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A nation-wide survey of neonicotinoid insecticides in agricultural land with implications for agri-environment schemes

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ABSTRACT

1. Neonicotinoids are the most widely used class of insecticides globally. However, the link between farming practices and the extent of contamination of soils and crops by neonicotinoid insecticides, as well as and the extent of such contamination in organic fields and ecological focus areas (EFAs) are currently unclear.

2. We measured the concentrations of five neonicotinoid insecticides (imidacloprid, clothianidin, thiamethoxam, thiacloprid, acetamiprid) in 702 soil and plant samples in 169 cultivated fields and EFAs from 62 conventional, integrated production and organic farms distributed over the entire lowland of Switzerland.

3. We detected neonicotinoids in 93% of organic soils and crops, and more than 80% of EFA soils and plants – two types of arable land supposedly free of insecticides. We also tested 16 samples of organic seeds, of which 14 were positive for neonicotinoids.

4. Finally, we calculated hazard quotients (HQs) and potentially affected fractions for 72 beneficial and 12 pest species. Under a field-realistic scenario, we found that between 5.3 and 8.6% of above-ground invertebrate species may be exposed to lethal concentrations of clothianidin, and 31.6 to 41.2% to sublethal concentrations, in "integrated production" and conventional fields. We also found that 1.3 to 6.8% (up to 12.5% based on HQs) of the beneficial invertebrate species may be exposed to sublethal concentrations of neonicotinoids in EFAs and organic fields. In contrast, no pest species would be exposed to lethal concentrations, even under a worst-case scenario.

5. *Synthesis and applications*. Our study suggests that diffuse contamination by neonicotinoids may harm a significant fraction of non-target beneficial species. The Use of neonicotinoids on crops may threaten biodiversity in refuge areas, while also potentially jeopardizing the practice of organic farming by impeding the biological control of pests. Based on our results, we call for a reduction in the dispersion and overuse of neonicotinoid insecticides in order to prevent any detrimental effects on biodiversity and ecosystem services associated with agroecosystems.

ZUSAMMENFASSUNG

1. Neonicotinoide sind weltweit die am meisten verbreitete Gruppe von Insektiziden. Es ist jedoch unklar inwiefern ein Zusammenhang zwischen den landwirtschaftlichen Praktiken und dem Ausmass der Kontimination von Böden und Erntegut durch Neonicotinoide besteht. Über das Ausmass einer solchen Kontamination auf biologischen Feldern und Ökologischen Ausgleichsflächen (ÖAF) ist ebenfall wenig bekannt.

2. Wir haben die Konzentrationen von fünf Neonicotinoid Insektiziden (Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid) in 702 Boden- und Pflanzenproben von 169 Ackerflächen und ÖAF von 62 konventionellen, IP (Integrierter Landbau) und biologischen Landwirtschaftsbetrieben im schweizer Mittelland gemessen.

3. Auf 93% der Böden und des Ernteguts der biologischen Betriebe als auch auf über 80% der Böden und Pflanzen der ÖAF haben wir Neonicotinoide festgestellt – zwei Arten von landwirtschaftlichen Flächen, welche vermeintlich frei von Insektiziden sein sollten. Wir haben ebenfalls 16 Proben von biologischem Saatgut untersucht, von denen 14 Neonicotinoide enthielten.

4. Schliesslich haben wir den Gefahrenquotienten (GQ) und den potentiel betroffenen Anteil von 72 Nützlingen und 12 Schädlingen berechnet. In einem feldrealistischen Szenario haben wir festgestellt, dass zwischen 5,3 bis 8,6% der oberirdischen Wirbellosenarten tödlichen Konzentrationen von Clothianidin und 31,6 bis 41,2% subletalen Konzentrationen in der IP- und konventionellen Feldern ausgesetzt sein können. Wir haben ebefalls festgestellt, dass 1,3 bis 6,8% (bis zu 12,5% basierend auf GQ) der Nützlinge subletalen Konzentrationen von Neonicotinoiden in ÖAF und biologischen Feldern ausgesetzt sein können. Im Gegensatz dazu würden Schädlinge selbst im schlimmsten Szenario keiner tödlichen Konzentration ausgesetzt sein.

5. *Synthese und Anwendungen*. Unsere Studie deutet darauf hin, dass eine diffuse Kontamination durch Neonicotinoide einen erheblichen Teil der nicht zu den Zielgruppen gehörenden Nützlingen schädigen kann. Die Verwendung von Neonicotinoiden auf Ackerflächen kann die biologische Vielfalt in Rückzugsgebieten bedrohen und gleichzeitig den biologischen Landbau gefährden, indem sie die biologische Schädlingsbekämpfung behindert. Auf Grundlage unserer Ergebnisse fordern wir eine Verringerung der Verbreitung

und des übermäßigen Einsatzes von Neonicotinoid-Insektiziden, um schädliche Auswirkungen auf die Biodiversität und die mit Agrarökosystemen verbundenen Ökosystemleistungen zu vermeiden.

INTRODUCTION

In recent decades, the worldwide loss of habitats together with an increasingly intensive agricultural production have resulted in the aggravated impoverishment of farmland biodiversity at all trophic levels (Tsiafouli et al., 2015, Hallmann et al., 2014, Geiger et al., 2010). In agricultural landscapes, agri-environment schemes (AESs) have been implemented by European governments to compensate for the impact of farming practices and to halt or slow down habitats and biodiversity erosions (Commission, 2010). AESs comprise two major tools: set-aside schemes, i.e. ecological focus areas (EFAs), which help maintain attractive landscapes and are extensively or low-intensively managed (e.g. hedgerows, ponds, extensive meadows) and in-production schemes that support environmentally-friendly crop production or pasture lands, i.e. organic farming and permanent pastures (Batáry et al., 2015). All member countries of the EU, as well as Switzerland and Norway, must provide direct payments to farmers who allocate a minimum of 5% of their arable land to EFAs (7% in Switzerland) and strict regulations apply to organic farming (Pe'er et al., 2014). These AESs are a very powerful tool to test whether a diversity of farming practices leads to a diversity of landscapes and therefore helps sustain habitat and species diversity. They also provide a remarkable framework to investigate how different farming practices are related to environmental contamination by pesticides.

