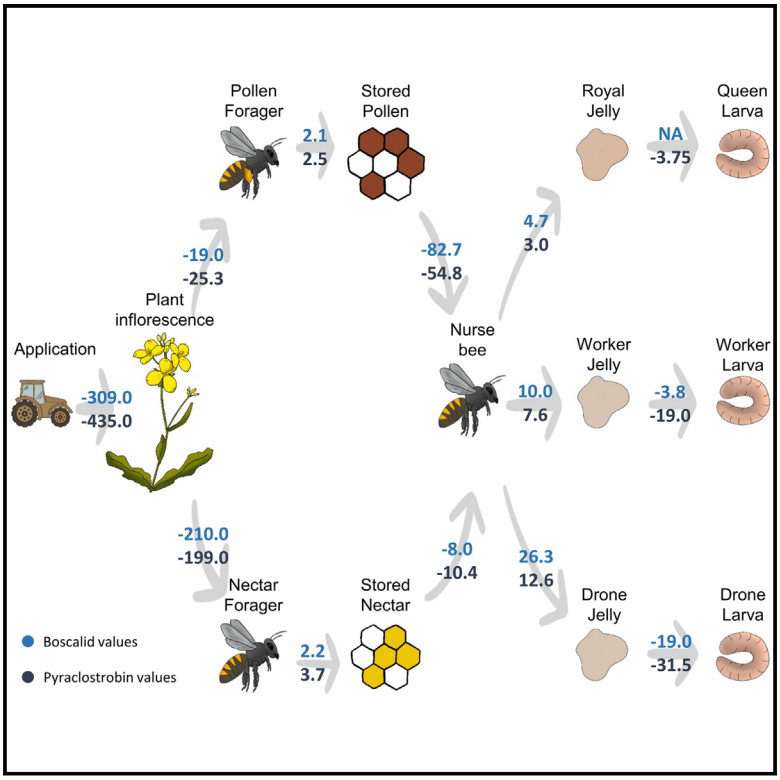
Report

Current Biology

Nurse honey bees filter fungicide residues to maintain larval health

Graphical abstract



Highlights

- Residues were analyzed along the entire transfer pathway from field to larvae
- Residue concentrations decrease along the transfer pathway
- Risk quotients for adult bees and larvae were below threshold values
- Nurse honey bees act as a filter in the transfer of contaminants to larvae

Authors

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In brief

Wueppenhorst et al. investigate the full transfer pathway of two fungicides, from in-field application to honey bee larval food jelly and the larvae. Residues were diluted along the pathway and, based on residue levels, low risks are suggested for adult bees and larvae, indicating a filtering function of the honey bee colony.

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Report

Nurse honey bees filter fungicide residues to maintain larval health

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SUMMARY

Residues of plant protection products (PPPs) are frequently detected in bee matrices[1–6] due to foraging bees collecting contaminated nectar and pollen, which they bring back to their hive. The collected material is further used by nurse bees to produce glandular secretions for feeding their larvae.[7] Potential exposure to PPPs occurs through direct oral ingestion, contact during foraging, or interaction with contaminated hive material.[8,9] Contaminants can pose health risks to adult worker bees,[10,11] queens,[12,13] drones (males),[14] or larvae,[15,16] potentially impacting colony health and productivity. However, residue concentrations can vary significantly between analyzed matrices, and potential accumulation or dilution steps have not been widely investigated. Although research has provided valuable insights into contamination risks, there remain gaps in our understanding of the entire pathway from field, via foragers, stored products, nurse bees, and finally to food jelly, i.e., royal, worker, and drone jelly, and the larvae, including all possible processing steps.[17] We collected samples of bee-relevant matrices following the in-field spray application of the product Pictor Active, containing the fungicides boscalid and pyraclostrobin. The samples were analyzed for residues along this entire pathway. Fungicide residues were reduced by a factor of 8–80 from stored product to nurse bees' heads, suggesting a filtering function of nurse bees. Furthermore, detected residues in larval food jelly resulted from added pollen and not from nurse bee secretions. Calculated risk quotients were at least twice as low as the threshold values, suggesting a low risk to honey bee colonies from these fungicides at the tested application rate.

RESULTS

In 159 of 241 samples from bee colonies placed at the edge of oilseed rape (OSR) fields treated with the fungicide formulation (Pictor Active), residues of the applied active substances were detected in concentrations of between 6×10^{-5} and 34.72 mg/kg. Boscalid and pyraclostrobin residues were detected in comparable concentrations in the same order of magnitude in each matrix. Samples from the untreated control sites showed positive trace values of, on average, 0.032 mg/kg (SD = 0.14 mg/kg) for at least one of the tested active substances in only 30% of 243 samples (Data S1A). Further, the plant inflorescence samples of the untreated control sites were tested for 288 active substances; the results are shown in Data S1B. Almost all samples were free of residues prior to application, with only four exceptions showing trace-level contamination (Figures 1 and 2). However, trace-level contamination is expected when conducting open-field experiments.[2]

Over time, residue concentrations of plant inflorescences, pollen baskets, and honey sacs decreased, with a negative logarithmic trend for both substances (Figures 1A and 1B). The derived dissipation time (DT_{50}) for boscalid or pyraclostrobin was 2.12 or 1.86 days for plant inflorescences, 0.93 or 1.11 days for pollen baskets, and 0.79 or 0.73 days for honey sacs. A decrease over time, albeit not statistically significant (linear mixed model [LMM]: $p > 0.05$), in residues was also detectable in stored pollen and nectar for both substances (Figure 1C). Concentrations in stored matrices, of approximately 0.2 mg/kg in pollen and

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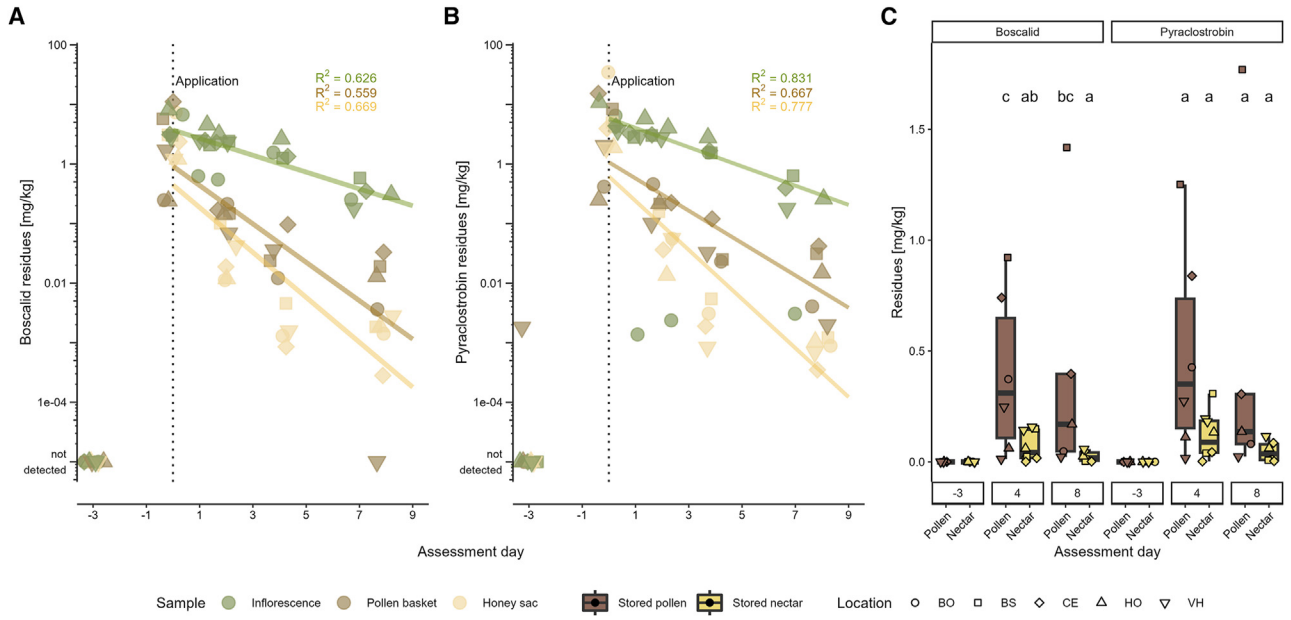

Figure 1. Fungicide residues detected in plant-related material and stored products

(A and B) The measured concentrations of active substances boscalid (A) and pyraclostrobin (B) in plant inflorescences (OSR), pollen baskets, and honey sacs from fungicide treatment sites with fitted logarithmic regression line (LMM: residue concentration ~ assessment day + sample type). Detected residues were plotted on a logarithmic scale (A and B).

(C) Residues of both substances detected in stored pollen and nectar. Boxes represent the first and third quartiles of data and the median, whiskers extend the range by values with a maximum of the 1.5-fold interquartile range. Different letters indicate significant differences in residue concentration between analyzed matrices (LMM: residue concentration ~ assessment day + sample type, $\alpha = 0.05$). Samples were collected in a field experiment at five different locations in Germany. Data points represent the measured concentration. BO, Bochum; BS, Braunschweig; CE, Celle; HO, Hohenheim; VH, Veitshöchheim.

See also Data S1 and Figure S1.

0.04 mg/kg in nectar, were in the same order of magnitude as the related plant-derived material (Figures 1 and 3; Data S1A). The concentrations in nurse bee heads, larval food jelly (Figure 2A), and the larvae remained constantly low, with a concentration of, on average, 0.029 mg/kg for boscalid and 0.028 mg/kg for pyraclostrobin (Figure 2B). Eight days after in-field exposure, the detectable residue concentrations were all below 1.8 mg/kg for both active substances (Figures 1 and 2). Significantly higher residue concentrations were only detectable in stored pollen, compared with stored nectar for boscalid (LMM: $p < 0.05$), on both assessment days after exposure (Figure 1C).

In contrast, residue concentrations in larval food jellies increased significantly—from the heads of nurse bees and royal jelly to worker food jelly and, finally, to drone food jelly. However, the detected concentrations were all below 0.3 mg/kg for both active substances (Figure 2A). Larvae of all castes and sexes showed the lowest residue concentrations: a maximum of 0.04 mg/kg for both active substances (Figure 2B). Further, the calculated risk quotient (RQ) for bees and larvae was at least 2 times lower than the threshold level of concern of acute exposure (threshold = 0.4) for pyraclostrobin and at least 15 times lower for boscalid. For chronic exposure (threshold = 1), larval RQ values were all at least 2 times lower as the threshold, with only two exceptions where pyraclostrobin RQ values were equal to the threshold values (Data S1C).

Samples of bee bread, honey, in-hive bees, and wax collected from treatment sites in autumn showed only trace-level residue concentrations, with a median concentration of 0.009 mg/kg for boscalid and 0.014 mg/kg for pyraclostrobin (Figure S1). In spring, bee bread, honey, and in-hive bees were collected and residues were only detected in 8 out of 15 samples, with a maximum concentration of 0.009 mg/kg for both substances. At the control sites, only 35% of the autumn samples showed detectable trace-level residues, with a median concentration of 0.003 mg/kg for both substances, and in spring 2023, all samples were absent of any residues, except of one sample with a trace-level concentration of 0.001 mg/kg (Figure S1).

The transfer factor of the residues was highest from applied solution to plant inflorescence, with a reduction of the residue concentration by a factor of 309–435, followed by the transfer from plants to nectar foragers by a factor of 200 (Figure 3). The storage of pollen and nectar led to an increase in concentration by a factor of 2 to 4 from foragers to the stored material, but concentrations found in the heads of nurse bees were again reduced by a factor of 8 to 80 from stored material to nurses. The median concentrations in larval food jelly ranged from 0.014 to 0.0079 mg/kg for both substances, and only traces of residues were transferred to larvae.

