig. 4). The largest amount reduction in AIs with H-statements from 2010 to 2019 was observed for acute inhalation toxicity (H330, H331, H332; -71%), reproductive toxicity (H360D/Df/FD & H361d/df;—58%) and specific target organ toxicity with repeated exposure (H372 & H373; – 47%). The number of potentially carcinogenic AIs (H351) decreased from 7 AIs in 2010 to 5 AIs in 2019; at the same time their amounts decreased by 31% from 126 t in 2010 to 86.5 t in 2019.

For humans (and other animals) it is important to point out that 12 AIs used between 2010 and 2019 are classified as potential endocrine disrupting (EDCs; penoxsulam, desmedipham, 2.4-D, 2.4-DB, metribuzin, pendimethalin, tribenuron-methyl, chlorotoluron, isoproturon, linuron, pyridate, glyphosate), and four AIs are confirmed EDCs (metamitron, bromoxynil-heptanoate/octanoate, ioxynil, picloram) [91].

Discussion

To our knowledge, this is one of the first studies to examine nationwide trends in herbicide use associated with toxic risks to honeybees, earthworms, birds, and humans. We found that the 24% decrease in herbicide amounts in Austria between 2010 and 2019 resulted in a 22% decrease in the total number of H-statements of herbicides used, a 24% decrease in the amounts of AIs used with at least one H-statement, and a 75% decrease for AIs with at least four H-statements. This indicates a shift towards AIs that are less hazardous to humans. However, over the same period, the decrease in herbicide amounts resulted in a 487% increase in persistence-weighted toxic loads for honeybees, 498% for earthworms, and 580% for birds. This result is relevant in that the reduction of herbicide amounts and risks to humans is partially close to the pesticide reduction targets set by the European Union under the "farm-to-fork"-strategy, but drastically missed the target of also reducing ecotoxicological risks for non-target organisms and biodiversity [108]. It is important to note that ecotoxicological risks are based on effects of toxicity versus exposure, while this study only considered toxic loads but not the exposure routes and factors that modify the actual exposure.

Herbicide usage patterns

The substantial 24% decrease in herbicide amounts between 2010 and 2019 can be attributed to several circumstances. First, in 2013, crop desiccation was banned in Austria for glyphosate, the most commonly used AI, mainly due to public pressure following herbicide-contaminated food and human urine samples [109]. Glyphosate was reduced by 38% from 408 tonnes in 2010 to 252 t in 2019. Second, Austrian Federal Railways, the second largest user of herbicides after agriculture, committed to reduce herbicide use [15]. Third, organically managed farmland with herbicide bans increased by 18% from 568,193 ha in 2010 to 669,921 ha in 2019 [69]. While organic farms typically use cover crops, tillage, and other mechanical methods to control weeds, some organically approved "burn-down" herbicides containing simple organic acids such as acetic acid, capric acid, caprylic acid, and pelargonic acid are available and are used in some crops. Indeed, in California, where pesticide use data are available, the use of several herbicide products containing caprylic and capric acids increased from zero kg used in 2008 to 53,072 kg (capric acid) and 77,949 kg (caprylic acid) used on 31,311 hectares in 2019 [110]. While such data are not available for Austria, the toxicity of these herbicides to humans and environmental receptors is rather limited due to their lack of systemic toxicity and rapid degradation in the environment, with typical half-lives of hours to days, depending on the degradation mechanism [111].

Comparing herbicide use trends with those of other countries is difficult because the need for herbicides varies with cropping systems. It can account for nearly 90% of pesticides used in sugar beet and less than 5% in vineyards [18]. However, herbicide use trends are available for 25 European countries showing an average increase in herbicide use of 13% between 2011 and 2019 [112]. However, trends vary considerably among countries, ranging from a 49% decrease over this period in Estonia to a 64% increase in Malta [112]. In Germany, amounts of top 11 herbicides increased by 17% between 2005 and 2015 [78], and the area treated with herbicides increased by 14.5% between 2011 and 2017 [113]. In the USA, herbicide use increased by 4182% from 1.11 million kg in 1991 to 46 million kg in 2018, especially in herbicide-tolerant genetically modified crops such as soybeans [114]. Italy's herbicide usage decreased by 27% from 28,129 t in 2010 to 20,559 t in 2019, with toxic and very toxic herbicides increasing by 62% (from 231 to 375 t) and their share by 122% [115].

