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Review

What about honey bee jelly? Pesticide residues in larval food jelly of the Western honey bee *Apis mellifera*

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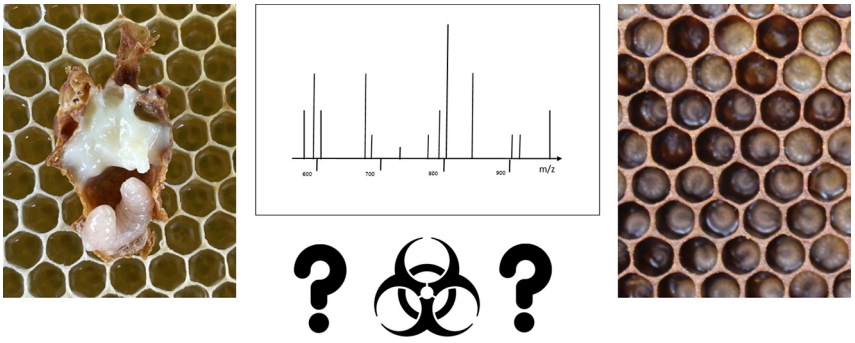
HIGHLIGHTS

- Review of current literature dealing with pesticide residues in food jelly of honey bees.
- Residues are detectable in royal and worker jelly, but no information available for drone jelly.
- Occurrence of residues mainly depends on application method and exposure scenario.
- Limited information about contamination pathway, accumulation or degradation of pesticides within different bee matrices, and effects on larval physiology.

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing loss of honey bee colonies is assumed to be caused by various factors such as habitat degradation, parasites, pathogens, or the exposure to environmental pollutants like pesticides in agriculture practice. Different bee-related products like honey, bee bread, wax, and pollen can be contaminated by pesticides and some of them might affect colony health. Stored nectar and pollen serve as nutritional sources for nurse bees to produce food jelly for queen, worker, and drone larvae and contaminants might be transferred. For the risk assessments, it is necessary to understand the occurrences of residues in larval food jelly and to evaluate factors influencing the concentration of contaminants. This review summarizes the current literature dealing with residue analysis of pesticides in food jelly to assess the pesticide transfer, to evaluate factors influencing pesticide appearance in jelly, and to deduce risk for larvae. Previous studies determined residues of different pesticides in royal jelly, and one in worker jelly. It was demonstrated that 30 out of 176 analyzed pesticides were detectable in different royal jelly samples. If residues remain in food jelly, this is mainly related to the used application and exposure method. It is shown that an artificial exposure (e.g., by forced feeding) results in higher detectable residues compared to field-realistic exposure scenarios (e.g., spray applications on plants). All detected concentrations were predominantly below the toxicity values for honey bee larvae, but sub-lethal effects should be considered. Moreover, it was demonstrated that there are still knowledge gaps about the contamination pathway of pesticides, dilution or accumulation factors within the hive, degradation time in bee-related matrices, and the impact on larval physiology. Filling those gaps is of major importance to consider realistic exposure scenarios in the risk assessment and to allow for sufficient protection level of honey bee brood.

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Contents

1. Introduction

Honey bees are essential for agriculture by providing pollination service and for the economy by their production of honey, propolis, wax and royal jelly. They forage on different plant products like nectar, honey dew, or pollen, which represent their nutritional resources. Collected food is brought back to the hive and stored as honey or bee bread. While foraging, bees are exposed to pollutants, pathogens, and parasites, which could lead to diseases and represent risks for colonies. In particular, habitat degradation, diseases, intoxication by pesticides, and malnutrition have been proposed as drivers of increasing numbers of colony losses (Osterman et al., 2021). Pesticides like insecticides, fungicides, and herbicides are applied to control insect pests, fungal diseases, undesirable vegetation, and to secure the crop yield. The prophylactic use of pesticides increases the risk of accumulation within the bee colony and environment (Goulson et al., 2015). Pesticides and their metabolites account for several lethal as well as sub-lethal effects such as reduced learning or colony performance of non-target pollinating insects, like honey bees, with insecticides proven to be the most harmful (Alkassab and Kirchner, 2017; Cullen et al., 2019; Desneux et al., 2007; Johnson, 2015). Hence, information about residue occurrence of pollutants such as pesticides in in-hive products are necessary to enable risk evaluation of colony and larval development. For a better understanding of the topic, different matrices have been evaluated for pesticide residues. Most of the studies focused on pollen, wax, honey, and detected single compounds or mixtures of different pesticides (El Agrebi et al., 2020; Mitchell et al., 2017).

