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Comparative toxicity of pesticides and environmental contaminants in bees: Are honey bees a useful proxy for wild bee species?

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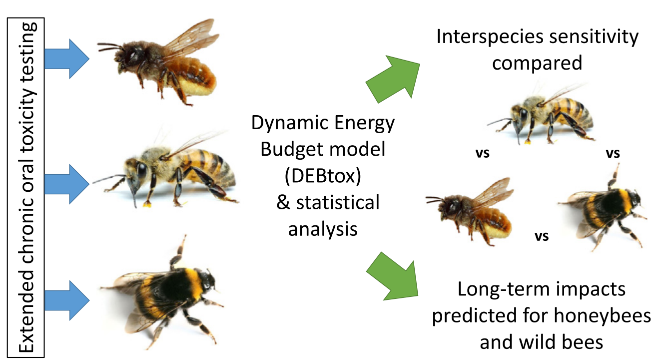
HIGHLIGHTS

- Comparison of honey bee susceptibility to toxicants with wild bee species using extended oral exposures
- Honey bees are a good proxy for other bee species, provided interspecific variation is accounted for.
- DEBtox predicts significant time dependent toxicity differences between bee species.
- Temporal changes in toxicity should be incorporated in bee risk assessments.

GRAPHICAL ABSTRACT



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ABSTRACT

Threats to wild and managed insect pollinators in Europe are cause for both ecological and socio-economic concern. Multiple anthropogenic pressures may be exacerbating pollinator declines. One key pressure is exposure to chemicals including pesticides and other contaminants. Historically the honey bee (*Apis mellifera* spp.) has been used as an 'indicator' species for 'standard' ecotoxicological testing but it has been suggested that it is not always a good proxy for other types of eusocial and solitary bees because of species differences in autecology and sensitivity to various stressors. We developed a common toxicity test system to conduct acute and chronic exposures of up to 240 h of similar doses of seven chemicals, targeting different metabolic pathways, on three bee species (*Apis mellifera* spp., *Bombus terrestris* and *Osmia bicornis*). We compared the relative sensitivity between species in terms of potency between the chemicals and the influence of exposure time on toxicity. While there were significant interspecific differences that varied through time, overall the magnitude of these differences (in terms of treatment effect ratios) was generally comparable (<2 fold) although there were some large divergences from this pattern. Our results suggest that *A. mellifera* spp. could be used as a proxy for other bee species provided a reasonable assessment factor is used to cover interspecific variation. Perhaps more importantly our results show significant and large time dependency of toxicity across all three tested species that greatly exceeds species differences (>25 fold within test). These are rarely considered in standard regulatory testing but may have severe environmental consequences, especially when coupled with the likelihood of differential species exposures in the wild. These insights indicate that further work is required to understand how differences in toxicokinetics vary between species and mixtures of chemicals.

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1. Introduction

Concerns over reductions in global pollination services encompass both losses of managed populations of insect pollinators, chiefly the Western honey bee (*Apis mellifera* spp.) (Laurent et al., 2015; Seitz et al., 2015), and declines in wild insect pollinators such as natural bee populations (Vanbergen and the I.P.I, 2013). Both eusocial and solitary wild bees have shown dramatic declines in range and diversity across Europe and North America over recent decades (Laurent et al., 2015; Seitz et al., 2015; Vanbergen and the I.P.I, 2013; Williams and Osborne, 2009). These declines have serious economic as well as conservation implications. Pollination, primarily by both managed and wild insects, provides direct commercial benefits to crop production (the value of insect pollination for world agriculture has been estimated >€150 billion p.a.) (Gallai et al., 2009; Lautenbach et al., 2012) and makes a key contribution to the dynamics and persistence of native plant species and communities (Fontaine et al., 2005).

Global threats to insect pollinators could arise from multiple environmental pressures which, singly and/or in combination may alter survival, behaviour and reproduction (Vanbergen and the I.P.I, 2013) and in turn jeopardize the delivery of pollination services to crops and wild plants. These environmental pressures include land-use intensification, pesticides, urbanization, invasive alien species, the spread of diseases and parasites and climate change.

One key pressure is exposure to chemicals (Goulson et al., 2015; Scott-Dupree et al., 2009; Whitehorn et al., 2012) through contact and consumption of contaminated nectar, pollen, water and guttation fluids, or via contact during foraging or nesting (e.g. in the air with contaminated dust particles, on crops and in soil with contaminated surfaces). This includes pesticide classes routinely applied to flowering crops and pesticides and environmental contaminants that may co-occur as a result of agrochemical use and diffuse or point source pollution (Botías et al., 2015; Long and Krupke, 2016; Samson-Robert et al., 2014). For example, over the last decade a median of >16 active ingredients (a.i) have been applied to an 'representative' UK arable field crop (proportion area treated 2014 = fungicides 40%, herbicides 31%, growth regulators 11%, seed treatments (often combinations of a.i's) 9%, insecticides 8%, molluscicides 2%; (unpublished analysis of FERA, 2016). Analysis of honey bees and hive products in North America and Europe have shown that most managed colonies contain a suite of chemical contaminants, including insecticides, acaricides, herbicides and fungicides (Bogdanov, 2006; Johnson et al., 2013; Mullin et al., 2010). It is highly likely that other pollinator species, foraging in similar habitats to honey bees, will be exposed to the same range of chemicals (Goulson et al., 2015).