There are growing concerns about the potential contamination of AESs by synthetic agrochemicals (Botías et al., 2016, Goulson, 2013). Among synthetic insecticides, neonicotinoids are the most widely used class of products, administered mainly prophylactically as seed coating, but also sprayed on some crops (Jeschke et al., 2011). The widespread use of neonicotinoids, the fact that neonicotinoid-containing dust is produced during sowing (Tapparo et al., 2012, Krupke et al., 2017, Stewart et al., 2014), their high solubility in water and their stability in soil (their half-life ranges from 3.4 to 1,000+ days depending on compound; (reviewed in Goulson, 2013 may lead to accumulation over time and spread over adjacent soils and vegetation (Botías et al., 2016, Bonmatin et al., 2015, Main et al., 2016). Consequently, neonicotinoid insecticides represent an environmental risk to adjacent non-treated land over distances that are so far little known, with potential consequences for non-target species. To date, some studies have assessed the contamination of different matrices (e.g. plants, pollen) and honeybees by neonicotinoids well beyond the fields where coated-seeds were sown, including isolated sites up to 140m from the nearest treated crops (Krupke et al., 2017, Mogren and Lundgren, 2016). However, the extent of contamination of agri-environment schemes (AESs) by neonicotinoid insecticides remains largely unknown. Worldwide and nation-wide studies exist, which have examined honey and bird feathers contamination by neonicotinoids (Mitchell et al., 2017, Woodcock et al., 2018, Humann-Guilleminot et al., 2019), but they do not provide information about contamination of soils and plants from agri-environment schemes, because the exact foraging sites of honey bees and birds are either unknown or likely situated within a cultivated field. Hence, data on the presence of neonicotinoids in organic soils and crops and in non-cultivated vegetation at a scale covering a whole country are lacking, and this prevents any accurate evaluation of the European policies for a sustainable agriculture management.

In this context, we sampled soil and vegetation of agricultural fields distributed over the entire lowland of Switzerland. Our aims were three-fold: 1) assess the levels of contamination by five neonicotinoid residues in the soil and plants of EFAs (no insecticides allowed under Swiss regulation) and cultivated fields, 2) compare these levels according to the type of agricultural practice (conventional, reduced use of pesticides and biodiversity enhancing measures called “IP-Suisse” and organic), and 3) evaluate the risk incurred by soil and above-ground non-target invertebrates under such contamination levels. It should be noted that, in the year of the study (2015), three of these neonicotinoid insecticides (imidacloprid, clothianidin and thiamethoxam) were under a moratorium declared by the EU and Switzerland in December 2013, and could not be used on specific crops (e.g. spring cereals and flowering crops).

MATERIALS AND METHODS

Sampling procedures

The field study was carried out between 20 April and 15 June 2015, after the planting of crops in cultivated fields. In total, we sampled 100 cultivated fields and 69 ecological focus areas from 62 farms (20 organic, 20 IP-Suisse labelled and 22 conventional farms) across the entire Swiss lowland agricultural area (Fig. 1).

We contacted the farmers and obtained their agreement to sample soil and vegetation based on a list provided by cantonal agricultural counsellors, by the Centre for Agricultural Advisory and Extension Services (Agridea), and by Bio-Suisse. Each farm included at least one extensively managed EFA (no insecticides allowed under Swiss regulation) eligible to subsidies by the Swiss Confederation. In our sample, all organic farms started to use organic practices at least ten years ago.

On each farm, we sampled in two different fields (preferably in two different crops, e.g. beets and cereals) and in one EFA. In each field and EFA, we took two separate samples, at least 10 m apart, and at least 3 m away from the edge (6 m in extensive meadows). Samples were taken in fields of beetroot, cereals (wheat, oat, rye, spelt, barley), potatoes, rapeseed, maize, peas, and flax, as well as in an EFA (Table S1). We did not sample agricultural plots that had been used as pastures in previous years. We had no knowledge about treatments with neonicotinoids or other pesticides during and before the study year. From each sample site, we measured the soil pH using a pH indicator paper. Additionally, we noted the exact GPS coordinates of the samples in order to obtain the average slope of the sampled field and the proportion of arable land within a circle of 1000 m that drains into the sampled field.

At each sampling site, we collected and homogenized between 300 and 500 g of soil in the top 10 centimetres using a manual auger. We also collected approximately 30 g of foliage (preferring the highest leaves) from the respective crop or from a variety of herbaceous plant species (annuals, biennials and perennials) in EFAs. All plant and soil samples were stored in closed plastic bags at -80°C at the end of each working day until analysis. Plants were collected in the immediate vicinity (± 20 cm) of the soil samples.

Distribution of farm and crop types

In total, we collected 351 samples in 169 different fields from 62 different farms, distributed as follows (the same distribution applies for both soil and plant samples): 40% of EFAs, 40% of cereals fields, 10% of beetroots (only from conventional farms), and 10% of diverse crops (see Table S1 for details). In 40 farms, we sampled soil and plants in two different cultivated fields and one EFA (occasionally two EFAs). In the twenty-two other farms, where the number of fields was limited, we sampled soil and plants in only one cultivated field and one EFA.

Neonicotinoid insecticides in organic seeds

In an attempt to identify possible routes of contamination, we conducted a study to test whether commercial organic seeds contained neonicotinoid insecticides. Three farmers kindly provided us with samples of seeds: one sample of barley and one sample of maize, three samples of wheat, two samples of spelt, two samples of rye, two samples of oat and two samples of peas, as well as two samples of commercial extensive meadow seeds mix. Commercial seeds came from various well-established resellers and mills. We also bought a 25kg bag of organic oat seeds from a well-established reseller for further testing.

