Chemosphere 232 (2019) 471–480

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

A two-year monitoring of pesticide hazard in-hive: High honey bee mortality rates during insecticide poisoning episodes in apiaries located near agricultural settings

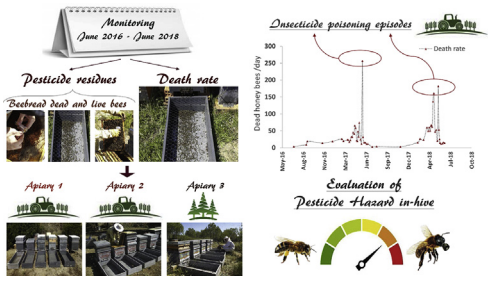
Pau Calatayud-Vernich [a, *], Fernando Calatayud [b], Enrique Simó [b], Juan Antonio Pascual Aguilar [c, d], Yolanda Picó [a]

[a] Environmental and Food Safety Research Group of the University of Valencia (SAMA-UV), Research Center on Desertification (CIDE, UV-CSIC-GV), Moncada-Naquera Road Km 4.5, 46113 Moncada, Valencia Spain
[b] Agrupación de Defensa Sanitaria Apícola (apiADS), Montroi-Turís Road, 46193, Montroi, Valencia, Spain
[c] Centro para el Conocimiento del Paisaje, Calle Rocha del Cine 41, 12415, Matet, Castellón, Spain
[d] Instituto Imdea Agua. Unidad de Geomática. IMDEA Water Institute, Avda. Punto Com 2, 28805, Alcalá de Henares, Spain

HIGHLIGHTS

- Pesticide residues in live honey bees were low and ranged from 2 to 56 ng g $^{-1}$.
- Relevant pesticide hazard in beebread was produced by insecticides used in crops.
- Beeswax was contaminated by miticides from present and past uses in beekeeping.
- Honey bee insecticide poisoning occurred in apiaries located near farmlands.
- Chlorpyrifos, dimethoate and imidacloprid were related to high mortality rates.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 March 2019
Received in revised form
17 May 2019
Accepted 19 May 2019
Available online 23 May 2019

Handling Editor: Klaus Kümmerer

Keywords:
Pesticide hazard
Honey bees
Poisoning
Insecticides
Mortality

ABSTRACT

Pesticide residues in beebread, live and dead honey bees, together with honey bee death rate were monitored from June 2016 to June 2018 in three apiaries, located near agricultural settings and in wildlands. Dead honey bees were only collected and analyzed when significant mortality episodes occurred and pesticide content in beeswax of each experimental apiary was evaluated at the beginning of the study. Samples were extracted by a modified QuEChERS procedure and screened for pesticides residues by liquid chromatography mass spectrometry (LC-MS/MS). Pesticide hazard in the samples was evaluated through the hazard quotient approach (HQ). Beebread was widely contaminated with coumaphos and amitraz degradate 2, 4-dimethylphenylformamide (DMF), miticides detected in 94 and 97% of samples respectively. However, insecticides sprayed during citrus bloom like chlorpyrifos (up to 167 ng g $^{-1}$) and dimethoate (up to 34 ng g $^{-1}$) were the main responsible of the relevant pesticide hazard in this matrix. Pesticide levels in live bees were mostly residual, and pesticide hazard was low. Beeswax of the apiaries, contaminated by miticides, revealed a low pesticide hazard to honey bee colonies. Acute mortality episodes occurred only in the two apiaries located near agricultural settings. Dead bees collected during these episodes revealed high levels (up to 2700 ng g $^{-1}$) of chlorpyrifos, dimethoate,

* Corresponding author.
E-mail address: pau.calatayud@uv.es (P. Calatayud-Vernich).

https://doi.org/10.1016/j.chemosphere.2019.05.170

omethoate and imidacloprid. HQ calculated in dead bees exceeded up to 37 times the threshold value considered as elevated hazard to honey bee health.

1. Introduction

Insect pollination increases yield of many crops (Andrikopoulos and Cane, 2018; Fijen et al., 2018; Perrot et al., 2018), and a 35% of fruit, vegetable and seed global production depends directly on pollinators (Klein et al., 2007). While global demand of pollinators in food production is increasing (Aizen and Lawrence, 2009), wild pollinators are disappearing from intensively farmed landscapes (Kosior et al., 2007; Garibaldi et al., 2011), and honey bee colonies are experiencing concerning loss rates (Potts et al., 2010; Kulhanek et al., 2017; Brodschneider et al., 2018). The increasing use of pesticides, habitat loss and lack of floral diversity, together with pathogens, is likely to be the explanation of pollinator loss documented worldwide (Goulson et al., 2015; Grassl et al., 2018).

Honey bees are exposed to multiple pesticides applied to crops, which are transferred to the hive by forager bees, due to that bees have been used as bioindicator of pesticides in agroecosystems (Porrini et al., 2014; Niell et al., 2017). In addition, honey bees are also in contact with acaricides used in beekeeping against *Varroa*, and analysis of beebread and beeswax have revealed contamination by several pesticide groups (Mullin et al., 2010; Calatayud-Vernich et al., 2018). As a result, bees are exposed to cocktails of pesticides inside and outside the hive (Traynor et al., 2016) that affect not only bee individuals but also colony viability. Risks may vary from acute toxicity that produces mortality in the short or middle term, to sub lethal effects in the long-term (Sanchez-Bayo and Goka, 2016). Acute and chronic exposure effects on bee health to a single or multiple pesticides are well documented, and can impair food transfer, sperm viability, alter learning and odour processes, enhance gene suppression, cause immune and nutritional stress, and cause mortality (Bevk et al., 2012; Andrione et al., 2016; Chaimanee et al., 2016; Gregore et al., 2018; Reeves et al., 2018; Siviter et al., 2018). Furthermore, high mortality rates of honey bees caused by insecticides used in plant protection have been reported around Europe (Calatayud-Vernich et al., 2015; Kiljanek et al., 2016a, 2017; Martinello et al., 2017).

Considering honey bees as the primary pollinator in agricultural landscapes, it is important to understand the magnitude of pesticide incidence in honey bee apiaries. The present study could be considered as a continuation of our previous pilot study **Calatayud-Vernich et al. (2016)**, in which pesticide concentration in dead bees samples and mortality of honeybees were monitored in different locations during blooming season. This study introduces innovative aspects since it reports results of a longer monitoring period, and analyze the most relevant matrices of beekeeping, the study was 2 years long, and the experimental apiaries were located not only near agricultural settings, but also in forest areas in order to compare whether high mortality episodes appear in both types of apiaries environment. Pesticide hazard was assessed not only in dead honeybees when acute mortality took place, but also periodically in live honey bees and beebread. Beeswax pesticide content was also analyzed to understand the contribution of this matrix to overall pesticide hazard in-hive.

2. Material and methods

2.1. Experimental apiaries

The three experimental apiaries were located in the east coast of Spain, in a typical Mediterranean climatic area. Apiary 1 and 2 were placed in intensive agriculture areas, while apiary 3 surroundings were predominantly wildlands with scattered rainfed crops like olive and carob trees. Apiary 1 was surrounded mainly by citrus orchards and apiary 2 was surrounded by citrus but also by other fruit trees like nectarines (Fig. 1).

Experimental apiaries consisted of five Dadant hives (10 frames of measures 42 × 27 cm). Colony health was evaluated throughout the study by periodic sanitary inspections. Analysis of pathogens including Deformed Wing Virus (DWV), Acute Paralysis Virus group (IAPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV) and *Nosema ceranae* were carried out following standard molecular biology approaches for reverse transcription quantitative real-time polymerase-chain reaction (RT-qPCR) (Herrero et al., 2019). Primer pairs used to detect and quantify each pathogen were either published elsewhere or designed *de novo* for this study (Table S9). Colonies were replaced if strength or viability was compromised. Screened bottom boards were used to monitor varroa infestation, and amitraz (Apitraz commercial product) was the only miticide applied in-hive against varroosis from September to December during the study.