DISCUSSION

Pesticide residues are often found in various concentrations within bee-related matrices, but the transfer pathway from plants to the hive remains unclear. The role of processing factors like

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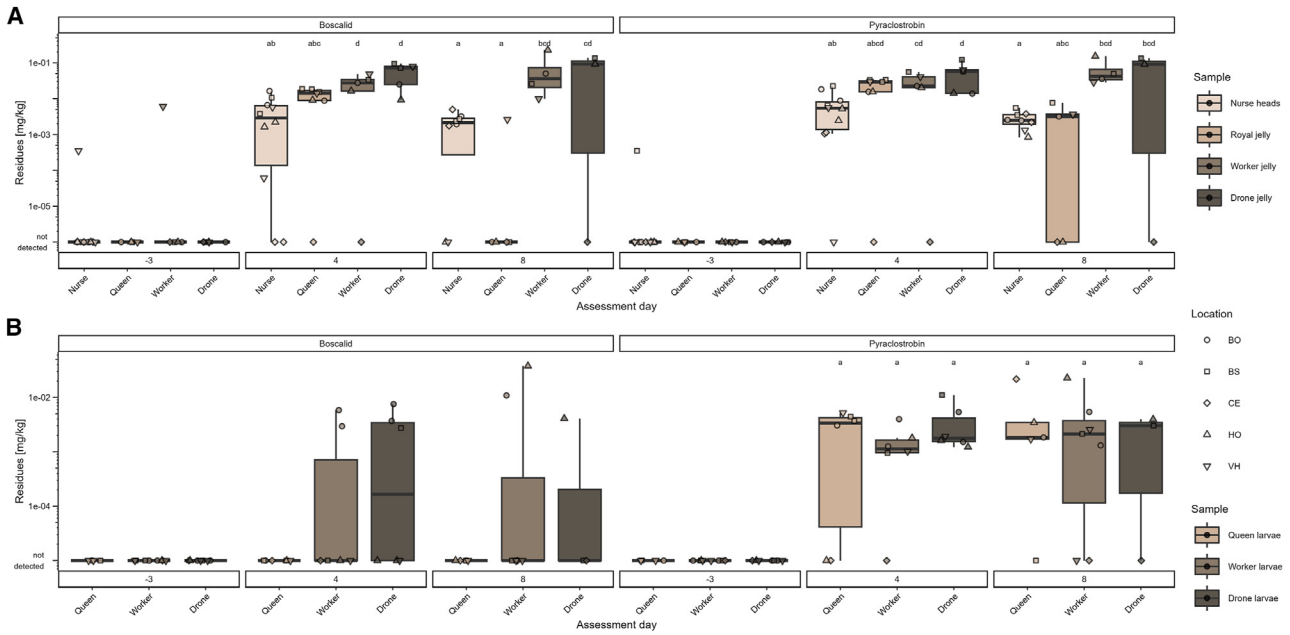

Figure 2. Fungicide residues detected in nurse bee heads and larval food jelly and larvae

(A) Nurse bee heads and larval food jelly. (B) Larvae. Figures show the measured concentrations of active substances boscalid and pyraclostrobin over time, collected from fungicide treatment sites. Detected residues were plotted on a logarithmic scale. Data points represent measured concentrations and boxes represent the first and third quartiles of data and the median, whiskers extend the range by values with a maximum of the 1.5-fold interquartile range. Different letters indicate significant differences in residue concentration between analyzed matrices (LMM: residue concentration ~ assessment day + sample type, $\alpha = 0.05$). BO, Bochum; BS, Braunschweig; CE, Celle; HO, Hohenheim; VH, Veitshöchheim.
See also Data S1 and Figure S1.

accumulation or dilution is not fully understood. In our field-realistic experiment, we collected samples along the entire transfer pathway and analyzed them for residues of boscalid and pyraclostrobin from the fungicide formulation Pictor Active.

Our findings provide critical insights into this transfer process. The residue content was reduced by a factor of 9.8 from stored pollen or 1.5 from nectar to larval food (Figures 1, 2, and 3), indicating that nurse bees may act as a filtering step in the following residue transfer from stored products to the larvae.[1,18,19] During digestion of consumed stored products, the antioxidative and immune system of nurse bees induces different enzymatic response mechanisms to metabolize and evacuate hazardous substances, such as plant protection products (PPPs).[20,21] Additionally, some PPPs may be excreted without passing through the digestive system and entering the hemolymph.[21] Within the body tissues, the accumulation of PPPs (and their modifications) will be partly determined by solubility, i.e., we therefore predict that lipophilic substances will accumulate in fat body rather than gland tissue. This could minimize contaminant concentrations when gland secretions are produced in the head glands of bees. Furthermore, nurse bees exchange food with each other, drones, and the queen in a process called trophallaxis, and contaminants can be transferred—but also diluted—through every mouth-to-mouth contact.[9] Even if residues are not directly transferred to larvae, indirect effects should be considered, as residues might negatively impact worker bees. Sub-lethal effects on workers may result in behavioral or physiological alterations, which could lead to a reduction in foraging activity or brood care.[22]

Previous studies support the hypothesis of a filtering function of nurse bees in reporting no or limited transfer of boscalid and pyraclostrobin residues from nurse bee to royal jelly in an artificial feeding scenario.[18,23] Nonetheless, a literature review showed that low concentrations of pesticides are transferred into royal jelly.[6] Only Böhme et al.[24] focused additionally on worker jelly and detected boscalid and pyraclostrobin in concentrations of up to 0.038 or 0.048 mg/kg in an artificial pollen-feeding scenario. The concentrations in worker jelly we detected are in the same order of magnitude (Figure 2A).

Furthermore, the lowest concentration of residues was detected in larvae, with a reduction factor from jelly to larvae of 3.8 to 31.5 (Figure 3). Honey bee larvae do not excrete during the larval stage of development until they pupate,[7] indicating that PPP residues in larval food jelly are not concentrated in larvae by ingestion. Consequently, our study showed that, under field-realistic conditions, the transfer from plant inflorescence to worker food would be approximately 1.3%, from collected pollen at around 29.9%, and from stored pollen up to 13%. The processing of collected material, which is realized by forager and in-hive bees, must be included in transfer calculations. Additionally, the improvement of colony strength, including many forager and nurse bees, may hence support bees' filtering function. Thus, it is to be expected that the more individuals that are present in a colony during application of plant protection products, the more efficiently they can filter and may buffer the impact of pesticide exposure. In contrast to social bee species, which could buffer the lethal or sub-lethal impacts of contaminants on an individual level by their colony strength, solitary bee

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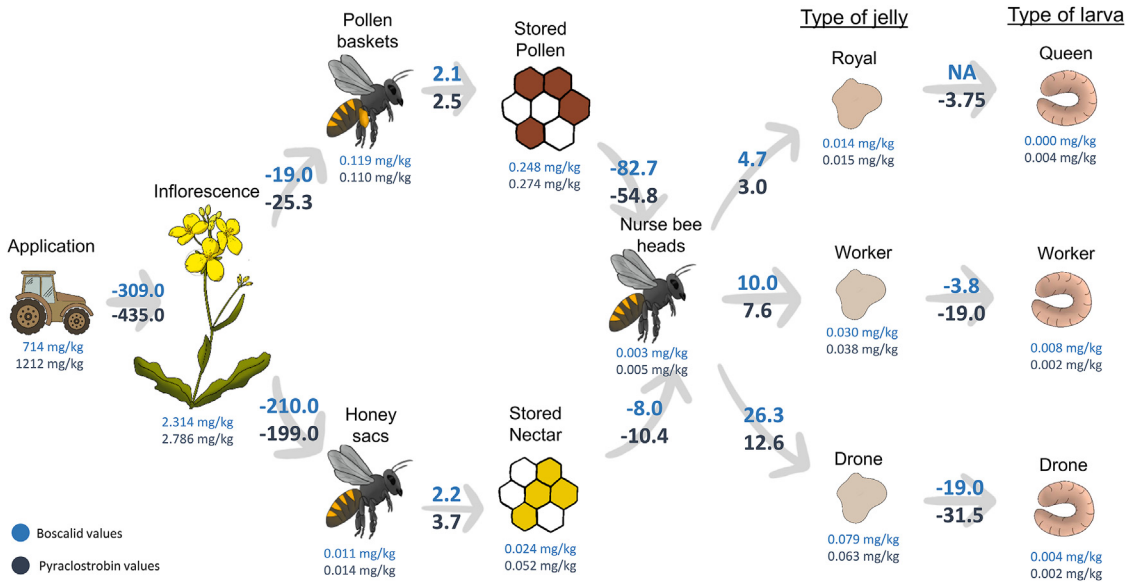

Figure 3. Transfer pathway of fungicide residues from in-field application to honey bee larvae

The figure shows the transfer factors from an analyzed matrix to another. Concentrations given below the icons are median concentrations measured among all time points, reflecting expected concentration levels independent of time. Values on the arrows are the transfer factors calculated from one stage to the next along the transfer pathway. Positive values represent a concentration factor, negative values a dilution factor. Light blue, boscalid; dark blue, pyraclostrobin. See also Data S1.

species may be more susceptible to these contaminants. If a solitary bee is poisoned by contaminants and dies, this will directly impact their offspring as there is no buffering capacity.[21] However, further research is still needed to compare exposure levels between different bee species in this context.

The filtering effect of nurse bees during food jelly production is further supported when comparing the results of the different food jellies. At both assessment days after exposure, worker and drone jellies contained significantly higher boscalid and pyraclostrobin concentrations than nurse bees' heads and, partly, than royal jelly (Figure 2A). Larval food jelly is a mixture of glandular secretions from the bees' heads, containing proteins and lipids.[25] In comparison with queen jelly, which consists only of these glandular secretions, worker and drone jellies contain additional pollen at later stages of their development.[26,27] Additional pollen in worker jelly correlates positively with higher PPP residue concentrations[24] (Figure 2A). This correlation is presumably related to the beneficial lipophilic character of pollen.[24] Boscalid and pyraclostrobin have a water solubility of 4.6 or 1.9 mg/L and a water partition coefficient of 2.96 or 3.99, indicating their low solubility and limited dissolution in water.[28] On the other hand, pollen has a high lipid content, including non-polar lipids.[29] The lipophilic character of pollen is hence beneficial for the solubility and accumulation of lipophilic fungicides. Nonetheless, higher exposure could also result from the fact that the stamens, and thus the pollen, are more exposed to the spray deposits, whereas the nectar is protected in the nectaries. Significantly higher residue concentrations in worker and drone jelly most likely resulted from the additional pollen added to the jelly at this later stage (L3–L4) of larval development and not from the nurse bee secretions. This is further supported by the fact that there are no significant differences in the residue concentrations of nurse bees' heads and royal jelly, which consists solely of the head gland secretions (Figure 2A). However, it is important to note that the bee head contains other tissues, such as the brain, trachea, and eyes, in addition to the food glands.[25] These non-glandular tissues may dilute the residues measured in the heads. Nonetheless, the head glands of nurse bees are particularly enlarged during the feeding period and show a positive correlation with head weight[30,31] (Figure S2).

Based on the residue unit doses (RUDs), residues in pollen are expected to be more than 10 times higher than in nectar, regardless of the active ingredients[32] (Data S1D). Here, we also showed this 10-times-higher median concentration in pollen products (pollen baskets and stored pollen) compared with nectar products (honey sac and stored nectar) (Figures 1A–1C), and the significantly higher boscalid concentration in stored pollen, compared with stored nectar at both assessment days after exposure (Figure 1C). However, the expected residues based on the median RUDs were overestimated when compared with the actual median residues detected in this study (Data S1D). The variation in residue concentrations across different matrices may be attributed to the different physicochemical properties of those matrices. The correlation between the lipophilic character of different pesticides and higher residue concentrations in pollen, bee bread, or beeswax was previously confirmed.[33,34] However, the storage of collected products in wax cells can, in turn, serve as a potential accumulation step.[34] Wax is a highly lipophilic matrix, with no polar content, and a source of accumulation for lipophilic substances. Finally, evaporation during nectar processing can also concentrate contaminants.[34–37] In this study, the concentration of residues from bees' collected material to stored products varied by a factor of 2.1–3.7, but a direct accumulation of the residues could not be shown (Figure 3).