Identification and quantification of neonicotinoids

Sample preparation and extraction

Five hundred milligrams of fresh plants were ground in liquid nitrogen to a fine powder with a pestle and mortar and weighed in a 15 ml Falcon tubes (± 0.1 g). 1.25 grams of soil were dried, homogenized, sieved (2 mm mesh), ground to a fine powder with a pestle and mortar and weighed in a 15 ml Falcon tube (± 0.1 g). Five hundred milligrams of seeds were ground using a Retsch mill. Neonicotinoids were extracted using a QuEChERS procedure (see Supplementary Materials for details).

Sample analysis

The quantification of five neonicotinoids (imidacloprid, clothianidin, thiamethoxam, thiacloprid, acetamiprid) was carried out by UHPLC-MS/MS using a method adapted from (Mitchell et al., 2017) (see Supplementary Materials for details, Table S2). One blank sample (i.e. solvent without matrix submitted to the entire extraction procedure) per batch of 16-36 samples was included and injected in the UHPLC-MS/MS to ensure that no contamination occurred during sample preparation. Samples in which neonicotinoid concentrations exceeded the upper quantification limits were diluted 100 times and reanalysed. Detection and quantification limits for each neonicotinoid molecule can be found in Table S3. The concentrations measured in the same soil and plant samples analysed twice over time (N=12) were highly repeatable (intra-class correlation coefficient: 0.995 and 0.977 for soil and plants, respectively).

Potential impacts of neonicotinoids on non-target species

We searched the literature and listed 84 species of terrestrial arthropods and annelid worms (Oligochaeta) for which the imidacloprid and clothianidin concentrations that would kill 50% of individuals by contact exposure (LC_{50}; acute toxicity) are known (Table S4). In this list, 12 species (3 orders) are agricultural pests and 72 species (13 orders) are pollinators, species used for biological control of pests or species of high biological value (e.g. earthworms). We included predatory invertebrates (e.g. adult parasitoids) because they may be exposed to neonicotinoid insecticides by ingestion of plant products [e.g. some adult parasitoids feed on nectar and pollen; (Jervis and Kidd, 1986)] or through residual contact by moving on contaminated leaves (Armer, Wiedenmann and Bush, 1998).

Hazard Quotients

As commonly used in ecotoxicological assessments (e.g. Botías et al., 2016, we calculated the exposure toxicity ratio (Hazard Quotient: HQ) as the mean or the maximum concentration detected in plant or soil samples divided by the lethal contact concentration (LC_{50}) of each species. HQ has the convenience of using the same unit for the numerator and the denominator to provide information on both lethality and sublethality. This enabled us to include species for which no tests of sublethal effects were available. HQ $\geq$ 1 indicates that the concentration found in the sample is equivalent or greater than the concentration that kills 50% of a population. We considered that HQ $\geq$ 0.01 (1% of LC_{50}) is indicative of potential sublethal toxicity. Such a threshold is reasonable since studies have reported sublethal effects at such low levels in several invertebrates (Earthworms: Capowiez et al., 2003, Alves et al., 2013; *Bombus impatiens*: Morandin and Winston, 2003, Czerwinski and Sadd Ben, 2017, Wu-Smart and Spivak, 2018; *Apis mellifera*: Brandt et al., 2016, Straub et al., 2016; *Orgilus* *lepidus*: Symington, 2003). Yet, aware that this is an arbitrary value, we also interpret our results using higher thresholds: HQ ≥ 0.05 (5% LC_{50}) and HQ ≥ 0.1 (10% LC_{50}), two levels at which a number of studies have reported detrimental effects in a variety of invertebrate species [*Apis mellifera*: 5 % LC_{50} (Yang et al., 2008, Decourtye, Lacassie and Pham-Delègue, 2003); *Apis mellifera, Bombus impatiens, Eretmocerus mundus*: 10 % LC_{50} (Pisa et al., 2017, Sohrabi et al., 2013)]. When several LC_{50} were available for a given species, we used the higher value.

Species Sensitivity Distributions and Potentially Affected Fractions

Species sensitivity distributions (SSD) are cumulative distributions describing the proportion of species affected by increasing log-concentrations of a given substance (Aldenberg and Jaworska, 2000). We used our Table 3 and Fig. S3 to estimate three SSDs for beneficial and pest species using median lethal concentrations (LC_{50}) for 1) soil invertebrates exposed to imidacloprid, 2) above-ground invertebrates exposed to imidacloprid and 3) above-ground invertebrates exposed to clothianidin. There were too few toxicity data to estimate an SSD for soil invertebrates exposed to clothianidin. We also computed the SSDs for the same three combinations of invertebrates and molecule using 10% and 1% of the median lethal concentrations. Observed SSDs computed using LC_{50} were tested against three theoretical distributions: normal, gamma and logistic. Observed SSDs computed using 10 % and 1 % of the LC_{50} were tested against the normal and the logistic distributions. We fitted and tested the distributions using the fitdistrplus R package (Delignette-Muller and Dutang, 2015) by comparing AICs. In all cases, the best fit was obtained with the normal distribution.

Since the observed SSDs followed a normal distribution [Fig. S3 (A)], we assessed the potentially affected fraction (PAF) of species that would be exposed to lethal (LC_{50}) or sublethal concentrations (10% or 1% of LC_{50}) when exposed to the mean or the maximum concentration found in our soil and plant samples from EFAs, organic, IP-Suisse and conventional fields.

Statistical analyses

The goodness-of-fit of parametric models was verified by comparing the AIC of the full model against the AIC of the null model (intercept only). Modeling assumptions of parametric models (normality and homoscedasticity of residuals) were verified by visual inspection of the residuals plotted against predicted values and using quantile-quantile plots. To perform the statistical analyses, all concentrations that were above the limits of detection but below the limits of quantification (< LOQ) were set to zero.

