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Translocation of pharmaceuticals from wastewater into beehives

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ABSTRACT

There has been a substantial research focus on the presence of pesticides in flowers and the subsequent exposure to honeybees. Here we demonstrate for the first time that honeybees can also be exposed to pharmaceuticals, commonly present in wastewater. Residues of carbamazepine (an anti-epileptic drug) up to 371 ng/mL and 30 μg/g were detected in nectar and pollen sampled from zucchini flowers (*Cucurbita pepo*) grown in carbamazepine spiked soil (0.5–20 μg/g). Under realistic exposure conditions from the use of recycled wastewater, carbamazepine concentrations were estimated to be 0.37 ng/L and 30 ng/kg in nectar and pollen, respectively. Incorporation of environmentally relevant carbamazepine residues in nectar and pollen into a modelling framework able to simulate beehive dynamics including the honeybee foraging activity at the landscape scale (BEEHAVE and BEESCOUT) enabled the simulation of carbamazepine translocation from zucchini fields into honeybee hives. Carbamazepine accumulation was modelled in 11 beehives across a 25 km^2 landscape over three years chosen to represent distinct climatic conditions. During a single flowering period, carbamazepine concentrations were simulated to range between 0 and 2478 ng per beehive. The amount of carbamazepine gathered not only varied across the simulated years but there were also differences in accumulation of carbamazepine between beehives within the same year. This work illustrates a fundamental first step in assessing the risk of pharmaceuticals to bees through realistic scenarios by demonstrating a method to quantify potential exposure of honeybees at the landscape scale. Pharmaceuticals are being inadvertently but increasingly applied to agricultural lands globally via the use of wastewater for agricultural irrigation in response to water scarcity problems. We have demonstrated a route of pharmaceutical exposure to honeybees via contaminated nectar and pollen. Given the biological potency of pharmaceuticals, accumulation of these chemicals in nectar and pollen suggest potential implications for honeybee health, with unknown ecosystem consequences.

1. Introduction

In recent years, there has been a substantial focus on the impact of exposure to chemicals on global honeybee populations (Klein et al., 2017; Potts et al., 2010). If direct exposure to such chemicals does not cause mortality or disorientate foraging honeybees immediately, these chemicals can be transferred back to the hive in contaminated nectar and pollen therefore presenting a risk to the honeybee colony. To date, plant protection products have been identified in honeybees, beebread and colony wax (Hrynko et al., 2019; Ostiguy et al., 2019; Traynor et al., 2016). The presence of insecticides, such as neonicotinoids in nectar and pollen and the subsequent exposure to honeybees potentially leads to adverse effects, even with low levels of contact (Blacquière et al., 2012; Goulson, 2013). The impact of plant protection products on pollinators however remains an active research area due to the complexity of food web interactions involved in pesticide exposure. Research has also demonstrated the presence of a number of other plant protection products (e.g. organochlorine pesticides), as well as persistent organic pollutants (e.g. polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDE)) in nectar and pollen (Roszko et al., 2016), although their effect on foraging insects is still being determined. Other organic contaminants, such as pharmaceuticals, have been shown to accumulate in edible plant organs with the potential impacts on human health being the focus of these studies (Carter et al., 2014; Malchi et al., 2014). However, the accumulation of pharmaceuticals in pollen and nectar, and subsequent exposure to foraging insects such as honeybees, has until now been over-looked. Nevertheless, a similar chemical profile and known ability to cross the root membrane and translocate within a plant via the xylem sap to distal tissues (Goldstein et al., 2014) would suggest that, like pesticides,

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pharmaceuticals also have the potential to accumulate in nectar and pollen.

Application of wastewater treatment by-products (e.g. treated wastewater and digested sludge) and animal manures are routes by which pharmaceuticals of human and veterinary origin can enter soils and accumulate (Dalkmann et al., 2012; Kinney et al., 2006). The input of pharmaceuticals in soils is expected to increase in the near future, with a drive to increase reuse of wastewater treatment by-products coupled with a need to reduce our dependence on freshwater irrigation and chemical based fertilisers. It is also expected that exposure to pharmaceuticals may be higher in developing countries where waste and chemical management capacity is not fully developed, yet agricultural systems routinely use raw or partially treated sewage as a source of irrigation. In the Food and Agricultural Organisation of the United Nations report "Water for Sustainable Food and Agriculture" recommendation 2 specifies that G20 members must invest in the treatment of urban wastewater and its reuse in agriculture in order to meet the projected 50% increase in food production by 2050. This recommendation directly supports the UN Sustainable Development Goal (SDG) 6 and SDG 13, whilst contributing to SDG 2 and SDG 15.

The persistence of pharmaceuticals in the environment is variable and dependent on a number of factors such as soil and pharmaceutical physico-chemical properties (Drillia et al., 2005). Nevertheless, research has shown that some pharmaceuticals are bio-available in soils, and can be taken up by non-target organisms, such as plants, and accumulate in terrestrial food webs (Shore et al., 2014). Consequently, wildlife across diverse ecosystems may be exposed to pharmaceuticals either directly or indirectly via food web transfer. However, the extent of exposure through this pathway remains unknown for most taxa and ecosystems (Arnold et al., 2014).

Accumulation of pharmaceuticals by non-target organisms poses a risk to species health because these chemicals are designed to interact with specific molecular targets in humans and domestic animals and these targets generally have orthologs that are conserved in other species, including hexapods (LaLone et al., 2016; Verbruggen et al., 2018). If pharmaceuticals are able to cross root membranes and accumulate in the nectar and pollen of flowering plants, visiting honeybees can be exposed to these residues via oral exposure, through ingestion of pollen into their corbiculae and extraction of nectar from flowers (Krupke et al., 2012). Through food web transfer, exposure to pharmaceuticals by non-target organisms has already been shown to alter behavioural and physiological responses in vertebrate wildlife (Bean et al., 2014), as well as causing a serious decline of the white back *Gyps* vulture species (Oaks et al., 2004). Honeybee populations are clearly under threat from a combination of physical, biological and chemical stressors in the environment, with habitat change and infectious diseases identified as key driving factors for population decline (Potts et al., 2010). However, the risk contributed by pharmaceuticals is largely unknown. There is therefore an urgent need to establish the potential for these biologically active chemicals to accumulate in nectar and pollen to understand the subsequent exposure to foraging honeybees and the colony.

