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Running title: Thermogenesis in bumble bees under pesticide exposure

The effect of dietary neonicotinoid pesticides on non-flight thermogenesis in worker bumble bees (*Bombus terrestris*)

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ABSTRACT

For bumble bees (genus *Bombus*), the capacity for non-flight thermogenesis is essential for two fundamental processes undertaken by adult workers, namely recovery from torpor after chilling and brood incubation. Farmland bees can be widely exposed to dietary residues of neurotoxic neonicotinoid insecticides that appear in the nectar and pollen of treated bee-attractive crops, which may harm them. An earlier study shows that dietary neonicotinoids cause complex alterations to thermoregulation in honey bees, but their effects on the thermogenic capabilities of individual bumble bees has been untested previously. We therefore conducted laboratory trials involving separate dietary exposures of bumble bees to two neonicotinoids, imidacloprid and thiamethoxam, and we measured their effects on the thoracic temperatures of bees during recovery from chilling. Specifically, we used thermal imaging to measure the rates of rewarming by individual bees after chill-induced torpor and to quantify their equilibrated thoracic temperatures post-recovery. We found that both toxicants caused dose-dependent decreases in the rates of rewarming and in the equilibrated thoracic temperatures. As previously found in honey bees, the dose-response relationship for imidacloprid exhibited a biphasic hormesis with low-dose stimulation and high-dose inhibition, for which we propose a mechanism. Our present study is among the first to detect ecologically relevant effects on bees in exposures involving dietary concentrations below 5 ppb. If the effects on thoracic temperatures that we observed over a short period were sustained, they could have ecologically significant impacts on farmland bumble bees.

1. Introduction

Non-flight thermogenesis (NFT) is fundamental to the ecology of many kinds of bees (Heinrich 1993), including honey bees (genus *Apis*), bumble bees (*Bombus*) and carpenter bees (*Xylocopa*). In individual bees, NFT principally involves the production of heat from the thorax by shivering of the flight muscles (Esch, Goller & Heinrich 1991), although it is sometimes supplemented to a small extent by non-shivering metabolic activity (Staples, Koen & Laverty 2004). In bees, NFT is employed in various functions related to thermoregulation. For example, NFT plays an important role both in pre-flight warm-up by individual bees (Heinrich 1975) and in brood incubation by colonies (Schultze-Motel 1991). NFT-related behaviours are regulated closely by nervous control (Heinrich & Kammer 1973), probably because of their critical functional significance and because of their high metabolic cost (Heinrich 1979; Silvola 1984). Consequently, the coordinated activation of NFT is expected to be susceptible to disruption when bees are exposed to a neurotoxin. Recently, this expectation has been confirmed by experiments made on African honey bees (*A. mellifera scutellata*) in which isolated adult workers were exposed by oral dosing to the neurotoxic insecticide, thiamethoxam (Tosi *et al.* 2016). In these experiments, dietary thiamethoxam caused complex alterations to thermoregulation by individual honey bees in which the dose-response relationship for thoracic temperature included a biphasic hormesis (Calabrese 2004), or a low-dose stimulation relative to undosed controls coupled with high-dose inhibition. Building on this previous research, the broad aims of our present study were: (1) to establish whether bumble bees were similarly susceptible to disruption of NFT-related thermoregulation by a dietary neurotoxin; and (2) to test whether neurotoxic exposure in bumble bees would generate similarly complex effects, such as hormesis.

Farmland bees can be exposed to the residues of neurotoxic insecticides, such as the neonicotinoid thiamethoxam, which appear in the nectar and pollen of treated bee-attractive

crops (David *et al.* 2016). For example, mass-blooming crops of oilseed rape and sunflower are widely treated systemically against insect pests with neonicotinoid seed dressings, whose active ingredient is neurotoxic (Nauen & Jeschke 2011). Various members of the neonicotinoid chemical family (including clothianidin and imidacloprid) are used worldwide as the active ingredients of seed dressings and they are currently notorious (Sluijs *et al.* 2013) as a potential threat to the health of farmland bees (Simon-Delso *et al.* 2015). In social bees, such as bumble bees and honey bees, dietary exposure to neoncotinoids can impair a wide range of behavioural and life history-related characteristics including homing behaviour (Henry *et al.* 2012), colony performance (Whitehorn *et al.* 2012) and foraging activity (Gill & Raine 2014). In Europe, concerns over neonicotinoids have led to regulatory prohibitions on their agricultural use based on accumulated scientific findings (Blacquière *et al.* 2012) that suggest potential harm to managed and wild bees.

One of the key goals in evaluating the risk to farmland bees from agrochemical neonicotinoids is to determine the severity of effects caused by environmentally relevant levels of exposure. The lifetimes of adult bees and the blooming periods of bee-attractive crops both extend over several weeks and the residue levels in nectar and pollen are fairly low (approximately 5 parts per billion, or ppb) (Godfray *et al.* 2014), which means that realistic dietary exposures are both sustained (multiple ingestion events) and low-level. The previous study of the effects of thiamethoxam intoxication on thermoregulation in honey bees (Tosi *et al.* 2016) employed short term (single meal) exposures to doses (i.e. it was conducted under the 'acute exposure' paradigm), but sustained exposure (the 'chronic exposure' paradigm) better simulates environmentally relevant scenarios. Additionally, the agricultural use of several members of the neonicotinoid family makes it valuable to establish whether effects on bees vary enough among the chemical variants to merit case-by-case treatment by regulators. In this context, therefore, our laboratory-based study had two specific objectives:

(1) to extend our current understanding of environmentally-relevant threats to bees by conducting dietary testing of toxicants under the 'chronic exposure' paradigm; and (2) by using NFT-related traits as ecologically relevant indicators of performance, to establish and compare the dose-response relationship between two focal neonicotinoids (thiamethoxam, imidacloprid) across an environmentally relevant range.