However, pesticide residues not only remain in honey, wax, or pollen, but are further transferred to other hive matrices like larval food jelly (Davis and Shuel, 1988; Kast and Kilchenmann, 2022). Contaminated nectar and pollen are collected by foraging honey bees, incorporated, and stored as honey and bee bread, where pesticide residues can accumulate (Dively et al., 2015). These products are the nutritional basis for nurse bees to produce larval food in their hypopharyngeal and mandibular glands (Winston, 1987). The ratio of glandular secretions within food jelly is dependent on the different larvae sexes, castes, and age. Queen larvae are continuously fed with a mixture of glandular secretions, called royal jelly, during their entire life cycle, whereas worker and drone larvae receive a different secretion mixture added with pollen and honey-sac content, which changes during larval development (von Planta, 1888; Winston, 1987).

Several reports demonstrated toxicity of different pesticides with effects on larval development (DeGrandi-Hoffman et al., 2013; Kast and Kilchenmann, 2022; Shi et al., 2020). For example, a cross-fostering experiment with the fungicide boscalid combined with the fungicide pyraclostrobin reduced survival of worker larvae (Fisher et al., 2021). Milone et al. (2021) reported an impact of a multiple-pesticide exposure on the nutritional composition of royal jelly. They detected changes in the metabolome, proteome or phytosterol composition in royal jelly of colonies treated with pesticides. In contrast, others did not detect any significant negative effects on larval development driven by pesticide exposure (Dai et al., 2019; Wood et al., 2020). All of them considered concentrations of pesticides at field relevant levels. Beyond the larval well-being, measuring residues in food jelly is of relevance in context of human food safety. By the consumption of honey or pollen and the use of royal jelly in traditional and modern medicine (Pasupuleti et al., 2017), humans might also be exposed to pesticide residues remaining in these products.

So far, the toxicological effects on adult bees as well as on larvae were assessed, and residues were described in different hive matrices. Primarily, detection was realized in the framework of food safety. However, literature is lacking information about: 1) Residue analysis of worker and drone jelly, 2) Main factors that have an impact on residue occurrence, 3) Which concentration of residues can be detected in food jelly? 4) Are the remaining residues toxic for larvae and can they lead to sub-lethal effects? 5) Impact of residues in larval food jellies on larval physiology, 6) Transmission pathways of pesticides within the colony, 7) Do pesticides accumulate in bee matrices or do residue concentrations decrease throughout the pathway from plant to jelly? and 8) Degradation time of pesticides in bee matrices like pollen, honey, and wax. Here, we address these open topics and aim to answer the first four questions using published data and discuss the remaining questions in context of risks for honey bee larvae.

2. Material and methods

The online library Web-of-Science was used to search for relevant literature in the field of pesticide residue analysis in honey bee food jelly. The literature search was performed using the following terms: [(royal, worker, and drone) jelly], [honey bee], [pesticide], [residue], and specific pesticide names of the commonly used pesticides within these studies (access date March 30th, 2022). Overall, 42 studies dealing with pesticides in jelly were found. Criteria for including resulting studies for further evaluations were 1) the detection of pesticide residues in either royal, worker or drone jelly and 2) method application on at least samples which had been produced for commercial purposes (e.g., royal jelly samples bought in supermarkets and drug stores). Articles with a focus on A) the development of a detection method for residue analysis without practical consideration, and B) of veterinary drugs, were excluded from further analyses (further information in Table S1). After filtering for the given conditions, 24 studies remained and 18 were excluded.

For discussing the effects and observations after pesticide treatment the following data were extracted from all relevant articles: type of food jelly (royal, worker, or drone jelly), substance (pesticide), sampling time point (day after initial exposure), application method and duration (acute and chronic), the initial concentration used for exposure [ng/g], and the detected concentration in food jelly [ng/g]. The exposure scenarios described within the studies can be divided into two distinct groups: artificial exposure or field-realistic exposure. Studies using artificial exposure were based on using spiked diets in form of syrup or pollen, which was applied *ad libitum* inside the hive over time. In turn, in field-realistic assays, substances were applied via fumigation inside the hive (beekeeping practice) or via spray application of plants (agricultural practice) based on the required application recommendations. To compare the different studies of different exposure scenarios the pesticide transfer was calculated. The

term is defined by dividing the detected residue concentration in food jelly by the initially applied concentration given as a percentage.

Statistical analyses were done using the open-source software R (Version 4.1.3) and the integrated development environment RStudio (Version 2022.02.0). Correlations between pesticide transfer and sampling time point after initial exposure, or the octanol-water-partition coefficient, were done using a linear regression analysis with a significance level of 0.05.