Beetroot plants are fragile and susceptible to insect pests, and we found high amounts of neonicotinoid insecticides in soils and plants from these fields. Therefore, we treated this type of crop separately and we did not include data on beetroots in the tables. However, since there was no difference in the mean total neonicotinoid concentrations between cereals and other types of crops (Kruskal-Wallis $\chi 2 > 0.02$, df = 1, all $p > 0.7$), we merged these two categories for all our analyses.

When comparing the concentrations of neonicotinoids found in cultivated fields and EFAs from different farm types, the data distribution (many low values and zeros and some very high values) precluded the use of generalized linear mixed-effect models (non-normality of residuals and strong heteroscedasticity; see supplementary material for details about the models explored). Therefore, we used Brown-Forsythe tests based on ranked data, because such statistics are robust to heteroscedasticity (Vargha and Delaney, 1998). For the same reasons, Kendall rank correlations were used to assess the relationships between levels of neonicotinoids and field characteristics (watershed, slope, pH, date) in cultivated soils and crops, and soil and plant samples from EFAs.

Since the neonicotinoids found in plants are likely to be absorbed, at least partly, from the soil via the root system, we investigated how concentrations in plants were related to concentrations in soil. We ran linear mixed-effect models including the neonicotinoid concentration (above LOQ) in plants as the dependent variable and the neonicotinoid concentration in soil (above LOQ) as the explanatory variable. We also included field identity as a random factor to account for the non-independence of the two replicates taken in the same field. We did not include data from beetroots in these analyses, because the very high values from this crop precluded us from fitting a statistical model to the data. We ran three separate models: one for imidacloprid alone, another for clothianidin alone and a third one for total neonicotinoids. The best model fit was obtained with a log10-log10 relationship. Additionally, we used the whole dataset (including values below LOQ set to 0) to run non-parametric Kendall rank correlations and test the association between concentrations in soils and concentrations in plants. Linear mixed models (only above LOQ) and non-parametric correlations (including below LOQ; see Supplementary Material) rendered qualitatively similar results. All statistical analyses were performed using the R v. 3.1.1 software (Ihaka and Gentleman, 1996).

RESULTS

The total neonicotinoid concentrations of the soil or plant samples taken from the same field were highly repeatable (intra-class correlation coefficient: 0.83 and 0.74 for soil and plant samples, respectively). This validates the representativeness of our samples for a given field.

Distribution of neonicotinoids in soils and plants

Cultivated fields

The total concentration of the five neonicotinoids measured in cultivated fields are reported in Table 1, and were highest in conventional fields, followed by IP-Suisse and organic fields (Brown-Forsythe tests based on ranked data (soils and crops respectively): $F_{2,61} = 40.71$, $p < 0.0001$, $F_{2,74} = 16.12$, $p < 0.0001$; conventional vs. IP-Suisse: $F_{1,44} = 10.56$, $p = 0.002$, $F_{1,49} = 6.55$, $p = 0.014$; conventional vs. organic: $F_{1,53} = 123.6$, $p < 0.0001$, $F_{1,52} = 34.34$, $p < 0.0001$; IP-Suisse vs. organic: $F_{1,38} = 23.9$, $p < 0.0001$, $F_{1,48} = 8.6$, $p = 0.005$; Fig. 1, Fig. S1, Table 1). All soils and crops of conventional and IP-Suisse fields, and 93% of organic soils and crops contained at least one neonicotinoid at a concentration above the limit of quantification (Fig. 1, S1). Imidacloprid and clothianidin clearly dominated in terms of presence and concentration in all samples from cultivated fields (Table 1). In both soil and crops, more than 46% of samples contained at least two neonicotinoids in concentrations above the LOQ. The imidacloprid-clothianidin combination was the most common in all cultivated soil samples and in all crop samples from conventional and IP-Suisse farms. In organic crops neonicotinoid combinations were more diverse (Table 2).

Ecological focus areas

The total concentration of the five neonicotinoids measured in EFAs are reported in Table 1. Concentrations were lower than in cultivated fields (Brown-Forsythe test based on ranked data: $F_{1,149} = 38.4$, $p < 0.0001$, $F_{1,146} = 438.2$, $p < 0.0001$; Fig. 1, Fig. S1), but 81% of soils and 93% of plants showed concentrations above the LOQ for at least one neonicotinoid of the five analysed. EFA soils of conventional farms exhibited higher concentrations than EFA soils of IP-Suisse and organic farms (Brown-Forsythe tests based on ranked data: $F_{2,60} = 6.83$, $p = 0.002$; conventional vs. IP-Suisse: $F_{1,40} = 6.09$, $p = 0.018$; conventional vs. organic: $F_{1,43} =$

14.6, $p = 0.0004$; IP-Suisse vs. organic: $F_{1,37} = 0.68$, $p = 0.42$; Fig. 1, Fig. S1, Table 1). Neonicotinoid concentrations in EFA plants did not differ according to cultivation practices (Brown-Forsythe test based on ranked data: $F_{2,66} = 0.48$, $p = 0.62$). Imidacloprid dominated in terms of presence and concentration in all EFA samples (Table 1). Remarkably, plant samples growing in EFAs of organic farms showed the highest percentage of contamination by neonicotinoids with 96% of all samples containing at least one neonicotinoid above the LOQ. In soils of EFAs, imidacloprid and clothianidin dominated in terms of presence and concentration, while imidacloprid dominated in plants of EFAs (Table 1). More than 21% of EFA soil samples contained at least two neonicotinoids, and more than 63% of EFA plant samples contained at least two neonicotinoids. As for cultivated fields, the imidacloprid-clothianidin combination was the most common in all EFA soil samples. In EFA plants neonicotinoid combinations were more diverse (Table 2).