The aim of this study was to demonstrate the potential for exposure of honeybees within hives to pharmaceutical residues via the accumulation of carbamazepine, an anti-epileptic pharmaceutical, in nectar and pollen sampled from zucchini (*Cucurbita pepo*) flowers. The plant uptake of carbamazepine has been previously well characterised due to its recalcitrance to wastewater treatment, its stability under environmental conditions and its physicochemical properties that are conducive to plant uptake (Li et al., 2013, 2018). Incorporation of environmentally relevant carbamazepine residues in nectar and pollen into a modelling framework able to simulate beehive dynamics including the honeybee foraging activity at the landscape scale (BEEHAVE and BEESCOUT (Becher et al., 2016, 2014)) enabled the simulation of carbamazepine translocation from zucchini fields into honeybee hives. A similar modelling approach has been recently adopted by Schmolke et al., (2019) whereby BEEHAVE was extended to represent colony exposure to the insecticide clothianidin via residues in pollen. A simulated southern Australian agricultural landscape (with a representative size and distribution of single fields) consisting of three habitat types (plants of high melliferous potentials) was used as a model to represent drier mid-latitude regions. In these regions, recycled water irrigation schemes are increasingly necessary for sustainable expansion of horticultural production where rain fed crop production is expected to become increasingly marginal (Turrant et al., 2011). This information provides a fundamental first step in assessing the risks of chemicals such as pharmaceuticals to bees through realistic scenarios to quantify potential exposure concentrations at the landscape scale.

2. Materials and methods

2.1. Pharmaceutical accumulation in nectar and pollen

Pollen and nectar were collected from zucchini flowers in an experiment described elsewhere where pharmaceutical residues were previously determined in zucchini leaf and fruit (Knight et al., 2018). In brief, zucchini (*C. pepo* var. Midnight F1 Container Garden) were grown from seed in a sandy soil (96.5% sand) spiked with carbamazepine (> 98% purity, CAS number 298-46-4; Sigma-Aldrich, Australia) in triplicate at a range of treatment concentrations (0.5, 1, 2, 5, 10, 20 μg/g). The soil had been collected from a non-irrigated cropping site in South Australia and, once air-dry, sieved < 2 mm to ensure homogeneity prior to use. The plants were grown for 14 weeks under controlled conditions. Upon flowering, pollen grains were brushed off the stamens directly into an Eppendorf tube. A 0.5–10 μL pipette was inserted into the ovaries to extract nectar from the carpels. Nectar and pollen collected from all replicates under the same treatment were stored as a single combined sample in an Eppendorf tube due to small sample volumes. Samples were stored at −80 °C until extraction and analysis of carbamazepine.

Carbamazepine was extracted from the harvested pollen in duplicate samples (25 ± 2 mg) per treatment following validated methods (83 ± 11% absolute recovery). Prior to extraction, pollen was spiked (0.1 μg/sample) with deuterated internal standard (carbamazepine-d10, 99.4% purity, TLC pharmachem, Canada) to account for recoveries and matrix interference. Samples were extracted by shaking the pollen with 2 mL of methanol for 30 min on a rotary shaker. Samples were centrifuged (30 min at 656*g*) and the supernatant removed. For each treatment, 10 μL of nectar was directly spiked into a HPLC vial containing a mixture of methanol, water and the internal standard (0.1 μg/sample). Quantification of carbamazepine in nectar and pollen samples was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using methods described elsewhere (Carter et al., 2014). Calculated limits of detection (LOD) and quantitation (LOQ) for carbamazepine residues in pollen were 3.1 and 10.4 μg/kg respectively. Calculated LOD and LOQ were 1.5 and 4.9 μg/L respectively for carbamazepine in nectar. In addition, LC-MS/MS was previously used to determine spiked soil and plant concentrations of carbamazepine (see Knight et al., 2018).

A linear relationship between measured soil concentration and measured concentration of carbamazepine in nectar and pollen (Figs. S1 and S2) enabled extrapolation between exposure concentrations used in the experiment and environmentally realistic carbamazepine exposure concentrations. A predicted soil concentration of carbamazepine was calculated based on measured carbamazepine concentrations in treated wastewater (44–3205 ng/L) in combination with known parameters for current recycled water irrigation schemes in southern Australia. Assumptions included that 9000 ha of agricultural land were irrigated with 37 GL/year, and that carbamazepine exhibits significant accumulation year on year due to its persistence in the environment (Williams and McLain, 2012) (see S1 for full calculation).

2.2. Pharmaceutical accumulation in beehives

The development of honeybee colonies and their foraging for nectar and pollen at the landscape scale was subsequently simulated using BEEHAVE (Becher et al., 2014) and its sub-modules BEEHAVE-Weather (Becher et al., 2014) BEESCOUT (Becher et al., 2016) to predict the transfer of carbamazepine from soil, via nectar and pollen, into beehives. Models were selected based on recommendations by the EFSA Panel on Plant Protection Products and their Residues (PPR) who concluded that BEEHAVE includes all important processes to simulate honeybee colony dynamics (EFSA, 2015). A recent model validation exercise on control colonies from honeybee field studies conducted in Germany underpins this conclusion with empirical data (Agatz et al., 2019).

Population numbers of total adult bees are provided in Fig. S4.

BEEHAVE (Becher et al., 2014) was developed to explore how various stressors (varroa mites, virus infections, impaired foraging behaviour, changes in landscape structure and dynamics, and pesticides) influence the dynamics of single colonies of honeybees. The model comprises of a colony module, a mite module, and a foraging module; overall simulating colony dynamics in a heterogeneous landscape for a defined period. The colony module describes in-hive processes like organism development, egg-laying and brood care, ultimately producing information on colony structure (number of eggs, larvae, pupae, and in-hive bees). Beekeeper activity, like varroa treatment, honey extraction, brood removal, and colony feeding, are implemented in the colony module. The mite module simulates the dynamics of a varroa mite population in the colony and interacts with the colony module via impacts on pupa and adult survival. The foraging module simulates foraging of adult bees on food sources defined as flower patches within specified distances to the hive. The foraging process during a day depends on the quality of food sources in the landscape, the weather, colony internal processes, and the current status of colony energy stores (i.e. pollen and nectar). Multiple foraging trips can be made by foragers within a single day if required, and if weather conditions allow. BEEHAVE-Weather creates weather files from air temperature and sunshine hour data to provide information for BEEHAVE on when, and the duration of suitable whether for potential foraging activity. BEESCOUT (Becher et al., 2016) creates BEEHAVE readable input files for complex landscapes that can be used as hive surroundings within the foraging module of BEEHAVE. Within BEESCOUT (Becher et al., 2016) scouting behaviour according to different theoretical scouting strategies or combinations of these can be used to characterize landscapes in terms of food availability (i.e., nectar and pollen availability across a landscape) and the probability of these food sources to be detected by scouting bees in proximity of a bee colony. The model allows use of landscape pictures (e.g. satellite images) or manually constructed landscapes to subsequently define the landscape and characterize the habitat-specific detection probability. Currently, the number of habitat types that can be parameterized is limited to four but the number of landscape attributes per habitat type is not limited. Food availability in BEESCOUT is defined by the start and end dates of food provision in a habitat type, the average nectar and pollen availability, the sugar content of nectar, and the average handling time of a forager bee to successfully collect full loads of nectar or pollen. BEHAVE and the submodels were implemented in the freely available software platform NetLogo (Wilensky, 1999).