Herbicidal toxic loads for honeybees, earthworms and birds

The increase in toxic loads for honeybees despite a decrease in pesticide use is consistent with other studies looking at honeybees in the UK [59] and the USA [62, 63], and more generally at insect pollinators and aquatic invertebrates in the USA [61]. However, none of those studies simultaneously evaluated ecotoxicological aspects of herbicides only, and none of them considered effects on earthworms. The impact of herbicides is relevant because their reduction potential appears to be high, because they are not only used for crop protection purposes as is the case with their use as desiccants, and because organic farming (at least in Europe) shows that farming without herbicides can be successful. Unpublished oral toxic herbicide loads for honeybees per acre in the USA (1992–2009) show an overall decline of toxic loads with herbicide amounts, mainly due to a decline of persistent AIs like diquat [63]. However, in contrast to Austria, glyphosate, which has low acute toxicity to honeybees and low persistence, increased in the USA over the years [116]. The increase of glyphosate in the USA is also associated with increasing toxicity to native plant species in soybean production [61], which negatively affects plant-pollinator-relationships. Although the acute toxicity of glyphosate to honeybees, earthworms and birds is rather low, several sublethal effects considering activity, embryonic development, reproduction, gut microbiota, foraging and navigation, have been reported [32, 33, 117, 118].

Our finding that toxic loads of birds to herbicides increases with decreasing herbicide amounts is in contrast to Tassin de Montaigu and Goulson [64], who found decreasing toxic loads with decreasing amounts. We explain this discrepancy in part by the different herbicides used in the UK and in Austria, which is due to the different crops grown in these countries. Moreover, in the UK study, the toxic load calculations were not adjusted for the respective persistence of AIs, as we did [64]. As a result of the inclusion of persistence in our evaluation, diquat was the most dominant AI for toxic loads of birds, honeybees and earthworms. Another AI with a high toxic load for birds was iron sulphate, which is only moderately toxic but also very persistent. Major contributors to bird toxic loads in birds in both Austria and the UK were chlorotoluron, MCPA, pendimethalin and glyphosate [64].

To our knowledge, this is the first study to evaluate persistence-weighted toxic loads of earthworms based on herbicide use. Toxic loads for earthworms were about 1000 times lower than for birds and 10,000 times lower than for honeybees. However, actual exposure is highly dependent on earthworm population density. Earthworm LC_{50} and NOEC-values are considered in the pesticide load calculations of others [60, 119], however both include several other parameters (human toxicity, environmental for other species, and environmental fate) in their assessments. Generally, earthworms are quite tolerant to pesticides while other soil biota such as springtails [120, 121] or insect larvae [122] are more sensitive to pesticides.

Our findings showed that herbicides can have high toxic loads because they are either toxic (e.g., diquat), very persistent (e.g., diquat, iron sulphate), or used in large amounts (e.g., glyphosate), or have a combination of these properties. The volatile, non-selective contact herbicide diquat accounted for only 2.8% of applied amounts in 2019 but had a toxic load fraction of > 92% for all species evaluated. It has been approved in Austria as foliar desiccant mainly in potatoes [79], but was also widely used throughout Europe as a desiccant on oilseed rape, sunflower, and pulses and as a herbicide on apple, citrus, pome fruit, stone fruit, tree nut, olive, grapevine, tomato, potato, carrot, chicory, sugar beet, and onions [123]. Depending on its use, exposure to honeybees, birds and earthworms will vary considerably. Additionally, it may affect non-target organisms by drifting onto these crops or adjacent flowering weeds and pose high exposure risk [27, 124]. Due to the high risk to human and bird health, it was for years a candidate for substitution in Europe and was finally withdrawn in May 2019 with a maximum grace period until February 2020 [107]. However, the high persistence of diquat in soil with a half-life of about 15 years, can lead to long-lasting, chronic poisoning of earthworms and their environment [47].