Neonicotinoids in organic seeds

We found that 14 out of the 16 (87.5%) commercial organic seed samples contained at least one neonicotinoid at a concentration above the LOQ. The mean ± SE and median of the total neonicotinoid concentrations were 7.7 ± 5.04 ppb and 0.9 ppb respectively, and levels ranged from 0 to 81.9 ppb. Clothianidin clearly dominated in terms of concentration in all samples. Clothianidin and imidacloprid were found in 62.5% of all seeds, while thiacloprid was found in 50% of all seeds followed by thiamethoxam and acetamiprid that were found in 43.7% of all seeds.

Relation between neonicotinoid concentrations in soil and plant samples

Concentrations of total neonicotinoids, imidacloprid and clothianidin in plant samples were positively correlated with the respective concentrations in soil samples ($r = 0.49$, $F_{1,166} = 56.7$; $p < 0.0001$, $r = 0.26$, $F_{1,114} = 12$; $p = 0.0008$ and $r = 0.55$, $F_{1,43} = 23.5$; $p < 0.0001$ respectively; Fig. S2). Plants showed higher neonicotinoid concentrations when growing in a soil with higher concentrations of neonicotinoids.

Field characteristics

The concentrations of neonicotinoids found in cultivated soils and crops were not correlated with soil pH, average slope of the field, the proportion of arable land in a watershed comprised within a 1000-m radius around the sampling point or sampling date (Kendall correlation, all $|\tau| < 0.1$, $|z| < 1.21$, $p > 0.23$). Similarly, we found no correlations between concentrations found in soils and plants from EFAs with the above-mentioned parameters (Kendall correlation, all $|\tau| < 0.11$, $|z| < 1.11$, $p > 0.27$).

Potential impacts of neonicotinoids on non-target species

Hazard Quotients

Hazard quotients for beneficial and pest invertebrates under a field-realistic (mean concentrations) and a "worst-case" (maximum concentrations) scenario are provided in Table S4. Using HQ $\geq$ 0.01 as a threshold, a level at which imidacloprid and clothianidin have been shown to have sublethal toxicity in several invertebrate species, and based on a field-realistic scenario whereby species would be exposed to the mean concentrations found in EFAs and organic fields, 12.5% of the species used for biological control of pests or of high biological value would be exposed to sublethal detrimental concentrations of imidacloprid and/or clothianidin. Using the same criteria, 12.5-19.4% of the beneficial species would be exposed to such concentrations in IP-Suisse or conventional fields and crops. These numbers rise to 19.4% for EFAs and organic fields and 19.4-34.7% in IP-Suisse and conventional fields under a "worst-case" scenario using the highest concentrations. We also found that, under a field-realistic scenario, contamination levels by imidacloprid and clothianidin as we found in our study may expose up to 2.8% of the beneficial species to a HQ $\geq$ 0.05 in organic soils and crops and in EFAs (up to 9.7% of the species under a worst-case scenario in EFAs and organic fields), and up to 11.1% of the species to a HQ $\geq$ 0.1 (up to 22.2% species under a worst-case scenario) in IP-Suisse and conventional fields and crops.

Potentially Affected Fractions

The potentially affected fractions (PAF) of species that would be exposed to lethal (LC_{50}) or sublethal (10% or 1% of LC_{50}) concentrations of imidacloprid (in plants or soil) or clothianidin (plants only) when exposed to the mean or the maximum concentration found in our soil and plant samples from EFAs, organic, IP-Suisse and conventional fields are provided in Table 3. In brief, we found that, under a field-realistic scenario, 5.3% and 8.6% of beneficial species may be subjected to lethal concentrations (LC_{50}) of clothianidin in crops growing in integrated-production and conventional farms, respectively. The percentage of beneficial species potentially exposed to plant or crop clothianidin concentrations equating to 1% of the LC_{50} reaches up to 6.8% in EFAs, and 31.6% and 41.2% in IP-Suisse and conventional farms.

DISCUSSION

In this large-scale field study covering the entire Swiss lowland agricultural area, we show that neonicotinoid insecticides are present in nearly all soil and vegetation samples, including organic fields and ecological focus areas (EFAs), two types of arable land, which were not expected to contain any insecticides. Moreover, we found that the most common neonicotinoid insecticide and the one with the highest concentrations both in plants and soils was imidacloprid, a molecule that was under a temporary ban in many of the crops we sampled (e.g. canola, maize, potatoes, spring cereals). We can propose several non-mutually exclusive explanations for the high prevalence of these substances in all types of fields despite the ban. First, when sowing insecticide-coated seeds, a small proportion (< 2%) of insecticides is lost as dust (Tapparo et al., 2012) and can be deposited on the soil and vegetation of field margins or neighbouring, possibly organic fields (Krupke et al., 2012, Krupke et al., 2017). However, off-field dust alone cannot explain the overall contamination of surrounding areas, because the way the five neonicotinoids we studied are distributed differs between organic and EFA fields on the one hand, and IP-Suisse (reduced use of pesticides) and conventional fields on the other hand (Table 1). For instance, acetamiprid, a neonicotinoid commonly sprayed on vegetables and stone fruits (OFAG), was more frequent in soil and plant samples from organic and EFA fields than in samples of IP-Suisse and conventional fields (Table 1). Also, acetamiprid, thiacloprid and thiamethoxam were more frequent in organic and EFA plants compared to organic and EFA soils, and the most frequent combination of neonicotinoids in plants was imidacloprid and thiacloprid, whereas imidacloprid and clothianidin was the most frequent combination in soils (Table 2). Finally, we found combinations of neonicotinoids in the plants, which were not found in the soils (Table 2). This points at a multiplicity of contamination routes besides off-field dust, such as runoff waters (Bonmatin et al., 2015, Chrétien et al., 2017) or aerial contamination via inadvertent spraying in adjacent fields during treatment (Krupke et al., 2012, Wood and Goulson, 2017). Given the very long half-lives of neonicotinoids (Wood and Goulson, 2017), crop rotation schemes could also be a factor explaining the presence of neonicotinoids in soils and plants that are normally poorly treated or subject to the moratorium implemented from December 2013 onwards by the EU (Regulation No. 485/2013) and Switzerland. The slopes of the $\log_{10}$-$\log_{10}$ relationships between concentration in the soil and concentration in the vegetation (Fig. S2) imply that concentrations in plants increased at low concentrations in soil, and then levelled off at higher concentrations, potentially reflecting a saturation process. Noticeably, the R-squares of the linear regressions were modest, again pointing at either variable holding capacities of soil, and/or at other routes of contamination and transport. Lastly, a sample of commercial organic seeds (N=16) collected in 2017 revealed that 87.5% were positive for neonicotinoid insecticides, mainly clothianidin. Although this last result is based on a modest sample size and deserves further investigation, it suggests that, concerning organic farms, field contamination may be due to seed contamination that may occur along the commercialization chain.