As an example application, the BEEHAVE model was parameterised for southern Australian conditions using an agricultural landscape representative of intensive horticultural production with reclaimed wastewater. Here, compound accumulation was assessed in relation to the amount of zucchini within the foraging range, the distance between the hive and the zucchini fields, and the weather conditions during zucchini flowering. A 2-D landscape was constructed representing a 25 km^2 agriculture rich region consisting of three habitat types as attractive foraging grounds for bees (zucchini fields (contaminated with carbamazepine), oil seed rape fields and trees) (Fig. 1). Accumulation of carbamazepine into 11 hives distributed across the landscape with varying distances to the foraging grounds (relative availability of zucchini for foraging bees varying between 0 and 100% when accounting for the maximum foraging range simulated by BEESCOUT using the search mode "colony" and a foraging distance of 2.5 km) was simulated. Simulations were conducted allowing for beekeeper intervention. The foraging strategy "colony" was used because this mode is described as a strategy bees would follow if placed in an unknown landscape (Becher et al., 2016). The varroa mite module in BEEHAVE was inactivated because varroa is not currently a threat to bees in Australia.

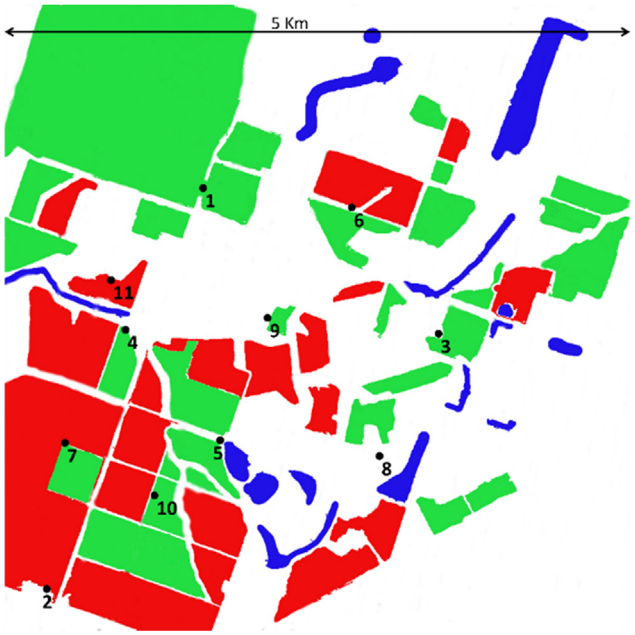

Fig. 1. Scaling of the landscape in BEESCOUT and colony placement (attractive tree (blue); zucchini (red); oil seed rape (green)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Simulations were conducted for 365 days across three different years with contrasting weather conditions (representing low, medium and high potential foraging activity) (Tables S4 and S5). Daily maximum air temperature and sunshine hours (2009–2018) to simulate the weather conditions was obtained from the Australian Bureau of Meteorology (Australian Bureau of Meteorology, 2019) to represent a typical "dry-summer subtropical" climate zone of southern Australia. The Köppen Climate Classification subtype for this climate is "Csb" (Mediterranean Climate). Missing data in the sunshine hours (maximum 3 consecutive days) were calculated by fitting a polynomial 4 parameter function through the individual data from 2009 to 2018 (see Fig. S5). The weather files created from these data for BEEHAVE included a temporal shift of 182 days to account for the fact that BEEHAVE was parameterised for European agricultural seasons whilst the growing season in Australia runs from September to March. Hence all data is presented over the period of two years (i.e. 2010–2011) representing one growing season running from 1st July to 30th June. Further model parameters for input into BEEHAVE and BEESCOUT were obtained from existing literature sources (e.g. handling time of nectar and pollen) (Table S2).

Adaptions of BEEHAVE and BEESCOUT were limited to the calculation of foraged carbamazepine and the determination of the relative habitat composition from the landscape used for foraging activity. Alterations did not alter any of the processes involved in the models but enabled the simulation of cumulative compound entry into each beehive throughout each modelled year. BEESCOUT alterations were

limited to the calculation of the relative habitat composition from the landscape used for foraging activity determination. For this an additional button was created in the interface of the model. Use of this button starts a sub-procedure that uses the defined maximum foraging distance to determine an area around the hive in which the proportion of each habitat type (in this case, individual plant species) is calculated. Further details on BEESCOUT model settings are provided in the Supporting Information (zip files).

Alterations to the BEEHAVE model (Version: BEEHAVE_BeeMapp2016; available at http://beehave-model.net/download/) were the addition of two inputs (Fig. S6), namely (1) a compound concentration in nectar (ng/L) and pollen (ng/g) for one habitat (here, red patches (see Fig. 1)); and (2) a process so that every foraging bee records whenever it collected food from a zucchini field to allow for the calculation of the cumulative amount of compound collected from pollen or nectar (assuming a full nectar or pollen load at each foraging trip). Further details on the BEEHAVE model settings is presented in the Supporting Information (Table S3). In addition the model and input files are provided as zip files.

3. Results

3.1. Pharmaceutical accumulation in nectar and pollen

Residues of carbamazepine were detected in nectar and pollen sampled from zucchini flowers (*C. pepo*) exposed to agricultural soil containing a range of carbamazepine concentrations (0.1–20 mg/kg soil). Concentrations found in nectar and pollen ranged between 1.75 and 371 ng/mL and 0.02–29.8 μg/g respectively and increased with increasing carbamazepine soil concentrations (linear relationship with $R^2 > 0.99$) (Figs. S1 and S2). Based on this relationship, it was estimated that the concentration of carbamazepine in nectar and pollen would be 0.37 ng/L and 30 ng/kg respectively, under a realistic exposure scenario where treated wastewater, containing carbamazepine residues, was applied to land resulting in a soil concentration of 20 ng/kg (see S1 for more details).

3.2. Pharmaceutical accumulation in beehives

Incorporation of these estimated carbamazepine residues into the BEEHAVE modelling framework enabled the simulation of foraging honeybees (*Apis mellifera*) gathering contaminated nectar and pollen to determine the potential transfer of carbamazepine to beehives at a landscape scale. The amount of carbamazepine gathered by each of eleven colonies scattered within a 25 km^2 landscape (Fig. 1), consisting of zucchini fields (contaminated with carbamazepine), oil seed rape fields and trees, increased exponentially during the flowering period of zucchini (Julian days 93–153, corrected for parameterisation of the model according to European agricultural seasons to remain relevant for the southern hemisphere) (Fig. 2).