3. Results and discussion

Overall, royal jelly samples were analyzed for 176 different pesticides and their metabolites, and finally 30 different substances were detected in 24 studies (Table 1). All studies assessed jelly samples for residues in a range of 1–16 different pesticides, despite one, which screened commercial royal jelly samples for 127 pesticides without detecting any residues (Martínez-Domínguez et al., 2014). Detected pesticide concentrations varied from 0.005 to 3,860.25 ppb. The most frequently detected substances were the acaricide coumaphos (eight times), the strobilurine fungicides azoxystrobin and pyraclostrobin (each three times), and the acaricide tau-fluvalinate (four times) (Table 1). Thirty-three percent of all studies used an artificial exposure scenario with 22 substances being detected, and 33% used a field-realistic exposure scenario resulting in seven cases of pesticide detection. Only coumaphos was detected in both exposure scenarios. The remaining 34% of the studies applied their specific detection method on commercial samples bought in supermarkets and only three substances out of 149 were detectable. Thus, 70% of the detected substances were related to an artificial exposure, 20% to a field-realistic exposure and 10% to commercial samples.

Only a single study focused on pesticide residues in worker jelly throughout larval development (Table 2) (Böhme et al., 2019). They fed contaminated pollen artificially inside the hive and screened the larval food for 13 pesticides and in 12 cases residues were detectable. The insecticide tau-fluvalinate was detected with the highest pesticide transfer, respectively 14%. To our knowledge, not any data for residues in drone jelly are currently available.

3.1. The impact of exposure time, duration, and application method on residue detectability

The methods of pesticide application varied from a field-realistic exposure via a spray application on plants like oil-seed rape (Li et al., 2017a, 2017b), to an artificial worst-case-like scenario of a continuous long-time oral exposure (Böhme et al., 2018; Dively et al., 2015; Milone et al., 2021; Milone and Tarpy, 2021; Ricke et al., 2021). In addition to spiked pollen patties, Milone and Tarpy (2021) reared queen larvae in contaminated wax cups. Thus, the larvae and royal jelly were double-exposed, which is assumed to have led to the higher amounts of pesticide residues (Table 1, Table S2). The used sampling or jelly production system influences the residue amount as well. It was shown that residue concentration was higher when sampling royal jelly from natural cells compared to sampling from artificial cells used for queen rearing (Karazafiris et al., 2022).

The pesticide transfer ranges in jelly from 0.00001% up to 58% (95% CI [1.91%, 9.49%]). To determine the main factors influencing if pesticides will remain detectable in jelly, these percentages were compared among application methods. Higher values were obtained for artificial exposure scenarios (Table 1, Table S2). For the field-realistic exposures, a pesticide transfer of maximum 0.13% was detected for residues, while samples of artificial exposures had a median of 1% with a range from 0.01% to 58.64% of residues. Artificial exposure, for example spiked pollen patties, which were directly applied inside the hive, strongly forced the exposure on larvae to the applied pesticide concentrations. The in-hive feeding facilitates the consumption of the contaminated food and minimizes the dilution of the residues. Furthermore, artificial exposure might cause accumulation in food jelly, which results in higher detections and residue concentrations. Exposure outside the hive represents a realistic dilution of residues and worker bees consequently might be exposed to lower concentrations while foraging. The sampling time point does not correlate with the pesticide transfer ($R = -0.0047, p = 0.3946$), which means that the application method and exposure scenario are the main factors influencing the occurrence and pesticide transfer in royal jelly (Fig. S1).

The highest pesticide concentrations were found in wax and pollen (El Agrebi et al., 2020; Mullin et al., 2010; Sanchez-Bayo and Goka, 2014), indicating that some pesticides can accumulate in lipophilic environments, for instance in wax due to its lipophilic physicochemical character (Giroud et al., 2019). Different analysis of wax samples from locations in Germany or Belgium showed that in 50% to 97.3% of the samples pesticide or veterinary drug residues were found in a range of 1 to 38 different substances each (Alkassab et al., 2020; El Agrebi et al., 2020). An analysis of adult bee, wax, pollen, and other bee-related matrices across North America showed that 60% of the in-hive samples were at least contaminated with one systemic pesticide (Mullin et al., 2010). Further, it was shown that capping wax, which is freshly produced by bees to seal honey cells, was significantly less contaminated than recycled wax of honey, brood, or pollen combs (El Agrebi et al., 2020). Wax obtained by commercial suppliers was found to be contaminated with the acaricide tau-fluvalinate although it was classified as pesticide-free or natural, and a transfer of tau-fluvalinate was detectable from treatment strips to larvae and pollen (Fulton et al., 2019). Thus, it can be concluded that residues remain longer in wax and wax recycling might lead to pesticide accumulation. Additionally, a positive correlation between residue concentrations in wax and in in-hive samples like beebread, honey, larvae, or pupae was proven (Alkassab et al., 2022). These high amounts of residues accumulated in wax might then be transferred in royal, worker, or drone jelly. However, the increasing pesticide transfer detected in royal jelly does not correlate with lipophilicity (Fig. S2), represented with the octanol-water partition coefficient of substances (pesticide transfer ~ octanol-water partition coefficient: $p = 0.2281$, $R = 0.0087$). Therefore, the outcome of this metadata analysis showed that the lipophilic character of a substance influences its residue occurrence in wax (Alkassab et al., 2022; Fulton et al., 2019) but not if residues will subsequently be transferred into jelly.