We have found high toxic loads for the selective, highly persistent, and relatively widely used iron sulphate which is applied to kill moss in lawns [91]. Because of its granular formulation, the risk of exposure to bees is considered low [125] but granular herbicides can affect earthworms [126] and birds [49]. We also have some reservations regarding the evaluation of iron sulphate because both iron and sulphate are essential elements that are part of the biological makeup, unlike many synthetic AIs. However, we took a pragmatic approach and relied on the same databases to evaluate the AIs. The third largest contributor to overall toxic loads in 2019 varied among organisms due to species-specific toxicities (Table 7). For honeybees, it was the non-selective, systemic terbuthylazine applied to arable land, orchards, vineyards. For earthworms, the selective, systemic aclonifen is applied pre-emergence on arable farmland. For birds, the selective, non-systemic chlorotoluron applied on arable land and orchards [79].

In general, insecticides are considered more toxic to honeybees, earthworms and birds than herbicides [4, 127, 128]. For example, the neonicotinoid insecticide imidacloprid is about 846 times more toxic to honeybees than the most orally toxic herbicide in the present study, aminopyralid [91]. For earthworms (*E. fetida*), the acute toxicity of the insecticide carbaryl is 3.9 times higher than the most toxic herbicide, flazasulfuron [91]. For birds (mallard ducks, *A. platyrhynchos*), the insecticide carbofuran is about 117 times more toxic than diquat [91]. Nevertheless, in our evaluation, one herbicide was classified as acutely highly toxic to honeybees (via contact exposure; quinoclamine), one was classified as chronically highly toxic to earthworms (flupyrsulfuron-methyl), and there were two herbicides with high acute toxicity to birds (ioxynil and diquat) and four with high chronic sublethal toxicity to birds (flufenacet, diquat, flurtamone, and prosulfuron) that were approved in Austria between 2010 and 2019.

Five herbicides—diquat, pendimethalin, chlorotoluron, glyphosate, metribuzin—that were among the top 10 contributors to honeybee, earthworm and bird toxic loads in 2019 are also classified as highly hazardous pesticides including environmental and human toxicity.

Human toxicology assessment

Herbicides are taken up by humans via ambient air, food, and skin [34]. Dietary uptake of herbicide residues on harvested products have been shown [129, 130], especially when herbicides (e.g., diquat) are used as desiccants prior to harvest. Such residues can be substantial, sometimes exceed maximal residue levels [131–133]. Using a qualitative analysis based on official hazard statements, we found a reduction in potential human toxicity risk from 2010 to 2019. However, despite this reduction, the amounts of AIs classified as highly hazardous pesticides did not show a decreasing trend [104]. Fortunately, 50% of the originally 14 used HHPs are no longer approved in Austria in 2022. However, of concern is that in 2019, five AIs used in 86.5 t are still suspected of causing cancer (H351; propyzamide, metazachlor, lenacil, chlorotoluron, aclonifen) and four of the AIs used in total of 47.6 t were suspected of damaging the unborn child (H361d; tembotrione, bromoxynil, fluazifop-P-butyl, chlorotoluron). Of all H-statements summarized for 2019, 8.2% were in the carcinogenicity class (cat. 2) and 6.6% in the reproductive toxicity class (cat. 2).

In principle, such a qualitative risk assessment is also carried out when evaluating the approval of a product: once a substance has been classified as carcinogenic, genotoxic or reproduction toxic, a quantitative risk assessment is usually no longer considered, since no approval may be granted for such pesticides [134, 135].