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Residue concentrations in OSR inflorescences, pollen baskets, and honey sacs decreased logarithmically over time, with a maximum detected concentration of 0.3 mg/kg 8 days after application (Figures 1A and 1B). The derived DT_{50} values for pollen baskets and honey sacs aligned with the expected dissipation rates of boscalid and pyraclostrobin in pollen or nectar, showing a dissipation time of less than 2 days.[32] Furthermore, residue concentrations decreased during overwintering in 2023 and were only detectable at a maximum of 0.009 mg/kg (Figure S1). Although boscalid and pyraclostrobin are frequently detected in bee matrices,[4,5,18,23,24,38–48] when samples are collected in a time-dependent interval after application, concentrations decrease over time[4,38–41] (Data S1E). However, metabolites of boscalid have been detected in adult bee samples and the resulting toxicological effects should also be considered.[48]

RQs were calculated based on the BeeREX model, with four different scenarios to evaluate potential threats for adult bees and the larvae.[49] The BeeREX was designed by the US Environmental Protection Agency (EPA) to assess the potential risk of residues in bee matrices by also considering the consumption of these matrices by bees of different castes, sexes, and developmental stages.[49] All calculated matrix-dependent RQs were below the levels of concern for acute and chronic exposure, which are of 0.4 and 1, respectively, except for two cases involving the larval chronic RQ for pyraclostrobin[50] (Data S1C). The default assumptions for boscalid generally overestimated the RQ values for adult bees and queen larvae, while underestimating RQ values for young worker larvae. Conversely, the default assumptions for pyraclostrobin showed the opposite pattern. The expected RQ values based on the RUD estimations were consistent with the default RQ values. Despite this, the RQ values were predominantly below the threshold levels. This low risk was further supported by our parallel field study in which we simultaneously monitored and assessed colonies within the same experimental setup. We observed only short-term effects related to the combined stressor effect of Pictor Active exposure and pollen restriction in nucleus colonies, such as alterations in colony development and brood ratio, but no impacts on the gut microbiome or brood termination. Effects were compensated over time and no long-term effects were observed in overwintering.[51] However, pyraclostrobin acts on bee mitochondria. It inhibits complex III of cellular respiration in fungi but also oxidative phosphorylation and ATP synthesis in bees.[52] Physiological alterations might impact honey bee health on an individual or colony level and should not be neglected.[53–57]

As honey bee queens and drones are the reproductive units,[7] impacts on their development and health could influence colony reproduction negatively. The queen lays thousands of eggs per day, from which the workers hatch and thus enable the colony to grow. Drone eggs are only laid and reared during the reproductive season, when queens are also reared.[14] Potential short-term effects include the impairment of the quality of larval food jelly,[16] while long-term effects could include a reduction in sperm quality or inhibition of ovarian development in queens.[14,58] Worker bees are responsible for many tasks within the colony, including nursing and feeding the brood, which is important for colony growth and strength. Therefore, understanding potential exposure routes and the transfer of residues within the honey bee colony is essential, as these can be considered in the risk evaluations.

Here, we have investigated the transmission pathway by evaluating all potential dilution and accumulation steps using the example of two globally used lipophilic fungicides. The transfer route from field to larvae showed that residue concentrations were diluted from one stage to another, with the exception of storing processes. Nurse bees act in this process as a biological filter that dilutes residue levels along the pathway. This key function may prevent queen, worker, and drone larvae from being exposed to high, potentially harmful concentrations of fungicides. Based on this, PPPs with active substances of different physicochemical characteristics and effects on other bee species should be assessed in future studies. Results may differ when polar substances are tested, as their chemical character tends to interact more with polar matrices such as nectar, or when solitary bees are used, as they may not rely on the filtering function of a colony.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Karoline Wueppenhorst (karoline.wueppenhorst@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All original data will be deposited at Open-Agrar and are publicly available as of the date of publication. DOI will be listed in the key resources table.
- This study did not generate any unique code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, K.W., A.T.A., H.B., I.I., W.H.K., R.O., and S.E.; methodology, K.W., A.T.A., H.B., G.B., U.E., E.F., I.I., J.K., W.H.K., R.O., and S.E.; formal analysis, K.W., A.T.A., R.O., and S.E.; writing – original draft, K.W.; writing – review & editing, K.W., A.T.A., H.B., G.B., U.E., E.F., I.I., M.J., J.K., W.H.K., R.O., and S.E.; project administration, S.E.; funding acquisition, A.T.A., H.B., I.I., M.J., W.H.K., R.O., and S.E.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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REFERENCES

1. Végh, R., Csóka, M., Mednyánszky, Z., and Sipos, L. (2023). Pesticide residues in bee bread, propolis, beeswax and royal jelly - A review of the literature and dietary risk assessment. Food Chem. Toxicol. *176*, 113806. https://doi.org/10.1016/j.fct.2023.113806.
2. Murcia-Morales, M., Heinzen, H., Parrilla-Vázquez, P., Gómez-Ramos, M.M., and Fernández-Alba, A.R. (2022). Presence and distribution of pesticides in apicultural products: A critical appraisal. Trends Anal. Chem. *146*, 116506. https://doi.org/10.1016/j.trac.2021.116506.
3. Odemer, R., Friedrich, E., Illies, I., Berg, S., Pistorius, J., and Bischoff, G. (2023). Potential risk of residues from Neonicotinoid-treated sugar beet in flowering weeds to honey bees (*Apis mellifera* L.). Environ. Toxicol. Chem. *42*, 1167–1177. https://doi.org/10.1002/etc.5602.
4. Zioga, E., White, B., and Stout, J.C. (2023). Honey bees and bumble bees may be exposed to pesticides differently when foraging on agricultural areas. Sci. Total Environ. *896*, 166214. https://doi.org/10.1016/j.scitotenv.2023.166214.
5. Zioga, E., White, B., and Stout, J.C. (2023). Pesticide mixtures detected in crop and non-target wild plant pollen and nectar. Sci. Total Environ. *879*, 162971. https://doi.org/10.1016/j.scitotenv.2023.162971.
6. Wueppenhorst, K., Eckert, J.H., Steinert, M., and Erler, S. (2022). What about honey bee jelly? Pesticide residues in larval food jelly of the Western honey bee *Apis mellifera*. Sci. Total Environ. *850*, 158095. https://doi.org/10.1016/j.scitotenv.2022.158095.
7. Winston, M.L. (1987). The Biology of the Honey Bee (Harvard University Press).
8. Crenna, E., Jolliet, O., Collina, E., Sala, S., and Fantke, P. (2020). Characterizing honey bee exposure and effects from pesticides for chemical prioritization and life cycle assessment. Environ. Int. *138*, 105642. https://doi.org/10.1016/j.envint.2020.105642.
9. Encerrado-Manriquez, A.M., Pouv, A.K., Fine, J.D., and Nicklisch, S.C.T. (2024). Enhancing knowledge of chemical exposures and fate in honey bee hives: Insights from colony structure and interactions. Sci. Total Environ. *916*, 170193. https://doi.org/10.1016/j.scitotenv.2024.170193.
10. Leska, A., Nowak, A., Nowak, I., and Górczyńska, A. (2021). Effects of insecticides and microbiological contaminants on *Apis mellifera* health. Molecules *26*, 5080. https://doi.org/10.3390/molecules26165080.
11. Tosi, S., Sfeir, C., Carnesecchi, E., Vanengelsdorp, D., and Chauzat, M.-P. (2022). Lethal, sublethal, and combined effects of pesticides on bees: A meta-analysis and new risk assessment tools. Sci. Total Environ. *844*, 156857. https://doi.org/10.1016/j.scitotenv.2022.156857.
12. Milone, J.P., and Tarpy, D.R. (2021). Effects of developmental exposure to pesticides in wax and pollen on honey bee (*Apis mellifera*) queen reproductive phenotypes. Sci. Rep. *11*, 1020. https://doi.org/10.1038/s41598-020-80446-3.
13. Walsh, E.M., Sweet, S., Knap, A., Ing, N., and Rangel, J. (2020). Queen honey bee (*Apis mellifera*) pheromone and reproductive behavior are affected by pesticide exposure during development. Behav. Ecol. Sociobiol. *74*, 33. https://doi.org/10.1007/s00265-020-2810-9.
14. Rangel, J., and Fisher, A. (2019). Factors affecting the reproductive health of honey bee (*Apis mellifera*) drones—a review. Apidologie *50*, 759–778. https://doi.org/10.1007/s13592-019-00684-x.
15. Tomé, H.V.V., Schmehl, D.R., Wedde, A.E., Godoy, R.S.M., Ravaiano, S.V., Guedes, R.N.C., Martins, G.F., and Ellis, J.D. (2020). Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. Environ. Pollut. *256*, 113420. https://doi.org/10.1016/j.envpol.2019.113420.
16. Milone, J.P., Chakrabarti, P., Sagili, R.R., and Tarpy, D.R. (2021). Colony-level pesticide exposure affects honey bee (*Apis mellifera* L.) royal jelly production and nutritional composition. Chemosphere *263*, 128183. https://doi.org/10.1016/j.chemosphere.2020.128183.
17. Sponsler, D.B., and Johnson, R.M. (2017). Mechanistic modeling of pesticide exposure: The missing keystone of honey bee toxicology. Environ. Toxicol. Chem. *36*, 871–881. https://doi.org/10.1002/etc.3661.
18. Johnson, R.M., and Percel, E.G. (2013). Effect of a fungicide and spray adjuvant on queen-rearing success in honey bees (Hymenoptera: Apidae). J. Econ. Entomol. *106*, 1952–1957. https://doi.org/10.1603/EC13199.
19. Lucchetti, M.A., Kilchenmann, V., Glauser, G., Praz, C., and Kast, C. (2018). Nursing protects honeybee larvae from secondary metabolites of pollen. Proc. Biol. Sci. *285*, https://doi.org/10.1098/rspb.2017.2849.
20. Gong, Y., and Diao, Q. (2017). Current knowledge of detoxification mechanisms of xenobiotic in honey bees. Ecotoxicology *26*, 1–12. https://doi.org/10.1007/s10646-016-1742-7.
21. Zhang, G., Dilday, S., Kuesel, R.W., and Hopkins, B. (2024). Phytochemicals, probiotics, recombinant proteins: Enzymatic remedies to pesticide poisonings in bees. Environ. Sci. Technol. *58*, 54–62. https://doi.org/10.1021/acs.est.3c07581.
22. Berenbaum, M.R., and Liao, L.-H. (2019). Honey bees and environmental stress: Toxicologic pathology of a superorganism. Toxicol. Pathol. *47*, 1076–1081. https://doi.org/10.1177/0192623319877154.
23. DeGrandi-Hoffman, G., Chen, Y., and Simonds, R. (2013). The effects of pesticides on queen rearing and virus titers in honey bees (*Apis mellifera* L.). Insects *4*, 71–89. https://doi.org/10.3390/insects4010071.
24. Böhme, F., Bischoff, G., Zebitz, C.P.W., Rosenkranz, P., and Wallner, K. (2019). From field to food II – will pesticide-contaminated pollen diet lead to a contamination of worker jelly? J. Apic. Res. *58*, 542–549. https://doi.org/10.1080/00218839.2019.1614727.
25. Haydak, M.H. (1970). Honey bee nutrition. Annu. Rev. Entomol. *15*, 143–156. https://doi.org/10.1146/annurev.en.15.010170.001043.
26. Rortais, A., Arnold, G., Halm, M.-P., and Touffet-Briens, F. (2005). Modes of honeybees exposure to systemic insecticides: Estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie *36*, 71–83. https://doi.org/10.1051/apido:2004071.
27. Von Planta, A. (1888). Ueber den Futtersaft der Bienen. Z. Physiol. Chem. *12*, 327–354. https://doi.org/10.1515/bchm1.1888.12.4.327.

28. European Union. EU Pesticides database. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances.

29. SANTE/11956/2016 SANTE (2019). Technical guidelines for determining the magnitude of pesticide residues in honey and setting maximum residue levels in honey.

30. Crailsheim, K., and Stolberg, E. (1989). Influence of diet, age and colony condition upon intestinal proteolytic activity and size of the hypopharyngeal glands in the honeybee (*Apis mellifera* L.). J. Insect Physiol. *35*, 595–602. https://doi.org/10.1016/0022-1910(89)90121-2.

31. Hrassnigg, N., and Crailsheim, K. (1989). Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera* L.) colonies. J. Insect Physiol. *44*, 929–939. https://doi.org/10.1016/S0022-1910(98)00058-4.

32. . EFSA3 14 Kyriakopoulou, K., Kandris, I., Pachiti, I., Kasiotis, K.M., Spyropoulou, A., Santourian, A., Kitromilidou, S., Pappa, G., and Glossioti, M. (2017). Collection and analysis of pesticide residue data for pollen and nectar – Final Report. https://doi.org/10.2903/sp.efsa.2017.EN-1303.