Commercial jelly samples lack information about their origin and exposure routes. These samples were screened mostly for a previously defined repertoire of the most used pesticides in beekeeping practice or agriculture such as acaricides or pyrethroid insecticides. The sample analysis was done without knowledge on the potential presence of the pesticides themselves. In commercial samples, 2.01% of the inspected pesticides were found. Consequently, those results should be taken with caution due to the lack of information on exposure, sampling conditions, and sample processing. This most likely explains the discrepancy between substances that were detected in samples of field or fumigation studies but not in commercial jelly samples (Table 1, Table S2).

As concluded the exposure scenario (i.e., artificial feeding of spiked samples) had the greatest influence on the detectability of residues in jelly. The exposure scenario should be considered depending on the research aim. An artificial exposure with spiked diets inside the hive or directly fed to the bees ensures the exposure to the active substance and potential effects on bees can be assigned. It represents a viable method in understanding the transfer routes of pesticides inside the hive, but the detected concentrations may not reflect residues in-field under natural conditions. If residue concentrations are aimed to be measured, a field-realistic scenario should be preferred. In agricultural practice, pesticides are applied to plants and bees might be exposed during their foraging flights. The foraging behavior and processing by the bees could lead to a decrease of residue concentrations and thus to lower pesticide transfer. Commercial jelly samples might be used only to validate detection methods, but conclusions should be taken with caution. In future studies, the methodology (exposure scenario) should be considered to enable comparisons with other studies. The pesticide transfer can differ significantly under different methodological conditions like larval age at time of jelly collection, storing method, sampling time point within the season, and honey bee sub-species. When samples are taken in early season during spring or directly after a *Varroa* treatment, the residue concentrations can be higher. A consistent experimental setup is mandatory over all studies.

Table 1
Summary of all pesticides and their metabolites found in royal jelly samples. All substances that have not been detected, but royal jellies have been screened for, are summarized separately in Table S2. The lipophilicity of each substance is given by their octanol-water partition coefficient, the higher the value the higher is their lipophilicity, or lower their hydrophilicity. Field-realistic exposure is defined as an application via fumigation or sprayed on plants based on the required agricultural or beekeeping practice. Artificial exposure is based on using spiked diets in form of syrup or pollen, which was applied *ad libitum* inside the hive over time. The pesticide transfer was calculated by dividing the detected concentration by the initial concentration given in percent. (NI — no information, NA — not available, ppb — parts per billion e.g. [ng/g], a.s. — active substance).