The role of NFT in the ecology of bumble bees

Bumble bees (*Bombus*) are eusocial insects whose individuals are capable of NFT by metabolically activating their thoracic muscles, which also power flight. The geographic range of bumble bees is dominated by the cool climates of temperate zones and it extends to the tundra (Williams 1994). Outside the bumble bee nest, foraging individuals therefore may encounter inclement weather, which they tolerate by landing and entering temporary torpor (Heinrich 1993). Later, they will resume flight after employing NFT to restore their previously chilled thoracic muscles to flight capability. Inside the bumble bee nest, both the queen and her adult workers employ NFT to maintain the developing brood at temperatures above 30 °C, irrespective of colder surroundings (Heinrich 1979). NFT may also play a role in producing social cues to modulate foraging activity (Mapalad, Leu & Nieh 2008). The importance of thermogenesis in the bumble bee's life history is indicated by the complex anatomy that has evolved to support it, which includes systems that enable finely controlled heat transfer between an individual bee's body segments (Heinrich 1976). Compared with honeybees and many other bees, disruption of NFT could be more detrimental to bumblebees because they typically occupy cool climates and forage during hours of the day when environmental temperatures can be low.

The capacity for post-torpor rewarming by NFT in bumble bees is well established. Individual bees enter torpor when chilled to approximately 7 °C (Goller & Esch 1990) and

rewarming initially is passive and depends on heat from the environment, probably because the respiratory enzymes function poorly at lower temperatures, but eventually non-flight thermogenesis is initiated. Subsequently, individual bees rewarm at rates of approximately 5 °C minute^{-1} (Heinrich 1975). After leaving torpor and before attempting flight, individuals achieve and maintain thoracic temperatures of approximately 35 °C (Heinrich 1975), which normally exceeds the ambient environmental temperature.

In many regions, bumble bees will experience dietary exposures to systemic insecticides, such as the neonicotinoids, because they are common visitors to bee-attractive insecticide-treated crops (e.g. oilseed rape and sunflower). The neurotoxic neonicotinoids are capable of causing evident symptoms of intoxication in bumble bees (Cresswell *et al.* 2012), which strongly suggests that NFT could be affected because shivering of the flight muscles is under nervous control.

1.1 Assaying non-flight thermogenesis in bumble bees

As an environmentally relevant testing paradigm for neurotoxic effects on thermogenesis, we studied the effect of dietary neonicotinoids on the ability of bumble bees to rewarm after cold-induced torpor. We assessed the rate of warming by the change in thoracic temperature, denoted ΔT (units of °C minute^{-1}), because the thorax is the seat of thermogenesis in bees. A second thermogenesis-related trait that we investigated was the 'equilibrated temperature', denoted T_E (units of °C), which we define as the steady-state thoracic temperature that a non-flying bee maintained after rewarming from a chilled state. The value of T_E is a product of both the individual's thermogenic capacity and its ability to appropriately regulate its thoracic temperature, either of which potentially could be disrupted by exposure to a neurotoxin.

2. Methods

2.1 Provenance and husbandry of bees

Bumble bees (*Bombus terrestris* L.) were obtained as colonies from a commercial supplier (Biobest, Westerlo, Belgium). Bees were placed individually in softwood cages (dimensions: 0.065 m × 0.05 m × 0.035 m) faced with fine plastic mesh (Cresswell et al., 2012). Each cage was supplied with a feeder containing sugar syrup (Attraker; Koppert B.V., Berkel en Rodenrijs, Netherlands) from which the bee fed *ad libitum*. Bees were maintained in a semi-controlled warmed environment (temperature c. 25 ◦C, relative humidity c. 40%, 12:12 h of dim light:darkness).

2.2 Exposure to dietary pesticide

Imidacloprid was obtained as a solution in acetonitrile (10 μg mL^{-1}, product code L14283700AL; Dr. Ehrenstorfer GmbH, Augsburg, Germany). The acetonitrile was removed completely by evaporation with a vacuum dryer (ScanVac MaxiVac Beta; Labogene, Lynge, Denmark) and the imidacloprid was dissolved in a small volume of water before addition to feeder syrup. Thiamethoxam was obtained in powder form (Pestanals; Sigma-Aldrich, Gillingham, UK) and similarly dissolved in water before addition to feeder syrup. For each toxicant, we produced nine experimental doses in feeder syrup by serial dilution at the following concentrations: 125.00; 50.00; 20.00; 8.00; 3.20; 1.28; 0.51; 0.20; 0.08 μg L^{-1} (or 98.4, 39.4, 15.8, 6.3, 2.5, 1.0, 0.4, 0.16, 0.06 μg kg^{-1}), which spans both the 'average maximum' values (1.9 μg kg^{-1} in nectar and 6.1 μg kg^{-1} in pollen) of environmentally realistic concentrations of neonicotinoid residues in nectar and pollen (Godfray *et al.* 2014) and also higher environmentally realistic levels for imidacloprid (e.g. 11 μg kg^{-1} in nectar and 19 μg kg^{-1} in pollen) and thiamethoxam (8 μg kg^{-1} in nectar and 35 μg kg^{-1} in pollen) (Sanchez-Bayo & Goka 2014). After three days of feeding on undosed control syrup to acclimate to

caged conditions, individual bees were randomly allocated to each dose and their syrup consumption was monitored for a further 72 h. Their thermogenic capabilities were assayed after 48-72 h of feeding on dosed syrup, because bumble bees show evident effects of toxicity after 48 h of exposure to dietary neonicotinoid (Cresswell *et al.* 2014). The experimental exposures to each toxicant, thiamethoxam and imidacloprid, across the full range of doses were first conducted in November-December 2015 and repeated in June-July 2016.