33. Perkins, J.A., Kim, K., Gut, L.J., Sundin, G.W., and Wilson, J.K. (2023). Fungicide exposure in honey bee hives varies by time, worker role, and proximity to orchards in spring. J. Econ. Entomol. *116*, 435–446. https://doi.org/10.1093/jee/toad008.

34. Alkassab, A.T., Bischoff, G., Thorbahn, D., Frommberger, M., and Pistorius, J. (2022). Transfer of xenobiotics from contaminated beeswax into different bee matrices under field conditions and the related exposure probability. Chemosphere *307*, 135615. https://doi.org/10.1016/j.chemosphere.2022.135615.

35. Dively, G.P., Embrey, M.S., Kamel, A., Hawthorne, D.J., and Pettis, J.S. (2015). Assessment of chronic sublethal effects of imidacloprid on honey bee colony health. PLoS One *10*, e0118748. https://doi.org/10.1371/journal.pone.0118748.

36. Kast, C., Kilchenmann, V., and Charrière, J.D. (2021). Long-term monitoring of lipophilic acaricide residues in commercial Swiss beeswax. Pest Manag. Sci. *77*, 4026–4033. https://doi.org/10.1002/ps.6427.

37. Luna, A., Flores, J.M., Miguel, E., Fernández-Alba, A.R., and Hernando, M.D. (2023). Coumaphos residue transfer to honey bee brood (*Apis mellifera*) in realistic scenarios. Res. Vet. Sci. *159*, 106–124. https://doi.org/10.1016/j.rvsc.2023.04.016.

38. Simon-Delso, N., San Martin, G.S., Bruneau, E., Delcourt, C., and Hautier, L. (2017). The challenges of predicting pesticide exposure of honey bees at landscape level. Sci. Rep. *7*, 3801. https://doi.org/10.1038/s41598-017-03467-5.

39. Kuivila, K.M., Judd, H., Hladik, M.L., and Strange, J.P. (2021). Field-level exposure of bumble bees to fungicides applied to a commercial cherry orchard. J. Econ. Entomol. *114*, 1065–1071. https://doi.org/10.1093/jee/toab051.

40. Schaad, E., Fracheboud, M., Droz, B., and Kast, C. (2023). Quantitation of pesticides in bee bread collected from honey bee colonies in an agricultural environment in Switzerland. Environ. Sci. Pollut. Res. Int. *30*, 56353–56367. https://doi.org/10.1007/s11356-023-26268-y.

41. Piechowicz, B., Szpyrka, E., Zaręba, L., Podbielska, M., and Grodzicki, P. (2018). Transfer of the active ingredients of some plant protection products from raspberry plants to beehives. Arch. Environ. Contam. Toxicol. *75*, 45–58. https://doi.org/10.1007/s00244-017-0488-4.

42. Daniele, G., Giroud, B., Jabot, C., and Vulliet, E. (2018). Exposure assessment of honeybees through study of hive matrices: Analysis of selected pesticide residues in honeybees, beebread, and beeswax from French beehives by LC-MS/MS. Environ. Sci. Pollut. Res. *25*, 6145–6153. https://doi.org/10.1007/s11356-017-9227-7.

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

44. Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., Vanengelsdorp, D., and Pettis, J.S. (2010). High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. PLoS One *5*, e9754. https://doi.org/10.1371/journal.pone.0009754.

45. Murcia-Morales, M., Díaz-Galiano, F.J., Vejsnæs, F., Kilpinen, O., van der Steen, J.J.M., and Fernández-Alba, A.R. (2021). Environmental monitoring study of pesticide contamination in Denmark through honey bee colonies using APIStrip-based sampling. Environ. Pollut. *290*, 117888. https://doi.org/10.1016/j.envpol.2021.117888.

46. Ostiguy, N., and Eitzer, B. (2014). Overwintered brood comb honey: colony exposure to pesticide residues. J. Apic. Res. *53*, 413–421. https://doi.org/10.3896/IBRA.1.53.3.10.

47. Piechowicz, B., Mróz, K., Szpyrka, E., Zwolak, A., and Grodzicki, P. (2018). Transfer of plant protection products from raspberry crops of Laszka and Seedling varieties to beehives. Environ. Monit. Assess. *190*, 135. https://doi.org/10.1007/s10661-018-6491-z.

48. Jabot, C., Daniele, G., Giroud, B., Tchamitchian, S., Belzunces, L.P., Casabianca, H., and Vulliet, E. (2016). Detection and quantification of boscalid and its metabolites in honeybees. Chemosphere *156*, 245–251. https://doi.org/10.1016/j.chemosphere.2016.04.135.

49. US Environmental Protection Agency (EPA) (2014). Guidance for assessing pesticide risks to bees. https://www.epa.gov/sites/default/files/2014-06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf.

50. Thompson, H.M. (2021). The use of the hazard quotient approach to assess the potential risk to honeybees (*Apis mellifera*) posed by pesticide residues detected in bee-relevant matrices is not appropriate. Pest Manag. Sci. *77*, 3934–3941. https://doi.org/10.1002/ps.6426.

51. Wueppenhorst, K., Alkassab, A.T., Beims, H., Ernst, U., Friedrich, E., Illies, I., Janke, M., Kirchner, W.H., Seidel, K., Steinert, M., et al. (2024). Honey bee colonies can buffer short-term stressor effects of pollen restriction and fungicide exposure on colony development and the microbiome. Ecotoxicol. Environ. Saf. *282*, 116723. https://doi.org/10.1016/ecoenv.2024.116723.

52. Nicodemo, D., Mingatto, F.E., De Jong, D., Bizerra, P.F.V., Tavares, M.A., Bellini, W.C., Vicente, E.F., and de Carvalho, A. (2020). Mitochondrial respiratory inhibition promoted by pyraclostrobin in fungi is also observed in honey bees. Environ. Toxicol. Chem. *39*, 1267–1272. https://doi.org/10.1002/etc.4719.

53. Christen, V. (2023). Different effects of pesticides on transcripts of the endocrine regulation and energy metabolism in honeybee foragers from different colonies. Sci. Rep. *13*, 1985. https://doi.org/10.1038/s41598-023-29257-w.

54. Zaluski, R., Bittarello, A.C., Vieira, J.C.S., Braga, C.P., Padilha, P.M., Fernandes, M.S., Bovi, T.S., and Orsi, R.O. (2020). Modification of the head proteome of nurse honeybees (*Apis mellifera*) exposed to field-relevant doses of pesticides. Sci. Rep. *10*, 2190. https://doi.org/10.1038/s41598-020-59070-8.

55. DeGrandi-Hoffman, G., Chen, Y., Watkins Dejong, E., Chambers, M.L., and Hidalgo, G. (2015). Effects of oral exposure to fungicides on honey bee nutrition and virus levels. J. Econ. Entomol. *108*, 2518–2528. https://doi.org/10.1093/jee/tov251.

56. Fisher, A., DeGrandi-Hoffman, G., Smith, B.H., Johnson, M., Kaftanoglu, O., Cogley, T., Fewell, J.H., and Harrison, J.F. (2021). Colony field test reveals dramatically higher toxicity of a widely-used mito-toxic fungicide on honey bees (*Apis mellifera*). Environ. Pollut. *269*, 115964. https://doi.org/10.1016/j.envpol.2020.115964.

57. Fisher, A., DeGrandi-Hoffman, G., Smith, B.H., Ozturk, C., Kaftanoglu, O., Fewell, J.H., and Harrison, J.F. (2021). Field cross-fostering and in vitro rearing demonstrate negative effects of both larval and adult exposure to a widely used fungicide in honey bees (*Apis mellifera*). Ecotoxicol. Environ. Saf. *217*, 112251. https://doi.org/10.1016/j.ecoenv.2021.112251.

58. Williams, G.R., Troxler, A., Retschnig, G., Roth, K., Yañez, O., Shutler, D., Neumann, P., and Gauthier, L. (2015). Neonicotinoid pesticides severely affect honey bee queens. Sci. Rep. *5*, 14621. https://doi.org/10.1038/srep14621.

59. Brooks, M., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M., and Bolker, B.M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. *9*, 378. https://doi.org/10.32614/RJ-2017-066.

60. R package version 0.4.6 Hartig, F. (2022). DHARMa: Residual diagnostics for hierarchical (multi-level / mixed) regression model. https://CRAN.R-project.org/package=DHARMa.

61. R package version 1.8.7 Lenth, R. (2023). Emmeans: Estimated marginal means, aka least-squares means. https://CRAN.R-project.org/package=emmeans.

62. Simon-Delso, N., San Martin, G., Bruneau, E., Hautier, L., and Medrzycki, P. (2017). Toxicity assessment on honey bee larvae of a repeated exposition of a systemic fungicide, boscalid. Bull. Insectology *70*, 83–89.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Brassica napus inflorescences	This paper	N/A
Honey bee (*Apis mellifera*) forager bees and in-hive bees	This paper	N/A
Hive-derived stored pollen and nectar	This paper	N/A
Hive-derived queen larvae and food jelly	This paper	N/A
Hive-derived worker larvae and food jelly	This paper	N/A
Hive-derived drone larvae and food jelly	This paper	N/A
Chemicals, peptides, and recombinant proteins		
Pictor Active	BASF, Ludwigshafen	N/A
Boscalid-d4	LGC Limited, Middlesex, UK	188425-85-6
Boscalid	LGC Limited, Middlesex, UK	2468796-76-9
Pyraclostrobin-d6	LGC Limited, Middlesex, UK	N/A
Pyraclostrobin	LGC Limited, Middlesex, UK	175013-18-0
Aceton	Th.Geyer GmbH&Co.KG, Renningen, Germany	67-64-1
Sodium chloride	Th.Geyer GmbH&Co.KG, Renningen, Germany	7647-14-5
Dichloromethane	Th.Geyer GmbH&Co.KG, Renningen, Germany	75-09-2
Methanol	Th.Geyer GmbH&Co.KG, Renningen, Germany	67-56-1
Acetonitrile	Th.Geyer GmbH&Co.KG, Renningen, Germany	75-05-8
Formic acid	Th.Geyer GmbH&Co.KG, Renningen, Germany	64-18-6
Ammonium formiate	Th.Geyer GmbH&Co.KG, Renningen, Germany	540-69-2
Abamectin	HPC Standards, Cunnersdorf, Germany	71751-41-2
Acephate	LGC Limited, Middlesex, UK	30560-19-1
Acetamiprid	HPC Standards, Cunnersdorf, Germany	135410-20-7
Acrinathrin	LGC Limited, Middlesex, UK	101007-06-1
Alachlor	LGC Limited, Middlesex, UK	15972-60-8
Alanycarb	HPC Standards, Cunnersdorf, Germany	83130-01-2
Aldicarb	LGC Limited, Middlesex, UK	116-06-3
Aldicarb-sulfone	LGC Limited, Middlesex, UK	1646-88-4
Aldicarb-sulfoxid	LGC Limited, Middlesex, UK	1646-87-3
Allethrin	LGC Limited, Middlesex, UK	584-79-2
alpha-Cypermethrin	HPC Standards, Cunnersdorf, Germany	67375-30-8
alpha-Endosulfan	LGC Limited, Middlesex, UK	115-29-7
alpha-HCH	LGC Limited, Middlesex, UK	319-84-6
Amisulbrom	HPC Standards, Cunnersdorf, Germany	348635-87-0
Amitraz	HPC Standards, Cunnersdorf, Germany	33089-61-1
Amitraz-metabolite BTS 27271	LGC Limited, Middlesex, UK	33089-74-6
Amitraz-metabolite BTS 27919	HPC Standards, Cunnersdorf, Germany	60397-77-5
Azadirachtin	HPC Standards, Cunnersdorf, Germany	11141-17-6
Azamethiphos	LGC Limited, Middlesex, UK	35575-96-3
Azinphos-ethyl	LGC Limited, Middlesex, UK	2642-71-9
Azinphos-methyl	LGC Limited, Middlesex, UK	86-50-0
Azoxystrobin	HPC Standards, Cunnersdorf, Germany	131860-33-8
Bendiocarb	LGC Limited, Middlesex, UK	22781-23-3
Benfuracarb	HPC Standards, Cunnersdorf, Germany	82560-54-1
Benthiavalicarb-isopropyl	HPC Standards, Cunnersdorf, Germany	413615-35-7