Substance	Class[1]	Sampling time point [days post application]	Application	Exposure scenario	Initial concentration (a.s.) [ppb]	Detected concentration [ppb]	Pesticide transfer [%]	Octanol-water partition coeff.	Reference
1,4-Dichlorobenzene	I	13	Exposed to fumigated wood	Field-realistic	30 g PDCB powder	82.2–1,520.6	NA	3.44	Tananaki et al., 2009
14C-Carbofuran	I	3	Feeding contaminated syrup	Artificial	12	1.97	16.42	1.80	Davis and Shuel, 1988
14C-Dimethoate	I	3	Feeding contaminated syrup	Artificial	12	2.87	13.91	0.75	Davis and Shuel, 1988
Acetamiprid	I	2	Feeding contaminated pollen (70 g)	Artificial	787.89	0.67–1.92	0.24	0.80	Böhme et al., 2018
Amitraz (metabolite)	A	48	Feeding contaminated diet (46 d)	Artificial	9–32,900	12.75–3,860.25	11.73	NA	Milone and Tarpy, 2021
Amitraz (metabolite)	A	3 days old larvae	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	NA	<7	NA	NA	Milone et al., 2021
Atrazine	H	48	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	25–61	3.94	6.46	2.70	Milone and Tarpy, 2021
Azoxystrobin	F	2	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	674.17	0.56–0.91	0.13	2.50	Böhme et al., 2018
Azoxystrobin	F	48	Feeding contaminated pollen (70 g)	Artificial	56–78	2.24	2.87	2.50	Milone and Tarpy, 2021
Bromopropylate	A	Commercial	NI	NI	NI	81	NA	5.40	Notardonato et al., 2014
Carbendazim	F	3–22	Exposed to sprayed plants	Field-realistic	432,000	77–550	0.13	1.48	Li et al., 2017b
Chlorantraniliprole	I	4	Feeding contaminated pollen	Artificial	26,000	45	0.17	2.86	Ricke et al., 2021
Chlorothalonil	F	48	Feeding contaminated diet (46 d)	Artificial	13,000–16,000	1,409.7–1,740.45	10.88	2.94	Milone and Tarpy, 2021
Chlorothalonil	F	3 days old larvae	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	7,700	<250	3.24	2.94	Milone et al., 2021
Chlorpyrifos	I	48	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	20–113	8.9–12.9	11.42	4.70	Milone and Tarpy, 2021
Coumaphos	A	7–56	Spray (50 ml/colony)	Artificial	0.64 g/l	10–92	NA	4.13	Balayannis, 2001
Coumaphos	A	1–42	Exposed to sprayed strips, chronic	Field-realistic (6 weeks)	100,000	0.269–0.477	0.0005	4.13	Karazafiris et al., 2022
Coumaphos	A	1–42	Exposed to sprayed strips, chronic	Field-realistic (6 weeks)	3,260	0.011–0.062	0.002	4.13	Karazafiris et al., 2022
Coumaphos	A	1–292	Exposed to sprayed strips and sampling after treatment	Field-realistic (42 days)	100,000	0.06–12.52	0.0125	4.13	Karazafiris et al., 2022
Coumaphos	A	48	Feeding contaminated diet (46 d)	Artificial	1,680–1,870	5.9–170	9.09	4.13	Milone and Tarpy, 2021
Coumaphos	A	3 days old larvae	Sprinkling onto bees	Field-realistic	4,700	5.93	0.16	4.13	Milone et al., 2021
Coumaphos	A	5–6	Sprinkling onto bees	Field-realistic	640,000	170–210	0.03	4.13	Skerl et al., 2010
Coumaphos	A	5–6	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	640,000	250–400	0.06	4.13	Skerl et al., 2010
Coumaphos (metabolite)	A	48	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	0–12	0.47–1.42	11.83	NA	Milone and Tarpy, 2021
DEET	I	48	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	0–92	7–8	8.69	2.18	Milone and Tarpy, 2021
Diflubenzuron	I	4	Feeding contaminated pollen	Artificial	66,000	367	0.56	3.89	Ricke et al., 2021
Dimoxystrobin	F	2	Feeding contaminated pollen (70 g)	Artificial	581.51	0.42–0.68	0.12	3.59	Böhme et al., 2018
Imidacloprid	I	40	Feeding contaminated diet (6 weeks)	Artificial	20–100	0.30–1.00	1.00	0.57	Dively et al., 2015
Metolachor	H	48	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	NA	25.2	NA	3.40	Milone and Tarpy, 2021
Metolachor	H	3 days old larvae	Feeding contaminated diet (46 d)	Artificial	NA	<25	NA	3.40	Milone et al., 2021
Propachlor	H	Commercial	NI	NI	NI	14.9	NA	1.60	Martínez-Domínguez et al., 2016

Table 1 (*continued*)