2.3 Quantification of non-flight thermogenesis

The individually-caged bees were placed in a refrigerator at approximately 5^0C for two hours to induce cold torpor. Each caged bee was then placed in a laboratory at room temperature (mean = 24 °C, SD = 1.3), where it warmed up over a period of approximately 30 minutes, which was sufficient for bees to reach an equilibrium temperature. In order to measure warming, we captured images of the thoracic surface temperature of each bee every two minutes with a thermal camera (Testo 870-1 Thermal Imager, Testo Ltd., Alton, UK). When taking pictures, the camera's emissivity scale was set to 0.97 (Stabentheiner & Schmaranzer 1987) and the reflected temperature compensation was set to 20 °C. In each thermal image, we estimated the surface temperature of the bee's thorax by taking the mean of the three hottest pixels in the dorsal intertegular region, which was the warmest part of the bee (Volynchik *et al.* 2006). The thermal images were analysed using Testo IRSoft v3.7.

By comparing the warming trajectories of live and dead bees, we established that active heating was reliably evident at $T_{thx} > 18$ °C (Fig. 1). Above this threshold, we quantified the maximum rate of heating achieved by each bee by the maximum value of ΔT in Eq 1 as follows.

$$\Delta T = \frac{T_j - T_i}{t_j - t_i} \qquad \text{Eq. 1}$$

In Eq 1, the increase in an individual's thoracic temperature (°C) between time $t = i$ and a later time $t = j$ is given by $(T_j - T_i)$ and the duration of the interval between two or more successive measurements of thoracic temperature (minutes) is given by $(t_j - t_i)$.

The second thermogenesis-related trait that we measured on each bee was the 'equilibrated temperature', denoted T_E °C. Specifically, in order to estimate the value of T_E for each bee, we calculated the mean thoracic surface temperature over an interval of at least six minutes after thoracic temperature had reached an evident plateau.

2.4 *Statistical analysis*

We fitted dose-response relationships for both endpoints, ΔT and T_E, by least-squares regression with the dietary concentration of the toxicant (x-axis) expressed on a logarithmic scale. For mathematical feasibility, we allocated the undosed controls a concentration of 0.008 μg L^{-1}, which is an order of magnitude (one log unit) below the lowest tested dose. For each dose-response relationship, we estimated the effect of two specified dietary concentrations: the highest dose tested (125 μg L^{-1}); and an environmentally relevant concentration (5 pbb, or 6.5 μg L^{-1}). We established 95% confidence intervals around the point estimates of these effects by using a Monte Carlo resampling procedure. Specifically, we produced 10,000 bootstrap datasets of equivalent dimension to the original by resampling with replacement within each dose; for each bootstrap dataset, we fitted the dose-response model and stored the relevant coefficients. Bootstrapped confidence intervals were established from the 2.5^{th} and 97.5^{th} percentiles of 10,000 solutions. Using the fitted dose-response relationships for both endpoints, ΔT and T_E, we estimated the half-effective dose, EC_{50}.

Differences in the temperature of body tissues can have a substantial effect on the rates of enzyme-mediated metabolic reactions. In order to better understand the potential ecological significance of toxicant-induced changes in equilibrated body temperature, T_E, we estimated the potential consequences for metabolic rates as follows. The temperature-sensitivity of a physiological process is conventionally indexed by Q_{10}, which is the metabolic rate ratio across a 10 °C increase in temperature (Willmer, Stone & Johnston 2004). Typical metabolic systems in insects have values of $2 < Q_{10} < 3$ (Hochachka & Somero 2002; Lardies & Bozinovic 2003). We therefore calculated potential changes in metabolic rate of bees, denoted M%, based on changes to their equilibrated thoracic temperature by assuming $Q_{10} = 2.5$.

We conducted exposures in separate experiments that each used different colonies of bumble bees and we observed that the thermogenic attributes of the bees differed clearly in the two separate experiments that involved exposures to thiamethoxam. In order to establish the dose-dependence of thermogenesis in the combined dataset, we therefore adjusted the data for one of the experiments by using $(\Delta T + 1)$ and $(T_E + 2)$ in place of the observed ΔT and T_E, respectively. We note that this adjustment does not bias our analysis of dose-dependence because the various doses were evenly represented in both experiments, including the controls, and it was not our objective here to test statistically for the presence of thermogenic variation among colonies.

3. Results

3.1 Dose-response relationships

The bees in our experiments consumed dose-dependent quantities of the toxicants that exceeded 10 ng bee^{-1} d^{-1} in the highest doses (Fig. 2) even though feeding rates declined with the increase of dietary concentration of both imidacloprid (Spearman's rank correlation, n = 28, rho = -0.46, $P < 0.01$; Fig. 2a) and thiamethoxam (Spearman's rank correlation, n = 30, rho = -0.34, $P < 0.05$; Fig. 2b). The ingestion rates of thiamethoxam by some of the experimental bumble bees in the present study (Fig. 2b) are similar to those of honey bees in a previous study, i.e. between 0.2 and 2.0 ng d^{-1} (Tosi et al. 2016).

Exposures of bumble bees to dietary imidacloprid and thiamethoxam both caused dose-dependent variation in post-torpor rates of warming (Table 1, Fig. 3; imidacloprid: $\Delta T = 1.57 - 0.10\log(dose) - 0.16\log(dose)^2$, regression analysis, $F_{1,69} = 4.9$, $p < 0.05$ and $F_{1,69} = 19.0$, $p < 0.001$ for the two coefficients respectively; thiamethoxam: $\Delta T = 2.19 - 0.19\log(dose)$, $F_{1,81} = 18.6$, $p < 0.001$). In the imidacloprid exposure, the dose-response relationship of warming rate, ΔT, exhibited hormesis with a maximum rate that was approximately 0.5 °C min^{-1} faster than the undosed controls (Fig. 3a) at a dietary dose of 0.5 µg L^{-1} (95% CI: 0.26, 0.76).

Exposures of bumble bees to dietary imidacloprid and thiamethoxam both caused dose-dependent declines in equilibrated thoracic temperature (Table 2, Fig 4; imidacloprid: $T_E = 25.39 - 0.60\log(dose)$, regression analysis, $F_{1,68} = 16.7$, $p < 0.001$; thiamethoxam: $T_E = 28.29 - 0.74\log(dose)$, $F_{1,81} = 21.0$, $p < 0.001$).