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Benzoximate	LGC Limited, Middlesex, UK	29104-30-1
beta-Cyfluthrin	HPC Standards, Cunnersdorf, Germany	1820573-27-0
beta-Endosulfan	LGC Limited, Middlesex, UK	33213-65-9
beta-HCH	LGC Limited, Middlesex, UK	319-85-7
Bifenazat	HPC Standards, Cunnersdorf, Germany	149877-41-8
Bifenox	HPC Standards, Cunnersdorf, Germany	42576-02-3
Bifenthrin	HPC Standards, Cunnersdorf, Germany	82657-04-3
Bromopropylate	LGC Limited, Middlesex, UK	18181-80-1
Bromoxynil	HPC Standards, Cunnersdorf, Germany	1689-84-5
Bromuconazole	HPC Standards, Cunnersdorf, Germany	116255-48-2
Buprofezin	HPC Standards, Cunnersdorf, Germany	69327-76-0
Butoxycarboxim	LGC Limited, Middlesex, UK	34681-23-7
Captan	HPC Standards, Cunnersdorf, Germany	133-06-2
Carbaryl	LGC Limited, Middlesex, UK	63-25-2
Carbendazim	HPC Standards, Cunnersdorf, Germany	10605-21-7
Carbofuran	LGC Limited, Middlesex, UK	1563-66-2
Carbophenothion	LGC Limited, Middlesex, UK	786-19-6
Carbosulfan	HPC Standards, Cunnersdorf, Germany	55285-14-8
Carboxin	HPC Standards, Cunnersdorf, Germany	5234-68-4
Carfentrazon-ethyl	HPC Standards, Cunnersdorf, Germany	128639-02-1
CEKAFIX	LGC Limited, Middlesex, UK	121227-99-4
Chlorantraniliprole	HPC Standards, Cunnersdorf, Germany	500008-45-7
Chlordimeform	LGC Limited, Middlesex, UK	19750-95-9
Chlorfenapyr	LGC Limited, Middlesex, UK	122453-73-0
Chlorfenvinphos	HPC Standards, Cunnersdorf, Germany	470-90-6
Chlorpyrifos	HPC Standards, Cunnersdorf, Germany	2921-88-2
Chlorpyrifos-methyl	HPC Standards, Cunnersdorf, Germany	5598-13-0
Chlorthalonil	HPC Standards, Cunnersdorf, Germany	1897-45-6
Chlorthiamid	LGC Limited, Middlesex, UK	1918-13-4
Clofentezine	HPC Standards, Cunnersdorf, Germany	74115-24-5
Clomazone	HPC Standards, Cunnersdorf, Germany	81777-89-1
Clothianidin	HPC Standards, Cunnersdorf, Germany	210880-92-5
Clothianidin-metabolite TZMU	HPC Standards, Cunnersdorf, Germany	634192-72-6
Clothianidin-metabolite TZNG	HPC Standards, Cunnersdorf, Germany	135018-15-4
Coumaphos	HPC Standards, Cunnersdorf, Germany	56-72-4
Cyantraniliprole	LGC Limited, Middlesex, UK	736994-63-1
Cyazofamid	HPC Standards, Cunnersdorf, Germany	120116-88-3
Cyflufenamid	HPC Standards, Cunnersdorf, Germany	180409-60-3
Cymiazole	LGC Limited, Middlesex, UK	61676-87-7
Cymoxanil	HPC Standards, Cunnersdorf, Germany	57966-95-7
Cypermethrin	HPC Standards, Cunnersdorf, Germany	52315-07-8
Cyphenothrin	HPC Standards, Cunnersdorf, Germany	39515-40-7
Cyproconazole	HPC Standards, Cunnersdorf, Germany	94361-06-5
Cyprodinil	LGC Limited, Middlesex, UK	121552-61-2
Cyromazine	HPC Standards, Cunnersdorf, Germany	66215-27-8
DEET	HPC Standards, Cunnersdorf, Germany	134-62-3
Deltamethrin	HPC Standards, Cunnersdorf, Germany	52918-63-5
Demeton-S-methylsulfone	LGC Limited, Middlesex, UK	17040-19-6
Diafenthiuron	HPC Standards, Cunnersdorf, Germany	80060-09-9
Dialifos	LGC Limited, Middlesex, UK	10311-84-9

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Diazinon	LGC Limited, Middlesex, UK	333-41-5
Dichlobenil	LGC Limited, Middlesex, UK	1194-65-6
Dichlorvos	LGC Limited, Middlesex, UK	62-73-7
Diethofencarb	LGC Limited, Middlesex, UK	87130-20-9
Difenoconazole	LGC Limited, Middlesex, UK	119446-68-3
Diflubenzuron	HPC Standards, Cunnersdorf, Germany	35367-38-5
Diflufenican	LGC Limited, Middlesex, UK	83164-33-4
Dimethachlor	HPC Standards, Cunnersdorf, Germany	50563-36-5
Dimethenamid-P	LGC Limited, Middlesex, UK	163515-14-8
Dimethoate	LGC Limited, Middlesex, UK	60-51-5
Dimethomorph	LGC Limited, Middlesex, UK	110488-70-5
Dimoxystrobin	LGC Limited, Middlesex, UK	149961-52-4
Dinotefuran	LGC Limited, Middlesex, UK	165252-70-0
Diuron	LGC Limited, Middlesex, UK	330-54-1
Empenthrin	LGC Limited, Middlesex, UK	54406-48-3
Endosulfansulfat	LGC Limited, Middlesex, UK	1031-07-8
Epoxiconazole	LGC Limited, Middlesex, UK	133855-98-8
Esfenvalerate	LGC Limited, Middlesex, UK	66230-04-4
Ethiofencarb	LGC Limited, Middlesex, UK	29973-13-5
Ethofumesate	LGC Limited, Middlesex, UK	26225-79-6
Ethoprophos	HPC Standards, Cunnersdorf, Germany	13194-48-4
Etofenprox	LGC Limited, Middlesex, UK	80844-07-1
Etoxazole	LGC Limited, Middlesex, UK	153233-91-1
Famoxadone	HPC Standards, Cunnersdorf, Germany	131807-57-3
Fenamidone	HPC Standards, Cunnersdorf, Germany	161326-34-7
Fenamiphos	HPC Standards, Cunnersdorf, Germany	22224-92-6
Fenarimol	LGC Limited, Middlesex, UK	60168-88-9
Fenazaquin	LGC Limited, Middlesex, UK	120928-09-8
Fenhexamid	LGC Limited, Middlesex, UK	126833-17-8
Fenitrothion	LGC Limited, Middlesex, UK	122-14-5
Fenoxaprop-P-ethyl	HPC Standards, Cunnersdorf, Germany	71283-80-2
Fenoxycarb	HPC Standards, Cunnersdorf, Germany	72490-01-8
Fenpropidin	HPC Standards, Cunnersdorf, Germany	67306-00-7
Fenpropimorph	HPC Standards, Cunnersdorf, Germany	67564-91-4
Fenpyroximate	HPC Standards, Cunnersdorf, Germany	111812-58-9
Fipronil	HPC Standards, Cunnersdorf, Germany	120068-37-3
Fipronil-carboxamid	HPC Standards, Cunnersdorf, Germany	205650-69-7
Fipronil-desulfinyl	HPC Standards, Cunnersdorf, Germany	205650-65-3
Fipronil-sulfid	HPC Standards, Cunnersdorf, Germany	120067-83-6
Fipronil-sulfon	HPC Standards, Cunnersdorf, Germany	120068-36-2
Flonicamid	HPC Standards, Cunnersdorf, Germany	158062-67-0
Flonicamid-metabolite TFNA	HPC Standards, Cunnersdorf, Germany	158063-66-2
Flonicamid-metabolite TFNG	HPC Standards, Cunnersdorf, Germany	207502-65-6
Fluazifop	LGC Limited, Middlesex, UK	69335-91-7
Fluazifop-P-butyl	HPC Standards, Cunnersdorf, Germany	79241-46-6
Fluazinam	HPC Standards, Cunnersdorf, Germany	79622-59-6
Fludioxonil	HPC Standards, Cunnersdorf, Germany	131341-86-1
Flufenacet	HPC Standards, Cunnersdorf, Germany	142459-58-3
Flufenoxuron	LGC Limited, Middlesex, UK	101463-69-8
Fluopicolide	HPC Standards, Cunnersdorf, Germany	239110-15-7

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Fluopyram	HPC Standards, Cunnersdorf, Germany	658066-35-4
Fluoxastrobin	HPC Standards, Cunnersdorf, Germany	361377-29-9
Flupyradifurone	HPC Standards, Cunnersdorf, Germany	951659-40-8
Fluquinconazole	HPC Standards, Cunnersdorf, Germany	136426-54-5
Fluroxypyr-1-methylheptylester	HPC Standards, Cunnersdorf, Germany	81406-37-3
Flurtamone	HPC Standards, Cunnersdorf, Germany	96525-23-4
Flusilazole	LGC Limited, Middlesex, UK	85509-19-9
Flutriafol	LGC Limited, Middlesex, UK	76674-21-0
Fluxapyroxad	HPC Standards, Cunnersdorf, Germany	907204-31-3
Folpet	HPC Standards, Cunnersdorf, Germany	133-07-3
Fonofos	LGC Limited, Middlesex, UK	944-22-9
Fosthiazate	HPC Standards, Cunnersdorf, Germany	98886-44-3
Fuberidazole	LGC Limited, Middlesex, UK	3878-19-1
Furathiocarb	HPC Standards, Cunnersdorf, Germany	65907-30-4
Heptenophos	LGC Limited, Middlesex, UK	23560-59-0
Hexachlorobenzene (HCB)	LGC Limited, Middlesex, UK	118-74-1
Hexaconazole	HPC Standards, Cunnersdorf, Germany	79983-71-4
Hexaflumuron	HPC Standards, Cunnersdorf, Germany	86479-06-3
Hexythiazox	HPC Standards, Cunnersdorf, Germany	78587-05-0
Icaridin	HPC Standards, Cunnersdorf, Germany	119515-38-7
Imazalil	HPC Standards, Cunnersdorf, Germany	35554-44-0
Imidacloprid	HPC Standards, Cunnersdorf, Germany	138261-41-3
Imidacloprid-5-hydroxy	HPC Standards, Cunnersdorf, Germany	155802-61-2
Imidacloprid-olefin	LGC Limited, Middlesex, UK	115086-54-9
Imiprothrin	HPC Standards, Cunnersdorf, Germany	72963-72-5
Indoxacarb	HPC Standards, Cunnersdorf, Germany	144171-61-9
Ioxynil	HPC Standards, Cunnersdorf, Germany	1689-83-4
Ipconazole	HPC Standards, Cunnersdorf, Germany	125225-28-7
Iprodion	HPC Standards, Cunnersdorf, Germany	36734-19-7
Iprovalicarb	HPC Standards, Cunnersdorf, Germany	140923-17-7
Isoproturon	HPC Standards, Cunnersdorf, Germany	34123-59-6
Isoxaben	HPC Standards, Cunnersdorf, Germany	82558-50-7
Kresoxim-methyl	HPC Standards, Cunnersdorf, Germany	143390-89-0
lambda-Cyhalothrin	HPC Standards, Cunnersdorf, Germany	91465-08-6
Lindane (gamma-HCH)	HPC Standards, Cunnersdorf, Germany	58-89-9
Lufenuron	HPC Standards, Cunnersdorf, Germany	103055-07-8
Malathion	HPC Standards, Cunnersdorf, Germany	121-75-5
Mandipropamid	HPC Standards, Cunnersdorf, Germany	374726-62-2
MCPA	HPC Standards, Cunnersdorf, Germany	94-74-6
Mecoprop	HPC Standards, Cunnersdorf, Germany	93-65-2
Mefentrifluconazole	LGC Limited, Middlesex, UK	1417782-03-6
Mepanipyrim	HPC Standards, Cunnersdorf, Germany	110235-47-7
Mepronil	LGC Limited, Middlesex, UK	55814-41-0
Metaflumizone	LGC Limited, Middlesex, UK	139968-49-3
Metalaxyl-M	HPC Standards, Cunnersdorf, Germany	57837-19-1
Metazachlor	HPC Standards, Cunnersdorf, Germany	67129-08-2
Metconazole	HPC Standards, Cunnersdorf, Germany	125116-23-6
Methamidophos	LGC Limited, Middlesex, UK	10265-92-6
Methidathion	LGC Limited, Middlesex, UK	950-37-8
Methiocarb	HPC Standards, Cunnersdorf, Germany	2032-65-7