The contamination by imidacloprid and clothianidin at concentrations found in cultivated fields, including organic, and in EFAs pose a threat to non-target beneficial invertebrates. Using hazard quotients (HQ) and potentially affected fractions (PAF) derived from species sensitivity distributions (SSD), we found that, under our field-realistic scenario, the percentage of beneficial species potentially exposed to detrimental concentrations of imidacloprid or clothianidin may be substantial (from 1.3 to 56.7%, see Results and Table 2). It is of particular concern that non-target beneficial species, especially those living in refuge areas (EFAs), may be chronically exposed to sufficiently high concentrations of neonicotinoids to induce sublethal to lethal effects (Pisa et al., 2015, Whitehorn et al., 2012, Hladik, Main and Goulson, 2018), because neonicotinoids have the potential to disrupt the food chains at many levels: within the soil, interfering with plant-arthropod relationships (Douglas, Rohr and Tooker, 2015), and at the predator level (e.g. predatory insects and birds) via a reduction in the biomass of arthropods eaten by predators (Hallmann et al., 2014, Hallmann et al., 2017).

Our assessment of lethality and sublethality to pests and non-target species considered imidacloprid and clothianidin separately. However, we found that between 59% and 71% of the contaminated plant samples from organic or EFAs contained at least two neonicotinoids and 26% and 43% of these samples contained at least three neonicotinoids. The fact that non-target species may be exposed to a mixture of neonicotinoids is worrisome, because the literature suggests that some combinations of neonicotinoid insecticides have detrimental additive or synergistic effects (Pavlaki et al., 2011, Loureiro et al., 2010, Maloney et al., 2017). In fact, our Table 2 shows that such pair-wise combinations occur at various frequencies, from 4.8 to 96.6%. Moreover, Maloney et al., 2018 have shown that synergistic effects also exist under chronic exposure. Therefore, synergistic and additive effects, especially under chronic exposure such as reported here, may increase the risk of toxicity to vulnerable species as well as the range of species possibly exposed to detrimental sublethal toxicity, and our percentages of vulnerable species are likely underestimated.

Remarkably, at the time we sampled the fields, none of the 12 pest species listed would be exposed to lethal or sublethal concentrations of imidacloprid and clothianidin under our field-realistic scenario. Under the "worst-case" scenario only two species (16.7%) would be exposed to sublethal concentrations (HQ ≥ 0.01) of neonicotinoids and no pest species would be exposed to a LC_{50} concentration (Table S4). Therefore, our data suggest that a balance has to be made between the number of pests and the number of predators affected by neonicotinoid insecticides, because exposing predators of pests may create a vicious circle leading to using ever more insecticides.

To conclude, our data suggest that the chronic exposure of non-target species in EFA and organic fields to mixtures of neonicotinoids could compromise organic farming by impeding the biological control of pests. It may also compromise the aim of EFAs that are designed to offset the loss of farmland biodiversity caused by intensive agriculture. The results of this study highlight the necessity to reduce the dispersion and overuse of neonicotinoid insecticides if we are to prevent any detrimental effects on biodiversity, ecological functions and ecosystem services associated with agroecosystems (Pisa et al., 2017).

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Authors' contributions SHG, FH, ŁB designed the study and collected the samples in the fields; SHG and GG analyzed the samples; GH created the map in Fig. 1; SHG and FH conducted the statistical analyses; SHG and FH wrote the manuscript and all authors commented on and approved the final version.

Data accessibility

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.9p7t664 (Humann-Guilleminot, Binkowski, Jenni, Hilke, Glauser & Helfenstein, 2019)