3.2 Magnitude of effects at cardinal doses

At the highest doses tested, individual bees consumed dietary neonicotinoids at rates in excess of 5 ng d^{-1} (Fig. 2), which reduced the warming rates (ΔT) of bumble bees by approximately one third relative to undosed controls (Table 1) and reduced the post-torpor equilibrated thoracic temperature by approximately 3 °C, which is capable in itself of

reducing metabolic rate by approximately 25% (Table 2). For both toxicant-endpoint combinations, the half-effective dose, EC_{50}, generally exceeded the highest dose tested, 125 $\mu g\ L^{-1}$ (Tables 1 & 2).

At environmentally relevant levels (approximately 5 ppb), dietary neonicotinoids altered the warming rates of bumble bees by approximately one quarter relative to undosed controls (Table 1); exposure to imidacloprid increased warming rates and thiamethoxam reduced them. However, both toxicants reduced the post-torpor equilibrated thoracic temperature in environmentally relevant exposures by approximately 2 °C, which is capable of reducing metabolic rate by approximately 15% (Table 2).

4. Discussion

Our findings demonstrate that the symptoms of intoxication in bumble bees that are caused by exposure to dietary neonicotinoids can include disruption of the recovery from cold torpor by non-flight thermogenesis (NFT). When taken together with previous evidence that dietary neonicotinoids impair thermoregulation in African honey bees (Tosi *et al.* 2016), our results begin to establish that neurotoxic insecticides can disrupt the coordinated control of thermogenesis in social bees in general. Below, we discuss: (1) the relative sensitivity of thermogenic traits to neonicotinoid exposure in comparison to other aspects of bee performance; (2) the relative potency of the focal neonicotinoids, thiamethoxam and imidacloprid; (3) the existence and possible mechanistic basis of complex dose-dependent effects (hormesis) involving NFT; and (4) the potential impact on farmland bees of dietary exposure to neonicotinoids through altered capacity for non-flight thermogenesis.

4.1 Relative sensitivity of thermogenic traits to neonicotinoid exposure

To function effectively, individual bees must perform a wide variety of physical and cognitive tasks that are controlled variously by the nervous and endocrine systems. Consequently, the apparent potency of a particular neurotoxin may differ when assessed by its effects on the performance of various tasks. In theory, such disparities may arise from differences in the extent to which the neurotoxin affects distinct loci of nervous control and by the amplification of these differential effects by the complex networks of the nervous and endocrine systems. One widely used index of sensitivity to a toxicant is the 'half-effect concentration', or EC_{50} (Walker 2014), for a specified aspect of performance, or 'endpoint'. As anticipated, the values for EC_{50} vary among the different endpoints that are measured after sustained dietary exposures of bees to neonicotinoids. For example, whereas the EC_{50} for exposure of bumble bees to dietary imidacloprid can be as low as 1 ppb when fecundity is the endpoint (Laycock & Cresswell 2013), the values that we estimated for thermogenic performance (> 100 ppb) were more than two orders of magnitude greater. This disparity initially suggests that thermogenic traits are not among the most sensitive and that, instead, they rank alongside traits such as locomotory activity in bumble bees and learning by honey bees in the proboscis-extension paradigm (Cresswell 2016).

However, whereas the EC_{50} describes the amplitude of the subject's response to a toxicant, it is also useful to consider the lowest threshold dose at which a response becomes apparent, which is measured by the 'no observable effect concentration', or NOEC (Walker 2014). In our present study, we detected a stimulation of post-torpor warming rates in bumble bees by imidacloprid at a dietary concentration of approximately 1 ppb. Imidacloprid is also capable of repressing bumble bee fecundity during a dietary exposure to imidacloprid at 1 ppb (Laycock & Cresswell 2013). Taken together, these results begin to establish that sustained exposures to even minute concentrations (< 5 ppb) of dietary imidacloprid can affect the performance of bumble bees. The capacity of imidacloprid to elicit low-dose effects in

sustained exposures does not stem from its gradual bioaccumulation to detrimental levels in a bee's body tissues, because the symptoms of intoxication rapidly disappear when dietary exposure ceases (Cresswell *et al.* 2014), presumably because the toxicant is rapidly eliminated (Suchail *et al.* 2004). Instead, the physiological basis for the singular low-dose potency of imidacloprid in bumble bees is currently unexplained and is therefore a target for future toxicokenetic/toxicodynamic research.

4.2 Relative potency of different neonicotinoids

Both toxicants that we studied were broadly similar in the magnitudes of their effects on thermogenic attributes of bumble bees. Specifically, at the highest doses tested both were capable of reducing post-torpor warming rates by about one third and of reducing equilibrated thoracic temperature by approximately 3°C. In our present study, however, we observed that low-level doses of imidacloprid stimulated NFT, but equivalent doses of thiamethoxam did not. Similarly, previous studies of microcolonies of orphaned worker bumble bees have also indicated that imidacloprid, but not thiamethoxam, has potent effects at levels below 5 ppb (or <6.3 $\mu g\ L^{-1}$ experimental feeder syrup; Fig. 5). Collectively, these findings suggest that chemically similar neonicotinoids (both imidacloprid and thiamethoxam are nitro-substituted artificial variants of nicotine) may possess different toxicity profiles at low doses. We are not aware of published a pharmacological basis for the different neurological impacts of the two neonicotinoid toxicants that we studied and more evidence from comparative toxicology is required to better understand the extent and basis of this differential potency.