Substance	Class[1]	Sampling time point [days post application]	Application	Exposure scenario	Initial concentration (a.s.) [ppb]	Detected concentration [ppb]	Pesticide transfer [%]	Octanol-water partition coeff.	Reference
Propiconazole	F	4	Feeding contaminated pollen	Artificial	88,500	109	0.12	3.72	Ricke et al., 2021
Prosulfocarb	H	2	Feeding contaminated pollen (70 g)	Artificial	634.4	0.52–0.9	0.14	4.48	Böhme et al., 2018
Pyraclostrobin	F	2	Feeding contaminated pollen (70 g)	Artificial	730.44	0.52–1.07	0.15	3.99	Böhme et al., 2018
Pyraclostrobin	F	4	Feeding contaminated pollen (600 g)	Artificial	51,000	47–52	0.10	3.99	Johnson and Percel, 2013
Tebuconazole	F	3 days old larvae	Rearing in contaminated wax	Artificial	412	80	19.40	3.70	Raimets et al., 2022
t-Fluvalinate	A	2	Feeding contaminated pollen (70 g)	Artificial	721.02	1.56	0.22	7.02	Böhme et al., 2018
t-Fluvalinate	A	1–42	Spray (50 ml/colony), chronic	Field-realistic (6 weeks)	206,000	0.005–0.028	0.00001	7.02	Karazafiris et al., 2022
t-Fluvalinate	A	48	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	NA	189–230.9	NA	7.02	Milone and Tarpy, 2021
Thiacloprid	I	2	Feeding contaminated pollen (70 g)	Artificial	480.38	0.93–2.16	0.45	1.26	Böhme et al., 2018
Thiamethoxam	I	commercial	NI	NI	NI	0.15–0.25	NA	−0.13	Giroud et al., 2019
Thymol	A	48	Feeding contaminated pollen, rearing in contaminated wax (35 d)	Artificial	89–2,200	2–1,290	58.64	3.30	Milone and Tarpy, 2021
Thymol	A	3 days old larvae	Feeding contaminated diet (46 d)	Artificial	NA	34–447	NA	3.30	Milone et al., 2021
Triadimefon	F	3–22	Exposed to sprayed plants	Field-realistic	216,000	4–10	0.004	3.18	Li et al., 2017a
Triadimenol (metabolite)	F	3–22	Exposed to sprayed plants	Field-realistic	NA	1–17	NA	NA	Li et al., 2017a

[1] A — acaricide, I — insecticide, F — fungicide, H — herbicide.

3.2. Variance among food jellies and transmission

Throughout their development, honey bee larvae of different castes and sexes receive individual feeding intensities. While queen larvae are fed about 1,600 times, resulting in an average total amount of 1.5 g royal jelly, worker larvae receive only about 4 to 47 feeding visits depending on larval developmental stage (Jung-Hoffmann, 1966; Lindauer, 1952; Seifert et al., 2020). The amount of food jelly increases with developmental stage of worker and drone larvae, but measured quantities fluctuate within and among studies. Overall, queen larvae receive about one to two orders of magnitude more food than worker and drone larvae. (Table S3). Böhme et al. (2018, 2019) measured both, the residues remaining in royal jelly and in worker jelly after exposure to pollen patties provided or fed *ad libitum* inside the hive that were spiked with 13 different pesticides. The comparison of the findings in both studies shows that the remaining pesticide residues were higher in worker jelly compared to the residues detected in royal jelly (Table 1, Table 2). The pesticide transfer in worker jelly was 2.39% to 13.99%, and in royal jelly 0.12% to 0.45% (Böhme et al., 2018, 2019).

As already mentioned, food jelly for queen and worker larvae is produced by glands of the nurse bees and consists of a mixture of protein containing clear and lipid containing milky secretions (Haydak, 1970). Contrary to queen larvae, worker and drone larvae are additionally feed with pollen starting from day three of their development (Rortais et al., 2005; von Planta, 1888). Five percent of the 50 or 79 mg protein needed for worker or drone larval development originates from pollen (Hrassnigg and Crailsheim, 2005). During worker development, 2 to 7 mg of pollen is added to worker jelly (Böhme et al., 2019). Royal jelly is free of pollen or contains only trace amounts (Haydak, 1970; von Planta, 1888). Consequently, worker and drone jelly possess an additional contamination source via pollen compared to royal jelly. The expected positive correlation between the amount of pollen grains and pesticide residues in worker jelly was proven recently (Böhme et al., 2019). The latter demonstrated that the more pollen grains were incorporated into worker jelly, the higher residues of pesticides were detected.

For a better understanding of the contamination pathway of food jellies, it is necessary to evaluate the in-hive distribution and possible resulting dilution of substances. Only a few studies have focused on additional in-hive matrices and thus investigated pesticide distribution (Table S4). After pesticide application to the field, foragers collect contaminated nectar and pollen, store it in form of honey and bee bread, and nurse bees consume the stored food for producing food jelly. Consequently, contaminates will be transferred from plants via foragers into the colony, and further via nurse bees into the food jelly. Most of the previous studies applied pesticides via spiked pollen or syrup within colonies, which does not correspond to a field-realistic pathway but to a worst-case scenario. However, pesticide concentrations decrease with every transmission vector and only small amounts, with a pesticide transfer median of 0.5%, remained detectable in royal jelly (Table S4). The dilution, (i.e., the decrease of pesticide transfer) is caused by the transfer of contaminated material throughout the hive. Pesticides are sprayed on plants and will contaminate nectar. The nectar will be consumed by foraging bees, brought into the hive, received by in-hive worker bees, and they will process nectar to honey which dilutes residue concentrations. Nurse bees will share and consume amounts of these to produce larval food in their head glands which dilutes again the residues. The same might be a scenario from contaminated and stored pollen, via the bee bread stage (including several microorganisms), to larval food producing nurse bees. Only in one case the percentage of remaining residues in royal jelly amounted to 19.4% (Raimets et al., 2022). In this study, queens were reared directly in prepared contaminated wax cells and royal jelly was sampled three days after acceptance, which corresponds to an artificial exposure scenario (Raimets et al., 2022). The transfer of contaminants within the hive was further assessed with alternative environmental toxins like plant secondary metabolites. Lucchetti et al. (2018) examined the transfer of the phytochemical echimidine from bee bread into royal jelly within an *in vitro* approach and demonstrated that echimidine was transferred from provided pollen into bee bread and via the nurse bees into royal jelly. Finally, they could show that the detected residues measured in royal jelly were three orders of magnitude lower than those measured in bee bread and below the sub-lethal concentration examined for bee larvae.