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Methiocarb-sulfon	LGC Limited, Middlesex, UK	2179-25-1
Methiocarb-sulfoxid	HPC Standards, Cunnersdorf, Germany	2635-10-1
Methomyl	HPC Standards, Cunnersdorf, Germany	16752-77-5
Methoxychlor	LGC Limited, Middlesex, UK	72-43-5
Methoxyfenozide	HPC Standards, Cunnersdorf, Germany	161050-58-4
Metofluthrin	LGC Limited, Middlesex, UK	240494-70-6
Metrafenone	LGC Limited, Middlesex, UK	220899-03-6
Metribuzin	HPC Standards, Cunnersdorf, Germany	21087-64-9
Mevinphos	LGC Limited, Middlesex, UK	7786-34-7
Milbemectin mixture of A3 and A4	HPC Standards, Cunnersdorf, Germany	51596-10-2/51596-11-3
Momfluorothrin	LGC Limited, Middlesex, UK	609346-29-4
Myclobutanil	HPC Standards, Cunnersdorf, Germany	88671-89-0
Naphthalin	HPC Standards, Cunnersdorf, Germany	91-20-3
Napropamid	HPC Standards, Cunnersdorf, Germany	15299-99-7
Nicotine	LGC Limited, Middlesex, UK	54-11-5
Nitenpyram	LGC Limited, Middlesex, UK	150824-47-8
Novaluron	HPC Standards, Cunnersdorf, Germany	116714-46-6
Omethoate	LGC Limited, Middlesex, UK	1113-02-6
Orange oil ((R) (+) Limonene)	LGC Limited, Middlesex, UK	8008-57-9
Oxydemeton-methyl	LGC Limited, Middlesex, UK	301-12-2
Paclobutrazol	LGC Limited, Middlesex, UK	76738-62-0
Paraoxon	HPC Standards, Cunnersdorf, Germany	311-45-5
Parathion	HPC Standards, Cunnersdorf, Germany	56-38-2
Parathion-methyl	HPC Standards, Cunnersdorf, Germany	298-00-0
Penconazole	HPC Standards, Cunnersdorf, Germany	66246-88-6
Pencycuron	HPC Standards, Cunnersdorf, Germany	66063-05-6
Pendimethalin	HPC Standards, Cunnersdorf, Germany	40487-42-1
Pentachlorobenzene	LGC Limited, Middlesex, UK	608-93-5
Pentachlorophenol	HPC Standards, Cunnersdorf, Germany	87-86-5
Permethrin	LGC Limited, Middlesex, UK	52645-53-1
Phenothrin	HPC Standards, Cunnersdorf, Germany	26002-80-2
Phosalone	LGC Limited, Middlesex, UK	2310-17-0
Phoxim	HPC Standards, Cunnersdorf, Germany	14816-18-3
Picoxystrobin	LGC Limited, Middlesex, UK	117428-22-5
Piperonylbutoxide	HPC Standards, Cunnersdorf, Germany	51-03-6
Pirimicarb	HPC Standards, Cunnersdorf, Germany	23103-98-2
Pirimicarb-desmethyl	HPC Standards, Cunnersdorf, Germany	30614-22-3
Pirimicarb-desmethyl-formamid	HPC Standards, Cunnersdorf, Germany	27218-04-8
Pirimiphos-methyl	HPC Standards, Cunnersdorf, Germany	29232-93-7
Prallethrin	HPC Standards, Cunnersdorf, Germany	23031-36-9
Prochloraz	LGC Limited, Middlesex, UK	67747-09-5
Prochloraz-metabolite BTS 40348	HPC Standards, Cunnersdorf, Germany	67747-01-7
Prochloraz-metabolite BTS 44596	HPC Standards, Cunnersdorf, Germany	139542-32-8
Procymidon	LGC Limited, Middlesex, UK	32809-16-8
Profenofos	LGC Limited, Middlesex, UK	41198-08-7
Propachlor	LGC Limited, Middlesex, UK	1918-16-7
Propamocarb	HPC Standards, Cunnersdorf, Germany	24579-73-5
Propaquizafop	LGC Limited, Middlesex, UK	111479-05-1
Propargite	HPC Standards, Cunnersdorf, Germany	2312-35-8
Propiconazole	LGC Limited, Middlesex, UK	60207-90-1

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Propoxur	LGC Limited, Middlesex, UK	114-26-1
Propyzamid	LGC Limited, Middlesex, UK	23950-58-5
Proquinazid	LGC Limited, Middlesex, UK	189278-12-4
Prosulfocarb	LGC Limited, Middlesex, UK	52888-80-9
Prothioconazol-desthio	LGC Limited, Middlesex, UK	120983-64-4
Pymetrozine	LGC Limited, Middlesex, UK	123312-89-0
Pyrazophos	LGC Limited, Middlesex, UK	13457-18-6
Pyrethrine	HPC Standards, Cunnersdorf, Germany	8003-34-7
Pyridaben	HPC Standards, Cunnersdorf, Germany	96489-71-3
Pyridalyl	LGC Limited, Middlesex, UK	179101-81-6
Pyrimethanil	HPC Standards, Cunnersdorf, Germany	53112-28-0
Pyriproxyfen	HPC Standards, Cunnersdorf, Germany	95737-68-1
Quinoclamine	HPC Standards, Cunnersdorf, Germany	2797-51-5
Quinoxyfen	HPC Standards, Cunnersdorf, Germany	124495-18-7
Rotenone	LGC Limited, Middlesex, UK	83-79-4
Sulfur	HPC Standards, Cunnersdorf, Germany	7704-34-9
Silthiofam	HPC Standards, Cunnersdorf, Germany	175217-20-6
Spinetoram (Mixture of Spinetoram J & L)	HPC Standards, Cunnersdorf, Germany	187166-40-1 / 187166-15-0
Spinosad (mixture of spinosy A & D)	HPC Standards, Cunnersdorf, Germany	131929-60-7/ 131929-63-0
Spirodiclofen	LGC Limited, Middlesex, UK	148477-71-8
Spiromesifen	LGC Limited, Middlesex, UK	283594-90-1
Spirotetramat	LGC Limited, Middlesex, UK	203313-25-1
Spiroxamine	HPC Standards, Cunnersdorf, Germany	118134-30-8
Sulfoxaflor	LGC Limited, Middlesex, UK	946578-00-3
tau-Fluvalinate	HPC Standards, Cunnersdorf, Germany	102851-06-9
Tebuconazole	HPC Standards, Cunnersdorf, Germany	107534-96-3
Tebufenozide	LGC Limited, Middlesex, UK	112410-23-8
Tebufenpyrad	LGC Limited, Middlesex, UK	119168-77-3
Teflubenzuron	LGC Limited, Middlesex, UK	83121-18-0
Tefluthrin	HPC Standards, Cunnersdorf, Germany	79538-32-2
Terbufos	LGC Limited, Middlesex, UK	13071-79-9
Tetrachlorvinphos	LGC Limited, Middlesex, UK	961-11-5
Tetraconazole	LGC Limited, Middlesex, UK	112281-77-3
Tetradifon	HPC Standards, Cunnersdorf, Germany	116-29-0
Tetrahydrophthalimide-cis-1,2,3,6	HPC Standards, Cunnersdorf, Germany	1469-48-3
Tetramethrin	LGC Limited, Middlesex, UK	7696-12-0
Thiabendazole	HPC Standards, Cunnersdorf, Germany	148-79-8
Thiacloprid	HPC Standards, Cunnersdorf, Germany	111988-49-9
Thiacloprid-amide	LGC Limited, Middlesex, UK	676228-91-4
Thiamethoxam	HPC Standards, Cunnersdorf, Germany	153719-23-4
Thymol	HPC Standards, Cunnersdorf, Germany	89-83-8
Tolclofos-methyl	LGC Limited, Middlesex, UK	57018-04-9
Tolylfluanid	LGC Limited, Middlesex, UK	731-27-1
Transfluthrin	LGC Limited, Middlesex, UK	118712-89-3
Triadimenol	LGC Limited, Middlesex, UK	55219-65-3
Triazamate	HPC Standards, Cunnersdorf, Germany	112143-82-5
Triazophos	HPC Standards, Cunnersdorf, Germany	24017-47-8
Triazoxid	LGC Limited, Middlesex, UK	72459-58-6
Tribenuron-methyl	HPC Standards, Cunnersdorf, Germany	101200-48-0
Trichlorfon	HPC Standards, Cunnersdorf, Germany	52-68-6

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Trifloxystrobin	HPC Standards, Cunnersdorf, Germany	141517-21-7
Triflumuron	HPC Standards, Cunnersdorf, Germany	64628-44-0
Trifluralin	HPC Standards, Cunnersdorf, Germany	1582-09-8
Triforine	LGC Limited, Middlesex, UK	26644-46-2
Triticonazole	HPC Standards, Cunnersdorf, Germany	131983-72-7
Vamidothion	LGC Limited, Middlesex, UK	2275-23-2
Vinclozolin	LGC Limited, Middlesex, UK	50471-44-8
Zeta-Cypermethrin	HPC Standards, Cunnersdorf, Germany	1315501-18-8
Zoxamide	HPC Standards, Cunnersdorf, Germany	156052-68-5
Deposited data		
Raw data	This paper	Open-Agrar: https://doi.org/10.5073/20240927-165036-0
Software and algorithms		
R (version 4.2.2)	R Core Team (2021)	https://www.r-project.org/
R Studio (version 2022.12.0)	Posit Software, PBC formely RStudio, PBC	https://posit.co/products/open-source/rstudio/
glmmTMB package (version 1.1.8)	Brooks et al.[59]	https://doi.org/10.32614/RJ-2017-066
DHARMa package (version 0.4.6)	Hartig[60]	https://CRAN.R-project.org/package=DHARMa
Emmeans package (version 1.8.7)	Lenth[61]	https://CRAN.R-project.org/package=emmeans
BeeREX assessment tool	US Environmental Protection Agency (EPA)[49]	https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment
Chromeleon 7.2.10 ES	ThermoFisher Massachusetts, USA	https://www.thermofisher.com/order/catalog/product/de/de/CHROMELEON7
Sciex OS 3.1.0.	AB Sciex Toronto Kanada	https://sciex.com/products/software/sciex-os-software

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The study design was affiliated to the experimental setup described in Wueppenhorst et al.[51] In brief, the study was conducted simultaneously in spring 2022 at one control and one treatment site of winter oilseed rape (OSR, *Brassica napus*) across five different geographical regions in Germany (Bochum, Braunschweig, Celle, Hohenheim (Stuttgart) and Veitshöchheim). Two to four full-sized, queen-right honey bee (*Apis mellifera* L.) colonies were placed at each control and treatment site to ensure sufficient resources for the subsequent samplings. The colonies were at least one year old and had already successfully overwintered once. Colonies were treated according to good beekeeping practice throughout the full experimental course.

METHOD DETAILS

Study design

Spray application of the formulation Pictor Active (150 g/L boscalid and 250 g/L pyraclostrobin) was carried out during full bloom of OSR (BBCH 64-65) at the treatment sites between 10 a.m. and 2 p.m., with visible foraging activity of bees ensuring adequate exposure. The highest application rate permitted in the European Union of one L (product) / ha and 200–250 L (water) / ha was applied in accordance with good agriculture practice.[51] The applied solutions were also tested for the active substances (a. i.). The maximum field recommended rate corresponds to a recovery rate of 750 ng/μl boscalid and 1250 ng/μl pyraclostrobin in the spray solution based on the weighted sample. The concentrations of boscalid and pyraclostrobin in the solutions were 98 and 138 ng/μl (13% and 11% potency of set value for a. i.) for Bochum, 714 and 1133 ng/μl (95% and 91%) for Braunschweig, 789 and 1368 ng/μl (106% and 109%) for Celle, 772 and 1357 ng/μl (103% and 109%) for Hohenheim and 650 and 1212 ng/μl (87% and 97%) for Veitshöchheim. The low level measured in the applied solution at Bochum was probably due to sampling error. The residues found on the plants were consistent with those found at the other sites, demonstrating the successful application. The day of application was defined as DAA0 (day after application).