4.3 Complex effects and hormesis

Like the only previous study of the effect of dietary neonicotinoids on NFT in bees (Tosi *et al.* 2016), our experiments revealed a biphasic hormesis in a dose-response relationship,

which exhibited low-dose stimulation coupled with high-dose inhibition. We propose a mechanistic explanation for these observations as follows. In bees, heat generation for NFT involves shivering of the thoracic flight muscles. In honey bees and bumble bees, the shivering is virtually undetectable by the unaided eye because the thoracic muscles are held in tetanic contraction (Heinrich 1993, p 233). Tetanus of the flight muscles is maintained by high-frequency discharges of the nerves serving the thoracic muscles (Heinrich & Kammer 1973). The neurotoxic mode of action of neonicotinoids is to produce an uncoordinated overstimulation of the insect nervous system (Deglise, Grunewald & Gauthier 2002). We therefore speculate that the stimulatory effect on NFT by imidacloprid arises because low-level dietary exposures cause heightened activity in the thoracic motorneurons. At higher doses, the generalised disruption of the nervous system causes systemic intoxication and so all fundamental processes of the bee, including NFT, are thereby disrupted and exhibit decreased performance. So far there is little evidence to support this speculation, mainly because the behaviour of bees has not been scrutinised sufficiently to establish fine variations in movement responses at lower and higher doses of chronic exposure and only rather crude measures of locomotory behaviours have been previously employed (e.g. Cresswell et al. 2012). Testing this speculation is a target for future research.

4.4 Potential impact on farmland bees of neonicotinoid exposure

According to our best-fit dose-response relationships, exposure to average maximum environmentally relevant levels (dietary residues of 5 ppb, Godfray *et al.* 2014) increases the post-torpor warming rate of bumble bees by approximately 0.3 °C min^{-1} if they are exposed to imidacloprid and reduces it by 0.5 °C min^{-1} if the exposure is to thiamethoxam. Assuming that a bee is required to warm from torpor at 5° to an equilibrated pre-flight thoracic temperature of 29 °C (Heinrich 1972), then an environmentally realistic exposure to dietary imidacloprid will speed up recovery by 4.6 minutes relative to unexposed bees and dietary

thiamethoxam will delay it by 2.4 minutes. Given that post-torpor thermal recovery takes between 15 and 20 minutes overall (Heinrich 1975), we propose that these small differentials in the time required for warming up have negligible ecological significance when taken in isolation, although they are likely to be indicative of a harmful, more generalised intoxication.

For bees exposed to 5 ppb, our best-fit dose-response relationship predicts a drop in thoracic temperature relative to unexposed bees of 1.7 °C due to dietary exposure to imidacloprid and 2.1 °C for bees exposed to thiamethoxam. A typical metabolic process in insects has a temperature-sensitivity of $Q_{10} = 2.5$, which implies that a 2°C decrease in tissue temperature will decrease reaction rates by approximately 20%. Consequently, the seemingly minor effect on thoracic temperature of an environmentally realistic exposure to dietary neonicotinoids may substantially impact on bumble bee metabolism and also potentially on fitness if the performance of the thoracic flight muscles is thereby compromised (Tosi, Burgio & Nieh 2017). Similarly, if these differentials in thoracic temperature are indicative of a general malfunction in thermogenesis, then these seemingly small differentials may have profound implications for brood development in this eusocial insect. Bumble bees have an annual cycle and their reproductive success depends on a rapid increase in the number of adult workers in the colony (Owen, Rodd & Plowright 1980). The rate of larval development in insects is highly temperature-dependent (Dixon *et al.* 2009) and so small decreases in the temperature of the thorax, which is the principal site of thermogenesis, may strongly influence colony size and reproduction by compromising the capacity of workers to incubate their colony's brood. However, we note that our experiments recorded thoracic temperatures only for an interval of approximately 10 minutes after rewarming, so the existence of long term effects on flight and brood incubation is currently a matter of speculation. Also, we did not investigate the flight performance or incubatory capacity of neonicotinoid-dosed bees.

Nevertheless, our findings indicate a need for further research to clarify the ecological significance of neonicotinoid exposure for bumble bees.

On the basis of our data, we estimated that the maximum stimulation of NFT (post-torpor warming rate) by imidacloprid occurred at a dietary concentration of approximately 1 ppb, which is the second time that such low-level exposures in the environmentally relevant range have been shown to disrupt ecologically important functions in bumble bees (Laycock & Cresswell 2013). We therefore raise the concern that, among the neonicotinoids, agricultural imidacloprid is singular in the severity of risk that its low-level residues could pose to farmland bumble bees.

4.5 Conclusions

In summary, our findings demonstrate the potential for realistic exposures to dietary neonicotinoids to affect and potentially disrupt two of the fundamental processes undertaken by worker bumble bees, namely flight and brood incubation, by compromising their capacity for non-flight thermogenesis. One of the toxicants that we studied, thiamethoxam, remains a market leader among plant protection products and is widely used as a systemic insecticide on bee-attractive crops (Simon-Delso *et al.* 2015). However, the actual implications of our results for the sustainability of farmland bumble bees are unclear because of limitations in our experimental approach, which do not fully explore environmentally realistic scenarios. However, future research is warranted for the thorough evaluation of the ecological significance of the disruption of non-flight thermogenesis in bumble bees by low-level dietary exposures to agrochemicals in general.

Conflict of interests

The authors declare that they have no competing interests

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Datasets in this study are available at: *http://hdl.handle.net/10871/29247*

Exposure	$\bar{X}$Control	R_{98}	R_{98}%	R_5	R_5%
IMI	1.1	-0.43	-40	+0.29	+27
	(0.93, 1.24)	(-0.15, -0.72)	(-14.5, -61.7)	(0.04, 0.54)	(3.3, 55.9)
TMX	2.6	-0.79	-30	-0.54	-21
	(2.47, 2.69)	(-0.61, -0.97)	(-23.6, -37.6)	(-0.47, -0.62)	(-18.4, -23.9)

Table 1.