FIGURES AND TABLES

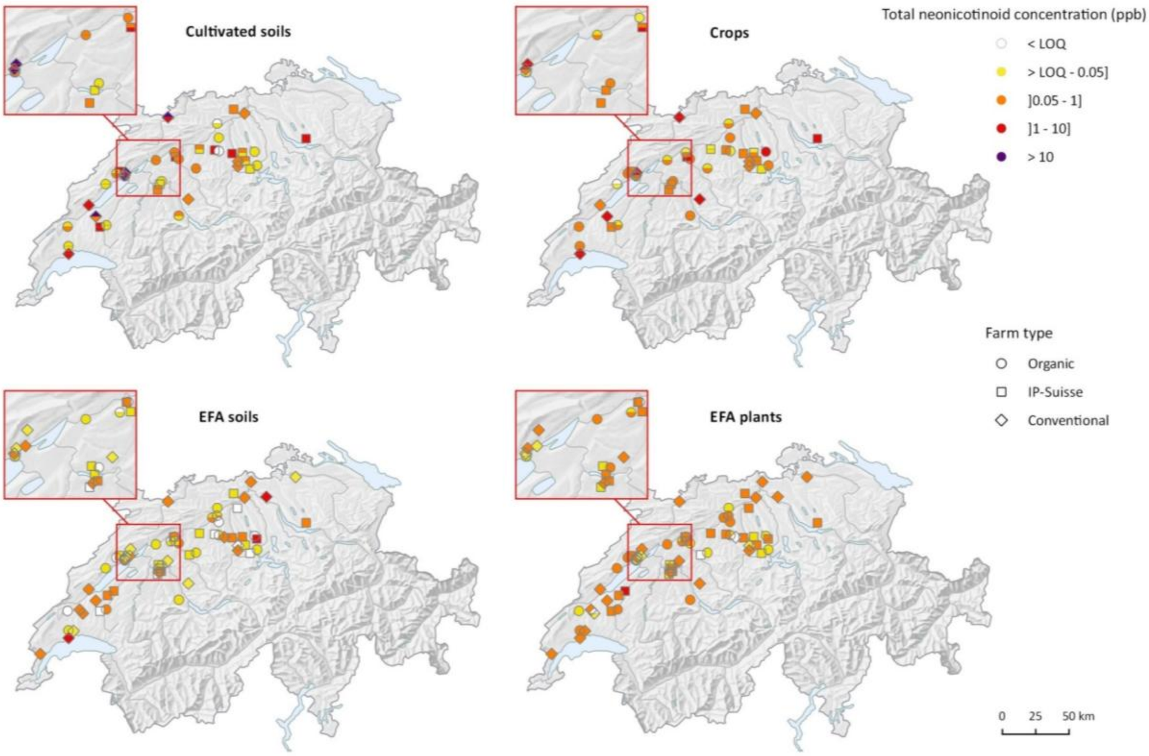

Fig. 1: Distribution of studied farms over the Swiss lowland agricultural area. Open symbols correspond to concentrations below the quantification limit (<LOQ) for total neonicotinoid concentrations. Colored symbols, from yellow to violet, correspond to increasing concentrations above LOQ for at least one neonicotinoid of the five analyzed. Symbols divided by a horizontal line indicate farms where two cultivated fields were sampled.

Table 1. Summary statistics for each neonicotinoid measured in soil (A) and plant (B) samples from the different fields in all farms.

Soil (A)		Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam	Total NNIs
		Neonicotinoid					
Cultivated fields							
	Total % fields > LOQ	13%	77%	94%	28%	27%	97%
Organic N=27	% fields > LOQ	7%	43%	82%	7%	0%	93%
	Maximum [ppb]	0.006	0.54	0.76	0.002	0	0.81
	Median [ppb]	0	0	0.02	0	0	0.27
	Average [ppb]	0.0003	0.04	0.05	0.00009	0	0.09
	SE [ppb]	0.0002	0.02	0.03	0.00008	0	0.034
IP-Suisse N=26	% fields > LOQ	15%	85%	100%	23%	31%	100%
	Maximum [ppb]	0.002	18.23	9.77	0.034	0.057	20.07
	Median [ppb]	0	0.1	0.07	0	0	0.96
	Average [ppb]	0.0003	1.50	1.19	0.003	0.008	2.71
	SE [ppb]	0.0001	0.73	0.51	0.0014	0.004	0.85
Conventional N=29	% fields > LOQ	17%	100%	97%	48%	52%	100%
	Maximum [ppb]	0.04	20.95	29.72	0.38	0.39	29.88
	Median [ppb]	0	0.28	1.8	0.0002	0	3.58
	Average [ppb]	0.003	2.72	3.58	0.026	0.033	6.36
	SE [ppb]	0.002	0.92	1.08	0.013	0.015	1.38
EFAs							
	Total % fields > LOQ	3%	46%	71%	13%	6%	81%
Organic N=24	% fields > LOQ	0%	21%	63%	8%	4%	71%
	Maximum [ppb]	0	0.049	0.17	0.001	0.016	0.17
	Median [ppb]	0	0	0.014	0	0	0.018
	Average [ppb]	0	0.004	0.027	0.00007	0.0007	0.031
	SE [ppb]	0	0.0022	0.0086	0.0001	0.0006	0.009
IP-Suisse N=21	% fields > LOQ	10%	43%	71%	19%	5%	76%
	Maximum [ppb]	0.028	0.11	5.55	0.021	0.016	5.63

	Median [ppb]	0	0	0.009	0	0	0.022
	Average [ppb]	0.001	0.013	0.32	0.001	0.0008	0.33
	SE [ppb]	0.001	0.005	0.26	0.001	0.0009	0.27
Conventional	% fields > LOQ	0%	74%	78%	13%	9%	96%
N=23	Maximum [ppb]	0	1.13	0.73	0.096	0.16	1.45
	Median [ppb]	0	0.01	0.058	0	0	0.1
	Average [ppb]	0	0.1	0.16	0.007	0.008	0.27
	SE [ppb]	0	0.06	0.044	0.05	0.007	0.086

Plant (B)		Neonicotinoid					
		Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam	Total NNIs
Cultivated fields							
	Total % fields > LOQ	34%	39%	87%	43%	19%	97%
Organic N=27	% fields > LOQ	37%	4%	81%	41%	15%	93%
	Maximum [ppb]	0.043	0.12	2.13	0.06	0.09	2.15
	Median [ppb]	0	0	0.072	0	0	0.08
	Average [ppb]	0.0036	0.0005	0.18	0.007	0.006	0.2
	SE [ppb]	0.002	0.0004	0.08	0.003	0.004	0.08
IP-Suisse N=25	% fields > LOQ	24%	48%	84%	44%	32%	100%
	Maximum [ppb]	0.008	6.67	0.91	0.093	0.1	6.79
	Median [ppb]	0	0	0.1	0	0	0.24
	Average [ppb]	0.001	0.59	0.17	0.01	0.015	0.79
	SE [ppb]	0.0004	0.29	0.05	0.004	0.006	0.31
Conventional N=27	% fields > LOQ	41%	67%	96%	44%	11%	100%
	Maximum [ppb]	0.011	8.06	2	0.3	0.16	9.35
	Median [ppb]	0	0.14	0.2	0	0	1.36
	Average [ppb]	0.002	1.66	0.41	0.031	0.008	2.11
	SE [ppb]	0.0008	0.47	0.096	0.015	0.006	0.48
EFAs							
	Total fields > LOQ	45%	12%	84%	59%	7%	93%
Organic N=24	% fields > LOQ	50%	13%	79%	58%	8%	96%
	Maximum [ppb]	0.063	0.065	0.82	0.04	0.015	0.86
	Median [ppb]	0.001	0	0.046	0.008	0	0.066
	Average [ppb]	0.005	0.005	0.1	0.011	0.0008	0.12
	SE [ppb]	0.003	0.003	0.04	0.002	0.0006	0.04
IP-Suisse N=21	% fields > LOQ	48%	14%	95%	57%	5%	95%
	Maximum [ppb]	0.022	0.41	1.14	0.057	0.086	1.14
	Median [ppb]	0	0	0.064	0.008	0	0.09
	Average [ppb]	0.005	0.027	0.14	0.012	0.004	0.19

