CENTRE FOR PESTICIDES IN THE ENVIRONMENT

Swedish Chemicals Agency

Funded by the Nordic Council of Ministers

Maj Rundlöf, Elin Eriksson, Julia Bergman, Sara Erling, Ove Jonsson, Mariana Ledesma, Laima Blažytė-Čereškienė, Bjørn Arild Hatteland, Glenn P Svensson, Kajsa Weslien, Ola Olsson and Mikaela Gönczi

Screening of pesticides in pollen collected by honey bees in the Northern zone

CKB rapport 2025:1 **Uppsala 2025**

SLU Centrum för kemiska bekämpningsmedel i miljön (CKB)
Sveriges lantbruksuniversitet

SLU Centre for Pesticides in the Environment (CKB)
Swedish University of Agricultural Sciences

Screening of pesticides in pollen collected by honey bees in the Northern zone

Maj Rundlöf[1], Elin Eriksson[2], Julia Bergman[2], Sara Erling[2], Ove Jonsson[2], Mariana Ledesma[3], Laima Blažytė-Čereškienė[4], Bjørn Arild Hatteland[5], Glenn P Svensson[1], Kajsa Weslien[2], Ola Olsson[1] and Mikaela Gönczi[2]

1. Department of Biology, Lund University, Sweden, 2. Department of Aquatic Sciences and Assessment, SLU Centre for Pesticides in the Environment, Swedish University of Agricultural Sciences, Sweden, 3. Swedish Chemicals Agency, Sweden, 4. Laboratory of Chemical and Behavioural Ecology, Nature Research Centre, Lithuania, 5. Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research, Norway.

Publisher:	Swedish University of Agricultural Sciences, SLU Centre for pesticides in the environment (CKB)
Year of publication:	2025
Place of publication:	Uppsala, Sweden
Cover picture:	Pollen sampling using pollen traps mounted on honey bee hives placed at one of the monitoring sites.
Copyright:	All featured images are used with permission from the copyright owner.
Title of series:	CKB rapport
Part number:	2025:1
ISBN:	978-91-8046-615-8
DOI:	https://doi.org/10.54612/a.5rg2oa0c6g

Updated version

This pdf was updated 2025-07-28 with an additional text in the Acknowledgement that was missing, about co-funding by the European Union's Horizon Europe research and innovation programme PollinERA.

Table of contents

Summary

In this project, financed by the Nordic Working Group for Chemicals, Environment and Health (NKE), pollen was collected from honey bees returning to their hives at six sites in Lithuania, Norway and Sweden during May–June 2024. The sites were located in agricultural areas expected to have high pesticide use. Weekly samples were taken over 3–4 weeks using pollen traps, with sampling beginning at the start of apple orchard bloom in the region.

The primary goal of the project was to increase knowledge of pesticide exposure of pollinating insects in agricultural landscapes within the European Northern Zone. We also explored the potential for using monitoring data to assess default values and outcomes of the regulatory risk assessment based on the Northern Zone interim risk assessment approach for bees. Another goal was to contribute to the establishment of a long-term monitoring program for pesticide residues in the terrestrial environment and evaluate risks to non-target terrestrial organisms.

The pollen was analysed for 173 pesticides using liquid and gas chromatography coupled with mass-spectrometry (LC-MS/MS and GC-MS) and 58 of these were detected in at least one sample. Each sample had 7–23 substances, with summed concentrations from 57 to 19213 ng/g. A risk index was calculated by summing pesticide concentrations weighted by the mean oral and contact LD_{50} (lethal dose to 50% of the test population) toxicity values for honey bees.

The most frequently detected substance was the insecticide acetamiprid (96% of the samples). The fungicides boscalid, captan, azoxystrobin and fluopyram showed the highest maximum concentrations, and they were also all among the 15 substances with the highest detection frequency. Despite being banned for use since 2022, the insecticide indoxacarb was the substance with the highest total risk index over all samples. There were no differences in summed pesticide concentrations or risk index between countries, but samples collected in the first week of the study showed higher values than those taken in the last week.

The plant origin of pollen was determined via machine learning image analysis. The most common pollen type was Brassicaceae, which includes cultivated oilseed rape. Both summed concentrations and risk index correlated positively with the proportion of crop pollen in the sample.

To test one of the assumptions in the risk assessment for honey bee larvae, we compared the measured concentrations of the pesticides acetamiprid, azoxystrobin and boscalid in pollen samples from this study with the calculated default values used in the current (interim) risk assessment scheme for the Northern Zone. The measured concentrations in pollen were nearly one order of magnitude higher than those based on calculated default concentrations, for all three substances.

The risk to honey bee larvae was calculated as a toxicity exposure ratio (TER) based on the measured concentrations as well as the calculated default concentrations. The risk is acceptable if the ratio between the toxicity endpoint for larvae (NOED, no observed effect dose) and the exposure dose through diet (pollen and nectar) is higher than 1. All calculated TER indicated acceptable risks. However, TER for one of the substances was lower when based on measured concentrations due mainly to the nectar values. This indicates that the current first step in the risk assessment for bee larvae might not be as protective as assumed and in some cases underestimate the exposure under field conditions. However, it should be emphasised that this risk assessment scheme for larvae is an interim solution until revised European guidance has been implemented.

Environmental monitoring to support the risk assessment of bees would benefit from determination of pesticide concentrations in both pollen and nectar, since pollen may contain higher pesticide concentration, but nectar is assumed to be consumed in largest amount. Alternatively, methods could be developed to explore the translation of concentrations in pollen to those in nectar for individual substances. Screening of pesticides in pollen collected by honey bees remains a promising approach based on the ease of collection and earlier shown relations between the pollen-based risk index and effects on the reproduction of wild bee species. However, the relevance of the pollen-based risk index for effects on pollinators beyond bees cannot be assumed without further evaluation.

In this study, we chose to select monitoring locations with high expected pesticide use and potential direct risk to bees. This could be a good strategy when resources are limited. For exploring trends in environmental pesticide residues and risks for non-target terrestrial organisms, there is a need for continuous data collection from the same locations year after year. For selecting more long-term monitoring sites, and evaluate exposure data in risk assessment, it would be valuable to have spatiotemporally resolved pesticide use data – something that is currently not publicly available in the EU. Coordinating pesticide monitoring sites with the planned monitoring of pollinators under the Nature Restoration Regulation would create opportunities to better understand how the two relate.

Svensk sammanfattning

I detta projekt, finansierat av den Nordiska arbetsgruppen för kemikalier, miljö och hälsa (NKE), samlades pollen in från honungsbin som återvände till sina bikupor på sex platser i Litauen, Norge och Sverige under maj–juni 2024. Platserna låg i jordbruksområden som förväntas ha hög användning av bekämpningsmedel. Veckoprover togs med pollenfällor under 3–4 veckor, med början när äppelodlingar i området började blomma.

Det primära målet med projektet var att öka kunskapen om bekämpningsmedelsexponering av pollinerande insekter i jordbrukslandskap inom den europeiska Norra Zonen. Vi undersökte också möjligheten att använda övervakningsdata för att bedöma standardvärden och utfall av den regulatoriska riskbedömningen baserad på Norra Zonens interimistiska riskbedömningsmetod för bin. Ett annat mål var att bidra till upprättandet av ett långsiktigt övervakningsprogram för bekämpningsmedelsrester och risker för icke-målorganismer i terrester miljö.

Pollenproven analyserades för 173 bekämpningsmedel med vätskekromatografi och gaskromatografi kopplad med masspektrometri (LC-MS/MS och GC-MS) och 58 av dessa påvisades i minst ett prov. Varje prov innehöll 7–23 ämnen, med summerade koncentrationer från 57 till 19213 ng/g. Ett riskindex beräknades genom att summera koncentrationer av bekämpningsmedel viktade med de genomsnittliga toxicitetsvärdena för oral och kontaktexponering (LD_{50}, dödlig dos för 50% av testpopulationen) för honungsbin.

Den vanligast detekterade substansen var insekticiden acetamiprid (96 % av proverna). Fungiciderna boskalid, kaptan, azoxystrobin och fluopyram hade de högsta maxkoncentrationerna, och de var också alla bland de 15 substanserna med högst fyndfrekvens. Insekticiden indoxakarb hade det högsta totala riskindexet sett till alla prover, trots att ämnet varit förbjuden för användning som växtskyddsmedel sedan 2022. Det fanns inga skillnader i summerade koncentrationer eller riskindex mellan länder, men prover som samlades in under första veckan av studien visade högre värden än de som togs under sista veckan.

Pollenprovernas växtursprung bestämdes med hjälp av bildanalys genom maskininlärning. Den vanligaste pollentypen var korsblommiga växter, Brassicaceae, som inkluderar odlad raps. Både summerade koncentrationer och riskindex korrelerade positivt med andelen grödpollen i provet.

För att testa ett av antagandena i den regulatoriska riskbedömningen för honungsbilarver jämfördes de uppmätta koncentrationerna av bekämpningsmedlen acetamiprid, azoxystrobin och boskalid i pollenprover från denna studie med de beräknade standardvärdena som används i det nuvarande (interimistiska) riskbedömningsschemat för den Norra Zonen. De uppmätta koncentrationerna i pollen var nästan en tiopotens högre än de beräknade utifrån standardvärden, för alla tre ämnena.

Risken för honungsbilarver beräknades som en toxicitets-exponeringskvot (TER) baserat på de uppmätta koncentrationerna samt de beräknade standardkoncentrationerna enligt den interimistiska riskbedömningen. Risken är acceptabel om förhållandet mellan toxicitetsvärdet för larver (NOED, no observed effect dose – dosen där ingen effekt observerats) och exponeringsdosen genom födan (pollen och nektar) är högre än 1. Alla TER indikerade acceptabla risker men TER för en substans var lägre när det baserades på uppmätta koncentrationer än på de beräknade standardvärdena. Detta indikerar att den nuvarande riskbedömningen för bilarver möjligen inte är så skyddande som antagits och i vissa fall skulle kunna underskatta exponeringen under fältförhållanden. Dock ska det påpekas att den

nuvarande riskbedömningsmetoden för larver är en interimistisklösning tills reviderade europeisk vägledningen har implementerats.

För en mer användbar återkoppling mellan miljöövervakningsdata och riskbedömningen av bin skulle det vara önskvärt med screening av bekämpningsmedel i både pollen och nektar. Alternativt skulle metoder kunna utvecklas för att relatera koncentrationer i pollen till de i nektar för enskilda ämnen. För närvarande är dock screening av pollen insamlat av honungsbin ett lovande tillvägagångssätt baserat på hur lätt det är att provta och det tidigare visade sambandet mellan det pollenbaserade riskindexet och effekter på reproduktionen hos vildbin. Metoder skulle kunna utvecklas för att utforska översättningen av koncentrationer i pollen till de i nektar för enskilda ämnen. När det gäller relevansen av det pollenbaserade riskindexet för effekter på andra pollinatörer än bin krävs det ytterligare utvärdering.

I denna studie valde vi övervakningslokaler med hög förväntad användning av bekämpningsmedel och potentiell direkt risk för bin. Detta kan vara en bra strategi när resurserna är begränsade. För att utforska trender i bekämpningsmedelsrester i miljön och risker för icke-målorganismer finns det ett behov av kontinuerlig datainsamling från samma platser år efter år. För att välja mer långsiktiga övervakningsplatser och utvärdera exponeringsdata i riskbedömning skulle det vara värdefullt att ha rumsligt upplösta data om användning av bekämpningsmedel – något som för närvarande inte är allmänt tillgängligt i EU. Att samordna övervakningsplatser för växtskyddsmedel med den planerade övervakningen av pollinatörer genom Naturrestaureringsförordningen skulle kunna skapa möjligheter att bättre förstå kopplingen mellan de två.

1. Introduction

Plant protection products (the term pesticide is used interchangeably in this report) are used to control pests and support agricultural production, but they can also have negative effects on pollinating insects and their ecosystem services. Monitoring of pesticides is performed in the aquatic environment throughout Europe e.g. following the Water Framework Directive, but, as far as we know, there is no long-term monitoring of pesticides in the terrestrial environment in Europe.

This project, financed by the Nordic Working Group for Chemicals, Environment and Health (NKE), was initiated by the Swedish Chemicals Agency and led by the SLU Centre for Pesticides in the Environment (CKB) at the Swedish University of Agricultural Sciences (SLU) in collaboration with Lund University (LU), and with participation from the Nature Research Centre (NRC), Lithuania, and the Norwegian Institute of Bioeconomy Research (NIBIO).

The aim of the project was to increase knowledge about pesticide exposure of pollinating insects in agricultural landscapes in the Northern Zone. There are three zones representing different agricultural and environmental conditions in Europe: the Northern, Central and Southern zone. The Northern Zone includes Denmark, Estonia, Finland, Iceland, Latvia, Lithuania, Norway and Sweden.

Honey bee (*Apis mellifera*) collected pollen was sampled from sites in Lithuania, Norway and Sweden, to represent the climatic and agricultural conditions in the Northern Zone. The pollen was analysed to determine pesticide concentrations and plant origin, with the latter giving insights into the sources of exposure. The project also aimed at facilitating future collaboration between research groups within the Northern Zone.

A better understanding of the exposure of pollinators to different pesticides is valuable in the authorisation process for plant protection products as a reality-check of the exposure predictions for the terrestrial environment. The residue and risk information that will be gained in this project are also useful as a reality-check to verify the estimations used for environmental risk assessments for the approval of active substances in EU.

The sampling sites in this project were located close to conventional agriculture systems. The data obtained in this project therefore gives a good insight into the actual combination of pesticides that bees are exposed to in the agricultural landscape. Information on pesticide exposure in the terrestrial environment could be used to identify opportunities for policy development to mitigate exposure to pollinators and other non-target organisms in the agricultural landscape.

Another goal of this project was to contribute to the establishment of a long-term system for monitoring contamination from pesticides in the terrestrial environment and potential risks to non-target terrestrial organisms.

1.1 Background

Insects, including pollinating insects, are declining globally (Wagner et al. 2021), including in the EU (Biesmeijer et al. 2006, Warren et al. 2021) and the Nordic countries (Dupont et al. 2011, Bommarco et al. 2012,). The decline does not have one single cause, but threats include land-use changes for agriculture, which results in the loss and degradation of habitats (Wagner et al. 2021). Pesticides have been pointed out as a threat that affect pollinators directly (insecticides and fungicides) and indirectly (herbicides) (e.g. Goulson et al. 2015, Potts et al. 2016). To be able to reduce and mitigate such

pesticide related threats, there is a need to better understand the exposure and risks of pesticide use for pollinating insects. This can be done by generating data on the pesticide residues that pollinating insects encounter in their environment.

The scope and results of this project are well in line with the knowledge gaps that need to be filled, and it also enables a follow-up on goals regarding the sustainable use of plant protection products (PPPs) in Directive 2009/128/EC (SUD). Considering the chosen sampling scheme that covers expected high pesticide use and risk locations and times in multiple countries, the project output should benefit the entire Northern Zone.

Pollinators, including bees, rely mainly on pollen and nectar to cover their nutritional needs (Woodard & Jha 2017). However, both pollen and nectar may contain residues of pesticides (e.g. Mullin et al. 2010, David et al. 2016, Kyriakopoulou et al. 2017, Böhme et al. 2018, Graham et al. 2021, Rundlöf et al. 2022, Végh et al. 2022, Jonsson et al. 2022, Knapp et al. 2023). Honey bees are generalist foragers, visiting a wide range of flowering plant species (e.g. Leonhardt & Blüthgen 2012, Böhme et al. 2018, Végh et al. 2022), which potentially makes their collected pollen a good general indicator for exposure of pesticides in the terrestrial environment (de Oliveira et al. 2016). Additionally, honey bees can forage over several kilometres, in different crop and non-crop habitats and bring back pollen and nectar to their central nest, which are reasons why sampling and analysis of pesticide residues in honey bee collected materials have been suggested as useful for environmental monitoring (Jonsson et al. 2013, de Oliveira et al. 2016). The pollen sampled from the central hive thus represents the pesticide residues at a (foraging) landscape scale, much like sampling a local water body to capture pesticide residues over the catchment area.

Pesticides are generally found in higher concentrations in pollen than nectar, and pollen can therefore be a particularly important route of exposure for insects that feed on pollen and nectar (Kyriakopoulou et al. 2017, Zioga et al. 2020, Knapp et al. 2023). This was also shown in a recent monitoring project in Sweden where pesticides were analysed in honey bees, pollen and nectar (Jonsson et al. 2022). Pollen was the matrix where the most substances were detected and where high summed concentrations and potential risk, calculated as the toxicity weighted concentrations (Stoner and Eitzer 2013, Rundlöf et al. 2022, Knapp et al. 2023), for pollinators occurred (calculated as a pollinator pesticide risk index, see Rundlöf et al. 2022 and Knapp et al. 2023). Both summed concentrations and risk indices for pollen, bee tissue and nectar were statistically related (Jonsson et al. 2022). The pollen-based summed concentration and risk can thus potentially also be used to estimate summed concentration and risk for nectar. However, the correlation between concentrations in pollen and nectar may vary among substances (for neonicotinoids, see Zioga et al. 2020). Furthermore, the risk index based on honey bee collected pollen was statistically related to the risk indices based on bumble bee (*Bombus terrestris*) and solitary bee (*Osmia bicornis*) collected pollen (Knapp et al. 2023) and related to reduced nesting and reproduction in wild bee species (Rundlöf et al. 2022, Nicholson et al. 2024a).