Responses of rates of rewarming from torpor (ΔT °C min^{-1}) in bumble bees to cardinal doses of either dietary imidacloprid (IMI) or thiamethoxam (TMX). Values (row-wise by toxicant) indicate:- $\bar{X}$Control: estimated mean warming rate (ΔT) of undosed controls; R_{98}: reduction of ΔT at highest dose tested (98.4 ppb, or 125 µg L^{-1}) relative to undosed controls; R_{98}%: percentage change in ΔT at highest dose tested; R_5: change in ΔT at environmentally relevant dose (5 ppb) relative to undosed controls; R_5%: percentage change in ΔT at environmentally relevant dose. The 95% CI (Monte Carlo resampling) on each estimate is indicated parenthetically below. Note: in one of the two experiments using thiamethoxam the data set was adjusted to (ΔT + 1); for the separate experiments, the unadjusted mean values of $\bar{X}$Control were 2.7 and 1.6.

Exposure	$\bar{X}$Control	R_{98}	M_{98}%	R_5	M_5%
IMI	26.7	-2.5	-21	-1.8	-15
	(26.3, 27.0)	(-1.9, -3.2)	(-16.0, -25.3)	(-1.5, -2.0)	(-12.9, -16.8)
TMX	29.9	-3.1	-25	-2.2	-18
	(28.3,29.0)	(-2.5, -3.7)	(-20.8, -28.6)	(-1.9, -2.4)	(-16.3, 19.6)

Table 2.

Responses of post torpor equilibrated thoracic temperature (T_E °C) in bumble bees to cardinal doses of either dietary imidacloprid (IMI) or thiamethoxam (TMX). Values (row-wise by toxicant) indicate:- $\bar{X}$Control: estimated mean equilibrated temperature (T_E) of undosed controls; R_{98}: reduction in T_E at highest dose tested (98.4 ppb, or 125 µg L^{-1}) relative to undosed controls; M_{98}%: percentage change in estimated metabolic rate relative to undosed control at highest dose tested; R_5: reduction in T_E at environmentally relevant dose (5 ppb) relative to undosed controls; M_5%: percentage change in estimated metabolic rate at environmentally relevant dose. The 95% CI (Monte Carlo resampling) on each estimate is indicated parenthetically below. Note: in one of the two experiments using thiamethoxam the data set was adjusted to (T_E + 2); for the separate experiments, the observed mean values of $\bar{X}$Control were 30.5 and 28.6.

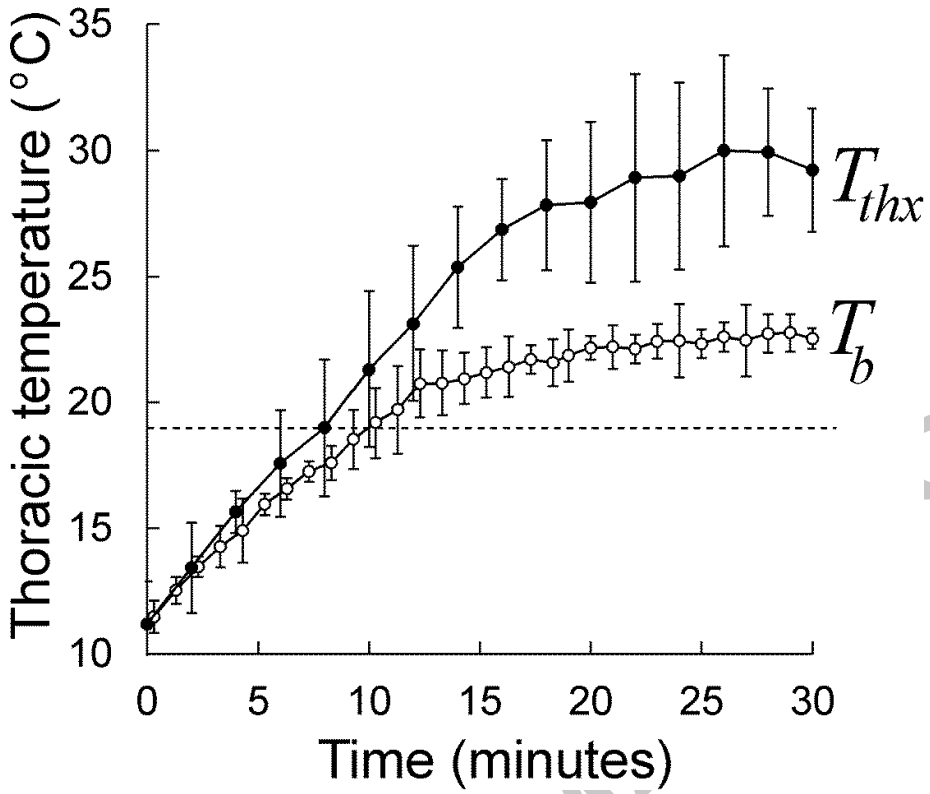

Fig. 1. Trajectories of thoracic temperature in live bumble bees that were fed with control feeder syrup (closed symbols, T_{thx}) and similar-sized corpses (open symbols, T_b) as they rewarm over time (minutes) at room temperature after removal from a refrigerated environment (c. 5 °C) at *time* = 0. Symbols indicate means (live bees: n = 9; corpses: n = 5) and error bars are 1.96 SD to reflect individual variation. Symbols are interpolated for inspection purposes only.