2.2. Monitoring mortality

During two years, from June 2016 to June 2018, honey bee mortality was monitored in the three apiaries (Table S2-S3-S4 Supplementary material). Mortality was calculated for each of the five colonies in the three apiaries, and the average value of the five colonies of each apiary was used to plot mortality curves. When significant mortality episodes occurred, collection of dead bees was carried out more frequently. A natural threshold death rate of 20 honey bees per day and colony was assumed according to the values proposed by Porrini et al. (2003). In spring season, there is a natural population growth in honey bee colonies, thus death rate should be considered moderately above 20 dead bees/day.

Death rate was quantified by collecting dead honey bees through basket traps (Accorti et al., 1991; Porrini et al., 2003). Traps consisted of a wooden box with a chain mail on top, placed under the hive entrance.

2.3. Sampling

2.3.1. Live and dead honey bees

Live bees (38 samples) from inside of the hives were collected periodically from the lateral combs to avoid recently born bees, and were a pool of bees from the five hives of each apiary (Table 1). Dead bees (17 samples) were collected when acute mortality signs appeared in the apiaries, this is piles of dead or dying bees at colony entrance. Dead bees were collected from front-door traps and pooled per apiary. The samples were transported to the laboratory

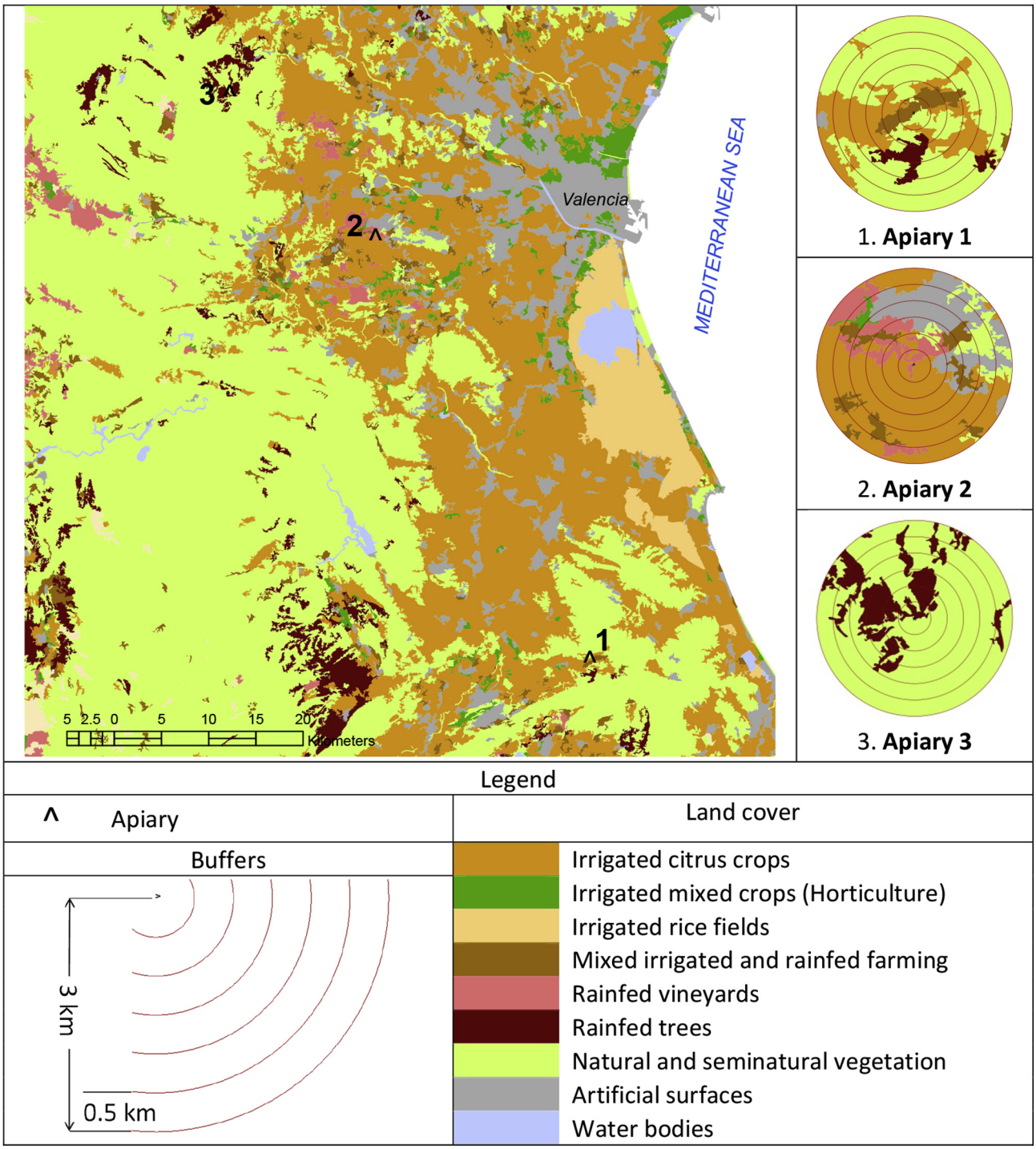


Fig. 1. Location of the experimental apiaries and land cover uses.

in an insulated cooler and stored at −20 °C until pesticide analysis.

2.3.2. Hive matrices

Beebread (33 samples) was collected periodically along with live honey bee samples. Beebread was collected from inside of a comb portion with a disposable wooden stick, and all beebread samples were pooled per location.

Three beeswax samples were collected and analyzed at the beginning of the monitoring period to be used as reference values for pesticide concentrations in wax from each apiary. Beeswax was obtained by cutting a portion of the comb free of beebread, honey or brood. The beeswax from each of the five colonies was mixed in a unique wax sample representative of each apiary.

2.4. Chemicals and reagents

High purity standards (98–99.9%) of the 60 selected pesticides together with the degradate products of amitraz; 2,4-dimethylaniline (DMA), 2,4-dimethylphenylformamide (DMF) and N-(2,4-dimethylphenyl)-N′-methylformamidine (DMPF) were from

Table 1
Sampling outline.

		Sample composition	Apiary 1 (Nº samples)	Apiary 2 (Nº samples)	Apiary 3 (Nº samples)	Time frame	Sampling dates
Honey bees	**Live bees**	5 g (c. 80 bees)	13	13	12	From June 2016 to June 2018	Each 1.5 or 2 months
	Dead bees	5 g (c. 80 bees)	11	6	–		During acute mortality episodes
Hive matrices	**Beebread**	5 g	11	11	11		Each 1.5 or 2 months
	Beeswax	2 g	1	1	1	June 2016	At the beginning of the study

Sigma-Aldrich (Steinheim, Germany) (listed in supplementary material Table S1). Individual standard solutions were prepared in methanol at a concentration of 1000 mg·L-1. The working standard solutions were prepared by mixing the appropriate amounts of individual standard solutions and diluting them with methanol to a final concentration of 1 and 10 mg L^{-1}. Solutions were stored in 15 mL vials at 4 °C in the dark. Magnesium sulfate was obtained from Alfa Aesar (Karlsruhe, Germany), ammonium formate, sodium chloride, acetonitrile and formic acid were purchased from Sigma-Aldrich (Steinheim, Germany). PSA and C18 sorbents, and PTFE (13 mm × 0.22 mm) filters were purchased from Análisis Vínicos S.L. (Tomelloso, Spain). Methanol was obtained from VWR chemicals (Radnor, Pennsylvania). Deionized water was from a MilliQ SP Reagent Water System (Millipore, Bedford, MA, USA).