1.2 Pesticide regulations in the EU and the Northern Zone

Active substances in plant protection products are jointly evaluated within the EU, and a decision on approval of an active substance is made at the EU level (Siviter et al. 2023). Companies seeking to market pesticides with a specific active substance must submit an application for approval for the active substance to a member state, which acts as the Rapporteur Member State (RMS). The RMS conducts a comprehensive evaluation, including a risk assessment for human health, the environment, and food residues. The assessment is then peer-reviewed by experts from other member states and the

European Food Safety Authority (EFSA). EFSA finalises its opinion, and lastly, the European Commission drafts a review report and regulation on approval. The draft regulation is discussed and voted on by the Standing Committee on Plant Protection Products, made up of representatives from all EU countries. The Commission then adopts and publishes the decision on approval.

Plant protection products are evaluated at the member state level and a decision on approval of products are made nationally. However, the plant protection product evaluation is supported by a zonal process to share the workload and ensure harmonized evaluation between member states. The EU member states are here divided into three zones that are assumed to have similar environmental conditions and pest management challenges: the Northern, Central and Southern zones. Non-EU countries in Europe can also participate in this zonal process. The Northern zone has developed a Guidance document on procedures and evaluation principles for the North Zone (Northern Zone 2024). This guidance is updated yearly as needed. The collaboration includes Denmark, Estonia, Finland, Iceland, Latvia, Lithuania, Sweden and Norway

The concept of the authorisation process is a stepwise approach where the first step is based on protective "realistic worst case" assumptions on exposure and effects. If the estimated risk based partly on default values in the first step exceeds set thresholds, the applying company can go to the next step and submit refined studies with more realistic conditions for the specific use in question, where exposure predictions and effect assessments differ from the first step.

The criteria for authorisation of plant protection products and the active substances in those products are specified in Regulation (EC) 1107/2009 along with agreed guidelines for the assessment of health and environmental risks. EFSA develops general guidance documents on the risk assessments to achieve harmonization. In 2013, new data requirements (Commission Regulations (EU) No 283/2013 and 284/2013) established that where bees are likely to be exposed, testing by both acute (oral and contact) and chronic toxicity, including sub-lethal effects, shall be conducted. This meant that besides default testing of acute effects on adult bees through oral and contact exposure, also data on more long-term effects, i.e. chronic effects, on bee adults and their larvae, must be provided whenever bees are likely to be exposed. To meet the adjusted data requirements for bees, activities were initiated to develop, validate and adopt new test guidelines (mainly OECD) and a new risk assessment scheme (EFSA). A new guidance document on risk assessment on bees, including *Apis mellifera, Bombus* spp. and solitary bees, was published initially in 2013 (EFSA 2013) and in revised form in 2023 (EFSA et al. 2023). Neither of the two guidance documents have yet been adopted by the European Commission and the member states, but the revised version is currently under consideration for endorsement.

In 2020, as an interim approach to handle the lack of adopted guidance, the Northern Zone added further guidance on risk assessment for bees to meet the new data requirements (Northern Zone 2020). The interim approach is used for risk assessments until an EU-wide guidance is adopted and summarizes available approaches, e.g. from ECPA, to calculate risks, i.e. with recommendations on acceptable methods to assess long term risk to honey bees, their larvae, as well as for bumble bees.

Although the Northern Zone shares the approach for plant protection product risk assessment, the individual countries make independent decisions on product approval within their countries and may also, for example, authorise use in different crops and different application rates. In addition, companies may apply for authorisation only for specific countries, which means that the availability of plant protection products in a member state depends on both the initial applications by companies and whether the member state grants approval. Because the data requirements have changed and guidelines are continuously updated, data requirements may vary depending on the date of application, i.e. earlier

authorisations may be based only on an acute toxicity risk assessment for honey bees, whereas recent authorisations may include risk assessment for bumble bees and chronic effects in honey bee adults and larvae. Furthermore, because different products can have different formulations even if they include the same active ingredient, different companies may present different toxicity data and refinements of the risk assessment based on these formulations.

2. Materials and methods

2.1 Sites and sampling of pollen

Pollen samples were collected from honey bee colonies at six sites located in Lithuania, Norway and Sweden, during four consecutive weeks in May-June 2024. Within each country, one person was responsible for coordinating the site selection, sample collection and shipping samples for further analysis.

2.1.1 Locations and timing

The sampling scheme consisted of six agricultural sites, with one located in Lithuania, one in Norway and four in Sweden. Selected sites represent agricultural landscapes that are expected to have high pesticide use and risk in relation to pollinating insects. This was achieved by selecting sites based on information from the landscape surrounding the site, including conventional rather than organic farm management, prior knowledge of pesticide use or risk to bees (e.g. Böhme et al. 2018, Jonsson et al. 2022, Knapp et al. 2023, Nicholson et al. 2024a, Nicholson et al. 2024b), presence of apple and other fruit orchard crops (Graham et al. 2022, Knapp et al. 2023, Nicholson et al. 2024a) and/or a high proportion of arable land and annual crops (Bednarska et al. 2022, Knapp et al. 2023). In addition, and for practical reasons, sites were preferentially chosen to be located close to the home institutions of the collectors or within a particular region to reduce travel time and where farmer and beekeeper contacts were already established. The initial plan was to co-locate the Swedish monitoring sites with locations of a recent 3-year Swedish pilot study for pollinator monitoring (Olofsson 2023, Guillermo Aguilera Nuñez, SLU, personal communication 2023). This was because taking samples at the same locations as the pollinator monitoring pilot project would give potential for synergies, as data from that project might facilitate a broader and deeper interpretation of the data in the project proposed here, and vice versa. However, the pollinator pilot monitoring sites were not fixed locations for future pollinator monitoring, so priority was instead given to the selecting of expected high pesticide use and risk locations that were accessible to the collectors.

The chosen sampling period was supported by results from the previous monitoring project in Sweden 2020-2021 (Jonsson et al. 2022), as well as by similar work done in 2019 (Knapp et al. 2023), showing that pollen and nectar from May-June had highest number of detected substances and the highest risk index, compared to samples from July and September-October. The pollen was sampled during four consecutive weeks, starting when apple trees were expected to start blooming in the region, i.e. early May in Lithuania and mid-May in Norway and Sweden. The timing of the start of the apple bloom was to achieve a consistent phenological stage across the countries and sites.

2.1.2 Sampling method

One or two honey bee colonies were placed or selected, if the site had a permanent apiary, at each monitoring location. If placed, colonies were placed at the location by the beekeeper at least one week

prior to the first sampling occasion. The use of more than one colony can be beneficial to get sufficient amount of pollen also when the colonies are less strong early in the season or the weather is less good, i.e. cold, cloudy or windy.

Pollen from foragers returning to the colonies was collected using pollen traps that were mounted on the entrance of the honey bee hives and tightly secured to the hive using nails and tape (Figure 1). Safety equipment such as bee suits and gloves were used when mounting pollen traps and collecting pollen. The traps were generally mounted and activated for pollen collection for less than 24 hours, with the exception of one occasion in Sweden where sampling had to be continued over three days to get a sufficient amount of pollen, due to weak colonies and windy conditions. The aim was to collect at least 10 g (approximately 25 ml) of pollen from a site during a sampling round. When pollen from more than one colony was collected at a site, the pollen was pooled into one sample per site and sampling round.

Figure 1. Typical pollen trap used in Sweden mounted at the entrance of a honey bee colony box and secured with nails and tape (left; photo Maj Rundlöf) and the collected pollen from four different honey bee colonies (right; photo Theresia Widhalm).

The pollen was collected and stored in clearly labelled (sample number, site and round) polypropylene tubes (Figure 2) and details on date, collector and amount of pollen (ml or g or both) were recorded. The sample was stored in a cooling box with plenty of frozen cooling elements in the field and then in a freezer at -20 °C as soon as possible and until shipment to the laboratories for analysis.

The pollen samples were used for both pesticide residue analysis (at SLU, Uppsala) and plant species (or group) identification (at Lund University). The division of samples was done in Lund for the samples collected in Sweden and in Uppsala for samples collected in Lithuania and Norway. From the pollen sample, 2 g (about 5 ml) of pollen was transferred to a separate tube. This was the sample used for pollen plant species identification and was kept in or sent to Lund. The remaining pollen was kept in or sent to Uppsala for pesticide residue analysis.

Figure 2. Pollen loads of different plant origin, collected using pollen traps mounted on honey bee colonies (photos Ove Jonsson).

2.2 Chemical analyses of pesticides

The pollen samples were stored at -20 °C prior to analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS) and gas chromatography mass spectrometry (GC-MS). In total 173 substances (fungicides, herbicides, insecticides, metabolites and plant growth regulators) were analysed, using three different multi methods.

All the analyses of pesticides were carried out at the OMK laboratory at SLU in Uppsala. The analysis methods have been developed at the OMK laboratory with funding from the Swedish Environmental Protection Agency and CKB and have been used in several projects (Jonsson et al. 2013, Jonsson & Kreuger, 2017, Jonsson et al. 2022, Knapp et al. 2023). Further method development to include as many relevant compounds as possible for pollinators in Europe was done within the EU Horizon project PollinERA, running in parallel.

In short, the sample preparation included homogenization of pollen baskets, weighing 0.2 g subsample, addition of internal standard solution for LC and GC methods, addition of 0.8 g drying agent (sodium sulphate) and then extraction using acetonitrile. The extraction was performed in two steps, first under vigorous mixing in Precellys tubes with ceramic beads, using a mixer from Bertin instruments, then, with fresh acetonitrile, by strong ultra sonication using a Vibra-Cell VCX 130 instrument from Sonics. The pooled extract was split in two, one part evaporated and reconstituted in a small volume acetonitrile for subsequent LC-MS/MS analysis (Figure 3), the second part, used for GC-MS analysis, was further cleaned using dispersive solid phase extraction with endcapped C18 and primary/secondary amine (PSA) adsorbents. After evaporation, extracts were reconstituted in a small volume of cyclohexane:acetone, 9:1 and injected on the GC-MS instrument.

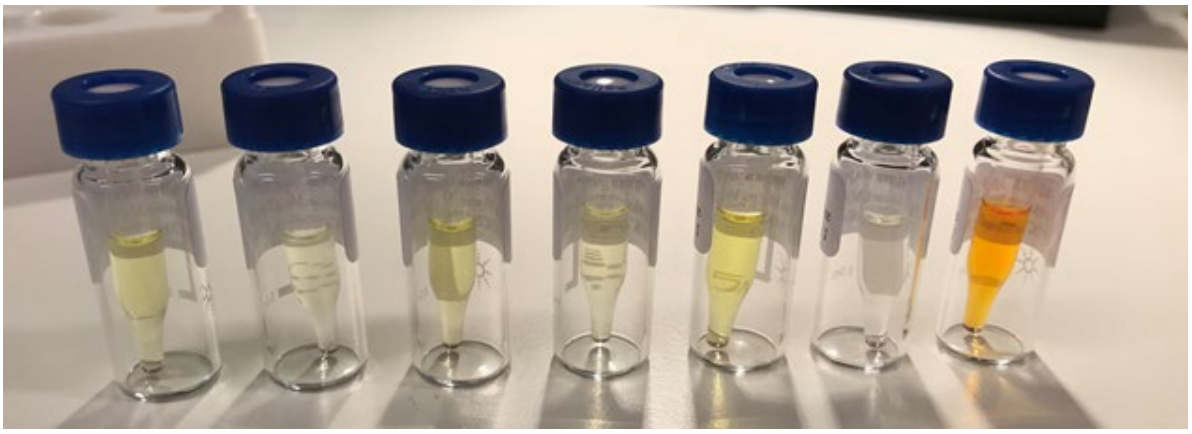

Figure 3. Pollen extracts ready for instrumental analysis (photo Ove Jonsson).

The pollen extracts were analysed using two multi methods based on LC-MS/MS with positive and negative electrospray ionization, respectively (Jansson & Kreuger 2010, modified for pollen samples and an extended number of compounds) and one method based on GC-MS with negative chemical ionization. All instrumentation was from Agilent Technologies and part of the accredited laboratory. The pollen methods, however, are not part of the accreditation. In total 173 substances were included (see Appendix 1). For method validation pollen samples known from previous analysis to have low or no levels of pesticides were used in spiking experiments at several different concentration levels. Solvent blanks, method blanks (methods run without pollen), matrix blanks (methods run with non-spiked pollen), as well as the spiked pollen samples were used to investigate interferences, selectivity, detection limits, absolute and relative recoveries, and precision. All results were corrected for relative recoveries from the spiking experiments.

2.3 Image analyses of plant origins

The pollen samples were also analysed for plant species or group origin. This was done using a semi-automated method where pollen mounted on microscope slides are scanned at high resolution and the pollen grains classified to pollen type using image analysis and machine learning (Olsson et al. 2021). The 2 g subsample of pollen was dissolved in 70% ethanol, using approximately one part pollen to eight parts ethanol based on weight. The solution was shaken until the pollen loads were dissolved in the ethanol. From the suspended pollen, 2 µl was transferred to a microscope slide already containing approximately a 2x2x2 mm cube of basic fuchsine coloured gel. The microscope slide was heated to approximately 50 ºC until the ethanol had evaporated and the gel had melted around the pollen grains and thereafter covered with a cover glass and left to cool (Figure 4). The edges of the cover glass were then sealed with clear nail polish to prevent drying.

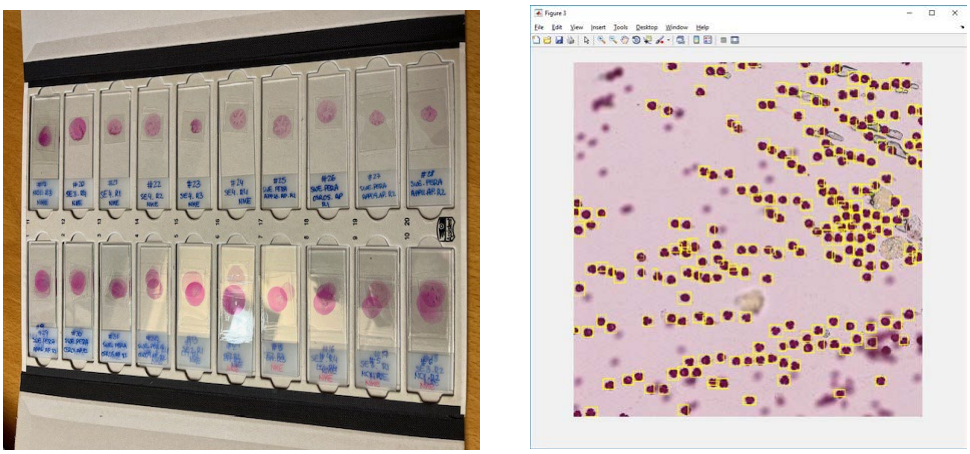

Figure 4. Pollen mounted in coloured gel on microscope slides ready for scanning (left; photo Maj Rundlöf) and the resulting scanned image file of the coloured pollen grains (right; photo Ola Olsson).

The microscope slides with the coloured and gel-mounted pollen were scanned using a Leica Aperio CS2 slide scanner at 0.25-µm resolution (40× magnification), repeated using five different focuses (Olsson et al. 2021). The scanned images were stored as svs files (8 bit RGB multi-layer JPEGs with metadata) and stacked over the five focuses using MATLAB to produce a single-layered JPEG image (Figure 4). MATLAB was also used to develop an algorithm that extracted single pollen grain images. These images were analysed using convolution neural networks (CNN) models that identifies 30 pollen grain types based on morphology (e.g. Beug 2015), trained on pollen from 205 identified plant species collected in southern Sweden. The output is the proportion of the pollen grains belonging to the 30 pollen types, adjusted by the frequency of the types in the sample because this resulted in higher accuracy in the pollen identification (Olsson et al. 2021).

Based on the pollen type classification, pollen types that contained crop plants that can be directly treated with pesticides were selected and summed to a group termed “crop pollen”. These included *Brassicaceae* covering oilseed rape and other cruciferous plants, *Fabaceae* types covering red clovers (*Trifolium pratense* and *T. medium*), other clovers (*Trifolium* spp. excluding *T. pratense* and *T. medium*) and *Vicia* spp. covering field beans and vetches, and *Rosaceae* of the *Sorbus* type covering apple and other fruit orchard crops as well as flowering wild trees and bushes, e.g. *Prunus* spp., *Pyrus* spp. and *Rubus* spp.