Although there are well established protocols for the testing of the acute toxicity of chemicals for pollinating insects this is almost exclusively focused on honey bees (OECD, 1998a,b; Medrzycki et al., 2013). This species is considered as highly sensitive to insecticides and fungicides and, although sensitivity it is generally less to herbicides, is considered a good environmental indicator of pesticide pollution. This is partly corroborated by the lower number of genes encoding xenobiotic detoxifying enzymes in the *A. mellifera* spp. genome compared with other insect species such as flies and mosquitoes (Claudianos et al., 2006). While some review studies have compared the relative sensitivity of *A. mellifera* spp. to other bees (Arena and Sgolastra, 2014; Tasei et al., 2000) and insect species (Hardstone and Scott, 2010), quantitative comparisons of differences in sensitivity, especially using the same experimental approaches are lacking (but see (Scott-Dupree et al., 2009)). In addition, most of the 'standard' tests conducted to date tend to be of short duration (48–96 h, e.g. (OECD, 1998a,b) with 'pulse' dosing frequently limited to topical exposures for testing contact toxicity. Policy decisions based on the assumption that honey bees are good proxies for other pollinating insects, including other bee species, have been challenged (Dicks, 2013) and there is a general consensus about a need to fully evaluate the importance of differing routes of exposure for different chemicals on non-*Apis* bee species (Carreck and Ratnieks, 2014; EFSA, 2012) over more realistic timeframes if they are to better inform environmental risk assessment and ecological understanding (Goulson et al., 2015; Rondeau et al., 2014).

The key question is how widely wild bees differ from honey bees in their responses to a range of chemicals that affect different metabolic pathways? In this study we developed both acute (short-term; up to 96 h) and chronic (extended up to 240 h) continuous feeding exposure tests to compare and predict the long term impacts of seven different chemicals on two wild bee species (*Bombus terrestris audax* and *Osmia bicornis*) and managed honey bees (*A. mellifera* spp.). We focused on oral exposure since recent evidence suggests this is often the most relevant and the most conservative approach for bees (EFSA, 2012). A priori our null hypothesis was that there would be no interspecific difference in sensitivity over time.

2. Material and methods

2.1. Study species

Three bee species were used to assess the potential hazards of the selected single chemicals. The honey bee *Apis mellifera* spp. is a eusocial species that is the most frequent managed pollinator in the world. Managed colonies are typically kept in hives containing thousands of individuals (brood and adults comprising thousands of female workers, hundreds of drones and a single queen) with well-defined castes, each with specific functions within the colony. Healthy, queen-right colonies persist for several years. For this study, honey bees were obtained as nucleus hives in spring 2014, from a commercial breeder in north Oxfordshire UK, each with a queen mated naturally the previous year. Eight hives were established and were regularly inspected and maintained to ensure that they were queen-right and maintained healthy brood and adult bees. Workers foraged freely but did not visit oilseed rape (which was not flowering) during the testing period (mid to late summer during peak colony strength). No chemical disease treatments were used for 4 months prior to test trials.

The bumblebee *Bombus terrestris audax* is a more primitive eusocial species with no clear caste system. It is a common wild pollinator which is also commercially reared for pollination in closed or semi-closed cultivation situations. In the temperate zone it is generally an annual species that lives in colonies that contain c. 100–150 female workers during the summer. Colonies of UK native *B. t. audax* were obtained as commercially reared colonies with c. 30 workers (NV Biobest, Belgium). On receipt, colonies were fed a pure 50% w/v sucrose food source, supplemented with fresh, disease free pollen.

The solitary bee *Osmia bicornis* is a non-eusocial wild pollinator species that nests in cavities. It is also produced at small scales for commercial pollination (Gruber et al., 2011). The species produces single nests containing c. 4–8 eggs that can only be harvested for testing over the spring months. Pupae used for hatching the adult bees to be used for this study were obtained from a managed field population collected at the end of the previous year i.e. <1 year old. The overwintered *O. bicornis* pupae were obtained from German commercial stocks (Dr Schubert Plant Breeding, Germany).

2.2. Chemical selection

Chemicals were selected to reflect both current concerns about the effects of agrochemicals on pollinators and the widespread presence of other trace pollutants, such as metals, in the environment. This was balanced with mechanistic considerations to ensure that different metabolisms (e.g. by cytochrome P450s, esterases, *p*-glycoproteins, melloproteins) and modes of action (e.g. neurotoxins, metabolic toxicant, reactive oxygen species production) were represented. This resulted in a list that included representatives from different insecticide, fungicide and herbicide classes, as well as a metalloid and a toxic non-essential metal (Table 1, dimethoate, an organophosphate insecticide

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