2.5. Analysis

Methodology used in the present study has been widely used to detect pesticide residues in beekeeping matrices (Herrera-López et al., 2016; Daniele et al., 2017). The samples were extracted by a slightly modified QuEChERS procedure and screened for 63 pesticides and its degradation products by liquid chromatography mass spectrometry (LC-MS/MS). The QuEChERS protocol using acetonitrile as extraction solvent and primary-secondary amine (PSA) and C18 as cleaner sorbents was applied to honey bees, beebread and beeswax samples (see Supplementary material for detailed information). Beeswax extraction procedure adapted from Niell et al. (2014), and methods used for beebread and honey bee extractions were validated in previous studies (Calatayud-Vernich et al., 2015, 2017, 2018). The chromatographic instrument was an HP1200 series LC equipped with an automatic injector, a degasser, a quaternary pump and a column oven-combined with an Agilent 6410 triple quadrupole (QQQ) mass spectrometer with an electrospray ionization (ESI) interface (Agilent Technologies, Waldbronn, Germany). Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokyo, Japan).

2.6. Hazard quotients (HQ)

Pesticide hazard to honey bees was calculated through the hazard quotient (HQ) scores (HQ = pesticide concentration in ng·g^{-1} ÷ pesticide topical/oral LD50 as μg/bee) proposed by Stoner and Eitzer (2013). This is, the sum of all pesticide residue concentrations detected (ng·g^{-1}) divided by their respective contact or oral LD50 in μg/bee for each residue in a given sample. The HQ score provides an estimate based on percentages of LD50 equivalents present in beebread, wax, and in honey bees themselves. Honey bees and beebread samples had a relevant HQ score when it was greater than 50, and the HQ score was considered as elevated when it was greater than 1000. In beeswax, pesticides are embedded in a lipophilic matrix and not all residues are in contact with honey bees. Only a fraction of the pesticide load is exposed to the individuals of the colony, so HQ in beeswax samples was considered as relevant when it was greater than 250. Samples with $HQ_{beeswax}$ > 5000 were considered to have an elevate pesticide load (Traynor et al., 2016). Pesticides LD50 used for the hazard quotient were taken from Sanchez-Bayo and Goka (2014), and University of Hertfordshire Pesticide Properties Database (Hertfordshire, 2018). Amitraz concentrations in the samples were calculated through its main breakdown products DMF and DMPF (Korta et al., 2001). Amitraz parent compound ecotoxicological data was used to HQ calculations when detected.

2.7. Data spatial integration and GIS information treatment analysis

Spatial distribution analysis was performed using GIS techniques with ARCGIS (V. 10.5). All digital layers were geographically positioned following national and regional mapping standards: Spatial reference system ETRS89 and Universal Transverse Mercator projection. Initial information consisted of a vector line layer with an update land use-cover for the year 2018 following a simplification of CORINE Land Cover nomenclature (Kosztra and Büttner, 2018). The original CORINE land cover nomenclature based in three levels was adapted into a single semantic legend considering the major land cover classes. Geometric and land cover type extraction was performed using the 2018 orthophoto provided by the Spanish Institute of Geography. As a result, nine land use cover groups were stablished, namely: Irrigated citrus crops, irrigated mixed crops, irrigated rice fields, mixed irrigated and rainfed farming, rainfed vineyards, rainfed trees, natural and seminatural vegetation, artificial surfaces and water bodies. Finally, ring maps were constructed from the point layer containing the location of the different apiaries. The buffer criteria applied was the creation of six circles of 0.5, 1, 1.5, 2, 2.5 and 3 km which center was each experimental apiary, with the assumption that the ring of 3 km radius would represent a typical honey bee foraging distance and would constitute a potential area of influence for incoming pesticides used in plant protection. Map overlay techniques were applied to land uses map and the rings to obtain the potential area of influence with land uses for each apiary and each buffer distance. Summarize relative values (percentages) for each land cover ring were obtained (Supplementary material S10).

3. Results and discussion

3.1. Monitoring pesticide hazard in-hive

3.1.1. Beeswax

Pesticide content in beeswax was assessed at the beginning of the study, and expected to be similar throughout the duration of the study, as several pervious studies already showed that pesticide levels were similar between wax from different seasons due to pesticides stability in this matrix and its low replacement rate (Calatayud-Vernich et al., 2017, 2018). Pesticides analysis of beeswax evidenced the high contamination of this matrix by miticides. Coumaphos and chlorfenvinphos were detected

simultaneously in the three apiaries. Coumaphos, not used as varroa treatment in the apiaries for many years, remain embedded in this matrix. It was found at concentrations of 880, 1935 and 5085 ng g $^{-1}$ in apiaries 3, 2 and 1, respectively. Chlorfenvinphos detections were 35, 295 and 320 ng g $^{-1}$ in apiaries 2, 1 and 3. This compound was not used in the experimental apiaries, so pesticide residues in wax come from the beeswax recycling process, where a mixed pool of wax from multiple beekeepers is melted to make new foundations sheets. These levels suggested the non-authorized use of this product in beekeeping (Regulation (EC), 2013). Previous surveys in Italy and Spain have also evidenced the use of this compound in beekeeping through detections in beeswax (Boi et al., 2016; Calatayud-Vernich et al., 2017; Perugini et al., 2018). Although Amitraz was the acaricide used in the experimental apiaries, amitraz degradate DMF was only detected in beeswax from apiary 3 with a concentration of 190 ng g $^{-1}$. This is explained by the DMF lower stability and affinity for beeswax (LogP = −1.1) (Hertfordshire, 2018). HQ $_{beeswax}$ scores were low (53 and 182) for apiaries 2 and 3, but relevant (326) for apiary 1. HQ $_{beeswax}$ calculated in this study was lower than those calculated in previous studies that showed average HQ in beeswax over 6000 points, and considered elevated (Calatayud-Vernich et al., 2018).

3.2. Beebread

Samples of beebread (n = 33) contained 17 different pesticide residues among miticides, insecticides, fungicides and herbicides (Table 2). Five samples from apiaries 1 and 2, located in agricultural landscapes, contained more than eight different pesticide residues simultaneously. An average of five pesticides per sample was detected in both apiaries, while beebread from apiary 3 was less contaminated with an average of three pesticides per sample (Supplementary material TableS5). Apiaries 1 and 2, located in areas with intensive agriculture surroundings, exhibited average HQ $_{beebread}$ between six and seven times higher than apiary 3, located in wildlands and with less agricultural settings in the surroundings. Apiary 3 exhibited a low pesticide hazard in more than 90% of samples. Beebread from apiaries 1 and 2 exhibited relevant pesticide hazard in more than 50% of samples. Therefore, apiaries surroundings influenced beebread HQ scores (Colwell et al., 2017). Amitraz and coumaphos were detected in most of the samples, 97 and 94% respectively. Both miticides had the highest mean concentrations, 71.2 and 31.6 ng g^{-1}, respectively. However, contributions to HQ $_{beebread}$ were low and did not exceeded 38 points (Table 2). Miticides not used in the apiaries like fluvalinate, chlorfenvinphos and acrinathrin were detected with mean concentrations below 2 ng g^{-1}, and their contributions to HQ $_{beebread}$ were low (<5 points) and did not pose substantial hazard to colonies health with the exception of acrinathrin, which showed low but also relevant contributions (>300 points) to hazard quotients in apiary 2. Hexythiazox was detected in 24% of samples with a mean concentration of 1 ng g^{-1}. So, while hexythiazox is used in fruit trees fields, and is likely to be transported to the hive through foraging activity, the main source of beebread contamination by miticides appears to be the wax matrix. Beeswax in our experimental apiaries was contaminated with amitraz degradate DMF, coumaphos and chlorfenvinphos, and previous surveys of Spanish beeswax have showed that acrinathrin and fluvalinte were also found in this matrix at high levels (Calatayud-Vernich et al., 2018).