2.4 Summed concentrations and calculation of risk index

The results are reported as concentrations of the different pesticides in the samples as nanogram of a specific substance per gram of pollen (ng/g, which is the same as µg/kg). Concentrations were also added over all substances found in a sample to give a summed concentration. However, the summed concentration does not take into account that different substances show different toxicity to pollinators. Therefore, a risk index was calculated, which combines concentrations and toxicity to give the potential risk of the pesticide mixture to bees and possibly other flower visiting insects (Rundlöf et al. 2022, Knapp et al. 2023). The risk index was calculated based on the concentration of a substance divided by its toxicity to honey bees determined in standard toxicity tests (Stoner & Eitzer 2013, Sanchez-Bayo & Goka 2014). The values were also summed for all substances found in the sample, with the assumption that the individual toxic effects are additive (EFSA 2019). Substance toxicity was based on data for honey bees because there is insufficient information across substances for other pollinator species. Furthermore, the toxicity was estimated based on honey bee LD_{50} (µg/bee) from the Pesticide Properties Database (PPDB, Lewis et al. 2016) averaged for acute oral and contact values,

because this reflects how bees and other pollinators may come in contact with pesticides when they feed on pollen, collect pollen from flowers, transport and store pollen and feed it to their offspring (Nicholson et al. 2024a). This index has been shown to correlate negatively to bee nesting and reproduction (Rundlöf et al. 2022, Nicholson et al. 2024a) but has not been evaluated for other pollinators.

2.5 Using monitoring data in risk assessment

In this report, we explored the possibility to use monitoring data to evaluate default values and outcomes of the regulatory risk assessment based on the Northern Zone interim risk assessment approach for bees (Northern Zone 2024).

2.5.1 Pollen concentrations and toxicity exposure ratio

The concentrations of different pesticides that are determined in honey bee collected pollen in this study could potentially be used to perform a "reality check" of some of the assumptions made in the interim bee risk assessment. The determined concentrations of individual substances in pollen samples in this study were compared to the calculated values used in the first step of the bee risk assessment, which is assumed to be protective and represent a "realistic worst case".

In the current (interim) risk assessment scheme for the Northern Zone (Northern Zone 2024), exposure via pollen is only accounted for in the chronic effects risk assessment for honey bee larva. The method is based on the bee risk assessment scheme for spray application suggested by ECPA, which is a modification of the EU agreed EPPO (2010) scheme and other new data and available approaches (ECPA 2017).

The larva risk assessment includes the following assumptions and variables:

Toxicity endpoint

The toxicity endpoint is the measured no observed effect dose of an active substance (a.s) during the developmental period of 22 days after five days of exposure (22 days NOED in µg a.s./larva).

Pesticide exposure dose

The pesticide exposure dose for an active substance is calculated as the combined exposure via consumption of pollen and nectar.

Exposure dose (µg a.s./larva) = Application rate (kg a.s./ha) * RUD (mg*ha/kg) * consumption (mg/larva in 5 days) / 1000

Where:

- Application rate is the intended dose of the product expressed as kg active substance per hectare (kg a.s./ha).
- RUD = residue per unit dose is set to default concentration of pollen and nectar for downward spray applications (EFSA 2013) based on a generic application rate of 1 mg/kg a.s. per hectare (used for all substances). RUD for pollen = 6.1 mg a.s./kg and RUD for nectar 2.9 mg a.s./kg.
- The default assumption of consumption is 2 mg pollen/larva and 198 mg nectar/larva (based on sugar intake via nectar of 59 mg, assuming 30% sugar content) during a five day period (Northern Zone 2024, based on Rortais *et al*, 2005)

The risk is considered acceptable if the toxicity endpoint for larvae (no observed effect dose, NOED) is higher than the exposure through the diet (pollen and nectar). The toxicity exposure ratio (TER; NOED/exposure dose) is calculated and should be higher than 1 to be accepted (ECPA 2017).

To compare the measured concentrations of individual substances in pollen samples from this study with the calculated values used in the risk assessment scheme for larvae, we selected the substances with the highest total risk index in the study, that are authorised for use in the Northern Zone. The maximum measured concentrations in pollen were compared to the calculated values for pollen concentrations (Application rate (kg a.s./ha) * RUD (mg*ha/kg)) for each substance. We used representative application rates of the substances for authorised products in Sweden, which are also relevant for other countries in the Northern Zone.

As a next step, the toxicity exposure ratios (TER) were calculated, based on the calculated values used in the risk assessment and for the maximum measured concentrations from this study. In this study only measured concentrations of pesticides in pollen were determined, whereas concentrations in nectar are also needed for the TER calculations. To this end, we used maximum concentrations measured in nectar from honey bees in 42 samples collected in the same region as the samples in this project, in 2019 (Knapp et al. 2023, Nicholson et al. 2024b).

The NOED:s used for azoxystrobin and boscalid were taken from registration reports for product formulations that have been authorised in Sweden, for acetamiprid the NOED corresponds to an EU agreed toxicity endpoint (EFSA 2016). NOED might not be the same for all products and are here used for illustration purpose only. Up to this date there are no EU-agreed NOED (larvae) for acetamiprid, azoxystrobin and boscalid.

2.5.2 Proportion crop pollen

Bees are central place foragers that can visit and forage in many patches and cropped fields in the landscape surrounding their colony. Landscape factors were introduced in the revised guidance document on the risk assessment of plant protection products on bees (EFSA 2023). They describe the proportion of the food intake of a bee colony or population that originates from the treated crop field. The landscape factor for the first step acute exposure assessments is 1 (100% of the collected pollen originates from the treated crop). This assumption is compared with the proportion of crop pollen in the samples collected in this study.

2.6. Statistical analyses

Summed concentrations of all substances and the toxicity weighted summed concentrations (the risk index) were related to country, sampling round and proportion of crop pollen using linear mixed models. Summed concentration and the risk index were $\log_{10}$ transformed before analysis to better fulfil the assumption of normally distributed residuals. The models also included site as a random factor to account for the non-independence of the weekly samples collected at the six independent sites. The differences among sampling rounds were explored using post hoc testing with Tukey adjustment of the P values. Least square means and accompanying 95% confidence limits were extracted from the models and used for plotting the summed concentration and risk index in relation to country and sampling round. Analyses were done in SAS version 9.4 (SAS Institute Inc.).

3. Results

All pollen samples were successfully collected except for the last one in Norway, due to difficult weather conditions and low bee activity. Thus, there were in total 23 pollen samples from the six locations and three to four sample weeks (Table 1).

Table 1. Timing of the four (three in Norway) sample rounds in the three countries and six sites.

Country	N sites	Round 1	Round 2	Round 3	Round 4
Lithuania	1	2 May	8 May	15 May	23 May
Norway	1	13 May	21 May	28 May	
Sweden	4	14 May	21-23 May	28 May	4 June

3.1 Detected substances, concentrations and risk index

Out of the 173 analysed substances, 58 were detected in one or more pollen samples. The number of substances detected per sample ranged between 7 and 23, and the summed pesticide concentration in single samples ranged between 57 ng/g and 19213 ng/g pollen and the corresponding risk index between 1 and 1800.

Most frequently detected was the insecticide acetamiprid (96%, i.e. in all but one sample), followed by the fungicides azoxystrobin and fluopyram (91 and 83% respectively) and the herbicide MCPA (also 83%) (Figure 5). All four are authorised for use in Lithuania, Sweden, and Norway. The fungicides boscalid, captan, azoxystrobin and fluopyram showed the highest concentrations in a single sample (11000, 8500, 6000 and 3600 ng/g pollen) respectively (Figure 6). Captan has not been authorized for use in Sweden since 2002 (except for emergency use in apples and pears in 2014), but was still detected at all Swedish sites, with the highest concentrations detected at one of the Swedish sites. In Lithuania, where it is still authorized for use, captan was found in three samples at concentrations of up to 1100 ng/g pollen.

Of the insecticides, which in general have the highest toxicity to bees, the substance with the highest concentration was acetamiprid (3600 ng/g pollen, i.e. the sixth highest concentration in the study). The insecticide indoxacarb, which is approximately 100 times more toxic to bees, was also found in five samples from Sweden (range 1.2 to 270 ng/g pollen), despite having been banned for use since September 2022. Indoxacarb had the highest risk index in a single sample followed by acetamiprid, boscalid, captan and azoxystrobin, the latter three mainly due to high concentrations (Figure 6). The neonicotinoid insecticides imidacloprid and thiacloprid were banned for outdoor use as plant protection product in EU and Norway in 2018 and 2020 respectively, yet thiacloprid was detected in several pollen samples from both Sweden and Lithuania while imidacloprid was detected in three samples from Lithuania. Imidacloprid was present in relatively low concentrations (44th highest concentration) but its high toxicity to pollinators resulted in the 7th highest risk index for one sample (Figure 6). It should be noted that imidacloprid is still used as biocide and in veterinary products.

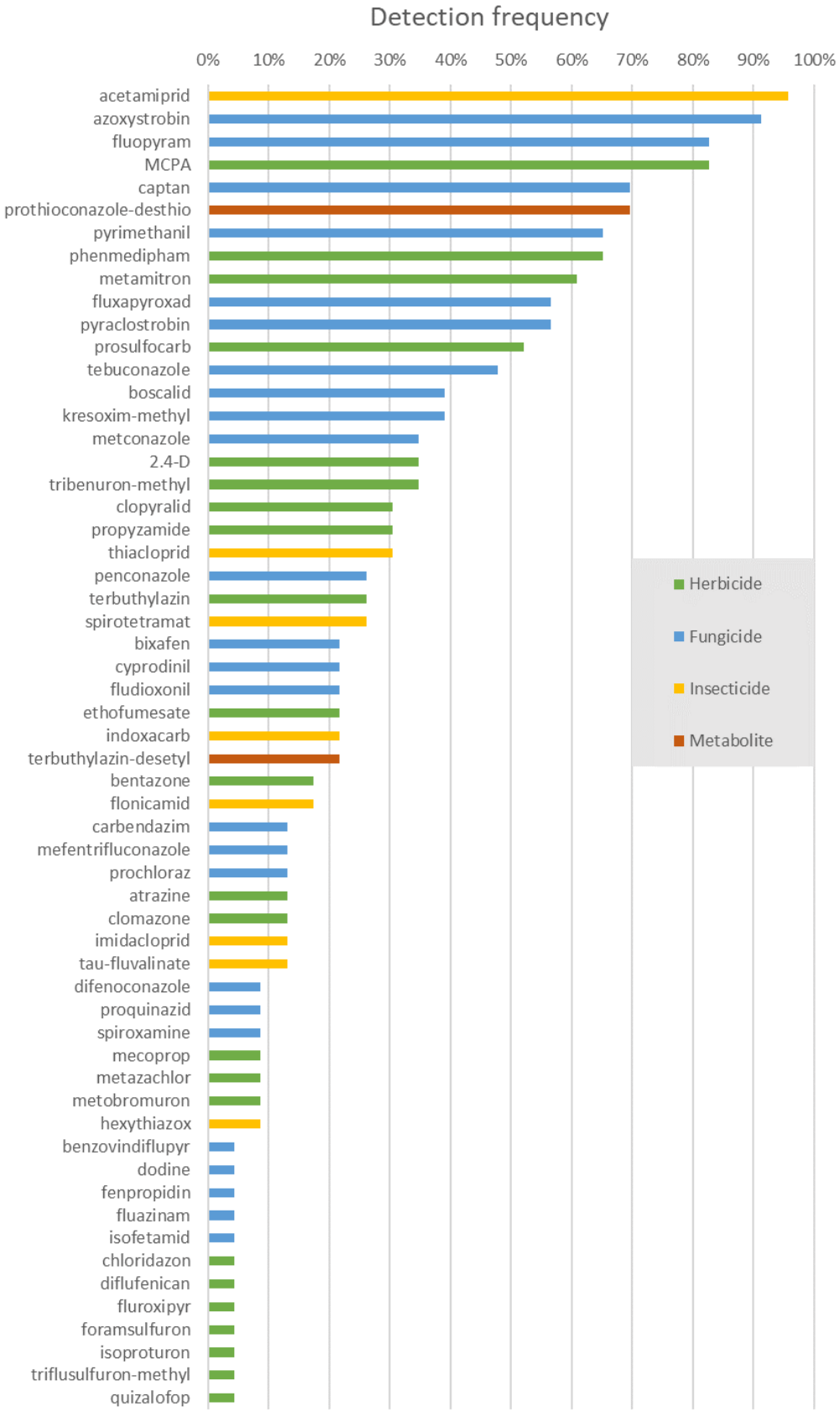

Figure 5. Detection frequency (%) of all detected substances in pollen samples from the six different sampling sites.

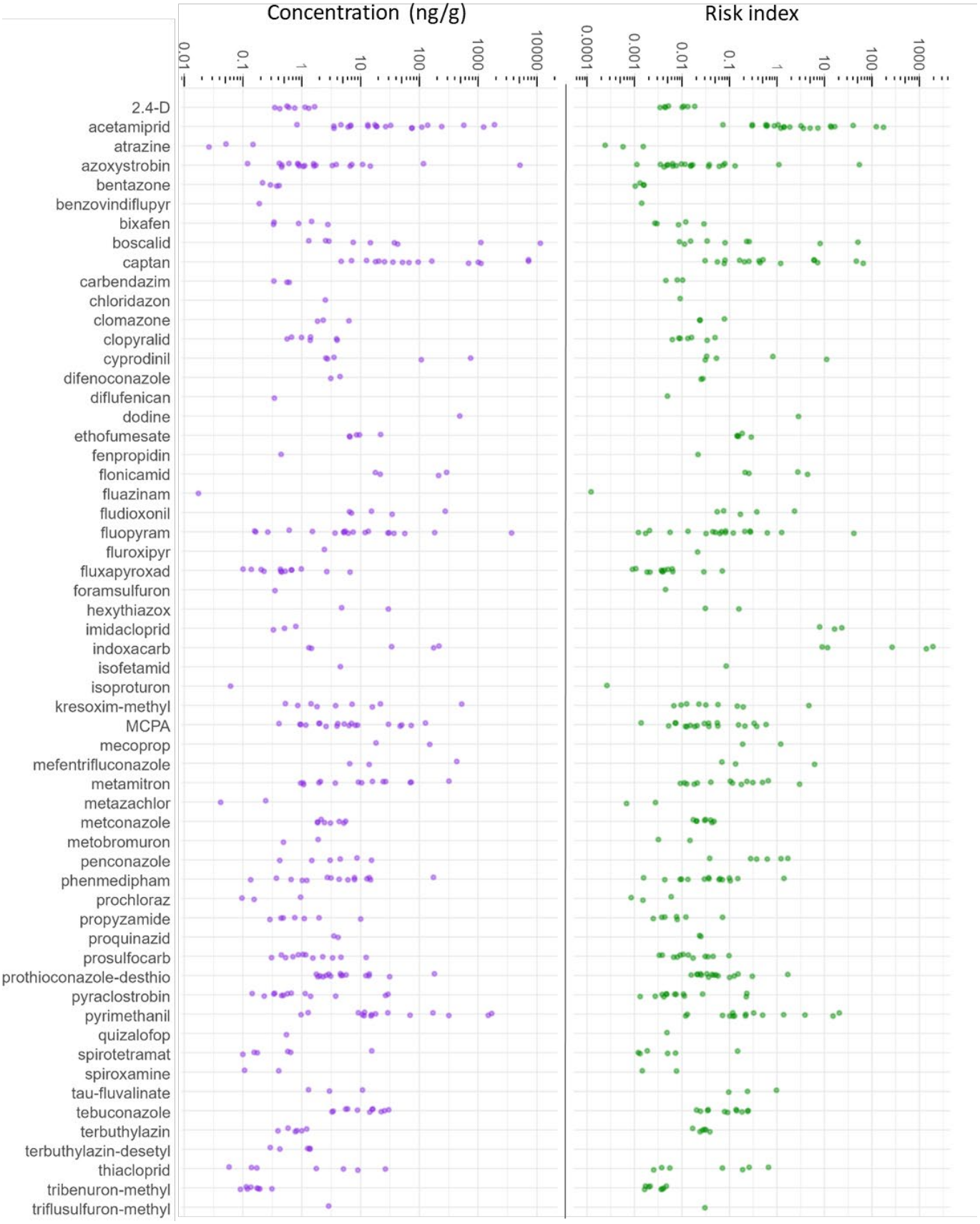

Figure 6. Concentrations (ng/g pollen) and risk indices for all detected substances in pollen samples from the six different sampling sites.