	SE [ppb]	0.0015	0.02	0.055	0.0035	0.004	0.06
Conventional	% fields > LOQ	38%	8%	79%	63%	8%	88%
N=24	Maximum [ppb]	0.007	0.031	0.44	0.16	0.044	0.47
	Median [ppb]	0	0	0.049	0.011	0	0.08
	Average [ppb]	0.001	0.0021	0.1	0.023	0.003	0.13
	SE [ppb]	0.0004	0.001	0.027	0.008	0.002	0.03

Cultivated fields

Organic

Soil	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	0.0%	7.1%	0.0%	0.0%
Clothianidin		35.7%	0.0%	0.0%
Imidacloprid			3.6%	0.0%
Thiacloprid				0.0%

Plant	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	3.7%	25.9%	22.2%	0.0%
Clothianidin		0.0%	3.7%	0.0%
Imidacloprid			33.3%	14.8%
Thiacloprid				7.4%

IP-Suisse

Soil	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	15.4%	15.4%	7.7%	3.8%
Clothianidin		84.6%	23.1%	30.8%
Imidacloprid			23.1%	30.8%
Thiacloprid				7.7%

Plant	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	8.0%	24.0%	12.0%	4.0%
Clothianidin		40.0%	24.0%	20.0%
Imidacloprid			32.0%	28.0%
Thiacloprid				16.0%

Conventional

Soil	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	17.2%	17.2%	6.9%	3.4%
Clothianidin		96.6%	51.7%	48.3%
Imidacloprid			48.3%	48.3%
Thiacloprid				27.6%

Plant	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	22.2%	40.7%	22.2%	3.7%
Clothianidin		63.0%	29.6%	7.4%
Imidacloprid			40.7%	7.4%
Thiacloprid				11.1%

EFAs

Organic

Soil	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	0.0%	0.0%	0.0%	0.0%
Clothianidin		20.8%	4.2%	0.0%
Imidacloprid			4.2%	0.0%
Thiacloprid				0.0%

IP-Suisse

Soil	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	0.0%	9.5%	4.8%	0.0%
Clothianidin		38.1%	14.3%	4.8%
Imidacloprid			19.0%	4.8%
Thiacloprid				4.8%

Conventional

Soil	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	0.0%	0.0%	0.0%	0.0%
Clothianidin		60.9%	8.7%	8.7%
Imidacloprid			8.7%	8.7%
Thiacloprid				4.3%

Plant	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	12.5%	37.5%	41.7%	0.0%
Clothianidin		12.5%	8.3%	0.0%
Imidacloprid			45.8%	8.3%
Thiacloprid				4.2%

Plant	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	14.3%	47.6%	38.1%	4.8%
Clothianidin		14.3%	9.5%	4.8%
Imidacloprid			57.1%	4.8%
Thiacloprid				4.8%

Plant	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Acetamiprid	0.0%	29.2%	37.5%	4.2%
Clothianidin		8.3%	4.2%	0.0%
Imidacloprid			54.2%	8.3%
Thiacloprid				4.2%

Table 2. Percentage of samples containing each combination of two neonicotinoids (above the LOQ) in each farm category from cultivated and EFA soil and plant samples.

Table 3. The potentially affected fractions (PAF) of species that would be exposed to lethal (LC50) or sublethal (10% or 1% of LC50) concentrations of imidacloprid (in plants or soil) or clothianidin (plants only) when exposed to the mean or the maximum concentration found in our soil and plant samples from EFAs, organic, IP-Suisse and conventional fields. PAF ≥ 0.05 appear in bold.

		Potentially Affected Fraction								
		Imidacloprid in plants			Imidacloprid in soils			Clothianidin in plants		
		LC_{50}	10% LC_{50}	1% LC_{50}	LC_{50}	10% LC_{50}	1% LC_{50}	LC_{50}	10% LC_{50}	1% LC_{50}
Field-realistic scenario (mean C°)	EFA	< 0.01	< 0.01	0.025	< 0.01	< 0.01	< 0.01	< 0.01	0.020	**0.068**
	Organic	< 0.01	< 0.01	0.035	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.013
	IP-Suisse	< 0.01	< 0.01	0.034	< 0.01	< 0.01	0.010	**0.053**	**0.147**	**0.316**
	Conventional	< 0.01	0.013	**0.058**	< 0.01	< 0.01	0.020	**0.086**	**0.214**	**0.412**
"Worst-case" scenario (max C°)	EFA	< 0.01	0.026	**0.100**	< 0.01	< 0.01	0.047	0.044	**0.127**	**0.285**
	Organic	< 0.01	0.039	**0.135**	< 0.01	< 0.01	< 0.01	0.022	**0.074**	**0.191**
	IP-Suisse	< 0.01	0.022	**0.090**	< 0.01	< 0.01	**0.116**	**0.154**	**0.327**	**0.548**
	Conventional	< 0.01	0.037	**0.131**	< 0.01	0.013	**0.402**	**0.166**	**0.344**	**0.567**