For the interpretation of human toxic risk, it is important to note that in our assessment, we used the official EU pesticide database, which classifies glyphosate as not carcinogenic. However, HHP analysis using other data sources classified glyphosate as potentially carcinogenic [34, 136].

Diquat, which showed increased use between 2017 and 2019, had the highest number of hazard-statements. According to the EFSA peer review of the pesticide risk assessment of diquat [123], it is harmful if swallowed (H302), fatal if inhaled (H330), causes skin and serious eye irritation (H315&319), may cause an allergic skin reaction (H317) and respiratory tract irritation (H335), and causes damage to organs through prolonged or repeated exposure (H372). It has been banned in Austria and throughout Europe with a grace period until February 2020 [79]. In addition, diquat has been linked to an increased risk of Parkinson's disease among French farmers [137]. In low- and middle-income countries, the easy availability of highly toxic pesticides such as diquat in farmers' households makes it the preferred means of suicide with an extremely high mortality rate [138].

Our finding that 12 AIs used between 2010 and 2019 are classified as potential and 4 are confirmed endocrine disrupting chemicals is important because endocrine disruption can occur at very low doses, contradicting classical dose–response relationships [139, 140]. Several of them can also be found in streaming waters, drinking water wells, and in comestible goods of herbal origin in all EU member countries [141, 142].

It is important to note that our toxic load assessment was based on parameters considered in official environmental risk assessments, such as LD_{50}, LC_{50}, and DT_{50}. However, real-world exposure and vulnerability to pesticides is more complex and involves many more interacting factors. Some examples include interactions between multiple pesticides [76] or different toxicities of AIs and co-formulants [32, 121]. Interactions in the field could potentially decrease overall herbicide toxicity, or enhance indirect effects by reducing food or shelter availability to non-target animals [25, 26]. We also did not include in our calculations degradation products of AIs and their inherent toxicity [143]. However, the complex situation in the field is not readily taken into account even in official environmental impact assessments, resulting in negative impacts on non-target organisms and biodiversity [144, 145]. Generally, pesticide use by kilograms applied does not provide a comprehensive estimate of toxicity loading to an ecosystem [63] and besides toxicity and persistence, application methods and timing, exposure routes, and mechanisms of dissipation from the application site all influence the net toxicity experienced by non-target organisms. However, the data needed to do an analysis that incorporates all of these factors is largely unavailable [63]. Therefore, our toxic load approach perhaps overestimates acute toxicity hazard to honeybees, birds and earthworms because of the simplifying assumptions used. Otherwise, our approach could also underestimates actual toxicity hazard because it does not account for sublethal effects, pesticide leaching, or potential synergistic impacts of pesticides used in combination in the field [63]. A detailed discussion of the toxic load approach is beyond the scope of this paper and can be found elsewhere [60, 63, 146]. Ecotoxicological assessments are usually performed on surrogate species, although there is interspecific sensitivity to pesticides [25]. However, we anticipate that the trends of increasing toxic risks calculated for our surrogate species due to increasing use of highly persistent AIs will most likely apply to other bee, earthworm and bird species and provide insights into the overall situation for non-target organisms and biodiversity [63].

Conclusions

Our findings highlight that a sole focus on a quantitative reduction of herbicides, neglecting the ecotoxicological properties of AIs, does not ensure the protection of non-target organisms and leads to poor policy decisions [147]. A strong focus on human toxicological parameters may lead to trade-offs between human health and environmental integrity, resulting in an even higher potential toxic load to non-target organisms. These trade-offs should be considered when implementing measures to reduce pesticide use to protect public health and biodiversity, such as the EU´s "farm-to-fork" strategy which aims to reduce the amounts and risks of synthetic pesticides [68]. One way to avoid such trade-offs would be to completely eliminate the use of herbicides, as is successfully practiced in organic agriculture [148], resulting in greater above and belowground biodiversity, lower economic costs and advantages for public health [149].