3.2 Comparison among countries and sampling rounds

Most substances were detected at the Swedish sites SE1 and SE3 (35 and 30 respectively). Fewest substances were detected at the Swedish site SE2 and the Norwegian site (25 and 13 respectively). However, only three samples were collected from the Norwegian site, whereas four were obtained from the other sites, which could affect the results (Figure 7). The total number of detected substances at all sampling sites remained relatively consistent across the four sampling rounds, with 41, 45, 41 and 38 compounds detected. Summed concentrations differed among sampling rounds ($F_{3,\ 13.1} = 3.45$, $P = 0.048$; Figure 8a) but not countries ($F_{2,\ 3.5} = 0.61$, $P = 0.59$; Figure 8b). Similarly, the risk index differed among sampling rounds ($F_{3,\ 13.0} = 4.01$, $P = 0.032$; Figure 8c) but not countries ($F_{2,\ 3.1} = 0.19$, $P = 0.84$; Figure 8d). For both summed concentrations ($t_{13} = 3.18$, $P = 0.032$) and the risk index ($t_{13.0} = 3.38$, $P = 0.022$), pairwise comparisons indicated higher values for sampling round 1 compared to round 4 while there were no differences among the other rounds (all $P > 0.05$).

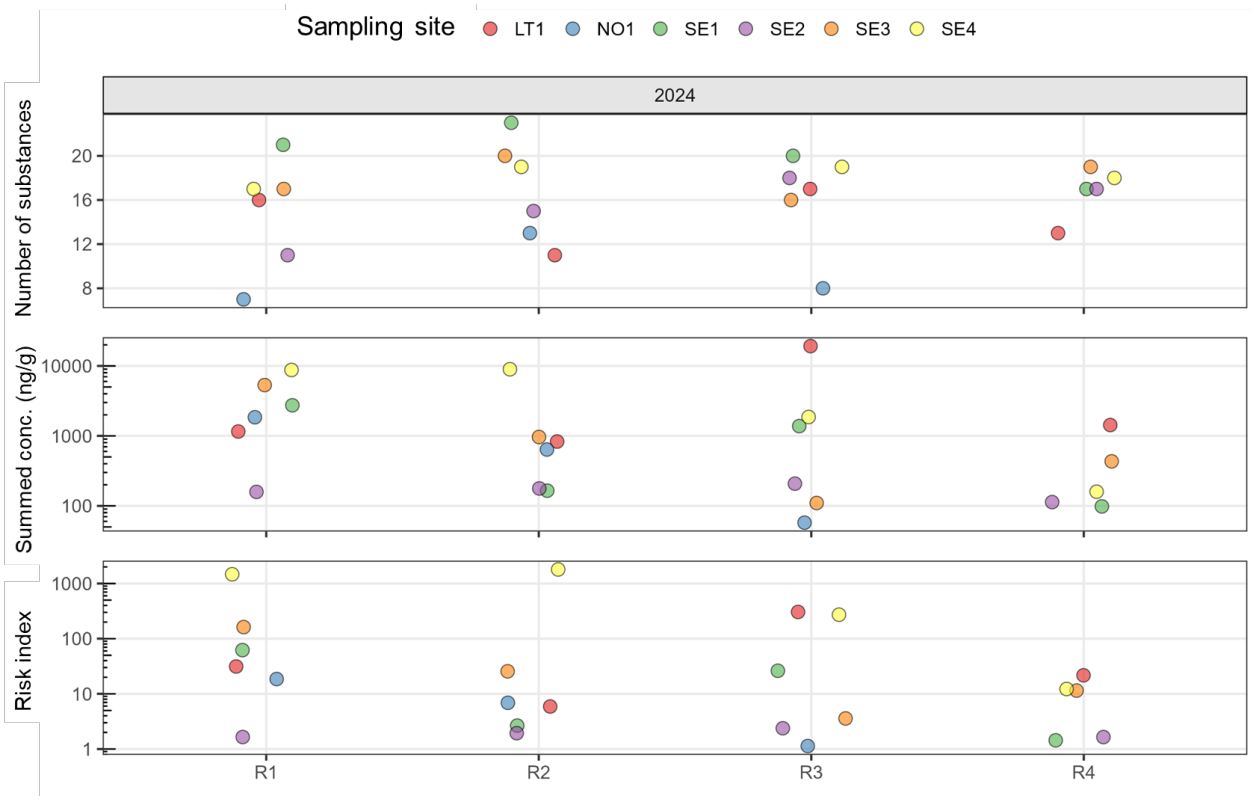

Figure 7. Number of detected substances, summed concentrations and risk index per sample for all six sampling sites in Lithuania (LT), Norway (NO) and Sweden (SE). The samples were collected over 3-4 sampling rounds (R1-R4) per site.

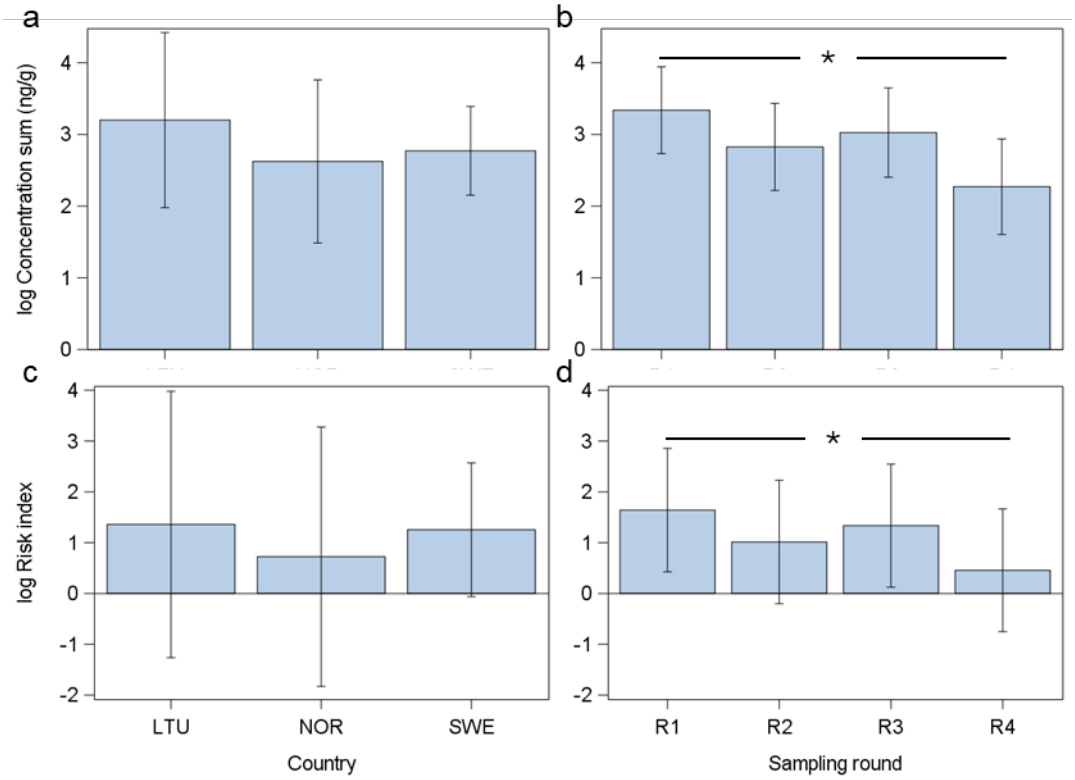

Figure 8. Summed concentrations of pesticides in pollen (a, b) and risk index (c, d) in relation to the country (LTU = Lithuania, NOR = Norway, SWE = Sweden) and sampling round from early-mid-May (R1) to late May-early June (R4). N = 6 with four sites in Sweden (four rounds) and one site each in Lithuania (four rounds) and Norway (three rounds). Bars show model estimated means and error bars 95% confidence intervals. * indicates the only confirmed statistical differences (P <0.05) between sampling rounds 1 and 4.

3.3 Plant origins and relations to concentration and risk

The commonest pollen type was Brassicaceae (mean 33% of the pollen grains in a sample, range: <1-96%), which includes cultivated oilseed rape but there are also many wild plants in this plant family (Figure 9). However, there was a large variation among countries, with on average 56% Brassicaceae in Lithuania, 1% in Norway and 33% in Sweden. The second most common pollen type was Rosaceae of the *Sorbus* type, which includes apple and other fruit orchard crops as well as flowering wild trees and bushes such as blackthorn, bird cherry and raspberry (25%, <1-71%; Lithuania 15%, Norway 44%, Sweden 24%). The third commonest pollen type was Fabaceae of the *Lupinus*/*Robinia* type (15%, 1-40%), which includes legumes such as common broom, lupin and black locust. Other pollen types compromised on average 27% of the pollen grains in a sample (3-78%). Plant species/groups with high proportions of pollen in individual samples were lacy phacelia *Phacelia tanacetifolia* (Sweden SE2:3, 65%), European horse-chestnut *Aesculus hippocastanum* (Sweden SE3:2, 38% and SE3:3, 16%) Rosaceae of the *Geum* type (Sweden SE1:4, 16%), including avens, and Plantaginaceae of the *Digitalis* type (Norway NO1:3, 15%) (Figure 9), including foxgloves.

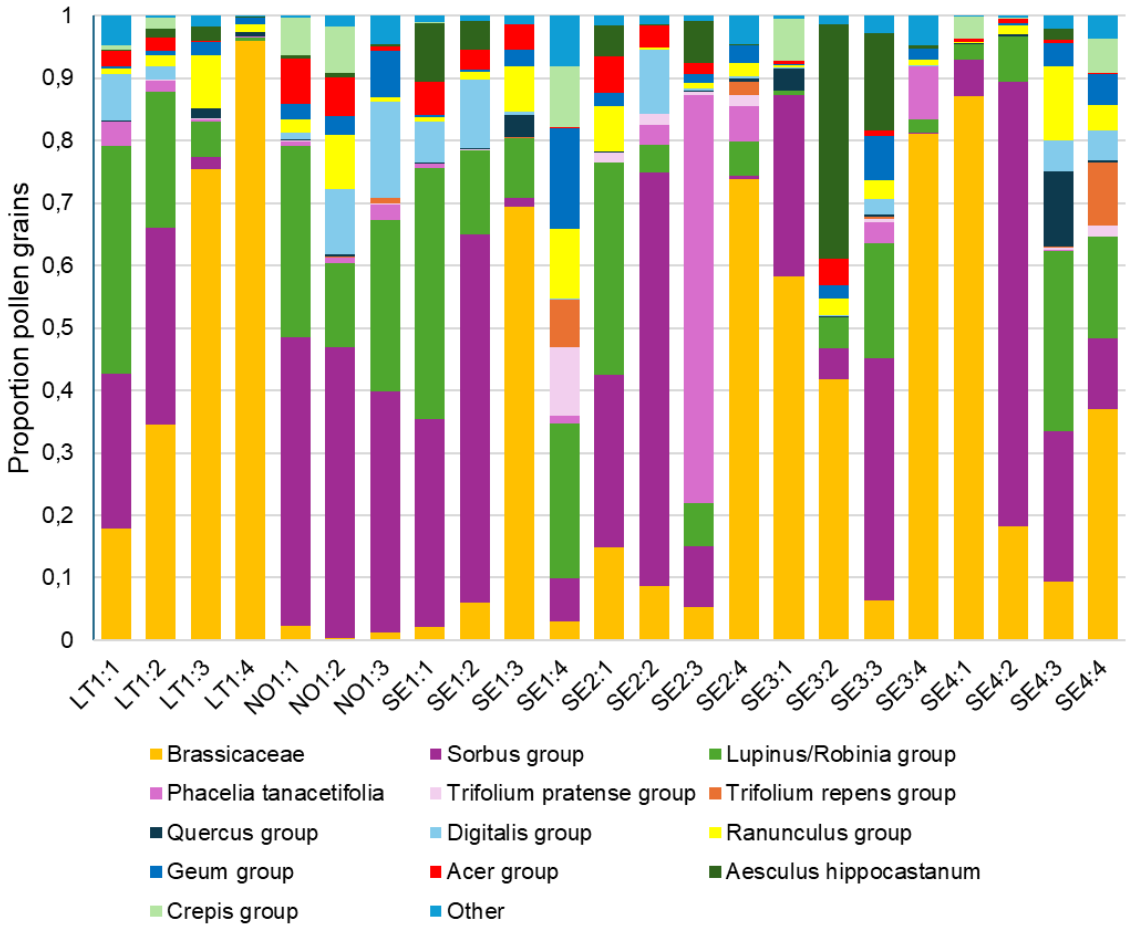

Figure 9. Proportion of pollen grains in the honey bee collected pollen identified to different plant species or groups at the six sites in Lithuania (LT), Norway (NO) and Sweden (SE) over the 3-4 sampling rounds (consecutive weeks) at each site. Sampling was done in 2024 and started May 2nd in Lithuania, May 13th in Norway and May 14th in Sweden. The plant species/group legend should be read from top left to the right and then down, with Brassicaceae as the most common pollen type, followed by the *Sorbus* group and thereafter *Lupinus*/*Robinia* group, *Phacelia tanacetifolia* and so on.

Both summed concentrations ($F_{1, 14.5} = 5.48$, $P = 0.034$) and the risk index ($F_{2, 13.4} = 5.67$, $P = 0.033$) correlated positively to the proportion of crop pollen in the sample (Figure 10).

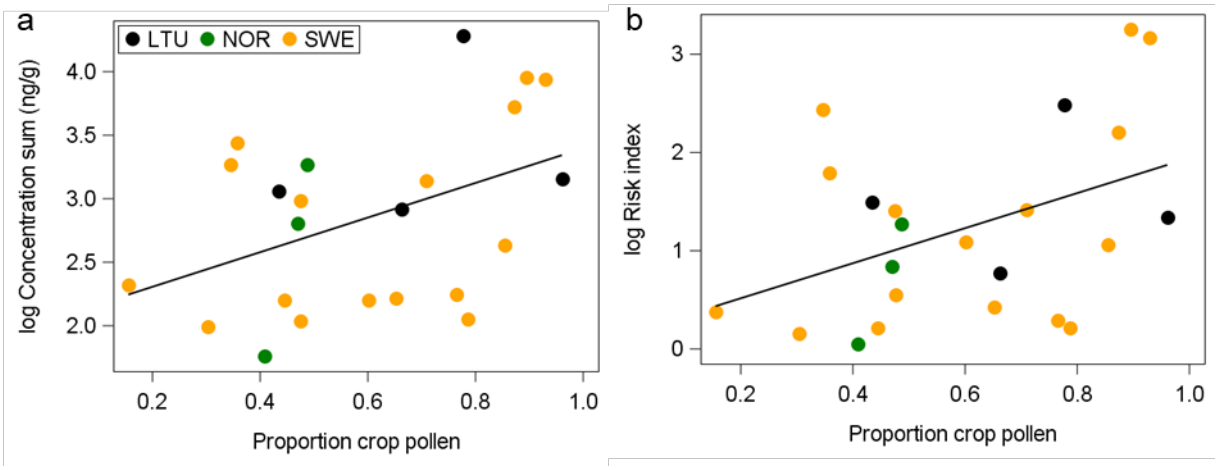

Figure 10. Summed concentrations of all substances (ng/g; a) and risk index (summed toxicity weighted concentrations; b) in honey bee collected pollen were positively related to the proportion of crop pollen (from the pollen type groups Brassicaceae, *Trifolium pratense*, *Trifolium repens*, *Vicia*, *Sorbus*) in the honey bee collected pollen samples from sites in Lithuania (LTU, 1 site, 4 rounds), Norway (NOR, 1 site, 3 rounds) and Sweden (SWE, 4 sites, 4 rounds). Figures are based on raw data with the black line assuming a linear relationship.

3.4 Highest risk substances

The top ten substances with the highest risk, when summing risk index per substance over the 23 samples, included insecticides and fungicides (Table 2). Two insecticides, Indoxacarb followed by acetamiprid, were at the top of the list, where the risk was mainly driven by high toxicity (Table 2). The next three compounds were the fungicides captan, boscalid and azoxystrobin, where high concentrations mainly contributed to the risk index (Table 2). These three fungicides have toxicity estimates based on so-called limit tests (Table 2), where the LD50 value was not determined exactly but lies above the tested dose. This means that the actual LD50 is unknown. The sixth most risky substance was imidacloprid (Table 2), banned for use as an outdoor plant protection product but still used as biocide and in veterinary products. Thereafter follow three more fungicides, fluopyram, pyrimethanil and cyprodinil, with relatively high summed concentrations and toxicity mainly based on limit tests (Table 2). The tenth riskiest compound was flonicamid, an insecticide where the toxicity is based on limit tests (Table 2).

Table 2. The ten substances with the highest risk index (summed toxicity, i.e. LD_{50}, weighted concentrations) over all the collected pollen samples, including information on the type of substance, main use (F= fungicide, H=herbicide, I=insecticide, M= metabolite), number of detections (max = 23 samples), LD_{50} for honey bees (µg/bee) averaged over acute contact and oral testing and summed, median and max concentrations (ng/g).