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Sampling

To evaluate the entire pathway, the following relevant samples were collected: OSR inflorescences, pollen and nectar foragers, stored nectar and pollen, nurse bees, larval food jelly (royal jelly, worker jelly and drone jelly), and queen, worker, and drone larvae. All samples were collected in sterile containers at both sites following protocols described below and stored at -20°C until further preparation and analysis.

OSR inflorescence samples were collected before exposure at DAA-3, during exposure at DAA0+1 h (one hour after application), DAA1, DAA2, DAA4, and after exposure at DAA7. Ten flowering inflorescences were collected along a diagonal or in zig-zag across the field minimizing the variance in concentration resulting from application. Headland or field edges were excluded from sampling.

Nectar and pollen foragers were collected using a vacuum cleaner with a modified collection container to avoid injuring the bees. Approximately 200 returning honey bees were collected. Sampling was carried out at DAA-3, DAA0+1 h, DAA2, DAA4, and DAA8. As only pollen baskets and honey sacs were used for further analysis, samples were prepared under sterile conditions by colony and date. Pollen baskets of pollen foragers were collected from their corbicula, and honey sacs were dissected from nectar foragers.

Plant inflorescence, pollen basket and honey sac samples were pooled per location, treatment site and time point for further analysis.

At least 1 gram of stored nectar and pollen, as well as approximately 100 in-hive bees directly from brood frames, were collected at DAA-2, DAA4, and DAA8. It was ensured that samples were not contaminated with wax during sampling. Worker bees perform different tasks during their early development and this suggests that bees are in different parts of the colony. We assume that nursing bees will predominantly be found on brood frames and thus we conclude that the collected in-hive bees are predominantly nursing bees. Heads of in-hive bees were dissected to evaluate their gland development and compared to a reference nursing bee (Figure S2). To evaluate the residue transfer from nursing bees to larval food jelly, only the heads of in-hive bees were used for the analysis, as the jelly is produced only in the hypopharyngeal glands and mandibular glands of the heads.

All (royal/queen, worker and drone) jelly and larvae samples were collected at DAA-3, DAA4, and DAA8. Cells containing larvae of stadium L3 to L4 were selected for worker and drone jelly collection, as they contain the largest quantity of food jelly during larval development.[24] Brood combs with age matching larvae were selected and on-sitting bees were carefully brushed off. Approximately 1,000-2,000 cells were sampled for one gram of worker and drone jelly. The larvae were pulled out of the cells without damaging them and were sampled in a collection tube. The larval jelly was then collected using a micro spoon without wax contamination. To ensure the availability of drone brood at the appropriate age, drone brood combs were provided to the colonies beforehand.

Royal jelly collection was performed using established queen rearing methods. Young L1 worker larvae were carefully removed from cells and were placed in polystyrene queen rearing cups. Around 20 cups were provided and placed in a rearing comb in the center brood frame and royal jelly was collected after three days as described above.

In autumn 2022, bee bread, honey and wax samples were collected to estimate the remaining residue load in colonies before overwintering. One gram of bee bread and honey was collected from each colony and one pool sample of beeswax per site and location. After overwintering, in spring 2023, bee bread and honey were collected again as well as approximately 100 in-hive bees to evaluate the remaining residues after overwintering.

Residue analysis

Sample preparation

Bee heads. The samples of bee heads (on average 0.6 g (BS, BO), 0.8 g (HO, VH) and 0.5 g (CE)) were weighed into a glass centrifuge tube. A surrogate standard solution (boscalid-d4, pyraclostrobin-d6, c = 2.5,1 ng/μL, 26.6 μL) and 20 mL of an acetone/water-mixture (2:1 *v/v*) were added to each sample. The tubes were closed and left to stand for 15 min. The samples were homogenized with an Ultra-Turrax for three minutes and subsequently centrifuged (10 min at 1690 × g). 15 mL of the supernatant were removed, and after adding 5 mL of sodium chloride-solution (20%) to this aliquot, transferred onto a disposable ChemElut cartridge (20 mL, unbuffered; Agilent, Santa Clara, CA, USA). 15 min later the samples were eluted twice with 50 mL dichloromethane. The combined eluates were rotary evaporated to approximately 2 mL (35°C water bath temperature), then transferred to a graduated tube and evaporated to dryness with nitrogen. The residual extract was re-dissolved with acetonitrile (1 mL) containing the internal standards using an ultrasonic device (10 s), and stored in the freezer (-18°C) over night. Eight of the internal standards are isotope-labeled. The concentrations in the measuring solution depend on the sensitivity of the measuring systems. Cold samples were filtered (syringe filter: PTFE 25 mm, 0.2 μm) into a sample vial and further stored in the freezer until measurement. Based on the described procedure, the measuring solution contains 75% of the sample weight.

Bees. The samples (30 bees: on average 4.3 g) were weighed into glass centrifuge tubes. The surrogate standard solution (see above, 100 μL) and 30 mL of an acetone/water-mixture (2:1 *v/v*) were added to each sample. The further course of sample processing up to the concentration and the final volume of the measuring solution (in this case: 1 mL) corresponds to the procedure described for bee heads. Based on the procedure described, the measurement solution contains 50% of the sample weight.

In hive samples. 0.8 g bee bread / pollen, 1 g nectar / honey sac, 0.9 g jelly or 0.9 g larvae were weighed into a glass centrifuge tube. The surrogate standard solution (see above, 26.6 μL) and 20 mL of an acetone/water-mixture (2:1 *v/v* bee bread / pollen and larvae, 3:1 *v/v* nectar / honey sac and jelly) were added to each sample. Subsequently, residue analysis was continued as described for the bee head samples. In this procedure, the measuring solution contains 75% of the sample weight.

Plant material. The samples (pre-homogenized by a knife-mill) (approx. 5 g) were weighed into glass centrifuge tubes and the surrogate standard solution (see above, 50 μL) and 30 mL of an acetone/water-mixture (3:1 *v/v*) were added. 15 mL of the supernatant

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were removed, and after adding 5 mL of sodium chloride-solution (20%) to this aliquot, transferred onto a disposable ChemElut cartridge (20 mL, unbuffered; Agilent, Santa Clara, CA, USA). 15 min later the samples were eluted twice with 50 mL dichloromethane. The combined eluates were rotary evaporated to dryness (35°C water bath temperature). The further course of the sample processing up to the concentration and the final volume of the measuring solution (in this case: 2.5 mL) corresponds to the procedure described for bee samples. Based on the procedure described, the measurement solution contains 50% of the sample weight. The treatment samples were diluted 1:100 with acetonitrile, as a high content was assumed.

In addition to the actual question of the project, the plant samples were also analyzed for 288 target substances using a full screening approach (Data S1B). The sample preparation was run analogously to the described plant preparation with additional surrogate standard solutions (acetamiprid-d3, chlorpyrifos-d10, pirimicarb-d6, c = 2, 2, 1 ng/μL, 50 μL). The full screen analysis included the following substances: Abamectin, Acephate,Acetamiprid, Acrinathrin, Alachlor, Alanycarb, Aldicarb, Aldicarb-sulfone, Aldicarb-sulfoxid, Allethrin, alpha-Cypermethrin, alpha-Endosulfan, alpha-HCH, Amisulbrom, Amitraz, Amitraz-metabolite BTS 27271, Amitraz-metabolite BTS 27919, Azadirachtin, Azamethiphos, Azinphos-ethyl, Azinphos-methyl, Azoxystrobin, Bendiocarb, Benfuracarb, Benthiavalicarb-isopropyl, Benzoximate, beta-Cyfluthrin,beta-Endosulfan, beta-HCH, Bifenazat, Bifenox, Bifenthrin, Bromopropylate, Bromoxynil, Bromuconazole, Buprofezin, Butoxycarboxim, Captan, Carbaryl, Carbendazim, Carbofuran, Carbophenothion, Carbosulfan, Carboxin, Carfentrazon-ethyl, CEKAFIX, Chlorantraniliprole, Chlordimeform, Chlorfenapyr, Chlorfenvinphos, Chlorpyrifos, Chlorpyrifos-methyl, Chlorthalonil, Chlorthiamid, Clofentezine, Clomazone, Clothianidin, Clothianidin-metabolite TZMU, Clothianidin-metabolite TZNG, Coumaphos, Cyantraniliprole, Cyazofamid, Cyflufenamid, Cymiazole, Cymoxanil, Cypermethrin, Cyphenothrin, Cyproconazole, Cyprodinil, Cyromazine, DEET, Deltamethrin, Demeton-S-methylsulfone, Diafenthiuron, Dialifos, Diazinon, Dichlobenil, Dichlorvos, Diethofencarb, Difenoconazole, Diflubenzuron, Diflufenican, Dimethachlor, Dimethenamid-P, Dimethoate, Dimethomorph, Dimoxystrobin, Dinotefuran, Diuron, Empenthrin, Endosulfansulfat, Epoxiconazole, Esfenvalerate, Ethiofencarb, Ethofumesate, Ethoprophos, Etofenprox, Etoxazole, Famoxadone, Fenamidone, Fenamiphos, Fenarimol, Fenazaquin, Fenhexamid, Fenitrothion, Fenoxaprop-P-ethyl, Fenoxycarb, Fenpropidin, Fenpropimorph, Fenpyroximate, Fipronil, Fipronil-carboxamid, Fipronil-desulfinyl, Fipronil-sulfid, Fipronil-sulfon, Flonicamid, Flonicamid-metabolite TFNA, Flonicamid-metabolite TFNG, Fluazifop, Fluazifop-P-butyl, Fluazinam, Fludioxonil, Flufenacet, Flufenoxuron, Fluopicolide, Fluopyram, Fluoxastrobin, Flupyradifurone, Fluquinconazole, Fluroxypyr-1-methylheptylester, Flurtamone, Flusilazole, Flutriafol, Fluxapyroxad, Folpet, Fonofos, Fosthiazate, Fuberidazole, Furathiocarb, Heptenophos, Hexachlorobenzene (HCB), Hexaconazole, Hexaflumuron, Hexythiazox, Icaridin, Imazalil, Imidacloprid, Imidacloprid-5-hydroxy, Imidacloprid-olefin, Imiprothrin, Indoxacarb, Ioxynil, Ipconazole, Iprodion, Iprovalicarb, Isoproturon, Isoxaben, Kresoxim-methyl, lambda-Cyhalothrin, Lindane (gamma-HCH), Lufenuron, Malathion, Mandipropamid, MCPA, Mecoprop, Mefentrifluconazole, Mepanipyrim, Mepronil, Metaflumizone, Metalaxyl-M, Metazachlor, Metconazole, Methamidophos, Methidathion, Methiocarb, Methiocarb-sulfon, Methiocarb-sulfoxid, Methomyl, Methoxychlor, Methoxyfenozide, Metofluthrin, Metrafenone, Metribuzin, Mevinphos, Milbemectin mixture of A3 and A4, Momfluorothrin, Myclobutanil, Naphthalin, Napropamid, Nicotine, Nitenpyram, Novaluron, Omethoate, Orange oil ((R) (+) Limonene), Oxydemeton-methyl, Paclobutrazol, Paraoxon, Parathion, Parathion-methyl, Penconazole, Pencycuron, Pendimethalin, Pentachlorobenzene, Pentachlorophenol, Permethrin, Phenothrin, Phosalone, Phoxim, Picoxystrobin, Piperonylbutoxide, Pirimicarb, Pirimicarb-desmethyl, Pirimicarb-desmethyl-formamid, Pirimiphos-methyl, Prallethrin, Prochloraz, Prochloraz-metabolite BTS 40348, Prochloraz-metabolite BTS 44596, Procymidon, Profenofos, Propachlor, Propamocarb, Propaquizafop, Propargite, Propiconazole, Propoxur, Propyzamid, Proquinazid, Prosulfocarb, Prothioconazol-desthio, Pymetrozine, Pyrazophos, Pyrethrine, Pyridaben, Pyridalyl, Pyrimethanil, Pyriproxyfen, Quinoclamine, Quinoxyfen, Rotenone, Sulfur, Silthiofam, Spinetoram (Mixture of Spinetoram J & L), Spinosad (mixture of spinosy A & D), Spirodiclofen, Spiromesifen, Spirotetramat, Spiroxamine, Sulfoxaflor, tau-Fluvalinate, Tebuconazole, Tebufenozide, Tebufenpyrad, Teflubenzuron, Tefluthrin, Terbufos, Tetrachlorvinphos, Tetraconazole, Tetradifon, Tetrahydrophthalimide-cis-1,2,3,6, Tetramethrin, Thiabendazole, Thiacloprid, Thiacloprid-amide, Thiamethoxam, Thymol, Tolclofos-methyl, Tolylfluanid, Transfluthrin, Triadimenol, Triazamate, Triazophos, Triazoxid, Tribenuron-methyl, Trichlorfon, Trifloxystrobin, Triflumuron, Trifluralin, Triforine, Triticonazole, Vamidothion, Vinclozolin, Zeta-Cypermethrin, Zoxamide

Wax. The samples (pre-homogenized by a knife-mill) (approx. 5 g) were weighed into glass centrifuge tubes and the surrogate standard solution (see above, 50 μL) and 30 mL of an acetone/water-mixture (3:1 *v/v*) added. The further course of the sample processing up to the concentration and the final volume of the measuring solution (in this case: 2.5 mL) corresponds to the procedure described for bee samples. Based on the procedure described, the measurement solution contains 50% of the sample weight.