Carbamazepine accumulation in a beehive during a single flowering period was simulated to range between 0 and 2478 ng, reaching a maximum concentration in colony 7 during the 2015–2016 exposure. Across all hives in the simulated landscape, the average amount of carbamazepine gathered per beehive (± 95% tolerance interval) was 899 (± 1905), 272 (± 1134), and 842 (± 2066) ng in 2010–2011, 2011–2012, and 2015–2016 respectively (Fig. 2). The large tolerance intervals reflect the fact that not all beehives accumulated cabamazepine.

The amount of carbamazepine gathered not only varied across the simulated years but there were also notable differences in accumulation of carbamazepine between beehives within the same year (Fig. 3). Similarly, the total number of adult bees throughout the year differed strongly between years and colonies (Fig. S4). Some simulated colonies collapsed during winter (Fig. S4). In 2010–2011, maximum carbamazepine accumulation was observed in colony 3 (2278 ng) with colony 4 having the lowest accumulation at 374 ng. The simulated year 2015–2016 resulted in a similar carbamazepine accumulation profile with concentrations ranging between 126 and 2478 ng however, in the growing season 2011–2012 carbamazepine was only predicted to be present in two beehives and reach a maximum of 1586 ng. Carbamazepine did not accumulate in the beehives across all three years when the percentage of zucchini within the site specific foraging range (398–641 m) (relative to characterised habitat) was less than 20% (colonies 1, 5, 8, 9) (Table S1). The accumulation of carbamazepine in the beehives was largely driven by carbamazepine residues in the pollen, which contributed 23–71 times more carbamazepine in each colony than the nectar on an annual basis (Fig. 4).

4. Discussion

Upon uptake by the plant, acropetal translocation enables chemical residues to move with the transpiration stream from the roots towards the leaves and accumulate in flowers. Carbamazepine is a neutral chemical under environmental conditions with a moderate lipophilicity (log K_{ow} 2.45) that has been previously demonstrated to be suitable for uptake into a range of plants (Carter et al., 2014; Malchi et al., 2014). Because pollen grains are relatively high in lipids, ranging from 1 to 10% by weight (Bogdanov, 2004), they are excellent storage vessels for organic chemicals, such as carbamazepine and other pharmaceuticals, which have been taken up by the plant. Given the widespread quantification of pesticide residues in pollen and nectar samples, including in *C. pepo* (Dively and Kamel, 2012; Stoner and Eitzer, 2012), it is therefore unsurprising that carbamazepine residues were also detected in zucchini nectar and pollen in this study.

In comparison to a previously published study, carbamazepine accumulated to a much lesser extent in the pollen (0.02–29.8 μg/g) than in the leaf of the corresponding zucchini plant (0.8–155.7 μg/g; “young leaf”), with a 5–38 fold difference in concentration across the exposure concentrations used (Knight et al., 2018). The greater concentration of carbamazepine in the leaves can be explained by transpiration processes occurring in the plant, moving water through the xylem to the place of demand. A larger transpiration rate from the leaves facilitates the translocation of carbamazepine to the leaves where it can accumulate at a faster rate, in comparison to the transpiration potential of flowers. In the highest carbamazepine treatment concentration (20 mg/kg) Knight et al. (2018) reported that the leaves displayed toxic symptoms, including the formation of dead spots and burnt leaf edges. This can influence the transpiration potential of the leaves, and offers an explanation as to why there was a smaller pollen:leaf carbamazepine ratio in the higher treatment concentrations as the movement of carbamazepine was altered, facilitating a smaller difference in the accumulation of carbamazepine between the leaf and the pollen.

Carbamazepine exposure has been previously quantified in aquatic food webs at higher concentrations than our environmentally relevant simulated scenario with detections in water bodies (< 6.8 ng/L), fish plasma (< 230 ng/L) and fish tissue (< 1.44 ng/g) (Bean et al., 2018; Ramirez et al., 2007). Similarly, based on our predicted environmentally relevant exposure, concentrations of carbamazepine in pollen are much lower than previously reported concentrations of neonicotinoids in global pollen samples (> 0.2–912 ng/g) (Blacquière et al., 2012). The exposure and extrapolation scenario considered in this analysis involved direct carbamazepine application to the soil, whereas spray irrigation represents a more direct exposure pathway for uptake into plants (Franklin et al., 2016). It is envisaged that potential surface adsorption onto pollen grains will have a notable influence on carbamazepine accumulation in the hive based on the significant role contaminated pollen had on carbamazepine accumulation in the hive in comparison to the proportion of carbamazepine associated with contaminated nectar (Fig. 4). In addition, spray irrigation presents a direct route of exposure for foraging bees *via* dermal exposure from spray drift (Sanchez-Bayo and Goka, 2014), thereby increasing pharmaceutical