Substance (type)	Detects	Risk sum	LD_{50} mean	LD_{50} contact	LD_{50} oral	Conc. sum	Conc. median	Conc. max
indoxacarb (I)	5	3402	0.156	0.08	0.232	531	38	270
acetamiprid (I)	22	415	11.3	8.09	14.53	4690	18	2000
captan (F)	16	133	150	200*	100*	20000	70.5	8500
boscalid (F)	9	67	183	200*	166*	12300	13	11000
azoxystrobin (F)	21	55	113	200*	25*	6170	1.5	6000
imidacloprid (I)	3	42	0.0424	0.081	0.0037	1.78	0.57	0.93
fluopyram (F)	19	39	101	100*	102.3*	3970	6.2	3600
pyrimethanil (F)	15	37	100	100*	100*	3740	18	1700
cyprodinil (F)	5	11	93.8	75*	112.5	1010	4.1	910
flonicamid (I)	4	7	80.3	100*	60.5*	560	106	330

*LD_{50} based on limit test or doses that did not result in 50% mortality in the test population.

3.5 Using monitoring data in risk assessment

3.5.1 Pollen concentrations and toxicity exposure ratio

As a reality-check of one of the assumptions in the risk assessment for honey bee larvae we compared the measured concentrations of three individual substances in pollen samples from this study with the calculated values used in the current (interim) risk assessment scheme for the Northern Zone (Northern Zone 2024). We selected the substances with the highest total risk index that are also authorised for use in the Northern Zone: acetamiprid, azoxystrobin and boscalid (Table 2).

The maximum concentrations were all measured in the same sample (LT1:3). Boscalid had the highest measured concentration in the sample whereas acetamiprid had the highest risk index. The sample contained 16 different compounds in total. A majority of the sampled pollen was from Brassicaceae (mostly oilseed rape) and the remaining pollen was from non-crop plants (Figure 9). All three substances are authorised for use in oilseed rape in the Northern Zone and the sampling was performed during the time window where oilseed rape is normally treated with pesticides.

We made the assumption that the concentrations of these substances in pollen are due to the use of pesticides in the oilseed rape fields nearby, at authorised application rates. The predicted exposure in the risk assessment for bees foraging on oilseed rape was thus considered comparable to the measured concentrations in pollen from the sample.

The maximum measured concentrations in pollen were compared to the calculated values for pollen concentrations for each substance (Table 3). Calculated nectar concentrations were compared to maximum concentrations measured in nectar from honey bees in samples collected in the same region as the samples in this project, but in 2019 (Knapp et al. 2023, Nicholson et al. 2024b) (Table 3).

The calculated values for pollen and nectar concentrations were based on representative application rates on oilseed rape of products authorised for use in Sweden (Table 3) (these are also relevant for other countries in the Northern Zone) and default residue per unit dose (EFSA 2013).

The measured concentrations in pollen were nearly an order of magnitude higher than the calculated concentrations for all three substances. For nectar, the measured concentrations were somewhat higher for acetamiprid and lower for azoxystrobin and boscalid.

Table 3. Calculated concentrations (mg substance/kg) of the three selected substances in pollen and nectar based on representative application rates (kg substance/ha) and default residue per unite dose (RUD; EFSA 2013) and maximum measured concentrations (mg substance/kg) in pollen from this study and for nectar from a study in the same region in 2019 (Knapp et al. 2023, Nicholson et al. 2024b).

Substance	Application rate	Calculated conc. in pollen	Measured conc. in pollen	Calculated conc. in nectar	Measured conc. in nectar
acetamiprid	0.050	0.31	2.0	0.15	0.23
azoxystrobin	0.15	0.92	6.0	0.44	0.045
boscalid	0.25	1.50	11	0.73	0.22

The risk to honey bee larvae was calculated as a toxicity exposure ratio (TER) (Table 4) based on the measured as well as calculated concentrations in Table 3. The risk is acceptable if the ratio between toxicity endpoint for larvae (NOED) and the exposure dose through diet (pollen and nectar) is higher than 1 (ECPA 2017).

For all three substances, the toxicity exposure ratio TER exceeded the trigger value of 1, indicating no unacceptable risk, both when using calculated exposure doses for a generic pesticide, according to the interim risk assessment scheme, but also considering measured concentrations in pollen and nectar under realistic exposure conditions.

Table 4. Toxicity exposure ratios (TER) of the three selected substances calculated as the toxicity endpoint NOED for 22 days development period (µg substance/larva) divided with the exposure dose for five days consumption; 2 mg pollen/larva and 198 mg nectar/larva (µg substance/larva) based on measured and calculated concentrations respectively. Maximum measured concentrations in pollen from this study and for nectar from a study in the same region in 2019 (Knapp et al. 2023, Nicholson et al. 2024b) are used. Calculated concentrations are based on representative application rates and default residue per unite dose (RUD; EFSA 2013). The resulting TER must exceed 1 to conclude on acceptable risk.

Substance	NOED	Exposure dose pollen	Exposure dose nectar	SUM pollen+nectar	TER
acetamiprid measured	1.3	0.0040	0.046	0.050	26
acetamiprid calculated		0.00061	0.029	0.029	44
azoxystrobin measured	8.9	0.012	0.0088	0.021	427
azoxystrobin calculated		0.0018	0.086	0.088	101
boscalid measured	0.30	0.022	0.043	0.065	5
boscalid calculated		0.0031	0.14	0.15	2

Besides acetamiprid, azoxystrobin and boscalid, the sample contained 13 other substances. The combined exposure to different substances is not accounted for in the risk assessment, unless included in the same plant protection product. Only in that case the TERs for each substance are added to the total risk assessment for the product.

3.5.2 Proportion crop pollen

In the EFSA et al. (2023) revised guidance on risk assessment for bees, the proportion of the food intake of a bee colony or population, that originates from the treated field, is set to 100%. In this study, the proportion of crop pollen varied between 16% and 96%, which shows that the default assumption of 100% can be seen as realistic based on field monitoring. In the samples with the highest proportion of pollen coming from crops the Brassicaceae type dominated with 96% of the pollen grains identified as this type. The Brassicaceae pollen type mainly consists of cultivated oilseed rape during this season. This can be seen by comparing the pollen identities in the sample from Norway, where oilseed rape is not grown in the region, and samples from the other locations, where winter-sown oilseed rape is commonly grown (Figure 9).

4. Discussion and conclusions

There were no differences among the countries in summed concentrations or risk, while these appeared to decrease from the first sampling in early May to the last in late May to early June. There was a large variation among samples in the number of substances detected (7-23), summed concentrations (57-19213 ng/g), and risk index (1-1800). Both the large variation and low number of sampled sites, particularly in Norway and Lithuania, could contribute to the lack of differences in summed concentration and risk among countries. However, there are few differences in the products and active substances registered in the different countries and the authorisation procedures are largely harmonized due to the common guidance (Northern Zone 2024). Thus, the variation in detections, concentrations and risk is probably related to which crops are grown and the related pest management challenges and resulting product use in the landscapes surrounding the monitoring sites (Nicholson et al. 2024b). The decrease in summed concentrations and risk from spring to summer has been observed in previous monitoring studies in Sweden (Jonsson et al. 2022, Knapp et al. 2023, Nicholson et al. 2024b) and this is also the case when exploring toxicity-scaled pesticide use statistics for Sweden (Rundlöf et al. 2024). From a practical point of view, the sampling method was easily implemented in all three countries and the collaboration between partners for pollen collection, identification and pesticide analyses worked well. In addition to the monitoring data, the project also contributed to new interactions between research groups within the Northern Zone, facilitating future joint studies and data sharing.

The insecticide acetamiprid was the substance with the highest detection frequency (96% of the samples). This was likely because acetamiprid is one of few insecticides still approved for use and it can be used both in oilseed rape and in apples, which were the dominant flowering crops in the studied sites. The high detection frequency can partly also be a result of the low detection limit for this compound (0.05 ng/g). The insecticide with the second highest detection frequency was thiacloprid (banned for use in all three countries in 2020), which was found in ca 30% of the samples. This is lower than the detection frequency found for thiacloprid in studies performed in the same region in Sweden in 2019 (100%; Knapp et al. 2023) and 2020-2021 (ca 65-70%; Jonsson et al. 2022) with sampling from May to July. For acetamiprid the trend is the opposite, with a detection frequency in pollen of 75% in 2019 (Knapp et al. 2023), somewhat greater than 40% in 2020, almost 80% in 2021 (Jonsson et al. 2022) and now 96% in 2024. The remaining substances with the highest detection frequencies were fungicides and herbicides: in total, 21 substances were detected with a frequency of 30% or higher.

The fungicides boscalid, captan, azoxystrobin and fluopyram showed the highest concentrations in a single sample out of all substances, and they were also all among the 15 substances with the highest detection frequency. Except for captan, which was a new compound included for this study and thus not analysed in the previous studies, these fungicides were also frequently detected in Sweden in 2019 (Knapp et al. 2023, Nicholson et al. 2024b) and 2020-2021 (Jonsson et al. 2022) and also then in high concentrations.

As mentioned for acetamiprid, detection frequency is a result not only of the actual presence of the substance in pollen but also of the detection limit of the analytical method. As reported in Appendix 1, detection limits vary over several orders of magnitude and concentrations in the environment may be underestimated for substances with high detection limits. This could be a problem in the estimation of risks, especially for compounds that are highly toxic to bees like, for example, some of the pyrethroids.

Highest risk substances and conditions

In this study we used the risk index as an estimate of the direct consequences that detected pesticide residues could have for bees, and possibly other pollinating insects, in agricultural landscapes. The toxicity values used in the index calculations were based on the LD_{50} (mean of oral and contact exposure) for honey bees because there is no such data for most substances for other bee species or pollinators. This risk index has been shown to correlate negatively to bee nesting and reproduction in a few earlier studies (Rundlöf et al. 2022, Nicholson et al. 2024a), although it has not been evaluated for effects on other pollinators. The risk index reflects direct toxic effects on bees and it is unlikely to capture indirect effects such as the potential impact of herbicides on flowering plants.

The risk index is a relative indicator for risk to bees that is best used to compare risk among locations, times and substances, because it is not known or decided what an unacceptable risk means in terms of this indicator. It builds on a similar principle as a hazard quotient, but for the hazard quotient the exposure is scaled by consumption or contact before it is divided by the toxicity for the particular type of exposure route (i.e. oral or contact for adult bees). However, the assumptions on consumption or contact are usually organism specific and with limited knowledge on sensitive organisms, it is challenging to generalize. The approach of dividing environmental concentrations or doses, or even applied pesticide amounts with an estimate of the toxicity for the organism group(s) in focus is generally used as an indication of pesticide environmental risk in both terrestrial and aquatic environments. For example, “total applied toxicity”, based on applied pesticides scaled by toxicity for different organism groups in terrestrial and aquatic environments, has been used to explore national risk trends over time (Schulz et al. 2021, Bub et al. 2023). The risk index we use in this study could be used in a similar way to follow trends in risk for bees through time.

High risk substances result from a combination of either high measured concentrations in pollen or high toxicity, or both. The top two substances with the highest risk index in total, over all samples in the study, were the insecticides indoxacarb and acetamiprid, which both have relatively low LD_{50} values (mean of LD_{50} oral and contact 0.16 µg and 11 µg per bee, respectively). Imidacloprid, which has the lowest LD_{50} of all detected substances (mean LD_{50} 0.042 µg per bee), had the sixth highest summed risk, despite very low concentrations in only three samples. This shows that the risk that pesticide exposure can pose to bees and other pollinators is highly dependent on the toxicity of the substances, which varies considerably among substances. The fact that the highly potent insecticide imidacloprid is still found in pollen, after being banned since many years, may be explained by its slow degradation in soil and its systemic properties. This means that it may be present in soil for a

long time and taken up by the rots of different plants and distributed to all parts, including pollen and nectar. Not surprisingly, insecticides are generally the most directly toxic to bees, although their LD_{50} values can vary across several orders of magnitude. The mean LD_{50} values for the detected insecticides in this study ranged between 0.042 and 156 µg/g per bee. The most toxic insecticides are no longer allowed for use as plant protection products but one of these (indoxacarb) still posed the highest summed risk to bees in this study. Of the active substances in plant protection products still registered for use, acetamiprid has the lowest mean LD_{50} of the ones detected in this study.

Of the ten substances with the highest summed toxicity over all samples in the study, six were fungicides. All of them have a mean LD_{50} over 90 µg per bee, so the main reason for their high risk is their high detection frequency and high concentrations. All of them have LD_{50} values that are based on so-called "limit-tests", where the highest tested dose did not result in 50% mortality in the test population, with the conclusion that the LD_{50} lies above the tested dose. In these cases, the highest tested dose was used as the LD_{50} value, which means that the toxicity of these substances may be overestimated.

No herbicides are on the top ten list of highest risk substances. The detected herbicide with the lowest LD_{50} value is terbuthylazine (27 µg per bee) which has been banned for 20 years. Of the herbicides still registered for use, ethofumesate has the lowest LD_{50} value (50 µg per bee). Both of these LD_{50} values are based on limit tests.

The summed risk index for all substances in each sample correlated positively to the proportion of crop pollen in the sample, which may indicate that crop pollen could be an important source of pesticide risk to bees and other pollinators. However, this relationship does not necessarily mean that it is the crop pollen that is most risky but may just as well indicate that more pesticides are used in areas with large cultivation of flowering crops. Studies indicate that although crop pollen can be a major source of pesticide exposure through pollen and nectar (Zioga et al. 2020), non-crop plant pollen, such as from cornflower *Centaurea cyanus*, poppy *Papaver* spp. and buttercup *Ranunculus* spp. can also lead to high pesticide exposure when growing in or close to cropland (Jonsson et al. 2022). The samples with the highest proportion of crop pollen contained mainly pollen from the groups Brassicaceae, including cultivated oilseed rape, and/or Rosaceae of the *Sorbus* type, including apple and other fruit orchard crops.

Using monitoring data in risk assessment

Based on the interim risk assessment scheme for product authorisation in the Northern Zone and using substance concentrations in pollen from this study and nectar concentrations from a study in the same region in 2019 (Knapp et al. 2023, Nicholson et al. 2024b) it can be concluded that the first step in this risk assessment is not fully protective for honey bee larvae. Although the TER, i.e. toxicity exposure ratios, do not fall below acceptability trigger 1 in the presented examples, one of the TER are lower when based on measured concentrations than the calculated default values. This indicates that the current risk assessment methodology for the first step might not as protective as assumed and in some cases underestimate the exposure under field conditions. When TER using default values is close to 1, TER based on measured concentrations could in reality result in unacceptable risk. However, this risk assessment scheme for larvae is an interim solution until revised European guidance is implemented.

In the revised guidance on the risk assessment of plant protection products for bees (EFSA et al. 2023) there is an assumption of a landscape factor of 1, i.e. assuming that 100% of the collected pollen originates from the treated crop. This situation is fully feasible when attractive crops are in bloom.

Although bees have a wide foraging area, in some of the samples pollen content had up to 96% Brassicaceae type pollen, which mainly consists of cultivated oilseed rape during this season.

In the interim risk assessment scheme for product authorisation in the Northern Zone, the pesticide exposure via pollen is marginal in relation to the exposure via nectar. The honey bee larvae intake of nectar is assumed to be 59 mg sugar, i.e. about 198 mg nectar using the assumed 30% sugar content, but only 2 mg pollen, during a period of five days. It would thus be valuable to know concentrations of individual substances also in nectar or, if a future monitoring program is based only on concentrations in pollen, to explore the potential to use a conversion factor between concentrations in the two materials. However, we feel that this study is a valuable first attempt to explore how the data obtained from post-approval monitoring could be used to evaluate default values and the outcome of the regulatory risk assessment.

Visions for long-term monitoring of pesticides in the terrestrial environment

There is no long-term monitoring of pesticides in the terrestrial environment in Europe. In the aquatic environment pesticides and other substances that can be harmful to the ecosystems are monitored based on the requirements in the Water Framework Directive (2000/60/EC). The monitoring system in the directive, however, aims to give a general picture of the status of the aquatic environment in Europe and is not specifically suited to follow risks from pesticides. Therefore, some European countries also have a more specific monitoring program for pesticides in the aquatic environment, e.g. Sweden and Switzerland (Spycher et al. 2024). A similar approach for monitoring pesticides in the terrestrial environment could be beneficial to increase knowledge about pesticide exposure in agricultural landscapes and to give valuable feedback to the authorisation process for plant protection products.