Table 2
Summary of all pesticides and their metabolites found in worker jelly samples. All substances that have not been detected, but worker jellies have been screened for, are summarized separately in Table S2. The lipophilicity of each substance is given by their octanol-water partition coefficient, the higher the value the higher is their lipophilicity, or lower their hydrophilicity. Field-realistic exposure is defined as an application via fumigation or sprayed on plants based on the required agricultural or beekeeping practice. Artificial exposure is based on using spiked diets in form of syrup or pollen, which was applied *ad libitum* inside the hive over time. The pesticide transfer was calculated by dividing the detected concentration by the initial concentration given in percent. (NI — no information, NA — not available, ppb — parts per billion e.g. [ng/g], a.s. — active substance).

Substance	Class[1]	Sampling time point [days post application]	Application	Exposure scenario	Initial concentration (a.s.) [ppb]	Detected concentration [ppb]	Pesticide transfer [%]	Octanol-water partition coeff.	Reference
Acetamiprid	I	3–6	Feeding contaminated pollen (60 g)	Artificial	9,021.8	99.5–871.0	9.65	0.80	Böhme et al., 2019
Azoxystrobin	F	3–6	Feeding contaminated pollen (60 g)	Artificial	835.4	3.6–76.9	9.21	2.50	Böhme et al., 2019
Boscalid	F	3–6	Feeding contaminated pollen (60 g)	Artificial	653.7	4.8–37.7	5.77	2.96	Böhme et al., 2019
Dimethenamid-P	H	3–6	Feeding contaminated pollen (60 g)	Artificial	908.5	3.8–21.7	2.39	1.89	Böhme et al., 2019
Dimoxystrobin	F	3–6	Feeding contaminated pollen (60 g)	Artificial	595.4	3.1–35.0	5.88	3.59	Böhme et al., 2019
Methiocarb	I	3–6	Feeding contaminated pollen (60 g)	Artificial	1,115.2	8.2–26.6	2.39	3.18	Böhme et al., 2019
Prosulfocarb	H	3–6	Feeding contaminated pollen (60 g)	Artificial	731.1	3.5–24.8	3.39	4.48	Böhme et al., 2019
Pyraclostrobin	F	3–6	Feeding contaminated pollen (60 g)	Artificial	772.6	2.9–47.5	6.15	3.99	Böhme et al., 2019
Tebuconazole	F	3–6	Feeding contaminated pollen (60 g)	Artificial	2,552.6	20.6–125.3	4.91	3.70	Böhme et al., 2019
t-Fluvalinate	I	3–6	Feeding contaminated pollen (60 g)	Artificial	469.5	8.9–65.7	13.99	7.02	Böhme et al., 2019
Thiacloprid	I	3–6	Feeding contaminated pollen (60 g)	Artificial	445.5	6.0–45.6	10.23	1.26	Böhme et al., 2019
Triadimenol	F	3–6	Feeding contaminated pollen (60 g)	Artificial	935.5	25.7–51.8	5.54	3.18	Böhme et al., 2019

[1] A — acaricide, I — insecticide, F — fungicide, H — herbicide.

A major problem in evaluating the risk of pesticides for bees is the lack of information on the degradation of pesticide residues in in-hive matrices like beebread (Roessink and van der Steen, 2021). Degradation depends on several factors such as temperature, humidity, half-life, pH, and UV-radiation. The degradation time in water is the only information available for detectable substances (Pesticide Properties Database (PPDB), 2021). However, extrapolation from water-based degradation to food jelly is not possible due to its different physicochemical character such as the more acidic pH. Nevertheless, the degradation of pesticides in pollen might be neglectable due to the seasonal fast consumption of stored pollen. When pollen rich sources are available, bees consume 75% of the stored pollen within a week after collection and overall 95% after two weeks (Roessink and van der Steen, 2021). Thus, it can be assumed for a worst-case scenario that bees consume the initial exposure concentration applied to pollen during the flowering season. Degradation of pesticides in bee bread, the processed, stored pollen, might be relevant only in late summer to autumn when bees prepare for overwintering and store pollen over months.