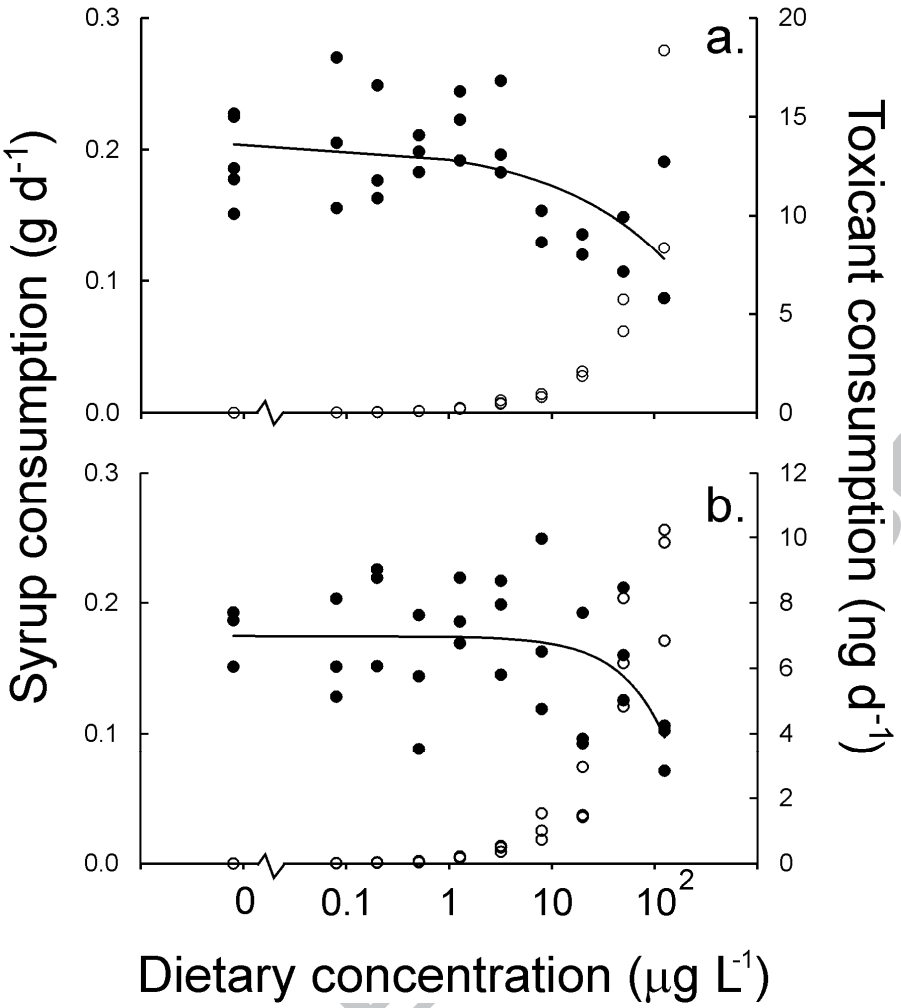

Fig. 2. Consumption rates of syrup (closed symbols, *y*-axis: g d^{-1}) and toxicant (open symbols, right-most *y*-axis: ng d^{-1}) by individual bumble bees (i.e. *per capita* rate) in relation to the dietary concentration of toxicant (*x*-axis: µg L^{-1}). Upper panel (a): exposure to imidacloprid; lower panel (b): thiamethoxam. Each solid curve indicates the least-squares regression of feeding rate on dose.

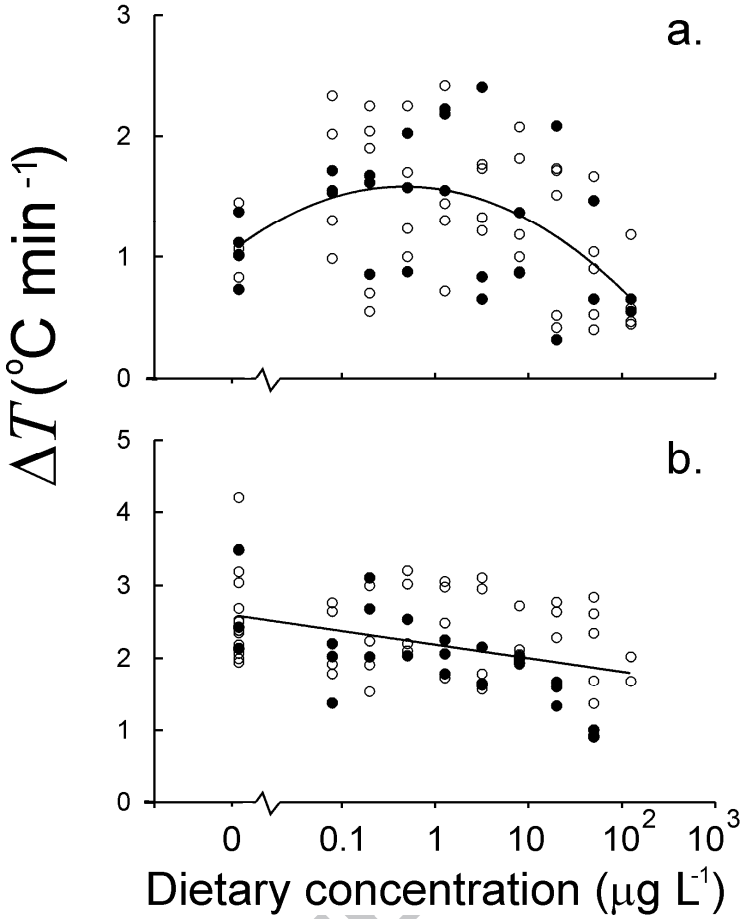

Fig. 3. Rates of rewarming from torpor (*y*-axis: ΔT °C min^{-1}) in individual bumble bees in relation to the dietary concentration of toxicant (*x*-axis: μg L^{-1}). Upper panel (a): exposure to imidacloprid; lower panel (b): thiamethoxam. The solid curves indicate the least-squares regressions of ΔT on dose. Closed and open symbols indicate data collected in experiments in 2015 and 2016, respectively.