The aim of the Swedish aquatic monitoring program for pesticides (Boye et al. 2019) is to track long-term trends in surface water and groundwater quality from pesticide application within agriculture. The sampling is conducted primarily in four small catchments with intensive cultivation (ca 90% arable land), each representing a major agricultural region in Sweden. The sampling locations are at the outlet of each catchment and due to the high proportion of arable land they can be seen as near edge-of-field. The results represent realistic worst-case conditions for Sweden. Also, in Switzerland small water bodies in agricultural landscapes are monitored.

In this project the focus has been on pesticide concentrations in pollen and risk to bees and other pollinating insects. Pollen sampled from a honey bee hive represents the pesticide residues at a (foraging) landscape scale. This is much like sampling a local water body to capture pesticide residues over the catchment area and is therefore also relevant as a general assessment of the pesticide exposure of non-target terrestrial organisms in the area (de Oliveira et al. 2016).

One option to track changes in pesticide-related risks to pollinators in space and time is to monitor pesticide residues in relevant materials and calculate related risk indicators that predict consequences for pollinators. Pesticide residues in materials collected from honey bees have been suggested as useful for environmental monitoring (Jonsson et al. 2013; de Oliveira et al. 2016). Using honey bee collected pollen is a simple and non-invasive method, which does not have a significant impact on the pollinator population. It is also convenient that bee keepers can provide with hives and also do the pollen collection themselves, which means that a citizen science cooperation would be possible. It has previously been shown that concentrations of pesticides in matched samples of pollen and nectar are correlated, both when collected directly from plants (Zioga et al. 2020) and from bees (Jonsson et al. 2022, Knapp et al. 2023), with higher concentrations and risk indices for pollen. The summed

concentrations and risk based on pollen in this study can thus be seen as a worst case that also represents nectar. However, less is known about correlations between nectar and pollen concentrations for individual substances. Positive correlations have been shown for individual neonicotinoids in pollen and nectar collected from plants, with a steeper slope and stronger relationship for crop plants compared to wild plants (Zioga et al. 2020). There are additional materials beyond pollen and nectar that are relevant for pollinators, for example plant material, soil and water (Topping et al. 2024), and it is currently unclear if pollen can also represent pesticide residues across pollinator-relevant materials more generally. Furthermore, the summed concentrations and risk based on pollen collected by different bee species (*A. mellifera, B. terrestris, O. bicornis*) are correlated (Knapp et al. 2023), indicating that concentrations and risk based on honey bee collected pollen could be representative also for other bee species. The pollen-based risk index has also been shown to be predictive of nesting and reproduction in wild bee species (Rundlöf et al. 2022, Nicholson et al. 2024a). Post-approval monitoring of pesticides based on honey bee collected pollen would thus also provide an indication for the consequences of pesticide use for other bee species in agricultural landscapes.

Another option for tracking pollinator pesticide exposure would be to analyze the insects for pesticide residues. That has been done for honey bees, where it was concluded that pesticide concentrations and risk index were correlated among bees, pollen and nectar (Jonsson et al. 2022). Other bees and butterflies have also been analyzed for pesticide residues (David et al. 2016, Graham et al. 2021, Knapp et al. 2023, Main et al. 2020, Rundlöf et al. 2022, Ward et al. 2022), as well as the ethanol from Malaise insect trapping in both Germany (Brühl et al. 2021) and Norway (Stenrød et al. 2023). It is not known how the detections and residues in these materials relate to residues in pollen or nectar or to consequences for bees or other pollinators in agricultural landscapes.

A further option is a coordinated pesticide and pollinator monitoring, where the timing and location of environmental monitoring of pesticide concentrations in materials relevant for pollinators are coupled with monitoring of the abundance and diversity of pollinators (Topping et al. 2024). Pollinator monitoring is one of the requirements of the Nature Restoration Regulation (NRR). The NRR (and following delegated acts) states that EU member states are obligated to monitor key groups of pollinators (bees, butterflies, hoverflies and moths) annually as of 2026/2027, following standardized and agreed methods (Potts et al. 2021, 2024). This creates an opportunity to align terrestrial environmental pesticide monitoring with the planned pollinator monitoring.

Whichever method is adopted, it remains a challenge to link the information from post-approval monitoring to the pre- and re-approval process regulating pesticide active substances and plant protection products. The reality-check of the harmonized risk assessment approach for honey bee larvae in the Northern Zone presented in this report is an attempt to use information from environmental monitoring to evaluate the realism of the default values used to estimate exposure. The sampling locations in Norway, Lithuania and Sweden were selected to be representative of the climatic and agricultural conditions in the Northern Zone, which would be a good point of departure for designing a common monitoring program across the zone. However, a more thorough evaluation of detections, concentrations and risk among countries in the zone and in relation to climatic and agricultural conditions would be beneficial to decide on an appropriate design for a common monitoring program. There is now a better understanding that pesticide exposure to bees, pollinators and other mobile organisms, is a landscape-scale process that acts over larger spatio-temporal scales than a one product-one crop environmental risk assessment can account for (Topping et al. 2020). In the Horizon Europe-funded project PollinERA, the overall aim is to move the environmental risk assessment for pollinators beyond the current one pesticide and honey bee focus, using a systems approach (Topping et al. 2024). Integrated modelling and monitoring is a key component of this

approach, where the unidirectional flow of information from risk assessors to risk managers and eventually farmers also flows in the other direction (Topping et al. 2020). In such a system, farmers could provide information on their pesticide use and agronomic practices and structured environmental monitoring could provide feedback on pesticide residues in the environment relevant for pollinators (Topping et al. 2024).

In this study, we chose to select monitoring locations with high expected pesticide use and potential direct risk to bees. This could be a good strategy when resources are limited and the intent is to have a type of warning system that informs on the outcome of the pesticide authorisation process in combination with the pesticide use. With more resources, locations could be selected to represent typical agricultural landscapes or the whole range of land use within a specified area. The design of a monitoring program should depend on the purpose of the program. For example, for exploring trends in environmental pesticide residues and risks for non-target terrestrial organisms, there is a need for continuous data collection from the same locations year after year. For selecting more long-term monitoring sites, it would be valuable to have spatiotemporally resolved pesticide use data – something that is currently not publicly available in the EU (Mesnage et al. 2021). Coordinating the sites with the planned monitoring of pollinators would create opportunities to better understand relations between the two.

Conclusions

- A high variation was found among samples in the number of substances detected, summed concentrations and in the calculated risk index.
- No differences were found among countries, while concentrations and the risk index appeared to decrease from the first sampling in early May to the last in late May to early June.
- The substance with the highest risk index in total, over all samples in the study, was the insecticide indoxacarb, which is no longer authorized for use. Of the substances authorized for use, the insecticide acetamiprid had the highest risk index and was also the most frequently detected substance.
- The summed risk index was positively correlated to the proportion of crop pollen in the sample.
- Using pollen concentrations from this study and nectar concentrations from a previous Swedish study, it can be concluded that the first step in the Northern Zone interim risk assessment is less protective for honey bee larvae than assumed.
- Concentrations in pollen alone are of limited use in an evaluation of the risk assessment for honey bees following the Northern Zone interim guidance, since pollen consumption is only accounted for in the risk assessment for larvae, and they are assumed to consume much more nectar than pollen (198 mg vs. 2 mg in 5 days).
- Environmental monitoring to support the risk assessment of bees would benefit from analyses of concentrations of substances in both nectar and pollen. Alternatively, methods could be developed to explore the translation of concentrations in pollen to those in nectar for individual substances.
- For future monitoring of pesticides in terrestrial environments, selecting locations with a large share of cropland and expected high pesticide use could be a suitable approach when resources are limited – similar to aquatic monitoring programs in Sweden and Switzerland. Spatio-temporally resolved information on use (product, dose, date) of pesticides in the surrounding fields would help in the selection of monitoring locations and also enable exploration of relationships with concentrations measured in different materials.

- For now, the analysis of pesticide concentrations in pollen collected by bees remains a promising approach to support risk assessments for bees based on the ease of collection and its relation to biological consequences for bee populations. However, the relevance for pollinators beyond bees cannot be assumed without further evaluation.

5. Acknowledgements

We would like to thank the funders the Nordic Working Group for Chemicals, Environment and Health (NKE), the landowners and beekeepers that were essential in enabling the pollen sampling, Martin Dolejs for collecting pollen at the Norwegian site and Inés Prieto Ruiz for preparing and scanning the pollen slides. We also would like to thank Tova Wennemo, Halldora Skarphedinsdottir and Johan Svensson at the Swedish Chemicals Agency authorisation department for help with the reality check, Vili Marttila, Leona Matssoff-Pakarinen and Sunita Hallgren for their feedback and Kristina Valionienė at the State Plant Service under the Ministry of Agriculture of Lithuania for information on unauthorised plant protection products.

This research was co-funded by the European Union's Horizon Europe research and innovation programme under grant agreement No.101135005 (PollinERA). Views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union (EU) or the European Research Executive Agency (REA). Neither the EU nor REA can be held responsible for them.

6. References

Bednarska, A. J., Mikołajczyk, Ł., Ziółkowska, E., Kocjan, K., Wnęk, A., Mokkapati, J. S. & Laskowski, R. 2022. Effects of agricultural landscape structure, insecticide residues, and pollen diversity on the life-history traits of the red mason bee Osmia bicornis. Science of The Total Environment 809: 151142. https://doi.org/10.1016/j.scitotenv.2021.151142

Beug, H.-J. 2015. Leitfaden der Pollenbestimmung für Mitteleuropa und angrenzende Gebiete. Verlag Dr. Friedrich Pfeil, München.

Biesmeijer, J. C., Roberts, S. P., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T. & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science, 313(5785), 351-354.

Boye, K., Lindström, B., Boström, G. & Kreuger, J. (2019). Long-term data from the Swedish national environmental monitoring program of pesticides in surface waters. Journal of Environmental Quality. https://doi.org/10.2134/jeq2019.02.0056.

Böhme, F., Bischoff, G., Zebitz, C. P. W., Rosenkranz, P. & Wallner, K. 2018. Pesticide residue survey of pollen loads collected by honeybees (Apis mellifera) in daily intervals at three agricultural sites in South Germany. PLoS ONE 13: e0199995. https://doi.org/10.1371/journal.pone.0199995

Bommarco, R., Lundin, O., Smith, H.G. & Rundlöf, M. (2012). Drastic historic shifts in bumble bee community composition in Sweden. Proceedings of the Royal Society B. Biological Sciences 279:309-315. https://doi.org/10.1098/rspb.2011.0647

Brühl, C. A., Bakanov, N., Köthe, S., Eichler, L., Sorg, M., Hörren, T., Mühlethaler, R., Meinel, G. & Lehmann, G. U. C. (2021). Direct pesticide exposure of insects in nature conservation areas in Germany. Scientific Reports, 11(1), 24144. https://doi.org/10.1038/s41598-021-03366-w

Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

David, A., Botías, C., Abdul-Sada, A., Nicholls, E., Rotheray, E. L., Hill, E. M., & Goulson, D. (2016). Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. Environment International, 88, 169-178. https://doi.org/10.1016/j.envint.2015.12.011

de Oliveira, R. C., Queiroz, S. C. d. N., da Luz, C. F. P., Porto, R. S., & Rath, S. 2016. Bee pollen as a bioindicator of environmental pesticide contamination. Chemosphere 163: 525-534. https://doi.org/10.1016/j.chemosphere.2016.08.022

Dupont, Y.L., Damgaard, C. & Simonsen, V. (2011). Quantitative historical change in bumblebee (*Bombus* spp.) assemblages of red clover fields. PLoS ONE 6: e25172. https://doi.org/10.1371/journal.pone.0025172

ECPA 2017: POS/17/LO/28028; modified EPPO for chronic RA for adult honeybees from spray applications

EFSA Scientific Committee, More, SJ., Bampidis, V., Benford, D., Bennekou, SH.,Bragard, C., Halldorsson, TI., Herández-Jerez, AF., Koutsoumanis, K., Naegeli, H., Schlatter, JR., Silano, V., Nielsen, SS., Schrenk, D., Turck, D., Younes, M., Benfenati, E., Castle, L., Cedergreen, N., Hardy, A., Laskowski, R., Leblanc, JC., Kortenkamp, A., Ragas, A., Posthuma, L., Svendsen, C., Solecki, R., Testai, E., Dujardin, B., Kass, GEN., Manini, P., Jeddi, MZ., Dorne, J-LCM. & Hogstrand, C. (2019) Guidance on harmonised methodologies forhuman health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal, 17:5634.

European Union, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy: water Framework Directive 327.

European Union, 2009. Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides

EFSA, 2013. Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees), revision of July 4th 2014).

EFSA (2016), Conclusion on the peer review of the pesticide risk assessment of the active substance acetamiprid. *EFSA Journal* 2016; 14(11):4610, 26 pp. https://doi.org/10.2903/j.efsa.2016.4610

EFSA, Adriaanse P, Arce A, Focks A, Ingels B, Jölli D, Lambin S, Rundlöf M, Süßenbach D, Del Aguila M, Ercolano V, Ferilli F, Ippolito A, Szentes Cs, Neri FM, Padovani L, Rortais A, Wassenberg J and Auteri D, 2023. Revised guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2023;21(5):7989, 133 pp. https://doi.org/10.2903/j.efsa.2023.7989

EPPO (2010) Environmental Risk Assessment scheme for plant protection products, Chapter 10: Honey bee PP 3/10 (3), OEPP/EPPO Bulletin 40, 323–331 https://doi.org/10.1111/j.1365-2338.2010.02419.x

Goulson, D., Nicholls, E., Botías, C. & Rotheray, E.L. (2015) Bee declines driven by combined stress from parasites, pesticides and lack of flowers. Science 6229. DOI: 10.1126/science.1255957

Graham, K. K., Milbrath, M. O., Zhang, Y., Baert, N., McArt, S., & Isaacs, R. 2022. Pesticide risk to managed bees during blueberry pollination is primarily driven by off-farm exposures. Scientific Reports 12: 7189. https://doi.org/10.1038/s41598-022-11156-1

Graham, K. K., Milbrath, M. O., Zhang, Y., Soehnlen, A., Baert, N., McArt, S., & Isaacs, R. (2021). Identities, concentrations, and sources of pesticide exposure in pollen collected by managed bees during blueberry pollination. Scientific Reports, 11(1), 16857. https://doi.org/10.1038/s41598-021-96249-z

Jonsson, O., Fries, I. & Kreuger, J. 2013. Utveckling av analysmetoder och screening av växtskyddsmedel i bin och pollen. CKB rapport 2013:1. Sveriges lantbruksuniversitet.

Jonsson, O., Rundlöf, M., Svensson, G., Forsberg, M., Lindström, B., Koch, A., Eriksson, E., Boström, G., & Gönczi, M. 2022. Pollinatörers exponering för växtskyddsmedel via pollen, nektar och luft i jordbrukslandskapet. CKB rapport 2022:1. SLU Centre for Pesticides in the Environment (CKB), Swedish University of Agricultural Sciences, Uppsala. https://www.slu.se/globalassets/ew/org/centrb/ckb/publikationer/ckb-rapporter/airbeesafe-ckb-rapport-2022_1.pdf

Knapp, J.L., Nicholson, C.C., Jonsson, O., de Miranda, J.R., & Rundlöf, M. 2023. Ecological traits interact with landscape context to determine bees' pesticide risk. Nature Ecology & Evolution 7: 547-556. https://doi.org/10.1038/s41559-023-01990-5

Kyriakopoulou, K., Kandris, I., Pachiti, I., Kasiotis, K.M., Spyropoulou, A., Santourian, A., Kitromilidou, S., Pappa, G. & Glossioti, M. (2017) Collection and analysis of pesticide residue data for pollen and nectar – final report. EFSA Supporting Publication 14.

Leonhardt, S.D. & Blüthgen, N. (2012) The same, but different: pollen foraging in honeybee and bumblebee colonies. Apidologie 43:449-464.

Lewis, K. A., Tzilivakis, J., Warner, D. J. & Green, A. 2016. An international database for pesticide risk assessments and management. Human and Ecological Risk Assessment 22 : 1050–1064. https://doi.org/10.1080/10807039.2015.1133242

Main, A. R., Hladik, M. L., Webb, E. B., Goyne, K. W., & Mengel, D. (2020). Beyond neonicotinoids – Wild pollinators are exposed to a range of pesticides while foraging in agroecosystems. Science of The Total Environment, 742, 140436. https://doi.org/10.1016/j.scitotenv.2020.140436

Mesnage, R., Straw, E.A., Antoniou, M.N., Benbrook, C., Brown, M.J., Chauzat, M.P., Finger, R., Goulson, D., Leadbeater, E., López-Ballesteros, A. and Möhring, N., 2021. Improving pesticide-use data for the EU. Nature Ecology & Evolution 5: 1560-1560.

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., van Engelsdorp, D. & Pettis, J.S. (2010) High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. Plos One, 5:e9754.