Equipment and measurement conditions

LC-MS/MS. Two different measurement systems were used. First, Nexera X2 HPLC system (SHIMADZU Corp., Kyoto, Japan) coupled to a triple quadrupole mass spectrometer Q TRAP 6500+ (SCIEX, Framingham, MA, USA) equipped with an electrospray ionization (ESI) source. The measurements were performed only in the positive mode.

The mass spectrometric parameters were used in the positive mode only for boscalid and pyraclostrobin as well as were as for the full screen analysis (see Data S1F).

The chromatographic separations were performed on a Raptor ARC-18 column (100 × 2.1 mm; 2.7 μm, 90 Å) with a pre-column Raptor ARC-18 column (5 × 2.1 mm; 2.7 μm) (both Restek). The column oven temperature was set to 40°C (boscalid and pyraclostrobin) or 50°C (full residue analysis) and the autosampler tray temperature was set to 15°C. The injection volume was 2 μl (boscalid and pyraclostrobin) or 5 μl (full residue analysis).

The samples were analysed with the mobile phases (A) methanol and (B) water; both solvents contained 2 mmol ammonium formiate and 0.2% formic acid. Flow rate and gradient are shown in Data S1G.

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The second LC-MS/MS-system used was Prominence UFLC XR HPLC system (SHIMADZU Corp., Kyoto, Japan) coupled to a triple quadrupole mass spectrometer 4000 QTRAP (SCIEX, Framingham, MA, USA) equipped with an electrospray ionization (ESI) source. The measurements were performed only in the negative mode for full screen analysis of plant material samples. The mass spectrometric parameters in the negative mode can be found in Data S1F.

The chromatographic separations were performed as described above for the full residue analysis. Flow rate and gradient are shown in Data S1G.

GS-MS/MS and GC-MS for full residue analysis of plant samples. Plant samples were screened additionally via GC-MS/MS and GC-MS, since not all 288 active substances of full screen can be detected with the LC-MS/MS.

The GC-MS/MS system used was a TSQ 8000 Evo (triple stage quadrupole mass spectrometer) with Trace GC 1310 and TriPlus RSH autosampler (Thermo Fisher Scientific Inc, Waltham, MA, USA). The measurements were run in the negative chemical ionization (NCI) and the electron ionization (EI) mode. The autosampler tray temperature was set to 10°C. A split/splitless-injector was used at a temperature of 250°C. The injection conditions were splitless with 1.5 min SL-time. The injection volume was 1 μL. The chromatographic separations were performed on a Zebron ZB-MultiResidue-1 column (30 m + 10 m Guardian × 0.25 mm × 0.25 μm; Phenomenex) with helium (5.0) as carrier gas (1.2 mL/min, constant flow). The oven temperature program in the NCI mode was: 90°C (2 min) > 10°C/min > 320°C (10 min). The oven temperature program in the EI mode was: 90°C (2 min) > 20°C/min > 320°C (2 min). The mass spectrometric parameters in the NCI mode and mass spectrometric parameters in the electron ionization (EI) mode can be found in Data S1F.

GC-MS. The GC-MS system used was an ISQ 7000 (single stage quadrupole mass spectrometer) with Trace GC 1310 and TriPlus RSH autosampler (Thermo Fisher Scientific Inc, Waltham, USA). The autosampler tray temperature was set to 10°C. A split/splitless-injector was used, the temperature was 250°C and the injection conditions were splitless with 1.5 min SL-time. The injection volume was 1 μL. The chromatographic separations were performed on a Zebron ZB-MultiResidue-1 column (30 m + 10 m Guardian × 0.25 mm × 0.25 μm; Phenomenex) with helium (5.0) as carrier gas (1.2 mL/min, constant flow) and the following oven temperature program: 90°C (2 min) > 5°C/min > 320°C (10 min). The mass spectrometric parameters in the negative chemical ionization (NCI) and mass spectrometric parameters in the electron ionization (EI) mode can be found in Data S1F.

Method validation

Validation studies were performed to determine recovery rates (REC), limits of detection (LOD) and limits of quantification (LOQ). Predominantly residue-free control samples from different years of bees (2020), bee heads (2020), honey (2018), pollen (2013, 2018), larvae (2020) and wax (2018) were spiked with different amounts of the fungicides (boscalid and pyraclostrobin) extracted in 5 replicates as described for the samples. Bees, bee heads, pollen (2018), and honey were spiked with 20 μg/kg and pollen (2013), larvae and wax with 50 μg/kg. However, this was not achievable for wax, as the available material contained residues of some of the target substances.

For the determination of recovery rates, detection and quantification limits, matrix matched reference standards were prepared with the following concentration levels: 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 pg/μl.

For the data obtained by LC-MS/MS, the LOD was determined as the lowest concentration at which at least two MRM or SRM were detectable whose signals were three times higher than the background noise of the chromatogram and whose ratio was within the range of the required criteria.[29]

The next highest concentration of calibration standards above the detection limit was determined to be the LOQ.

It should be noted that the available material for the matrix-matched standards is usually different from the real samples. These differences in matrix properties may lead to slight differences in the LOD and LOQ determined using the matrix-matched standards measured along with the samples.

LOD and LOQ values were calculated in μg/kg for each individual sample based on the actual weights (Data S1A).

According to the SANTE document,[29] mean recoveries from initial validation for all analytes within the application range of a method should be in the range of 70-120%, with a corresponding repeatability RSD $\leq$ 20% (RSD = relative standard deviation). In exceptional cases, mean recoveries outside the range of 70-120% may be accepted if they are consistent (RSD $\leq$ 20%), but the mean recovery must not be less than 30% and not exceed 140%.

In the project, surrogate standards (surrogates) were added to each sample at the beginning of the preparation. Due to availability, the isotopically labelled (deuterated) "twins" of the target substances were used in this project.

Assuming that these substances behave very similar throughout the analysis, it is possible to convert the results for the target substances to 100% using the recovery rate for the surrogate standards. In this way, the matrix effects are compensated for in the measurement and the recovery rate flows directly into the results.

The additional experiment (method validation) was carried out once in fivefold repetitions, measured repeatedly in advance and in each case with the samples of the five locations and should show to what extent the results for the target substances and the deuterated surrogate standards actually agree and which variations can be assumed.

The graphical summaries of the results for the individual locations show how well this procedure worked (Figure S3). The recovery rates for the target substances and the deuterated surrogate standards are almost identical in all matrices. It is important that the surrogate standards follow the "swings" of the recovery rate up and down, which was consistently the case.

The mean recovery rates for pyraclostrobin varied only slightly across matrices and locations and lie between just under 70% and a good 80%. In this case, the relative standard deviations were also below 10% across matrixes and locations.

The mean recovery rates for boscalid varied considerably more across matrices and location and lie overall - with one exception - between 70% and 120%. The relative standard deviations also varied considerably more - with the exception of the matrix "honey". This was especially true (and almost as expected) for the matrix "pollen", but also for the matrix "bee heads".

For the matrix "bee heads" and the active ingredient boscalid, comparatively low mean recovery rates of about 50% were achieved for the location "Hohenheim", for the target substance and the deuterated twin. The evaluation of the measurement data was checked several times. There is no metrological explanation for these results. It cannot be due to the additive itself, as the results for pyraclostrobin do not differ from the other locations.

With this procedure, there is no under- or overestimation of the results and a consideration of the data taking into account separately achieved recoveries is not necessary.

QUANTIFICATION AND STATISTICAL ANALYSIS

LC-MS/MS-systems (Q trap 6500+ and Q trap 4000, SCIEX, Framingham, MA, USA) were used for the identification and quantification of boscalid and pyraclostrobin in the samples. The LC-MS/MS and the GC-MS(/MS)-systems (TSQ 8000 Evo, Thermo Fisher Scientific Inc, Waltham, MA, USA) were also used for the identification and quantification of the active substances in the full screen analysis of plant samples. All substances were identified by their retention time and three MRM-transitions (multiple reaction monitoring). For quantification, the internal standard method (relative peak areas) with matrix-matched calibration standards (concentrations: 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 5, 10, 20, 50, 100, 200, 500 pg/μL acetonitrile) was used. If extracts had to be diluted (1:100 (v/v)) the substance contents in the samples were determined using reference standards in the solvent. At the beginning of the residue analysis, isotope-labelled surrogate standards matching the target substances were added to each sample. This method takes the matrix effect and the recovery rate of the target substances for each individual sample into account, as these unlabeled or deuterated target substances behave very similarly in the analytical process. Therefore, this study presents the results for the target substances corrected using the recovery of the surrogate standard. The results shown are means of duplicate injections of sample extracts.

All statistical analyses were performed in R (version 4.2.2) with the user interface RStudio (version 2022.12.0). A detailed overview of the sample sizes can be found in Data S1A. For further analysis and data plotting all samples with no detection (ND) or detections below the limit of quantification or detection (<LOQ, <LOD) were set to zero. Mean values or median values reported in this article were calculated only from samples with positive detection. Regression analysis for residue data of plant inflorescence, pollen basket and honey sac data was analyzed using the *glmmTMB* function of *glmmTMB* package (version 1.1.8)[59] and model fit was visually inspected using the *DHARMa* package.[60] Best model fit was achieved with log 10 transformed data. Based on this model, the dissipation time (DT_{50}) was determined. Residue data of stored pollen, stored nectar, bee heads, jelly samples and larvae were compared using the *glmmTMB* and *DHARMa* packages. Sampling date and sample type were defined as explanatory factors for residue concentrations with a Gaussian distribution. Estimated marginal mean comparison of model output was calculated using the *emmean* function from *emmeans* packages[61] with a Bonferroni-Holm adjustment for multiple testing.

Risk quotients were calculated based on the US Environmental Protection Agency (EPA) BeeREX[49] assessment tool described in Thompson, 2021[50] (Data S1C). In short, risk quotient was calculated by a division of the exposure in μg/bee by the ecotoxicological endpoint values LD_{50} (lethal doses) for acute exposure or NOED (no observed effect dose) for chronic exposure in μg/bee if available. Four different evaluations were performed: 1) with the default settings of the BeeREX model, 2) with the pollen and nectar data measured in the bee collected material (pollen baskets and honey sacs), 3) with the pollen and nectar data detected in the stored pollen and nectar, and 4) with the expected residue concentrations derived from residue unit doses (RUDs)[32] (Data S1D). The RQ for larvae in evaluations 2 and 3 was performed using the individual residue concentration measured in each jelly type. The exposure was set to the concentration in the analyzed matrix in μg/kg multiplied by the consumption rate in μg/bee. Ecotoxicological LD50 endpoints for boscalid and pyraclostrobin derived from the Pesticide Database of the European Union,[28] the database of the United States Environmental Protection Agency,[49] and Simon-Delso et al.[38,62] Risk quotients were calculated only if ecotoxicological data were complete and no extrapolation was carried out.