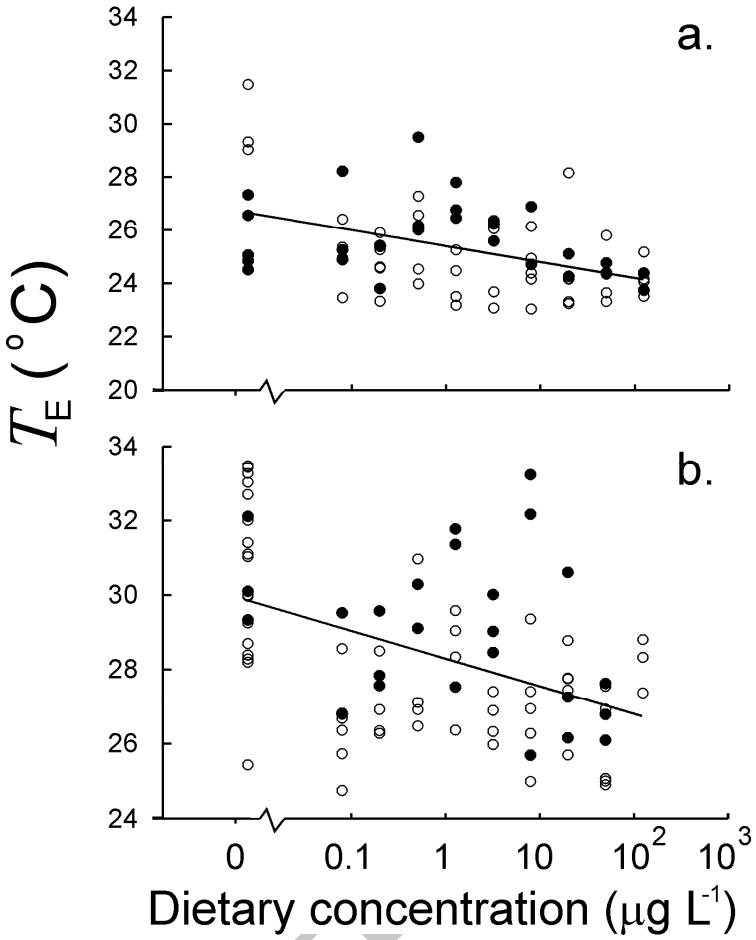

Fig. 4. Equilibrated thoracic temperature after rewarming from torpor (*y*-axis: T_E °C) in individual bumble bees in relation to the dietary concentration of toxicant (*x*-axis: μg L^{-1}). Upper panel (a): exposure to imidacloprid; lower panel (b): thiamethoxam. The solid curves indicate the least-squares regressions of T_E on dose. Closed and open symbols indicate data collected in experiments in 2015 and 2016, respectively.

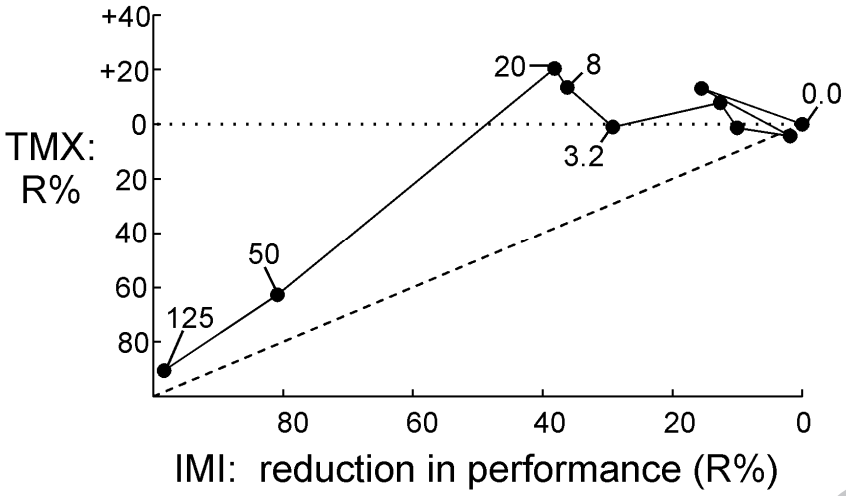

Fig. 5. Comparative toxicity of imidacloprid and thiamethoxam to bumble bees over a range of equivalent doses where data from two previous studies of the dose-dependence of pollen consumption by microcolonies (Laycock *et al.* 2012; Laycock *et al.* 2014) are displayed as a 'response-response' plot (Cresswell & Laycock 2012). Axes indicate the percentage response (R% reduction in performance relative to undosed controls) by bumble bee microcolonies under dietary exposure to either imidacloprid (*x*-axis, denoted 'IMI') or thiamethoxam (*y*-axis, 'TMX'). The dashed diagonal indicates the hypothetical relationship when equivalent responses to each toxicant occur at equivalent doses. The response variable, pollen consumption, is a correlate of fecundity and the experimentally observed responses at equivalent doses are shown by closed symbols. When a symbol lies off the dashed line, this indicates differential toxicity at equivalent doses. The number attached to a data symbol denotes the experimental dose in $\mu g\ L^{-1}$. For example, exposure to a dietary concentration of 3.2 $\mu g\ L^{-1}$ imidacloprid reduced pollen consumption by approximately 30% relative to undosed controls, but the equivalent dose of thiamethoxam caused no response. The dataset comprises variation in *per capita* pollen consumption (mg $bee^{-1}\ d^{-1}$) in microcolonies of bumble bees due to separate exposure to two dietary neonicotinoids (*y*-axis: thiamethoxam; *x*-

axis: imidacloprid) at a series of equivalent dietary doses (125.00; 50.00; 20.00; 8.00; 3.20; 1.28; 0.51; 0.20; 0.08 μg L^{-1}); symbols are interpolated for inspection purposes only in the preceding dose order (i.e. the control dose, denoted '0.0' is connected to 0.08 μg L^{-1}, 0.08 connects to 0.20, etc.)

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Neonicotinoid insecticides affect rates of recovery from cold-torpor in bumble bees

Dietary neonicotinoid insecticides disrupt thermogenesis in worker bumble bees

Imidacloprid caused low-dose stimulation of thermogenesis in bumble bees

In laboratory exposures of bumble bees to two neonicotinoids, imidacloprid and thiamethoxam, both toxicants caused dose-dependent decreases in the rates of rewarming and in the equilibrated thoracic temperatures.