Nicholson, C. C., Knapp, J., Kiljanek, T., Albrecht, M., Chauzat, M-P., Costa, C., De la Rúa, P., Klein, A-M., Mänd, M., Potts, S. G., Schweiger, O., Bottero, I., Cini, E., de Miranda, J. R., Di Prisco, G., Dominik, C., Hodge, S., Kaunath, V., Knauer, A., Laurent, M., Martínez-López, V., Medrzycki, P., Pereira-Peixoto, M.H., Raimets, R., Schwarz, J.M., Senapathi, D., Tamburini, G., Brown, M.J.F., Stout, J.C. & Rundlöf, M. 2024a. Pesticide use negatively affects bumble bees across European landscapes. Nature 628: 355–358. https://doi.org/10.1038/s41586-023-06773-3

Nicholson, C. C., Lonsdorf, E. V., Andersson, G. K. S., Knapp, J. L. Svensson, G. P., Gönczi, M., Jonsson, O., de Miranda, J. R., Williams, N. M., & Rundlöf, M. 2024b. Landscapes of risk: A comparative analysis of landscape metrics for the ecotoxicological assessment of pesticide risk to bees. Journal of Applied Ecology, early online. https://doi.org/10.1111/1365-2664.14622

Northern Zone, 2020. Guidance document on work-sharing in the Northern zone in the authorisation of plant protection products. Version 9, June 2020. https://eng.mst.dk/media/kuwf5xsl/nordzone-vejledning-v-9-2020.pdf

Northern Zone, 2024. Guidance document on work-sharing in the Northern zone in the authorisation of plant protection products. Version 12, July2024.https://eng.mst.dk/media/wtbl5xnb/nzgd_2024_version_12_final_rev.pdf

OECD (1998), Test No. 213: Honey Bee (Apis Mellifera) Larval Toxicity Test, Single Exposure, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264070165-en.

OECD (2013), Test No. 237: Honey Bee (Apis Mellifera L.), Chronic Oral Toxicity Test (10-Day Feeding), OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264203723-en.

OECD (2017), Test No. 245: Honey Bee (Apis Mellifera L.), Chronic Oral Toxicity Test (10-Day Feeding), OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264284081-en.

Olofsson, C. (2023) Snopet avslut för pilotsatsning om pollinatörer. Extrakt. Published 7 March 2023, Updated 8 March 2023. https://www.extrakt.se/snopet-avslut-for-pilotsatsning-om-pollinatorer/?utm_campaign=unspecified&utm_content=unspecified&utm_medium=email&utm_source=apsis

Olsson, O., Karlsson, M., Persson, A.S., Smith, H.G., Varadarajan, V., Yourstone, J. & Stjernman, M. (2021) Efficient, automated and robust pollen analysis using deep learning. Methods in Ecology and Evolution 12: 850-86.

Potts, S.G., Bartomeus, I., Biesmeijer, K., Breeze, T., Casino, A., Dauber, J., Dieker, P., Hochkirch, A., Hoye, T., Isaac, N., Kleijn, D., Laikre, L., Mandelik, Y., Montagna, M., Montero Castano, A.,

Öckinger, E., Oteman, B., Pardo Valle, A., Polce, C., Povellato, A., Quaranta, M., Roy, D., Schweiger, O., Settele, J., Ståhls-Märkelä, G., Tamborra, M., Trooost, G., Van Der Wal, R., Vujic, A. & Zhang, J. 2024. Refined proposal for an EU pollinator monitoring scheme. Publications Office of the European Union, Luxembourg. doi:10.2760/2005545, JRC138660.

Potts, S.G., Dauber, J., Hochkirch, A., Oteman, B., Roy, D.B., Ahrné, K., Biesmeijer, K., Breeze, T.D., Carvell, C., Ferreira, C., FitzPatrick, Ú., Isaac, N.J.B., Kuussaari, M., Ljubomirov, T., Maes, J., Ngo, H., Pardo, A., Polce, C., Quaranta, M., Settele, J., Sorg, M., Stefanescu, C., Vujić, A., (2021) Proposal for an EU Pollinator Monitoring Scheme, EUR 30416 EN, Publications Office of the European Union, Ispra, ISBN 978-92-76-23859-1, doi:10.2760/881843, JRC122225. https://doi.org/doi/10.2760/881843

Potts, S.G., Imperatriz-Forseca, V., Ngo, H.T., Aizen, M.A., Biesmeijer, J.C., Breeze, T.D., Dicks, L.V., Garibaldi, L.A., Hill, R., Settele, J. & Vanbergen, A.J. (2016) Safeguarding pollinators and their values to human well-being. Nature, 540, 220-229.

Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EE https://eur-lex.europa.eu/eli/reg/2009/1107/oj

Rortais, A., Arnold, G., Halm, M.-P. & F. Touffet-Briens (2005): Modes of honeybees exposure tosystemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. - Apidologie 36: 71–83

Rundlöf, M., Stuligross, C., Lindh, A., Malfi, R. L., Burns, K., Mola, J. M., Cibotti, S., & Williams, N. M. (2022). Flower plantings support wild bee reproduction and may also mitigate pesticide exposure effects. Journal of Applied Ecology, 59, 2117-2127.

Rundlöf, M., von Post, M., Klatt, B., Andersson, G., Nicholson, C. & Herbertsson, L. 2024. Minska och spåra riskerna med växtskyddsmedelsanvändning för pollinerande insekter i jordbrukslandskap. Lunds universitet, Lund. https://jordbruksverket.se/download/18.3d62482418f74ddeba5ae52/1715848722667/Minska-och-spara-riskerna-med-vaxtskyddsmedelanvandning-for-pollinerande-insekter-i-jordbrukslandskap-tga.pdf

Sanchez-Bayo, F. & Goka, K. (2014) Pesticide Residues and Bees – A Risk Assessment. PLoS ONE, 9:e94482.

SANCO, 2002. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document, SANCO/10329/2002 rev 2 final, 17 October 2002).

Schulz, R., Bub, S., Petschick, L.L., Stehle, S. & Wolfram, J., 2021. Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops. Science 372: 81-84.

Siviter, H., Linguadoca, A., Ippolito, A., & Muth, F. (2023). Pesticide licensing in the EU and protecting pollinators. Current Biology, 33(2), R44-R48.

Spycher, S., Kalf, D., Lahr, J., Gönczi, M., Lindström, B., Pace, E., Botta, F., Bougon, N., Staub,P-F., Hitzfeld, KL., Weisner, O., Junghans, M. & Kroll, A. (2024) Linking chemical surface water monitoring and pesticide regulation in selected European countries . Environ Sci Pollut Res 31, 43432–43450 (2024). doi.org/10.1007/s11356-024-33865-y

Stenrød, M. et al. 2023. Plantevernmidler i etanol fra insektfeller (Malaisefeller) i semi-naturlig mark Relevans for å vurdere påvirkning av miljøgifter på insektfunn. NIBIO rapport, Ås.

Stoner, K.A., Eitzer, B.D., 2013. Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in Connecticut. PloS One 8, e77550. https://doi.org/10.1371/journal.pone.0077550.

Topping CJ, Bednarska AJ, Benfenati E, Chetcuti J, Delso N, Duan X, Focks A, Laskowski R, Lombardo A, Marcussen L, Metodiev T, Rubinigg M, Rundlöf M, Sgolastra F, Stoyanova C, Sušanj G, Williams J, Ziółkowska EM (2024) PollinERA: Understanding pesticide-Pollinator interactions to support EU Environmental Risk Assessment and policy. Research Ideas and Outcomes 10: e127485. https://doi.org/10.3897/rio.10.e127485

Topping, C. J., Aldrich, A. & Berny, P. 2020. Overhaul environmental risk assessment for pesticides. Science 367: 360–363.

Végh, R., Sörös, C., Majercsik, N., & Sipos, L. (2022). Determination of Pesticides in Bee Pollen: Validation of a Multiresidue High-Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry Method and Testing Pollen Samples of Selected Botanical Origin. Journal of Agricultural and Food Chemistry, 70(5), 1507-1515. https://doi.org/10.1021/acs.jafc.1c06864

Wagner, D. L., Grames, E. M., Forister, M. L., Berenbaum, M. R., & Stopak, D. (2021). Insect decline in the Anthropocene: Death by a thousand cuts. Proceedings of the National Academy of Sciences, 118(2), e2023989118.

Ward, L. T., Hladik, M. L., Guzman, A., Winsemius, S., Bautista, A., Kremen, C., & Mills, N. J. (2022). Pesticide exposure of wild bees and honey bees foraging from field border flowers in intensively managed agriculture areas. Science of The Total Environment, 831, 154697.

Warren, M. S., Maes, D., van Swaay, C. A., Goffart, P., Van Dyck, H., Bourn, N. A., Wynhoff, I., Hoare, D., & Ellis, S. (2021). The decline of butterflies in Europe: Problems, significance, and possible solutions. Proceedings of the National Academy of Sciences, 118(2), e2002551117.

Woodard, S.H. & Jha, S. (2017) Wild bee nutritional ecology: predicting pollinator population dynamics, movement, and services from floral resources. Current Opinion in Insect Science, 21:83-90.

Zioga, E., Kelly, R., White, B. & Stout, J.C. (2020) Plant protection product residues in plant pollen and nectar: a review of current knowledge. Environmental Research, 189:109873.

7. Appendices

Appendix 1. Analysed substances

Appendix 2. Detected substances and their toxicity values for honey bees

Appendix 3. Measured concentrations of all detected substances

Appendix 1 – Analysed substances

All substances determined in pollen samples using three different methods (LC-MS/MS with positive or negative ionization and GC-MS with negative chemical ionization). Substance types: F= fungicide, H=herbicide, I=insecticide, M= metabolite and PGR= plant growth regulator. (Some of the compounds have more than one use, the main use is listed). The method limits of detection (LOD) are based on spiking experiments of pollen at different concentration levels, findings in study samples as well as calibration sample responses. Sample weight is always based on wet weight (i.e. fresh weight, not dry weight).

Additional compounds that were tested but not included, since they did not work in any of the three multi methods were: 1,2,3,6-Tetrahydrophthalimide (THPI) (CAS No 85-40-5), amisulbrom (CAS No 348635-87-0), dithianon (CAS No 3347-22-6), ethofenprox (CAS No 80844-07-1), HCH (hexachlorocyclohexane) alpha, beta, delta, and gamma (CAS No 319-84-6, 319-85-7, 58-89-9 and 319-86-8, respectively) and pymetrozine (CAS No 123312-89-0).

	Substance name	Type	CAS No	Method	LOD (ng/g)
1	1-naphthylacetamide	PGR	86-86-2	LC-MS/MS pos	0.05
2	2,6-dichlorobenzamide (BAM)	M	2008-58-4	LC-MS/MS pos	2.5
3	2.4-D	H	94-75-7	LC-MS/MS neg	0.35
4	6-benzyladenine	PGR	1214-39-7	LC-MS/MS pos	0.05
5	acetamiprid	I	135410-20-7	LC-MS/MS pos	0.05
6	aclonifen	H	74070-46-5	GC-MS NCI	35
7	alachlor	H	15972-60-8	LC-MS/MS pos	2.5
8	alpha-cypermethrin	I	67375-30-8	GC-MS NCI	1.6
9	alpha-endosulfan	I	959-98-8	GC-MS NCI	3
10	amidosulfuron	H	120923-37-7	LC-MS/MS pos	5
11	atrazine	H	1912-24-9	LC-MS/MS pos	0.02
12	atrazine-desethyl	M	6190-65-4	LC-MS/MS pos	5
13	atrazine-desisopropyl	M	1007-28-9	LC-MS/MS pos	25
14	azoxystrobin	F	131860-33-8	LC-MS/MS pos	0.01
15	bentazone	H	25057-89-0	LC-MS/MS neg	0.2
16	benzovindiflupyr	F	1072957-71-1	LC-MS/MS pos	0.1
17	beta-endosulfan	I	33213-65-9	GC-MS NCI	0.6
18	bifenox-acid	M	53774-07-5	LC-MS/MS neg	2.5
19	bitertanol	F	55179-31-2	LC-MS/MS pos	5
20	bixafen	F	581809-46-3	LC-MS/MS pos	0.3
21	boscalid	F	188425-85-6	LC-MS/MS pos	1
22	bupirimate	F	41483-43-6	LC-MS/MS pos	2.5
23	captan	F	133-06-2	GC-MS NCI	3
24	carbendazim	F	10605-21-7	LC-MS/MS pos	0.1
25	carfentrazone	H	128621-72-7	LC-MS/MS neg	25
26	carfentrazone-ethyl	H	128639-02-1	LC-MS/MS pos	1
27	chlorantraniliprole	I	500008-45-7	LC-MS/MS pos	5

28	chloridazon	H	1698-60-8	LC-MS/MS pos	2
29	chlorpyrifos	I	2921-88-2	GC-MS NCI	0.44
30	cinnerin I	I	25402-06-6	LC-MS/MS pos	5
31	cinnerin II	I	121-20-0	LC-MS/MS pos	5
32	clethodim	H	99129-21-2	LC-MS/MS pos	2.5
33	clomazone	H	81777-89-1	LC-MS/MS pos	1.5
34	clopyralid	H	1702-17-6	LC-MS/MS neg	0.5
35	clothianidin	I	210880-92-5	LC-MS/MS pos	0.5
36	cyantraniliprole	I	736994-63-1	LC-MS/MS pos	2.5
37	cyazofamid	F	120116-88-3	LC-MS/MS pos	5
38	cycloxidim	H	101205-02-1	LC-MS/MS pos	25
39	cyflufenamid	F	180409-60-3	LC-MS/MS pos	5
40	cyfluthrin	I	68359-37-5	GC-MS NCI	0.2
41	cymoxanil	F	57966-95-7	LC-MS/MS pos	2.5
42	cyprodinil	F	121552-61-2	LC-MS/MS pos	2.5
43	deltamethrin	I	52918-63-5	GC-MS NCI	6
44	dichlorprop	H	120-36-5	LC-MS/MS neg	2.5
45	difenoconazole	F	119446-68-3	LC-MS/MS pos	0.1
46	diflufenican	H	83164-33-4	LC-MS/MS pos	0.4
47	dimethenamid-p	H	163515-14-8	LC-MS/MS pos	0.1
48	dimethoate	I	60-51-5	LC-MS/MS pos	0.5
49	dimethomorph	F	110488-70-5	LC-MS/MS pos	0.05
50	diuron	H	330-54-1	LC-MS/MS pos	0.5
51	dodine	F	2439-10-3	LC-MS/MS pos	300
52	endosulfan sulfate	M	1031-07-8	GC-MS NCI	0.6
53	epoxiconazole	F	135319-73-2	LC-MS/MS pos	25
54	esfenvalerate	I	66230-04-4	GC-MS NCI	0.9
55	ethofumesate	H	26225-79-6	LC-MS/MS pos	5
56	fenbuconazole	F	114369-43-6	LC-MS/MS pos	12.5
57	fenpropidin	F	67306-00-7	LC-MS/MS pos	0.4
58	fenpropimorph	F	67564-91-4	LC-MS/MS pos	0.25
59	fenpyrazamine	F	473798-59-3	LC-MS/MS pos	0.25
60	flonicamid	I	158062-67-0	LC-MS/MS neg	15
61	florasulam	H	145701-23-1	LC-MS/MS pos	0.25
62	fluazinam	F	79622-59-6	LC-MS/MS neg	0.01
63	fludioxonil	F	131341-86-1	LC-MS/MS pos	5
64	flufenacet	H	142459-58-3	LC-MS/MS pos	0.05
65	fluopicolide	F	239110-15-7	LC-MS/MS pos	5
66	fluopyram	F	658066-35-4	LC-MS/MS pos	0.1
67	fluoxastrobin	F	361377-29-9	LC-MS/MS pos	0.5
68	flupyradifurone	I	951659-40-8	LC-MS/MS pos	1.25
69	flupyrsulfuron-methyl-sodium	H	144740-54-5	LC-MS/MS pos	0.05
70	fluroxipyr	H	69377-81-7	LC-MS/MS neg	2.5
71	flurtamone	H	96525-23-4	LC-MS/MS pos	0.05