The comparison of the pesticide transfer between the different food jellies highlights that most studies focus on the residue analysis in royal jelly. Only one dealt with residues in worker jelly and none with drone jelly. Further evaluations should investigate all types of jellies and compare their pesticide transfer as there are known differences in jelly composition and feeding routines. Further, the contamination pathway starting with the exposed plants, via the foraging honey bee to stored material, and the nursing bees who feed the larvae, should also be investigated. This would gain a better understanding on the distribution of contaminants, respectively pesticides, within the hive and enable more specific prevention measures.

3.3. Larval risk

Pesticide residues were detected in a wide range of concentrations and thus it should be investigated whether these residues can be toxic to bee larvae. The toxicity of chemicals to honey bee larvae are calculated based on the mortality of the test organisms given as lethal dose (LD_x) or no observed effect dose (NOED), specified in μg (substance) per larva. To determine the individual risk for larvae, the approximate incorporated amount of the substance per larva (dose) was calculated here. Thereto, the detected residue concentrations (ppb) were multiplied by 1.5 g, which corresponds to the average total amount of royal jelly consumed by a single queen larva (Jung-Hoffmann, 1966). Altogether, the calculated dose based on the detected concentrations in food jelly were predominantly below the toxicity values (median: 46.46-times lower) determined for bee larvae independent of the exposure scenario (Table S5). Consequently, larvae might not be harmed lethally due to the remaining residues in food jelly, considering a worst-case assumption of 1.5 g consumption. However, in agricultural practice, a mixture of different pesticides and pesticide adjuvants are applied (Wernecke et al., 2019, 2021). Thus, bees are exposed to a cocktail of those substances. Different modes of action or the physicochemical character of the active substances may affect their interaction and detoxification, and consequently could lead to additive or synergistic effects on bees (Wernecke et al., 2019, 2021).

Indirect sub-lethal effects related to queen development have been reported. For instance, the protein profile of royal jelly was affected when bees were exposed to the glyphosate-containing product Roundup®. In particular, Major royal jelly protein 3, which is related to social immunity and signaling among bees, was downregulated under treatment indicating sublethal effects on disease resistance of bees (Chaves et al., 2021). Nevertheless, there are other proteins proven for their antimicrobial activity in food jelly, which might be beneficial in context of larval social immunity, for instance in alternative plant protection by using potentially harmful microorganisms (Erler and Moritz, 2016; Erler et al., 2022). Another sublethal effect of pesticide exposure is the degradation of hypopharyngeal glands of the nursing bees (Berenbaum and Liao, 2019; Hatjina et al., 2013; Zaluski et al., 2017). Alterations of the hypopharyngeal glands by pesticides like neonicotinoids can affect the quantity and quality of food jelly and consequently lead to larval mortality (Schott et al., 2021; Wessler et al., 2016).

4. Conclusion

The assessment of pesticide residues in food jellies is complex and different interacting parameters must be considered. For instance, residues are transferred vertically via different transmission steps from the source of exposure into jelly, and horizontally within the honey bee colony. Accumulation for instance in pollen or wax, or a decrease of concentrations throughout the transmission pathway are possible.

Foraging bees bring contaminated nectar and pollen in the colony and thus different matrices like honey, bee bread, or wax will be contaminated. Nursing bees produce glandular secretions after consumption of these contaminated stored products and further feed the larvae. Honey bee queen, worker, and drone larvae might be exposed to the remaining residues. It was shown in several studies that residues of pesticides can be detected in food jelly, in a wide range. The pesticide transfer is influenced by the exposure scenario, respectively an artificial scenario leads to higher amounts. A consistent experimental setup among studies is mandatory when residues are to be compared. Although, the risk evaluation showed that residues are predominantly below the toxicity values concluding no direct lethal effects, sublethal effects should not be underestimated. However, there is still a lack of information on the distribution, transfer, and degradation of pesticides within the colony. Further studies have to be conducted filling those data gaps.

CRediT authorship contribution statement

Karoline Wueppenhorst: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Jakob H. Eckert:** Writing – review & editing. **Michael Steinert:** Writing – review & editing. **Silvio Erler:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Supervision.

Data availability

The data that has been used can be found in the manuscript and supplementary.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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