Chlorpyrifos and dimethoate (organophosphates insecticides) were detected in 45 and 24% of the samples, and mean concentrations were 16.2 and 3.4 ng g^{-1}. Both compounds are the most used in citrus crops during bloom, and so, they were detected at high levels in beebread from apiaries 1 and 2. Chlorpyrifos is the most frequently detected insecticide in hive matrices worldwide, and levels in pollen and beebread have reached level of concern for bee health (Mullin et al., 2010; Tosi et al., 2017). In apiary 1, chlorpyrifos was responsible of the highest contributions (up to 696 points) to pesticide hazard found in 2016 and 2017, while dimethoate showed a relevant contribution to HQ $_{beebread}$ (200 points) during nectar flow in 2018 (Fig. 2). In apiary 2, both insecticides had substantial contributions to pesticide hazard during bloom in 2018. Dimethoate, applied in scattered olive trees orchards close to apiary 3, appeared in three beebread samples from this apiary. HQ $_{beebread}$ scores from apiary 3 were low with the exception of one sample in June 2016, with a relevant contribution of dimethoate to HQ $_{beebread}$ (82 points). As olive trees are rarely visited by honey bees, dimethoate found in beebread from apiary 3 came most likely from non-cultivated plants in olive field margins contaminated by spray drift. Contamination by pesticides of non-

Table 2
Summary of pesticide residues detected in beebread samples.

Beebread (n = 33)							
Pesticide	Oral LD_{50}(μg·bee^{-1})	Use	Detection (%)	Range (ng·g^{-1})	Mean [a] (ng·g^{-1})	HQ score	
						Lowest	Highest
DMF (Amitraz)[b]	50	Miticide	32 (97%)	2–496	71.2	<0.1	20
Coumaphos	4.6	Miticide	31 (94%)	4–174	31.6	0.9	38
Chlorpyrifos	0.24	Insecticide	15 (45%)	2–167	16.2	8	696
Carbendazim	50	Fungicide	10 (30%)	2–29	2.0	<0.1	0.6
Acetamiprid	14	Insecticide	9 (27%)	1–19	1.7	0.1	1
Fluvalinate	45	Miticide	9 (27%)	1–20	1.5	<0.1	0.4
Dimethoate	0.17	Insecticide	8 (24%)	2–34	3.4	12	200
Hexythiazox	200	Miticide	8 (24%)	1–14	1.1	<0.1	<0.1
Chlorfenvinphos	0.55	Miticide/Insecticide	6 (18%)	1–2	0.2	2	4
Acrinathrin	0.12	Miticide/Insecticide	6 (18%)	3–40	2.0	29	333
Pyriproxyfen	100	Insecticide	4 (12%)	1–5	0.5	<0.1	<0.1
Imidacloprid	0.0037	Insecticide	4 (12%)	1	0.1	270	270
DMPF (Amitraz)[b]	50	Miticide	3 (9%)	8–22	1.4	<0.1	20
Methiocarb	0.08	Insecticide	3 (9%)	2–28	1.4	25	350
Tebuconazole	83.05	Fungicide	2 (6%)	1–3	0.1	<0.1	<0.1
Buprofezin	164	Insecticide	1 (3%)	2	<0.1	<0.1	<0.1
Terbuthylazine	22.6	Herbicide	1 (3%)	2	<0.1	<0.1	<0.1

[a] If a compound was not detected in a sample, concentration value was considered as 0.
[b] DMF and DMPF are the degradation products of the amitraz pesticide.

cultivated habitats adjacent to agricultural areas can represent a high pesticide risk to honey bees (Botias et al., 2015, 2016; Long and Krupke, 2016; McArt et al., 2017). Imidacloprid and Methiocarb, detected in 12 and 9% of samples respectively, were involved in relevant HQ $_{beebread}$ scores (up to 350 points). Methiocarb was detected in beebread samples, from apiary 2, collected in May and August 2017. Imidacloprid was found in beebread from both apiaries collected during citrus bloom in 2017 and 2018, and in February 2018. Low levels of this neonicotinoid, as detected in this study, were proved to alter honey bee physiology and reduce foraging motivations in other pollinator species (Lamsa et al., 2018; Cook, 2019). Acetamiprid and pyriproxyfen were detected in 27 and 12% of samples respectively, with mean concentrations below 2 ng g^{-1}. Insecticide buprofezin, together with herbicide terbuthylazine were found in less than 10% of samples and mean concentrations did not exceeded 1.4 ng g^{-1}. Fungicides Carbendazim and tebuconazole, detected in 30% and 6% of beebread samples, contributed less than one point to HQ $_{beebread}$ scores in positive samples for these compounds. In general, fungicides toxicity to honey bees is considered low, and in the HQ approach used in this study, indirect effect of fungicides on the colony are not contemplated. However, fungicides reduce the population of beneficial symbiotic fungi present in pollen that are crucial in the maturation of pollen into beebread. Therefore, nutritional value of beebread contaminated by fungicides is adversely affected and honey bee colony weakened (Yoder et al., 2012; Steffan et al., 2017).

3.2.1. Live honey bees

Live honey bees samples (n = 38) were less contaminated in both, number and quantity of pesticide residues. Ten samples (26%) were free of pesticides and an average of one pesticide per sample was detected (Supplementary material TableS6). Honey bees were contaminated mostly by compounds used in beekeeping against varroosis. Chlorpyrifos and dimethoate insecticides, involved in poisoning episodes, were only detected in one and two samples, respectively, but contributions to pesticide hazard were relevant in