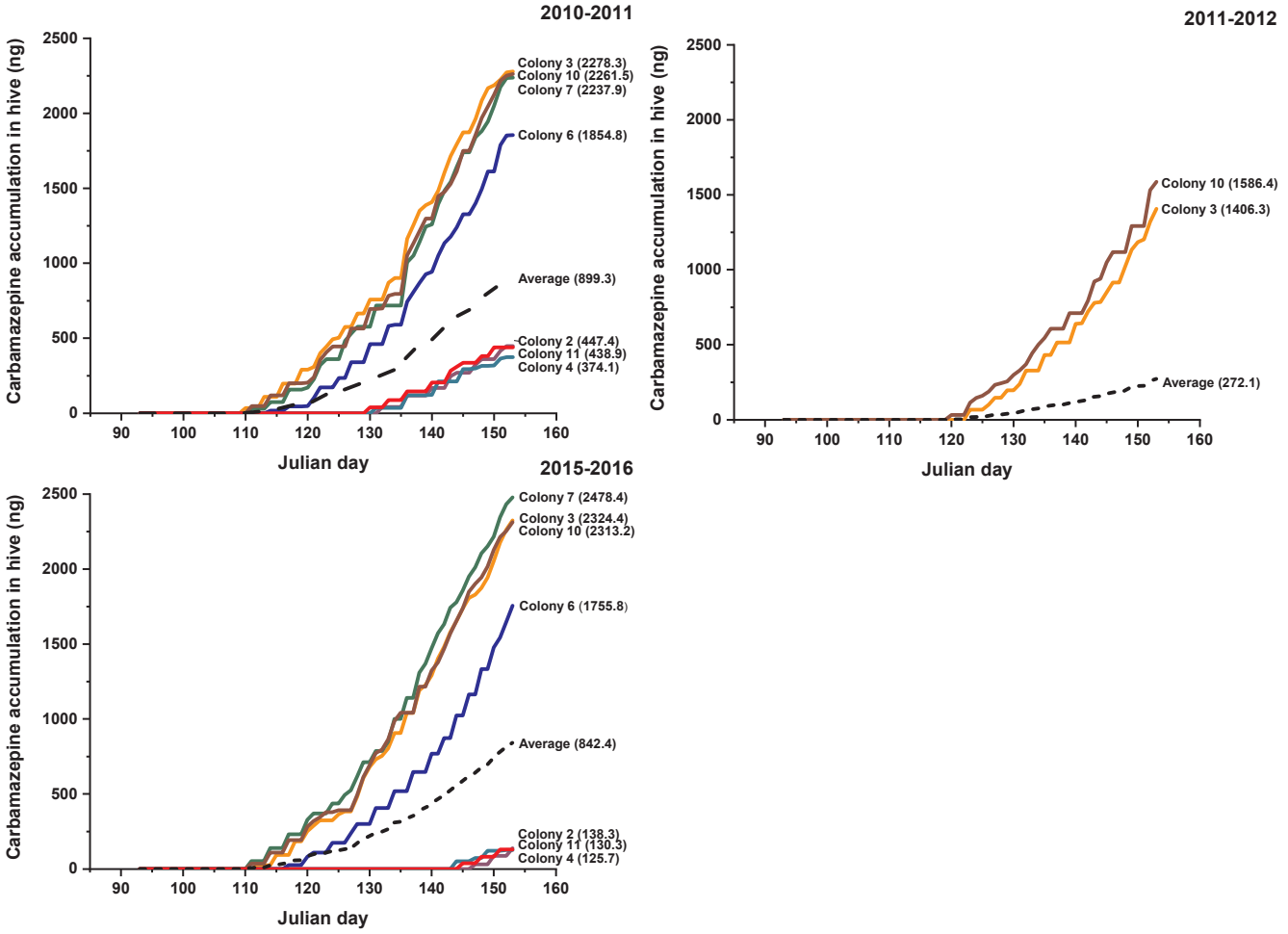

Fig. 2. Cumulative carbamazepine accumulation from contaminated nectar and pollen in individual beehives across a 25 km^2 landscape (ng/beehive). Modelled accumulation presented for three simulated years (2010–2011, 2011–2012 and 2015–2016) together with an average accumulation (dotted line). Horizontal axis truncated to display accumulation during flowering period of zucchini only (Julian day 93–153).

exposure to bees.

The foraging behaviour of honeybees is dependent on a number of factors including the location of the hive, the weather and time. The location of the hive influences the availability of the pollen and nectar sources in the foraging area and the temperature and number of sunshine hours influences the amount of times a honeybee leaves the hive to forage. Time influences which flowers are available for the honeybees to harvest nectar and pollen from, with differences in nectar and pollen availability and nutritional content driving the options foragers have and use. Lastly, the actual condition of the hive (population structure and pollen and nectar stores) influences honeybee foraging activity by directing gathering activity towards nectar or pollen to meet colony demands. Variation in honeybee foraging behaviour results in differences of honey production between hives, which in turn alters foraging activity. An average honey production between 49.7 and 91.8 kg/hive across Australia was reported for the season 2014–2015 (van Dijk et al., 2016). Under the scenarios modelled in BEEHAVE, the maximum amount of honey harvested was 105.6 kg (2010–2011), with an average of 41.2, 19.1 and 50.4 kg/hive for 2010–2011, 2011–2012 and 2015–2016 respectively. This supports the use of a realistic exposure scenario in this analysis based on typical Australian beekeeping practices.

As the simulated landscape scenario reflected seasonal and locational factors that influence the foraging activity of honeybees, the accumulation of carbamazepine in the hives was therefore a factor of attributes related to the hive and simulated year (Figs. 2 and 3). A clear pattern was not evident between the availability of each food source within the site-specific foraging range (relative to characterised habitat) and the total accumulated compound on the last day of each year (Fig. S3). The surrounding landscape in combination with in-hive dynamics determine whether, and how many, foragers are active and what resource these foragers collect and thus prevents a clear relationship between carbamazepine accumulation and availability of a single food source.

Concurrent flowering of zucchini and oilseed rape (Table S2) allows the actual colony strength, hive resources (pollen and honey stores) and colony composition (number of foragers, in-hive bees and brood) to drive the foraging activity throughout the zucchini flowering phase (Table S3). Potential foraging activity (i.e. whether conditions that allow for foraging activity) was not a good predictor of carbamazepine accumulation in the hive. Even though 2011–2012 was selected to simulate a year with 'average' climate conditions, the numbers of foraging honeybees was very low throughout the flowering period of zucchini which ultimately lead to less in-hive accumulation of carbamazepine than in the unseasonably cold and dark year of 2010–2011 (Table S3). This demonstrates that irrespective of the percent of habitat containing contaminated zucchini, a small number of foraging honeybees (< 1500) (that results from colony performance in relation to the surrounding landscape prior to zucchini flowering) will reduce the capacity for carbamazepine to be collected and brought back to the hive (Table S3 and Fig. 3). Comparatively, as shown by the cluster of points to the right of Fig. 3, carbamazepine accumulation in

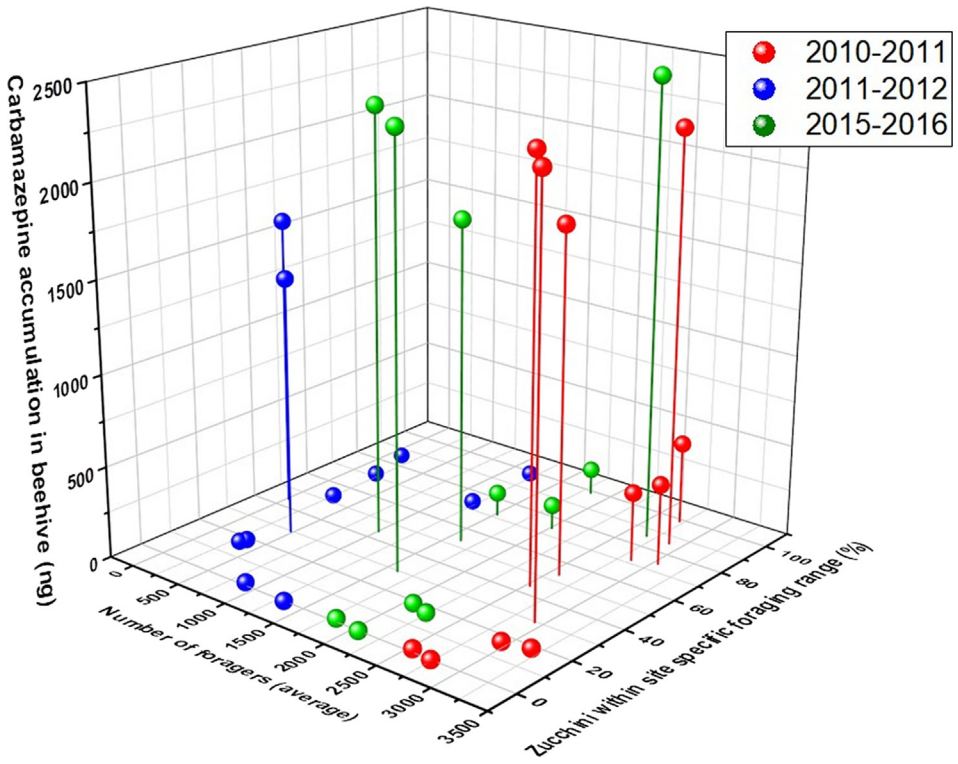

Fig. 3. Relationship between average carbamazepine accumulation in beehives, percent of zucchini within site specific foraging range (relative to characterised habitat) and average number of foragers during zucchini flowering period (Julian day 93–153) for three simulated years (2010–2011, 2011–2012 and 2015–2016).