The toxic load approach shows the potential risk of acute poisoning instead of actual exposure or fatalities. The use of nationwide sales data provides only a rough estimate of toxicological exposure, and certainly field-specific, spatially referenced use data would provide a more accurate risk assessment [150]. Since professional applicators are legally required to keep record of all pesticides used, date of application, area and crops treated, these data could be centrally collected, anonymized, and made openly available to improve transparency and facilitate future research [150].

Further, integrative monitoring programs to assess biodiversity and human toxicological parameters in the agricultural landscape are essential to assess the long-term impacts of pesticides and other factors of agricultural intensification.

Abbreviations
TL: Toxic load; EW: Earthworms; AI: Active ingredient; GHS: Globally Harmonized System of Classification and Labeling of Chemicals; HHP: Highly hazardous pesticide; bw: Bodyweight; H-statement: Hazard statement; LD50: Median lethal dose; LC50: Median lethal concentration; NOEC: No observed effect concentration; NOEL: No observed effect level, EDC: endocrine disruptive compound/chemical.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12302-022-00622-2.

Additional file 1: Table S1. Honeybee, earthworm, bird toxicity & DT_{50} of AIs approved 2000-2019. (*): HHP. Bird columns: (A): A. platyrhynchos, (Cj): C. japonica, no abbreviation: C. virginianus. R (Rating) columns: n.t.: non-toxic, m.t.: moderately toxic, h.t.: highly toxic, n.p.: non-persistent, m.p.: moderately persistent, p.: persistent, v.p.: very persistent. **Table S2.** Ranking of AIs approved during 2000–2019 most toxic to honeybees (*A. mellifera*, oral + contact toxicity combined), earthworms (*E. fetida*, acute (LC_{50}), chronic (NOEC)) and birds (*S. serinus*, acute LD_{50}, chronic NOEL) and most persistent. AIs with * were not approved in Austria anymore in 2021 (BAES 2021). **Table S3.** %-share, change & linear model output of contact TLs for honeybees (A. mellifera, $LD_{50\ contact}$) from herbicides 2010–2019, *P*-values (in bold print if significant), arrows indicate the direction of trends. **Table S4.** TL ranking of herbicide AIs used in 2019 for honeybees (*A. mellifera*) via contact exposure, %-share on total TL , %-change from 2010–2019, LD50 & HL (Lewis et al. 2016), *P*-values (in bold print if significant), arrows indicate the direction of trends. **Table S5.** Herbicidal AIs approved in Austria in 2000–2019 classified as highly hazardous pesticides (HHP), reasons for classification (PAN 2021). * not approved in Austria in 2021 anymore (BAES 2021). GHS… Globally Harmonized System of Classification and Labeling of Chemicals, IARC…International Agency for Research on Cancer, EPA…United States Environmental Protection Agency. For AIs in bold used amounts were provided for 2010–2019. **Table S6.** H-statements ((EC)1272/2008) of herbicide AIs (n=101) (1: classified as) approved in AT 2000–19. Last column: sum of H-statements per AI. Penultimate line: sum AIs per H-statement. **Fig. S1.** TLs of herbicidal chemical classes used from 2010–2019 in Austria for (a, b) honeybees (*A. mellifera*) via contact exposure ($LD_{50\ contact}$). Left column (a) considering all herbicide classes, right column (b) without bipyridylium herbicides.

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Author contributions
RMC, SJ, JGZ conceptualized the study, curated data and conducted the formal data analyses; KH contributed human toxicological aspects, SK helped with toxic load calculations, FL provided expertise in statistical analyses. RMC, SJ, JGZ wrote the original draft. All authors read and approved the final manuscript.

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The original datasets from authorities cannot be made available due to business secrecy. All other data are available in the Additional file 1.

Declarations

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Competing interests
The authors declare no financial and non-financial competing interests.

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