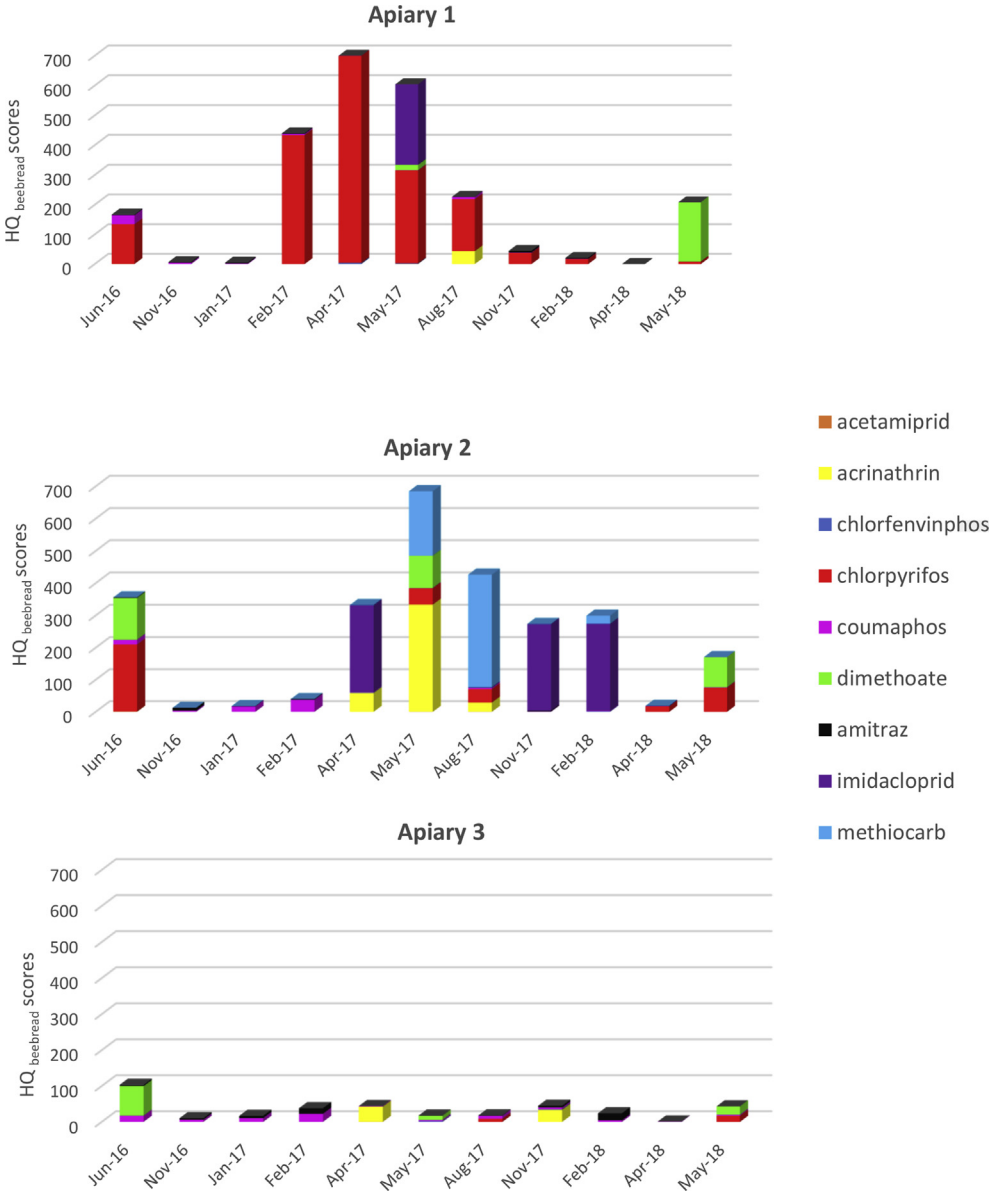


Fig. 2. Evolution of Hazard Quotients (HQ) calculated in beebread samples in the three monitored apiaries. Contribution of each pesticide detected to HQ scores is illustrated. Pesticides contributing less than 0.5 points to HQ scores are not included in the figures.

the three samples. Coumaphos was detected in 50% of samples, mostly at residual concentrations (Table 3). This miticide remains trapped in wax matrix and can contaminate honey bees years after its last application to the colonies. Amitraz, detected in the samples through its degradate DMF, was the miticide applied for *varroa* control in the colonies from September to December in 2017 and 2018. Results showed how hazard posed by amitraz decreased gradually since the application date (Fig. 3). Furthermore, amitraz contributions to HQs in the samples were insignificant because this product is relatively safe for bees compared to other synthetic acaricides (Gashout et al., 2018). Carbendazim fungicide and fluvalinate acaricide were detected in one sample at residual concentrations. On five occasions, the date of collection of dead and live bees coincided. Whereas dead bees from mortality traps were highly contaminated, analysis of live in-hive bees showed a remarkably low pesticide load (Supplementary Material Table S8). Guard bees that prevent the entry of poisoned bees with abnormal behaviors to the colony, the hygienic behavior of honey bees - like the fast intervention of undertaker bees in removing poisoned dead bees from inside the hive-, and honey bees' detoxifying enzymes are probably the main reasons that could explain the reduced pesticide load of live in-hive bees compared to dead bees collected outside the hive.

3.3. High mortality rates during pesticide poisoning episodes of honey bees

Mortality traps underestimate death rates of honey bee colonies because deaths outside the hives are not quantified. Furthermore, honey bees with high doses of pesticides that die while foraging, or disoriented poisoned bees unable to find the way back to the colonies are not analyzed, thus underestimating the magnitude of poisoning episodes occurred in the apiaries. Nevertheless, poisoning symptoms were observed in apiaries 1 and 2, located near agricultural settings. Honey yield of the bee colonies affected by poisoning events was significantly reduced, and population of forager bees decreased, thus debilitating the colonies, but not killing them. Apiary 3, surrounded by wildlands and with less agricultural pressure, was free of pesticide poisoning episodes. Death rate in apiary 3 followed a natural pattern throughout the monitoring period. Mortality was around 20 dead bees/day during periods of low activity, summer (July–August) and winter (December–January), and higher during periods of high activity like citrus (April–May) and rosemary (February–March) blooming seasons (Fig. 3). During flowering, hive population grows and honey bees intensify foraging flights, thus reducing their lifespan. As a result, there is a natural growth in mortality.

In apiary 1 and 2, elevated pesticide hazard appeared during and immediately after spraying and decreased after application periods, as also reported by Beyer et al. (2018). Dead honey bees collected in mortality traps were mostly contaminated by dimethoate (76.5%), its metabolite omethoate (52.9%) and chlorpyrifos (41.2%), confirming the high exposure of foragers (Supplementary material TableS7). Chlorpyrifos (found up to 2700 ng g^{-1}) and dimethoate (up to 338 ng g^{-1}) were detected in dead honey bees with the highest mean concentrations, 232.9 and 89.9 ng g^{-1}, respectively (Table 3). Fluvalinate (35.3%) was found at residual concentrations in most of the samples (6–10 ng g^{-1}). Imidacloprid neonicotinoid was found in two samples (11.8%), at 22 and 476 ng g^{-1} in apiary 2. Amitraz degradate DMF (5.9%), hexythiazox (17.6%), and coumaphos (5.9%), together with the insecticides pyriproxifen and acetamiprid (11.8%), were detected in the samples and contribution to pesticide HQ were insignificant.

3.3.1. Apiary 1

Considering a natural death rate of 20 dead bees/day, three important acute mortality peaks occurred during the monitoring period. The highest mortality peaks were found in May 2017 (up to 256 dead bees/day) and May 2018 (up to 160 and 180 dead bees/day) during citrus bloom, and dead bees were poisoned with the organophosphate insecticides chlorpyrifos and dimethoate (Fig. 4), as also occurred in previous studies (Calatayud-Vernich et al., 2015; Kiljanek et al., 2017). Both compounds were also identified as responsible of poisoned honey bees from other European countries

Table 3
Summary of pesticide residues detected in live and dead honey bee samples.

Live honey bees (n = 38)							
Pesticide	Contact LD_{50}(µg·bee^{-1})	Use	Detection (%)	Range (ng·g^{-1})	Mean[a] (ng·g^{-1})	HQ score	
						Lowest	Highest
Coumaphos	20	Miticide	21 (55.3%)	2–34	5.2	0.1	2
DMF (Amitraz)[b]	50	Miticide	16 (42.1%)	2–56	11.5	<0.1	2
Dimethoate	0.12	Insecticide	2 (5.3%)	12–36	1.3	100	300
Chlorpyrifos	0.072	Insecticide	1 (2.6%)	22	0.6	306	306
Carbendazim	50	Fungicide	1 (2.6%)	3	<0.1	<0.1	<0.1
Fluvalinate	8.7	Miticide	1 (2.6%)	2	<0.1	0.2	0.2

Dead honey bees (n = 17)							
Pesticide	Contact LD_{50} (µg·bee^{-1})	Use	Detection (%)	Range (ng·g^{-1})	Mean[a] (ng·g^{-1})	HQ score	
						Lowest	Highest
Dimethoate	0.12	Insecticide	13 (76.5%)	4–338	89.9	33	2817
Omethoate	0.05	Insecticide	9 (52.9%)	10–48	13.8	200	960
Chlorpyrifos	0.072	Insecticide	7 (41.2%)	2–2702	232.9	28	37528
Fluvalinate	8.7	Miticide	6 (35.3%)	6–180	19.4	0.7	21
Hexythiazox	200	Miticide	3 (17.6%)	4–266	16.2	<0.1	1
Pyriproxyfen	100	Insecticide	2 (11.8%)	4–558	33.1	<0.1	6
Imidacloprid	0.061	Insecticide	2 (11.8%)	22–476	29.3	361	7803
Acetamiprid	7.9	Insecticide	2 (11.8%)	6–14	1.2	0.8	2
DMF (Amitraz)[b]	50	Miticide	1 (5.9%)	47	2.8	0.9	0.9
Coumaphos	20	Miticide	1 (5.9%)	2	0.1	0.1	0.1

[a] If a compound was not detected in a sample, concentration value was considered as 0.
[b] DMF and DMPF are the degradation products of the amitraz pesticide.