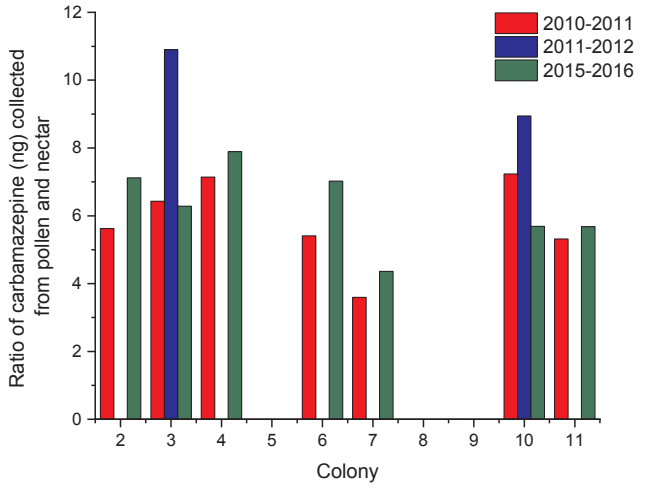

Fig. 4. Contribution of carbamazepine accumulation in 11 colonies on last day of zucchini flowering from contaminated nectar and pollen, expressed as a ratio of carbamazepine (ng) in pollen and nectar.

the hive was typically greatest when there was a high percentage of zucchini within the site-specific foraging range and a large number of foraging honeybees. However, there are a number of anomalies to this trend, driven by alterations in colony dynamics deriving from beekeeper intervention (feeding of colonies) and partial colony loss in winter (Fig. S4), demonstrating the complexity of the exposure scenario (Fig. 3). There is no simple solution to account for the complexity of in-hive dynamics and its interplay with the environment (weather and landscape) to predict the potential exposure of a colony in an environmentally realistic manner. However, mechanistic modelling approaches, such as those considered in this analysis, offer a potential platform to achieve this.

Currently there are no regulations pertaining to the environmental risk assessment of pharmaceuticals to honeybees. Mechanistic understanding of in-hive dynamics and the interplay with the environment would enable the generation of an environmentally relevant exposure scenario for use in the terrestrial risk assessment of pharmaceuticals. As outlined in the exposure assessment for plant protection products, multiple factors can influence honeybee exposure to chemicals, and applied safety (or uncertainty) factors are needed to account for this variability (EFSA, 2016). For example, in our study a difference in excess of 2000 ng was observed for carbamazepine accumulation between hives within a given year (Fig. 2), and applied safety factors would need to account for this in an attempt to balance realism and conservatism when generating exposure scenario estimates. Other variables (e.g. exposure concentrations, crop type) are expected to significantly alter accumulation of pharmaceuticals in nectar and pollen and therefore an appropriate safety factor will need to be determined and applied when considering risk assessment scenarios. Under normal bee keeping practices, the maximum total load of carbamazepine in a single hive was estimated to be approximately 80 ng (Fig. 2). However, this does not account for potential loss of carbamazepine via chemical degradation or metabolism. Future model development needs to account for potential changes in pharmaceutical parent compound concentration, including the potential metabolism of carbamazepine by honeybees as this will ultimately influence exposure in the beehive.

In humans, the metabolism of carbamazepine is catalysed by enzymes belonging to the cytochrome P450 family (Kerr et al., 1994) which are also one of the superfamilies of enzymes responsible for the metabolism and detoxification of toxins in insects, including honeybees (Li et al., 2007). However sequencing and annotation of the honeybee genome revealed a 50% or greater reduction in the number of genes encoding for these enzyme families relative to other insect genomes (Claudianos et al., 2006). Whilst it appears that honeybees are no more vulnerable to insecticides than other insects (du Rand et al., 2015), the smaller number of detoxification genes may limit the capacity of honeybees to metabolize multiple toxins simultaneously, causing bees to be more sensitive to synergistic interactions of chemicals e.g. competitive

inhibition of P450s (Johnson et al., 2012, 2009). Nevertheless, even with a reduction in the number of enzymes responsible for the metabolism of toxins, the metabolism of pesticides in bees has been reported, including the metabolism of the neonicotinoid, imidacloprid (Dively et al., 2015; Suchail et al., 2004). In this case, it has been suggested that 5-hydroxyimidacloprid and olefin metabolites of imidacloprid (Suchail et al., 2004) have a high affinity for the honeybee nicotinic acetylcholine receptors (nAChR), thereby contributing to extending the action of the parent compound in honeybees (du Rand et al., 2015; Johnson et al., 2012, 2009; Nauen et al., 2001). More work is therefore needed to understand the extent of metabolism of pharmaceuticals by honeybees and to assess the potency of metabolites relative to the parent compound. Following improvements to take into account factors such as degradation, metabolism, effects on foragers and effects on in-hive bees (and subsequent effects on in-hive dynamics and foraging activity), the use of mechanistic modelling approaches will provide valuable information regarding exposure estimates for the terrestrial risk assessment of pharmaceuticals. Future risk assessments concerning honeybee exposure to pharmaceuticals should make use of tools to evaluate cross-species sensitivity to interrogate potential molecular targets of active pharmaceuticals in honeybees due to the conservation of drug targets, using tools such as SeqAPASS and ECOdrug (LaLone et al., 2016; Verbruggen et al., 2018).