72	fluxapyroxad	F	907204-31-3	LC-MS/MS pos	0.1
73	foramsulfuron	H	173159-57-4	LC-MS/MS pos	0.3
74	gamma-cyhalothrin	I	76703-62-3	GC-MS NCI	0.6
75	halauxifen-methyl	H	943831-98-9	LC-MS/MS pos	0.05
76	hexazinone	H	51235-04-2	LC-MS/MS pos	0.05
77	hexythiazox	I	78587-05-0	LC-MS/MS pos	4
78	imazalil	F	35554-44-0	LC-MS/MS pos	2.5
79	imazamox	H	114311-32-9	LC-MS/MS neg	12.5
80	imidacloprid	I	138261-41-3	LC-MS/MS pos	0.25
81	indoxacarb	I	173584-44-6	LC-MS/MS pos	1
82	iodosulfuron-methyl-sodium	H	144550-36-7	LC-MS/MS pos	5
83	ipconazole	F	125225-28-7	LC-MS/MS pos	0.5
84	iprodione	F	36734-19-7	GC-MS NCI	15
85	isofetamid	F	875915-78-9	LC-MS/MS pos	1
86	isoproturon	H	34123-59-6	LC-MS/MS pos	0.05
87	isopyrazam	F	881685-58-1	LC-MS/MS pos	0.05
88	jasmolin I	I	4466-14-2	LC-MS/MS pos	10
89	jasmolin II	I	1172-63-0	LC-MS/MS pos	5
90	kresoxim-methyl	F	143390-89-0	LC-MS/MS pos	0.5
91	linuron	H	330-55-2	LC-MS/MS pos	5
92	malathion	I	121-75-5	LC-MS/MS pos	0.25
93	mandipropamid	F	374726-62-2	LC-MS/MS pos	0.05
94	MCPA	H	94-74-6	LC-MS/MS neg	0.3
95	mecoprop	H	7085-19-0	LC-MS/MS neg	20
96	mefentrifluconazole	F	1417782-03-6	LC-MS/MS pos	5
97	mepanipyrim	F	110235-47-7	LC-MS/MS pos	1.25
98	mesosulfuron-methyl	H	208465-21-8	LC-MS/MS neg	5
99	mesotrione	H	104206-82-8	LC-MS/MS pos	25
100	metaflumizone	I	139968-49-3	LC-MS/MS pos	12.5
101	metalaxyl	F	57837-19-1	LC-MS/MS pos	0.05
102	metamitron	H	41394-05-2	LC-MS/MS pos	0.8
103	metazachlor	H	67129-08-2	LC-MS/MS pos	0.04
104	metconazole	F	125116-23-6	LC-MS/MS pos	1.5
105	methabenzthiazuron	H	18691-97-9	LC-MS/MS pos	0.05
106	methiocarb	I	2023-65-7	LC-MS/MS pos	5
107	methoxyfenozide	I	161050-58-4	LC-MS/MS pos	0.5
108	metobromuron	H	3060-89-7	LC-MS/MS pos	0.4
109	metolachlor	H	51218-45-2	LC-MS/MS pos	5
110	metrafenone	F	220899-03-6	LC-MS/MS pos	5
111	metribuzin	H	21087-64-9	LC-MS/MS pos	0.25
112	metsulfuron-methyl	H	74223-64-6	LC-MS/MS pos	0.5
113	myclobutanil	F	88671-89-0	LC-MS/MS pos	12.5
114	napropamide	H	15299-99-7	LC-MS/MS pos	0.5
115	oxathiapiprolin	F	1003318-67-9	LC-MS/MS pos	5

116	penconazole	F	66246-88-6	LC-MS/MS pos	0.25
117	pendimethalin	H	40487-42-1	LC-MS/MS pos	25
118	penthiopyrad	F	183675-82-3	LC-MS/MS neg	2.5
119	permethrin	I	52645-53-1	GC-MS NCI	15
120	phenmedipham	H	13684-63-4	LC-MS/MS pos	0.1
121	phosmet	I	732-11-6	LC-MS/MS pos	1.25
122	picloram	H	1918-02-1	LC-MS/MS neg	125
123	picolinafen	H	137641-05-5	LC-MS/MS pos	2.5
124	picoxystrobin	F	117428-22-5	LC-MS/MS pos	0.05
125	pinoxaden	H	243973-20-8	LC-MS/MS pos	0.01
126	pirimicarb	I	23103-98-2	LC-MS/MS pos	0.05
127	prochloraz	F	67747-09-5	LC-MS/MS pos	0.05
128	propamocarb	F	24579-73-5	LC-MS/MS pos	5
129	propaquizafop	H	111479-05-1	LC-MS/MS pos	0.25
130	propiconazole	F	60207-90-1	LC-MS/MS pos	25
131	propoxicarbazone-sodium	H	181274-15-7	LC-MS/MS neg	2.5
132	propyzamide	H	23950-58-5	LC-MS/MS pos	0.25
133	proquinazid	F	189278-12-4	LC-MS/MS pos	2
134	prosulfocarb	H	52888-80-9	LC-MS/MS pos	0.3
135	prothioconazole-desthio	M	120983-64-4	LC-MS/MS pos	1.5
136	pyraclostrobin	F	175013-18-0	LC-MS/MS pos	0.1
137	pyraflufen-ehtyl	H	129630-19-9	LC-MS/MS pos	1
138	pyrethrin I	I	121-21-1	LC-MS/MS pos	160
139	pyrethrin II	I	121-29-9	LC-MS/MS pos	6
140	pyrimethanil	F	53112-28-0	LC-MS/MS pos	1
141	pyriofenone	F	688046-61-9	LC-MS/MS pos	0.5
142	pyroxsulam	H	422556-08-9	LC-MS/MS pos	0.5
143	quinmerac	H	90717-03-6	LC-MS/MS pos	0.5
144	quizalofop	H	76578-12-6	LC-MS/MS neg	0.5
145	rimsulfuron	H	122931-48-0	LC-MS/MS pos	0.05
146	sedaxane	F	874967-67-6	LC-MS/MS pos	0.5
147	silthiofam	F	175217-20-6	LC-MS/MS pos	0.5
148	simazine	H	122-34-9	LC-MS/MS pos	0.5
149	spinosad	I	168316-95-8	LC-MS/MS pos	0.4
150	spirotetramat	I	203313-25-1	LC-MS/MS pos	0.1
151	spiroxamine	F	118134-30-8	LC-MS/MS pos	0.05
152	sulfosulfuron	H	141776-32-1	LC-MS/MS pos	0.5
153	tau-fluvalinate	I	102851-06-9	GC-MS NCI	1
154	tebuconazole	F	107534-96-3	LC-MS/MS pos	2.5
155	tefluthrin	I	79538-32-2	GC-MS NCI	3
156	terbuthylazin	H	5915-41-3	LC-MS/MS pos	0.4
157	terbuthylazin-desetyl	M	30125-63-4	LC-MS/MS pos	0.2
158	terbutryn	H	886-50-0	LC-MS/MS pos	2.5
159	tetraconazole	F	112281-77-3	LC-MS/MS pos	25

160	thiacloprid	I	111988-49-9	LC-MS/MS pos	0.05
161	thiamethoxam	I	153719-23-4	LC-MS/MS pos	0.05
162	thiencarbazone-methyl	H	317815-83-1	LC-MS/MS pos	1.25
163	thifensulfuron-methyl	H	79277-27-3	LC-MS/MS pos	0.5
164	thiophanate-methyl	F	23564-05-8	LC-MS/MS pos	5
165	tri-allat	H	2303-17-5	LC-MS/MS pos	25
166	tribenuron-methyl	H	101200-48-0	LC-MS/MS pos	0.1
167	trifloxystrobin	F	141517-21-7	LC-MS/MS pos	0.5
168	trifloxystrobin-acid	M	252913-85-2	LC-MS/MS pos	0.25
169	triflusulfuron-methyl	H	126535-15-7	LC-MS/MS pos	3
170	trinexapac-acid	M	104273-73-6	LC-MS/MS neg	125
171	trinexapac-ethyl	PGR	95266-40-3	LC-MS/MS pos	5
172	triticonazole	F	131983-72-7	LC-MS/MS pos	5
173	tritosulfuron	H	142469-14-5	LC-MS/MS neg	2.52

Appendix 2 - Detected substances and their toxicity values for honey bees

Detected substances in the pollen samples, including information on the type of substance, main use (F= fungicide, H=herbicide, I=insecticide, M= metabolite), number of detections (max = 23), maximum concentrations (ng per gram pollen) and LD_{50} for honey bees (µg/bee) averaged over acute contact and oral testing (indicating determined values by = and limit testing by >).

Substance	Type (main)	Detects	Max conc.	LD_{50} mean	LD_{50} contact	LD_{50} contact type	LD_{50} oral	LD_{50} oral type
2.4-D	H	8	1.6	97	100	>	94	=
acetamiprid	I	22	2000	11.31	8.09	=	14.53	=
atrazine	H	3	0.13	100	100	>	100	>
azoxystrobin	F	21	6000	112.5	200	>	25	>
bentazone	H	4	0.34	200	200	>	200	>
benzovindiflupyr	F	1	0.16	104.5	100	>	109	>
bixafen	F	5	2.9	110.7	121.4	>	100	>
boscalid	F	9	11000	183	200	>	166	>
captan	F	16	8500	150	200	>	100	>
carbendazim	F	3	0.66	75	50	>	100	>
chloridazon	H	1	2.1	200	200	>	200	>
clomazone	H	3	5.2	82.915	89.5	>	76.33	>
clopyralid	H	7	4.7	99.05	98.1	>	100	>
cyprodinil	F	5	910	93.75	75	>	112.5	=
difenoconazole	F	2	3.7	138.5	100	>	177	>
diflufenican	H	1	0.41	103.7	100	>	107.4	>
dodine	F	1	390	172.5	145	=	200	>
ethofumesate	H	5	18	50	50	>	50	>
fenpropidin	F	1	0.5	28	46	=	10	>
flonicamid	I	4	330	80.25	100	>	60.5	>
fluazinam	F	1	0.02	150	200	>	100	>
fludioxonil	F	5	230	100	100	>	100	>
fluopyram	F	19	3600	101.15	100	>	102.3	>
fluroxipyr	H	1	2.6	108.55	180	>	37.1	=
fluxapyroxad	F	13	7.5	105.45	100	>	110.9	>
foramsulfuron	H	1	0.38	105.05	100	>	110.1	>
hexythiazox	I	2	26	156	200	>	112	>
imidacloprid	I	3	0.93	0.04235	0.081	=	0.0037	=
indoxacarb	I	5	270	0.156	0.08	=	0.232	=
isofetamid	F	1	5.2	65	100	>	30	>
isoproturon	H	1	0.06	197.5	200	=	195	=
kresoxim-methyl	F	9	450	105	100	>	110	>
MCPA	H	19	110	200	200	>	200	>
mecoprop	H	2	120	100	100	>	100	>
mefentrifluconazole	F	3	500	100	100	>	100	>
metamitron	H	14	340	98.6	100	>	97.2	>
metazachlor	H	2	0.22	86.1	100	>	72.2	>
metconazole	F	8	5	92.5	100	>	85	=

metobromuron	H	2	2.1	159.55	200	>	119.1	=
penconazole	F	6	13	7.1	3	>	11.2	>
phenmedipham	H	15	170	102.4	100	>	104.8	>
prochloraz	F	3	0.82	121.15	141.3	=	101	>
propyzamide	H	7	8.6	118	136	>	100	>
proquinazid	F	2	4.1	161	197	>	125	>
prosulfocarb	H	12	11	91.7	80	>	103.4	=
prothioconazole-desthio	M (F)	16	150	103.25	100	>	106.5	>
pyraclostrobin	F	13	24	105	100	>	110	>
pyrimethanil	F	15	1700	100	100	>	100	>
quizalofop	H	1	0.52	100	100	>	100	>
spirotetramat	I	6	16	103.65	100	>	107.3	>
spiroxamine	F	2	0.41	52.1	4.2	=	100	>
tau-fluvalinate	I	3	13	12.3	12	=	12.6	=
tebuconazole	F	11	32	141.525	200	>	83.05	>
terbuthylazin	H	6	1	27.3	32	>	22.6	>
terbuthylazin-desetyl	M (H)	5	1.6					
thiacloprid	I	7	22	28.07	38.82	=	17.32	=
tribenuron-methyl	H	8	0.26	53.75	98.4	>	9.1	>
triflusulfuron-methyl	H	1	3.3	100	100	>	100	>

Appendix 3 – Measured concentrations of all detected substances

Concentration values (ng per gram pollen) of all substances detected at least once at the six different sampling sites (LT1, NO1, SE1, SE2, SE3 and SE4) across the four sampling rounds.

Substance	LT1:1	LT1:2	LT1:3	LT1:4	NO1:1	NO1:2	NO1:3	SE1:1	SE1:2	SE1:3	SE1:4
2.4-D	0.53		1.1	0.42				0.36		1.4	
acetamiprid	19	3.4	2000	70	0.9	13		470	16	34	5.6
atrazine				0.13					0.03		
azoxystrobin	1.5	6.2	6000	110		0.47		0.57	0.85	9.2	3.52
benzovindiflupyr								0.16			
bixafen								1.1	1.4		0.32
boscalid			11 000	1 200		1.4		39	7.6	37	2.5
captan	1100	800	200					8.4	35		
carbendazim	0.66										
clopyralid	3.8		1.1							1.4	
cyprodinil			2.8		95	2.9	4.1			910	
difenoconazole	3.7	3.1									
dodine								390			
flonicamid											22
fluazinam									0.02		
fludioxonil					42	6.6	5.5			230	16
fluopyram					0.21	0.15	0.17	6.2	6.2	12	5.1
fluroxipyr										2.6	
fluxapyroxad	0.2	0.12						0.48	2.9		0.38
hexythiazox								26	4.7		
imidacloprid	0.93		0.57	0.28							
indoxacarb										1.5	
isoproturon											0.06
kresoxim-methyl								450	7.1		3.4
MCPA	0.34				4.9	110	9.3	1.3	7	60	4.3
mecoprop						120	22				
metamitron	1			1				10	25	10	1

metazachlor		0.05	0.22								
penconazole						0.34	4.6				
phenmedipham						0.13		2.5	5.4	6.6	0.75
prochloraz	0.09		0.18					0.82			
propyzamide									0.25	1.1	
proquinazid					4.1	3.7					
prosulfocarb			1.9						1.2	0.7	2.9
prothioconazole-desthio	4.4	2.8	1.6					2	13		2.7
pyraclostrobin		0.43	0.12					24	3.4	23	0.74
pyrimethanil	19	14			1 700	380	11	1 300	8.9	12	27
quizalofop	0.52										
spirotetramat						0.11	0.8	0.5	0.15		
spiroxamine		0.41	0.09								
tau-fluvalinate				13						1.1	
tebuconazole			2.9	32				4	18	26	
terbuthylazin				0.9					0.7		
terbuthylazin-desetyl				1.5							
thiacloprid			0.12	0.07							
tribenuron-methyl	0.1	0.11	0.18	0.12					0.12	0.19	

Substance	SE2:1	SE2:2	SE2:3	SE2:4	SE3:1	SE3:2	SE:3	SE3:4	SE4:1	SE4:2	SE4:3	SE4:4
2.4-D			0.8	1.6	0.49							
acetamiprid	3.6	5.5	8	8	1400	200	31	80	15	130	160	17
atrazine		0.06										
azoxystrobin	1.4	1	0.48	0.15	0.56	6.6	2	4.8	13	2	0.84	0.86
bentazone			0.32	0.34		0.22	0.24					
bixafen			2.9	0.36								
boscalid								3.3	13			
captan	59	83		15	82	12	32	5.5	7900	8500	1100	23
carbendazim						0.63				0.37		
chloridazon						2.1						
clomazone					1.9	2.5		5.2				
clopyralid			4.7	1.3	0.68	0.69						
diflufenican			0.41									
ethofumesate			6.8		10	18	7	8.6				
fenpropidin										0.5		
flonicamid								190	330	18		
fluopyram	10	5.5	34	60	3600	160	9	26	27	3.2	0.51	1.3
fluxapyroxad		0.81	0.63	0.59			0.54	7.5		0.13	0.41	0.22
foramsulfuron									0.38			
indoxacarb									220	270	38	1.2
isofetamid						5.2						
kresoxim-methyl		0.65	1						15	1.3	2	21
MCPA	4.4	2.4	83	10	40	7.2	3.1	32	2.4	0.86	1.2	
mefentrifluconazole								12			500	6.3
metamitron	60	60	19	2.3	22	340	1.7	3.9				
metconazole				4.9	2	3.5		5	3	1.7	2.1	2.6
metobromuron							2.1	0.41				
penconazole									3	1.6	9.5	13
phenmedipham	16	13	7.3	4	11	170	1.2	3.1		1.2		0.4
propyzamide	0.53								8.6	0.9	0.56	1.6
prosulfocarb	0.86	2.5	11	0.37	5.2		1.2				0.63	0.28

prothioconazole-desthio	2.2		26	3.8	150	12	12	6.8		3.6	4.2	2.4
pyraclostrobin	0.4	1.1		0.26	0.41	0.68		0.57	1.4			
pyrimethanil		1.3			1.2				170	13	18	60
spirotetramat											16	0.17
tau-fluvalinate									3.3			
tebuconazole						11		16	31	17	5.7	6.2
terbuthylazin		0.42	1				0.9				0.7	
terbuthylazin-desetyl				0.24			1.6			0.47	1.5	
thiacloprid					0.17	8.4	4.3	22				1.7
tribenuron-methyl		0.23	0.26									
triflusulfuron-methyl						3.3						