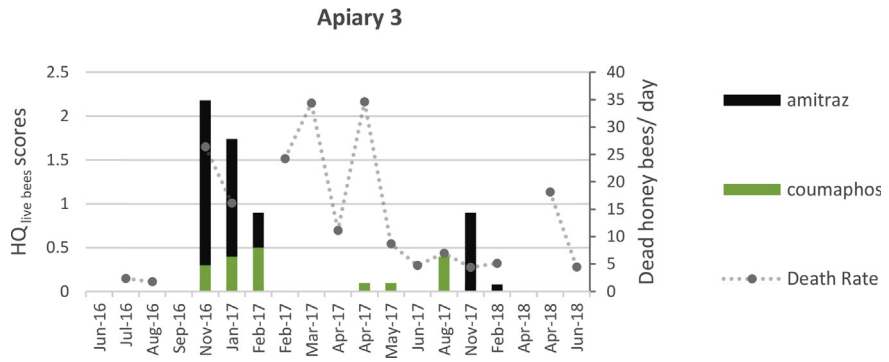


Fig. 3. Evolution of death rate and contribution of each pesticide detected to Hazard Quotients (HQ) scores in live honey bees from apiary 3.

(Barnett et al., 2007; Porrini et al., 2014; Kiljanek et al., 2016b). HQ $_{\text{dead bees}}$ in May 2018 and 2017 exceeded from 3 to 37 times the threshold value considered as elevated hazard to honey bee health, respectively. At the beginning of April 2017, mortality started to rise up to 65 dead bees/day. During this increase, we collected one dead bee sample that was free of pesticides. Two weeks later, chlorpyrifos, dimethoate and omethoate were detected in dead bees and were responsible of the elevated HQ $_{\text{bees}}$ (>15000 points). During March–April 2018, mortality was slightly above natural death rate (up to 65 dead bees/day), and pesticide analysis revealed that two dead bee samples collected during this period were free of pesticides. In spite of a good spring buildup of bee population, black bees with hairless syndrome, a typical sign of chronic bee paralysis virus (CBPV), were detected in traps, and virus analysis of live bees revealed an infection by CBPV that could be responsible of rise in mortality during this period. Presence of hairless black bees ceased in the middle of April, and in early May 2018 (up to 160 dead bees/day), dead bee samples were contaminated by dimethoate contributing to a relevant hazard to bees (HQ $_{\text{bees}} = 67$ points). However, such pesticide hazard is unlikely to be the only factor involved in the high mortality observed, so the undetected presence of others pesticides not included in our methodology, the degradation of dimethoate in traps, and a higher pesticide susceptibility of exhausted forager bees at the end of bloom, could be contributing to this acute mortality event.

3.3.2. *Apiary 2*

Poisoning symptoms were observed during nectarine (February 2017) and citrus bloom (April–May 2017 and 2018). Dead honey bees collected in February 2017 were contaminated with

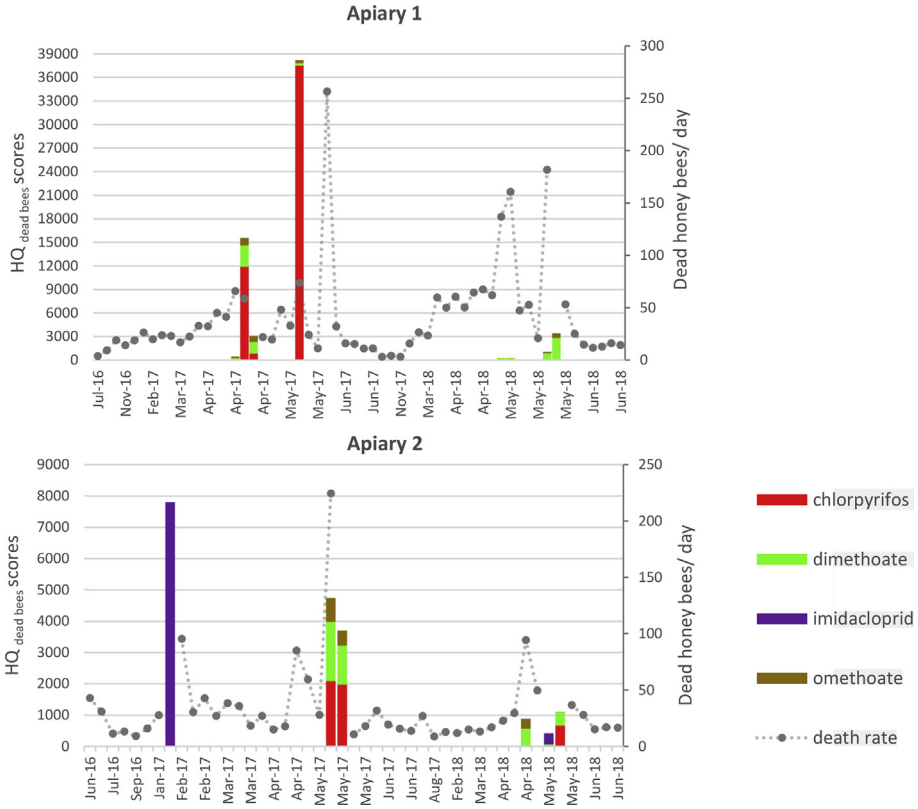


Fig. 4. Evolution of death rate and contribution of each pesticide detected to Hazard Quotients (HQ) scores in dead bees samples collected during acute mortality episodes. Pesticides contributing less than 21 points to HQ scores are not included in the figures.

imidacloprid, used in nectarine orchards near to the apiary. Sprayings of this neonicotinoid during bloom was banned in 2013, and since 2018, the use outdoors is completely prohibited by European Union (EU regulation 2018/783). Therefore, detections of this neonicotinoid suggest a violation of EU regulation. Levels detected of this compound and its high toxicity to honey bees were responsible of the rise in mortality (up to 95 dead bees/day). Contribution to HQ_{bees} was elevated and exceeded 7000 points (Fig. 4). Death rate increased the second half of April, and in May 2017 mortality reached the highest value (>200 dead bees/day). As occurred in apiary 1, chlorpyrifos, dimethoate and omethoate insecticides were sprayed in citrus orchards during blooming season, thus poisoning forager honey bees. Analysis of dead bees revealed that these compounds were responsible of the elevated pesticide hazard found in honey bee samples (HQ_{bees} > 4700 points). In April 2018, mortality increased up to 95 dead bees/day, forager bees were poisoned with the compounds fraudulently applied during citrus bloom (chlorpyrifos, dimethoate and omethoate). Imidacloprid was also found in poisoned bees during this mortality peak and had a relevant contribution (360 points) to pesticide hazard calculated in one sample collected during this mortality episode. Furthermore, two samples from apiary 2 contained 120 and 180 ng g^{-1} of fluvalinate, such concentrations were not residual and could not be acquired by honey bees through contact with contaminated beeswax. Both samples were collected during May 2017, so fluvalinate residues came most likely from citrus spraying with this compound. Fluvalinate, only detected in one live honey bee sample at 2 ng g^{-1}, and with a residual mean concentration lower than 0.1 ng g^{-1}, support this explanation (Table 3).