5. Conclusions

Ultimately, given the biological potency of pharmaceuticals, the accumulation of these chemicals in nectar and pollen presents a significant potential risk to honeybee colonies when these residues accumulate within a beehive. This work describes a fundamental first step in the quantification of pharmaceuticals in nectar and pollen, which has enabled us to demonstrate the potential for honeybee colonies to be exposed to bioactive pharmaceutical residues. There remains an urgent need to evaluate the potential for a wider suite of wastewater-derived pharmaceuticals to accumulate in these matrices and the potential risk of this to honeybee health given the increasing necessity for wastewater irrigation practices in water stressed regions. A detailed understanding of the potential transfer of pharmaceutical residues to the beehive will enable scientists and regulators to be able to design experiments to consider the risk of this exposure (e.g. larval toxicity) by considering realistic exposure doses of pharmaceuticals. There is also a need to understand how pharmaceutical-induced toxicity can affect bee foraging, the results of which can be incorporated into models to simulate the development of bee colonies and their foraging for nectar and pollen analogous to the recent approach by Prado et al. (2019) which simulated the impact of pesticide exposure on bee foraging. Bees are essential to maintaining functioning ecosystems and the current decline of bee populations is of global concern. Habitat loss and fragmentation, changes to weather patterns (including climate change), and exposure to chemical pollutants such as pesticides all present a risk to honeybee populations. Here we demonstrate that exposure to pharmaceutical residues increases the range of complex and interacting threats towards honeybee health and warrants urgent further investigation.

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 material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105248.

References

Agatz, A., Kuhl, R., Miles, M., Schad, T., Preuss, T.G., 2019. An EVALUATION of the BEEHAVE model using honey bee field study data: insights and recommendations. Environ. Toxicol. Chem. etc.4547. https://doi.org/10.1002/etc.4547.

Arnold, K.E., Brown, A.R., Ankley, G.T., Sumpter, J.P., 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Philos. Trans. R. Soc. B-Biological Sci. 369. https://doi.org/10.1098/rstb.2013.0569.

Australian Bureau of Meteorology, 2019. South Australian Weather and Warnings. Daily Weather Observation Tables [WWW Document]. URL http://www.bom.gov.au/sa/observations/index.shtml?ref=hdr (accessed 1.28.19).

Bean, T.G., Boxall, A.B.A., Lane, J., Herborn, K.A., Pietravalle, S., Arnold, K.E., 2014. Behavioural and physiological responses of birds to environmentally relevant concentrations of an antidepressant. Philos. Trans. R. Soc. B-Biological Sci. 369. https://doi.org/10.1098/rstb.2013.0575.

Bean, T.G., Rattner, B.A., Lazarus, R.S., Day, D.D., Burket, S.R., Brooks, B.W., Haddad, S.P., Bowerman, W.W., 2018. Pharmaceuticals in water, fish and osprey nestlings in Delaware River and Bay. Environ. Pollut. 232, 533–545. https://doi.org/10.1016/J.ENVPOL.2017.09.083.

Becher, M.A., Grimm, V., Knapp, J., Horn, J., Twiston-Davies, G., Osborne, J.L., 2016. BEESCOUT: A model of bee scouting behaviour and a software tool for characterizing nectar/pollen landscapes for BEEHAVE. Ecol. Modell. 340, 126–133. https://doi.org/10.1016/J.ECOLMODEL.2016.09.013.

Becher, M.A., Grimm, V., Thorbek, P., Horn, J., Kennedy, P.J., Osborne, J.L., 2014. BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure. J. Appl. Ecol. 51, 470–482. https://doi.org/10.1111/1365-2664.12222.

Blacquière, T., Smagghe, G., van Gestel, C.A.M., Mommaerts, V., 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotoxicology 21, 973–992. https://doi.org/10.1007/s10646-012-0863-x.

Bogdanov, S., 2004. Quality and Standards of Pollen and Beeswax. Apiacta 38, 334–341.

Carter, L.J., Harris, E., Williams, M., Ryan, J.J., Kookana, R.S., Boxall, A.B.A., 2014. Fate and uptake of pharmaceuticals in soil-plant systems. J. Agric. Food Chem. 62, 816–825. https://doi.org/10.1021/jf404282y.

Claudianos, C., Ranson, H., Johnson, R.M., Biswas, S., Schuler, M.A., Berenbaum, M.R., Feyereisen, R., Oakeshott, J.G., 2006. A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. Insect Mol. Biol. 15, 615–636. https://doi.org/10.1111/j.1365-2583.2006.00672.x.

Dalkmann, P., Broszat, M., Siebe, C., Willaschek, E., Sakinc, T., Huebner, J., Amelung, W., Grohmann, E., Siemens, J., 2012. Accumulation of pharmaceuticals, enterococcus, and resistance genes in soils irrigated with wastewater for zero to 100 years in Central Mexico. PLoS One 7, 10. https://doi.org/10.1371/journal.pone.0045397.

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

Dively, G.P., Kamel, A., 2012. Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. J. Agric. Food Chem. 60, 4449–4456. https://doi.org/10.1021/jf205393x.

Drillia, P., Stamatelatou, K., Lyberatos, G., 2005. Fate and mobility of pharmaceuticals in solid matrices. Chemosphere. https://doi.org/10.1016/j.chemosphere.2005.01.032.

du Rand, E.E. du, Smit, S., Beukes, M., Apostolides, Z., Pirk, C.W.W., Nicolson, S.W., 2015. Detoxification mechanisms of honey bees (Apis mellifera) resulting in tolerance of dietary nicotine. Sci. Rep. 5, 11779. https://doi.org/10.1038/srep11779.

EFSA, 2016. Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA J. 11. https://doi.org/10.2903/j.efsa.2013.3295.

EFSA, 2015. Statement on the suitability of the BEEHAVE model for its potential use in a regulatory context and for the risk assessment of multiple stressors in honeybees at the landscape level 1. EFSA J. 13, 1–92. https://doi.org/10.2903/j.efsa.2015.4125.

Franklin, A.M., Williams, C.F., Andrews, D.M., Woodward, E.E., Watson, J.E., 2016. Uptake of three antibiotics and an antiepileptic drug by wheat crops spray irrigated with wastewater treatment plant effluent. J. Environ. Qual. 45, 546–554. https://doi.org/10.2134/jeq2015.05.0257.

Goldstein, M., Shenker, M., Chefetz, B., 2014. Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. Environ. Sci. Technol. 48, 5593–5600. https://doi.org/10.1021/es5008615.

Goulson, D., 2013. REVIEW: an overview of the environmental risks posed by neonicotinoid insecticides. J. Appl. Ecol. 50, 977–987. https://doi.org/10.1111/1365-2664.12111.

Hrynko, I., Łozowicka, B., Kaczyński, P., 2019. Comprehensive analysis of insecticides in melliferous weeds and agricultural crops using a modified QuEChERS/LC-MS/MS protocol and of their potential risk to honey bees (Apis mellifera L.). Sci. Total Environ. 657, 16–27. https://doi.org/10.1016/J.SCITOTENV.2018.11.470.

Johnson, R.M., Mao, W., Pollock, H.S., Niu, G., Schuler, M.A., Berenbaum, M.R., 2012. Ecologically appropriate xenobiotics induce cytochrome P450s in Apis mellifera. PLoS One 7, e31051. https://doi.org/10.1371/journal.pone.0031051.

Johnson, R.M., Pollock, H.S., Berenbaum, M.R., 2009. Synergistic interactions between in-hive miticides in Apis mellifera. J. Econ. Entomol. 102, 474–479.

Kerr, B.M., Thummel, K.E., Wurden, C.J., Klein, S.M., Kroetz, D.L., Gonzalez, F.J., Levy,

R., 1994. Human liver carbamazepine metabolism: role of CYP3A4 and CYP2C8 in 10,11-epoxide formation. Biochem. Pharmacol. 47, 1969–1979. https://doi.org/10.1016/0006-2952(94)90071-X.
Kinney, C.A., Furlong, E.T., Werner, S.L., Cahill, J.D., 2006. Presence and distribution of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water. Environ. Toxicol. Chem. 25, 317–326. https://doi.org/10.1897/05-187r.1.
Klein, S., Cabirol, A., Devaud, J.-M., Barron, A.B., Lihoreau, M., 2017. Why bees are so vulnerable to environmental stressors. Trends Ecol. Evol. 32, 268–278. https://doi.org/10.1016/j.tree.2016.12.009.
Knight, E.R., Carter, L.J., McLaughlin, M.J., 2018. Bioaccumulation, uptake, and toxicity of carbamazepine in soil-plant systems. Environ. Toxicol. Chem. 37, 1122–1130. https://doi.org/10.1002/etc.4053.
Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G., Given, K., 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7, e29268. https://doi.org/10.1371/journal.pone.0029268.
LaLone, C.A., Villeneuve, D.L., Lyons, D., Helgen, H.W., Robinson, S.L., Swintek, J.A., Saari, T.W., Ankley, G.T., 2016. Editor's Highlight: Sequence alignment to predict across species susceptibility (SeqAPASS): a web-based tool for addressing the challenges of cross-species extrapolation of chemical toxicity. Toxicol. Sci. 153, 228–245. https://doi.org/10.1093/toxsci/kfw119.
Li, J., Dodgen, L., Ye, Q., Gan, J., 2013. Degradation kinetics and metabolites of carbamazepine in soil. Environ. Sci. Technol. 47, 3678–3684. https://doi.org/10.1021/es304944c.
Li, M., Ding, T., Wang, H., Wang, W., Li, J., Ye, Q., 2018. Uptake and translocation of 14C-Carbamazepine in soil-plant systems. Environ. Pollut. 243, 1352–1359. https://doi.org/10.1016/J.ENVPOL.2018.09.079.
Li, X., Schuler, M.A., Berenbaum, M.R., 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol. 52, 231–253. https://doi.org/10.1146/annurev.ento.51.110104.151104.
Malchi, T., Maor, Y., Tadmor, G., Shenker, M., Chefetz, B., 2014. Irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks. Environ. Sci. Technol. 48, 9325–9333. https://doi.org/10.1021/es5017894.
Nauen, R., Ebbinghaus-Kintscher, U., Schmuck, R., 2001. Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites inApis mellifera (Hymenoptera: Apidae). Pest Manage. Sci. 57, 577–586. https://doi.org/10.1002/ps.331.
Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M., Mahmood, S., Ali, A., Khan, A.A., 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature 427, 630–633. https://doi.org/10.1038/nature02317.
Ostiguy, N., Drummond, F., Aronstein, K., Eitzer, B., Ellis, J., Spivak, M., Sheppard, W., Ostiguy, N., Drummond, F.A., Aronstein, K., Eitzer, B., Ellis, J.D., Spivak, M., Sheppard, W.S., 2019. Honey bee exposure to pesticides: a four-year nationwide study. Insects 10, 13. https://doi.org/10.3390/insects10010013.
Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345–353. https://doi.org/10.1016/J.TREE.2010.01.007.
Prado, A., Pioz, M., Vidau, C., Requier, F., Jury, M., Crauser, D., Brunet, J.-L., Le Conte, Y., Alaux, C., 2019. Exposure to pollen-bound pesticide mixtures induces longer-lived but less efficient honey bees. Sci. Total Environ. 650, 1250–1260. https://doi.org/10.1016/J.SCITOTENV.2018.09.102.
Ramirez, A.J., Mottaleb, M.A., Brooks, B.W., Chambliss, C.K., 2007. Analysis of Pharmaceuticals in Fish Using Liquid Chromatography-Tandem Mass Spectrometry. https://doi.org/10.1021/AC062215I.
Roszko, M.Ł., Kamińska, M., Szymczyk, K., Jędrzejczak, R., 2016. Levels of selected persistent organic pollutants (PCB, PBDE) and pesticides in honey bee pollen sampled in Poland. PLoS One 11, e0167487. https://doi.org/10.1371/journal.pone.0167487.
Sanchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees–a risk assessment. PLoS One 9, e94482. https://doi.org/10.1371/journal.pone.0094482.
Schmolke, A., Abi-Akar, F., Hinarejos, S., 2019. Honey bee colony-level exposure and effects in realistic landscapes: an application of BEEHAVE simulating clothianidin residues in corn pollen. Environ. Toxicol. Chem. 38, 423–435. https://doi.org/10.1002/etc.4314.
Shore, R.F., Taggart, M.A., Smits, J., Mateo, R., Richards, N.L., Fryday, S., 2014. Detection and drivers of exposure and effects of pharmaceuticals in higher vertebrates. Philos. Trans. R. Soc. B-Biological Sci. 369. https://doi.org/10.1098/rstb.2013.0570.
Stoner, K.A., Eitzer, B.D., 2012. Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (Cucurbita pepo). PLoS One 7, e39114. https://doi.org/10.1371/journal.pone.0039114.
Suchail, S., Debrauwer, L., Belzunces, L.P., 2004. Metabolism of imidacloprid in Apis mellifera. Pest Manage. Sci. 60, 291–296. https://doi.org/10.1002/ps.772.
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, 1–16. https://doi.org/10.1038/srep33207.
Turrant, H., Burke, J., Faures, J.-M., 2011. FAO Water Reports: climate change, water and food security. Rome.
van Dijk, J., Gomboso, J., Levantis, C., 2016. Australian Honey Bee Industry: 2014–15 Survey Results. Commonwealth of Australia, Canberra, Australia.
Verbruggen, B., Gunnarsson, L., Kristiansson, E., Österlund, T., Owen, S.F., Snape, J.R., Tyler, C.R., 2018. ECOdrug: a database connecting drugs and conservation of their targets across species. Nucleic Acids Res. 46, D930–D936. https://doi.org/10.1093/nar/gkx1024.
Wilensky, U., 1999. NetLogo, center for connected learning and computer-based modeling.
Williams, C.F., McLain, J.E.T., 2012. Soil persistence and fate of carbamazepine, lincomycin, caffeine, and ibuprofen from wastewater reuse. J. Environ. Qual. 41, 1473. https://doi.org/10.2134/jeq2011.0353.