4. Conclusions

Beeswax was contaminated exclusively with acaricides used in beekeeping, and exhibited products not used in the apiaries for years, thus pointing out the stability of pesticides in this matrix. Miticides used in beekeeping were the most frequent pesticides in beebread from the three apiaries, whereas insecticides were responsible of the highest contributions to pesticide hazard. Live honey bees collected from inside the colonies were remarkably less contaminated. Pesticide poisoning episodes only took place in the two apiaries located near agricultural settings, and dead honey bees analyzed revealed high levels of chlorpyrifos, dimethoate and imidacloprid, used in the surrounding crops. In view of our results, the use of less contaminated sources of beeswax is needed to dilute pesticides accumulated in wax and prevent future pesticide transferences from this matrix to honey bees and beebread. Sustainable management practices like reducing applications of persistent pesticides in-hive and the use of organic acids against *varroa* should be implemented in beekeeping in order to reduce miticides levels in honey bee colonies. It is important to consider the location of the apiaries to avoid poisoning events, and reduce pesticide hazard in honey bee colonies. Nevertheless, reliance on pesticides of modern agriculture should be reconsidered, and wild and managed pollinators should be valued as essential components in agroecosystems in order to develop a more sustainable management of the agroenvironments.

Acknowledgements

This work has been suported by the Spanish Ministerio de Agricultura, Alimentación y Medio Ambiente through the project 20160020000834: “Análisis de la Pérdida de Viabilidad y Despoblamiento de las Colonias de Abejas (Apis mellifera L.) Mediante el Mapeo de Xenobióticos” and through the project ANTROPOCEN@ (PROMETEO/2018/155) by Generalitat Valenciana. Pau Calatayud-Vernich acknowledges the Foundation “Tatiana Pérez de Guzmán el Bueno” for the financial support through PhD Environmental Fellowship Program. We thank the Agrupación de Defensa Sanitaria Apícola (apiADS) for their help with counseling and sampling. We also thank ERI BIOTECMED, Department of Genetics from University of Valencia for their analysis of honey bee pathogens.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.05.170.

References

Accorti, M., Luti, F., Tarducci, F., 1991. Methods for collecting data on natural mortality in bee. Ethol. Ecol. Evol. 3, 123–126.
Aizen, M.A., Harder, L.D., 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr. Biol. 19, 915–918.
Andrikopoulos, C.J., Cane, J.H., 2018. Comparative pollination efficacies of five bee species on raspberry. J. Econ. Entomol. 111, 2513–2519.
Andrione, M., Vallortigara, G., Antolini, R., Haase, A., 2016. Neonicotinoid-induced impairment of odour coding in the honeybee. Sci. Rep. 6, 38110.
Barnett, E.A., Charlton, A.J., Fletcher, M.R., 2007. Incidents of bee poisoning with pesticides in the United Kingdom, 1994-2003. Pest Manag. Sci. 63, 1051–1057.
Bevk, D., Kralj, J., Cokl, A., 2012. Coumaphos affects food transfer between workers of honeybee Apis mellifera. Apidologie 43, 465–470.
Beyer, M., Lenouvel, A., Guignard, C., Eickermann, M., Clermont, A., Kraus, F., Hoffmann, L., 2018. Pesticide residue profiles in bee bread and pollen samples and the survival of honeybee coloniesa case study from Luxembourg. Environ. Sci. Pollut. Res. 25, 32163–32177.
Boi, M., Serra, G., Colombo, R., Lodesani, M., Massi, S., Costa, C., 2016. A 10 year survey of acaricide residues in beeswax analysed in Italy. Pest Manag. Sci. 72, 1366–1372.
Botias, C., David, A., Hill, E.M., Goulson, D., 2016. Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target insects. Sci. Total Environ. 566, 269–278.
Botias, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E., Goulson, D., 2015. Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. Environ. Sci. Technol. 49, 12731–12740.
Brodschneider, R., Gray, A., Adjlane, N., Ballis, A., Brusbardis, V., Charriere, J., Chlebo, R., Coffey, M.F., Dahle, B., de Graaf, D.C., Drazic, M.M., Evans, G., Fedoriak, M., Forsythe, I., Gregorc, A., Grzeda, U., Hetzroni, A., Kauko, L., Kristiansen, P., Martikkala, M., Martin-Hernandez, R., Aurelio Medina-Flores, C., Mutinelli, F., Raudmets, A., Ryzhikov, V.A., Simon-Delso, N., Stevanovic, J., Uzunov, A., Vejsnaes, F., Woehl, S., Zammit-Mangion, M., Danihlik, J., 2018. Multi-country loss rates of honey bee colonies during winter 2016/2017 from the COLOSS survey. J. Apic. Res. 57, 452–457.
Calatayud-Vernich, P., Calatayud, F., Simo, E., Suarez-Varela, M.M., Pico, Y., 2015. Influence of pesticide use in fruit orchards during blooming on honeybee mortality in 4 experimental apiaries. Sci. Total Environ. 541, 33–41.
Calatayud-Vernich, P., Calatayud, F., Simó, E., Picó, Y., 2018. Pesticide residues in honey bees, pollen and beeswax: assessing beehive exposure. Environ. Pollut. 241, 106–114.
Calatayud-Vernich, P., Calatayud, F., Simó, E., Picó, Y., 2017. Occurrence of pesticide residues in Spanish beeswax. Sci. Total Environ. 605, 745–754.
Chaimanee, V., Evans, J.D., Chen, Y., Jackson, C., Pettis, J.S., 2016. Sperm viability and gene expression in honey bee queens (Apis mellifera) following exposure to the neonicotinoid insecticide imidacloprid and the organophosphate acaricide coumaphos. J. Insect Physiol. 89, 1–8.
Colwell, M.J., Williams, G.R., Evans, R.C., Shutler, D., 2017. Honey bee-collected pollen in agro-ecosystems reveals diet diversity, diet quality, and pesticide exposure. Ecol. Evol. 7, 7243–7253.
Cook, S.C., 2019. Compound and dose-dependent effects of two neonicotinoid pesticides on honey bee (Apis mellifera) metabolic physiology. Insects 10.
Daniele, G., Giroud, B., Jabot, C., 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.
Fijen, T.P.M., Scheper, J.A., Boom, T.M., Janssen, N., Raemakers, I., Kleijn, D., 2018. Insect pollination is at least as important for marketable crop yield as plant quality in a seed crop. Ecol. Lett. 21, 1704–1713.
Garibaldi, L.A., Steffan-Dewenter, I., Kremen, C., Morales, J.M., Bommarco, R., Cunningham, S.A., Carvalheiro, L.G., Chacoff, N.P., Dudenhoeffer, J.H., Greenleaf, S.S., Holzschuh, A., Isaacs, R., Krewenka, K., Mandelik, Y., Mayfield, M.M., Morandin, L.A., Potts, S.G., Ricketts, T.H., Szentgyoergyi, H., Viana, B.F., Westphal, C., Winfree, R., Klein, A.M., 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. Ecol. Lett. 14, 1062–1072.
Gashout, H.A., Goodwin, P.H., Guzman-Novoa, E., 2018. Lethality of synthetic and

natural acaricides to worker honey bees (Apis mellifera) and their impact on the expression of health and detoxification-related genes. Environ. Sci. Pollut. Res. Int. 25, 34730–34739.
Goulson, D., Nicholls, E., Botias, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347, 1255957.
Grassl, J., Holt, S., Cremen, N., Peso, M., Hahne, D., Baer, B., 2018. Synergistic effects of pathogen and pesticide exposure on honey bee (Apis mellifera) survival and immunity. J. Invertebr. Pathol. 159, 78–86.
Gregore, A., Alburaki, M., Rinderer, N., Sampson, B., Knight, P.R., Karim, S., Adamczyk, J., 2018. Effects of coumaphos and imidacloprid on honey bee (Hymenoptera: apidae) lifespan and antioxidant gene regulations in laboratory experiments. Sci. Rep. 8, 15003.
Herrera López, S., Lozano, A., Sosa, A., Hernando, M.D., Fernández-Alba, A.R., 2016. Screening of pesticide residues in honeybee wax comb by LC-ESI-MS/MS. A pilot study. Chemosphere 163, 44–53.
Herrero, S., Coll, S., González-Martínez, R.M., Parenti, S., Millán-Leiva, A., González-Cabrera, J., 2019. Identification of new viruses specific to the honey bee mite Varroa destructor. Biorxiv, 610170.
Hertfordshire, U., 2018. PPDB - Pesticides Properties DataBase [online] Sitem.herts.ac.uk. Available at: https://sitem.herts.ac.uk/aeru/ppdb/. (Accessed 30 November 2018).
Kiljanek, T., Niewiadowska, A., Gawel, M., Semeniuk, S., Borzecka, M., Posyniak, A., Pohorecka, K., 2017. Multiple pesticide residues in live and poisoned honeybees Preliminary exposure assessment. Chemosphere 175, 36–44.
Kiljanek, T., Niewladowska, M., Posyniak, A., 2016a. Pesticide poisoning of honeybees: a review of symptoms, incident classification, and causes of poisoning. J. Apic. Sci. 60, 5–24.
Kiljanek, T., Niewiadowska, A., Semeniuk, S., Gawel, M., Borzecka, M., Posyniak, A., 2016b. Multi-residue method for the determination of pesticides and pesticide metabolites in honeybees by liquid and gas chromatography coupled with tandem mass spectrometry-Honeybee poisoning incidents. J. Chromatogr., A 1435, 100–114.
Klein, A., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. P. Roy. Soc. B-Biol. Sci. 274, 303–313.
Korta, E., Bakkali, A., Berrueta, L.A., Gallo, B., Vicente, F., Kilchenmann, V., Bogdanov, S., 2001. Study of acaricide stability in honey. Characterization of amitraz degradation products in honey and beeswax. J. Agric. Food Chem. 49, 5835–5842.
Kosior, A., Celary, W., Olejniczak, P., Fijal, J., Krol, W., Solarz, W., Plonka, P., 2007. The decline of the bumble bees and cuckoo bees (hymenoptera : apidae : bombini) of western and central Europe. Oryx 41, 79–88.
Kosztra, B., Büttner, G., 2018. Proposal for Enhancement of CLC Nomenclature Guidelines. European Environmental Agency.
Kulhanek, K., Steinhauer, N., Rennich, K., Caron, D.M., Sagili, R.R., Pettis, J.S., Ellis, J.D., Wilson, M.E., Wilkes, J.T., Tarpy, D.R., Rose, R., Lee, K., Rangel, J., vanEngelsdorp, D., 2017. A national survey of managed honey bee 2015-2016 annual colony losses in the USA. J. Apic. Res. 56, 328–340.
Lamsa, J., Kuusela, E., Tuomi, J., Juntunen, S., Watts, P.C., 2018. Low dose of neonicotinoid insecticide reduces foraging motivation of bumblebees. P. Roy. Soc. B-Biol. Sci. 285, 20180506.
Long, E.Y., Krupke, C.H., 2016. Non-cultivated plants present a season-long route of pesticide exposure for honey bees. Nat. Commun. 7, 11629.
Martinello, M., Baratto, C., Manzinello, C., Piva, E., Borin, A., Toson, M., Granato, A., Boniotti, M.B., Gallina, A., Mutinelli, F., 2017. Spring mortality in honey bees in northeastern Italy: detection of pesticides and viruses in dead honey bees and other matrices. J. Apic. Res. 56, 239–254.
McArt, S.H., Fersch, A.A., Milano, N.J., Truitt, L.L., Boroczky, K., 2017. High pesticide risk to honey bees despite low focal crop pollen collection during pollination of a mass blooming crop. Sci. Rep. 7, 46554.
Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., Pettis, J.S., 2010. High levels of miticides and agrochemicals in north American apiaries: implications for honey bee health. PLoS One 5, e9754.
Niell, S., Jesús, F., Pérez, N., Pérez, C., Pareja, L., Abbate, S., Carrasco-Letelier, L., Díaz, S., Mendoza, Y., Cesio, V., Heinzen, H., 2017. Neonicotinoids transference from the field to the hive by honey bees: towards a pesticide residues biomonitor. Sci. Total Environ. 581–582, 25–31.
Perrot, T., Gaba, S., Roncoroni, M., Gautier, J., Bretagnolle, V., 2018. Bees increase oilseed rape yield under real field conditions. Agric. Ecosyst. Environ. 266, 39–48.
Perugini, M., Tulini, S.M.R., Zezza, D., Fenucci, S., Conte, A., Amorena, M., 2018. Occurrence of agrochemical residues in beeswax samples collected in Italy during 2013-2015. Sci. Total Environ. 625, 470–476.
Porrini, C., Caprio, E., Tesoriero, D., Di Prisco, G., 2014. Using honey bee as bioindicator of chemicals in Campanian agroecosystems (South Italy). Bull. Insectol. 67, 137–146.
Porrini, C., Gloria Sabatini, A., Girotti, S., Ghini, S., Medrzycki, P., Grillenzoni, F., Bortolotti, L., Gattavecchia, E., Celli, G., 2003. Honey bees and bee products as monitors of the environmental contamination. APIACTA 38, 63–70.
Potts, S.G., Roberts, S.P.M., Dean, R., Marris, G., Brown, M.A., Jones, R., Neumann, P., Settele, J., 2010. Declines of managed honey bees and beekeepers in Europe. J. Apic. Res. 49, 15–22.
Reeves, A.M., O'Neal, S.T., Fell, R.D., Brewster, C.C., Anderson, T.D., 2018. In-hive Acaricides alter biochemical and morphological indicators of honey bee nutrition, immunity, and development. J. Insect Sci. 18, 8.
Regulation (EC), 11 October 2013. No 1107/2009 of the European Parliament and of the Council of 21 October 2009 Concerning the Placing of Plant Protection Products on the Market and Repealing Council Directives 79/117/EEC and 91/414/EEC.
Regulation (EU) 2018/783 of, 29 May 2018. Amending Implementing Regulation (EU) No 540/2011 as Regards the Conditions of Approval of the Active Substance Imidacloprid.
Sanchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees - a risk assessment. PLoS One 9, e94482.
Sanchez-Bayo, F., Goka, K., 2016. Impacts of pesticides on honey bees. In: Tech (Ed.), Beekeeping and Bee Conservation. Advances in Research.
Siviter, H., Koricheva, J., Brown, M.J.F., Leadbeater, E., 2018. Quantifying the impact of pesticides on learning and memory in bees. J. Appl. Ecol. 55, 2812–2821.
Steffan, S.A., Dharampal, P.S., Diaz-Garcia, L., Currie, C.R., Zalapa, J., Hittinger, C.T., 2017. Empirical, metagenomic, and computational techniques illuminate the mechanisms by which fungicides compromise bee health. Jove-J. Vis. Exp., e54631
Stoner, K.A., Eitzer, B.D., 2013. Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (Apis mellifera) in Connecticut. PLoS One 8, e77550.
Tosi, S., Costa, C., Vesco, U., Quaglia, G., Guido, G., 2017. A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides. Sci. Total Environ. 615, 208–218.
Traynor, K.S., Pettis, J.S., Tarpy, D.R., Mullin, C.A., Frazier, J.L., Frazier, M., vanEngelsdorp, D., 2016. In-hive Pesticide Exposome: Assessing risks to migratory honey bees from in-hive pesticide contamination in the Eastern United States. Sci. Rep 6, 33207.
Yoder, J.A., Nelson, B.W., Jajack, A.J., Sammataro, D., 2017. Fungi and the effects of fungicides on the honey bee colony. In: Vreeland, R.H., Sammataro, D. (Eds.), Beekeeping – from Science to Practice, vol. 7.