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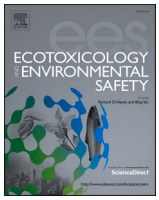

Repeated exposure of bumblebee workers to field-realistic doses of di-2-ethylhexyl and di-n-butyl phthalates lowers worker survival and induces obesity-related phenotypes in workers and brood

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ABSTRACT

Wild bees, like many other insects, are declining. Multiple drivers have been identified, but the potential effects of endocrine disruptors such as phthalates, remain poorly studied. Yet, di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DnBP), two of the most common phthalates that pervade the environment, are known to affect key hormonal and metabolic pathways in invertebrates even at field realistic doses. These ubiquitous pollutants are semi-volatile compounds used to increase the flexibility of plastic materials, or as solvents for certain detergents, pesticides, and other products. Bees are notably exposed to these lipophilic molecules through their cuticle, with exposure varying in both quantity and chemical composition. To investigate the potential impacts of phthalate exposure, *Bombus terrestris* workers were repeatedly exposed (once per week) to nominal field-realistic doses of DEHP and DnBP and maintained in microcolonies during 35 days. Worker mortality and male emergence were recorded throughout the experiment. Offspring production was assessed in terms of number and mass, and abdominal lipid ratio was assessed in adults. Repeated exposure to DEHP increased worker mortality up to 50%, while DnBP increased worker lipid stores and offspring mass, suggesting potential metabolic disruption. These findings indicate that the two most common phthalates can have both lethal and sublethal effects on bees, likely through disruption of crucial physiological pathways, with cascading consequences for individual health and colony development even at environmental concentrations.

1. Introduction

The current global decline in insect populations has been well documented with several anthropogenic drivers identified, including land-use intensification, climate change and chemical pollution (Wagner et al., 2021). Bees are no exception, and chemical pollution is frequently reported as one of the many pressures contributing to their decline (Michez et al., 2026). Given their essential role in the pollination flowering plants (Ollerton et al., 2011), bees have become model organisms for environmental risk assessment and for evaluating the impacts of pollutants, particularly pesticides, on pollinators (EFSA et al., 2023). However, the effects of other pollutants remain poorly understood.

Phthalates are used as plasticisers and solvents and are commonly found in agricultural and urban environments (Billings et al., 2021), but also in regions remote from anthropogenic activity (Lenoir et al., 2016). They have been detected at varying concentrations in soil, water and the atmosphere (Billings et al., 2021). Di−2-ethylhexyl phthalate (DEHP)

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and di-n-butyl phthalate (DnBP) are among the most widely used and frequently detected phthalates worldwide (Billings et al., 2021). For example, Tran et al. (2015) reported phthalate concentrations in urban soils of Paris (France) ranging from 92.5 ng/g soil for DnBP to 310 ng/g for DEHP. In another European city, Novi Sad (Serbia), soil concentrations ranged from 30 ng/g and 145 ng/g for DnBP and from 129 ng/g and 2.04 μg/g for DEHP (Škrbić et al., 2016). In these matrices, although free phthalates are rapidly degraded, they can persist for years when bound to organic matter (Kickham et al., 2012). Moreover, these lipophilic pollutants can adsorb onto insect cuticles, be absorbed into the insect body (Lenoir et al., 2014), and subsequently be transported into lipid-rich nest structures (Gómez-Ramos et al., 2016). The chemical composition of both their cuticle and nest structures therefore makes bees particularly susceptible to daily exposure to these contaminants. DEHP has been detected on honeybee worker cuticles (Gómez-Ramos et al., 2019), and in wax combs (Gómez-Ramos et al., 2016) while DnBP has been detected in pollen loads at concentrations ranging from below detection limit to 2.41 μg/g (Martín-Gómez et al., 2024). More recently, over 70% of *Bombus terrestris* workers collected along an urbanisation gradient in the European Metropole of Lille (France) contained two or more phthalates, with DnBP and DEHP being the most frequently detected compounds. Average cuticular concentrations reached 108 ng/g body weight (b.w.) and 1389 ng/g b.w. for DnBP and DEHP, respectively, with measured concentrations ranging from the ng/g to the μg/g level for both compounds (Unpublished results). Together, these findings indicate that bees are routinely exposed to phthalates in their environment and that contact exposure may represent an important, yet understudied, route of exposure.

DnBP and DEHP are recognised as endocrine-disrupting compounds (EDCs) in vertebrates and aquatic invertebrates (Gore et al., 2015), but only limited data are available for terrestrial insects. Investigation into the long-term effects of phthalate exposure in insects have primarily focused on the non-social species *Drosophila melanogaster* and *Spodoptera littoralis* (Avilès et al., 2019; Williams et al., 2016). In these models, DnBP altered lipid metabolism through disruption of the insulin-signalling pathway (Williams et al., 2016), while DEHP affected olfaction-related behaviours and developmental duration through alteration of the ecdysteroid-related cascade (Avilès et al., 2019). In insects, both pathways play key roles in reproduction, development, and metabolism (Antonova et al., 2012; Bonneton and Laudet, 2012). In eusocial bee species, insulin signalling is involved in key processes underlying caste-specific behavioural and physiological regulation (Jedlička et al., 2016). Differential activation of ecdysteroid signalling during larval development also contributes to caste determination (Pinto et al., 2002). Moreover, 20-hydroxyecdysone influences olfactory memory in pollen foragers of the honeybee *Apis mellifera* (Geddes et al., 2013), as well as nestmate recognition and aggressive behaviours (Pandey and Bloch, 2015). Finally, both signalling directly regulate juvenile hormone production, a crucial determinant in bumblebee offspring production (Shpigler et al., 2014), and a regulator of metabolic rate in *B. terrestris* (Shpigler et al., 2021). Consequently, a disruption of these pathways by phthalates could alter key traits linked to colony performance and fitness in bumblebees.

The present study aims to investigate the lethal and sublethal effects of long-term exposure to environmentally relevant concentrations of phthalates on a social species, *B. terrestris*. This species is among the most abundant bumblebee in Europe and is widespread in both urban and agricultural environments (Rasmont et al., 2021), where phthalate contamination is pervasive due to plastic pollution, industrial activity, and agricultural runoff (Billings et al., 2021). In a previous study, we reported lethal effects of DnBP, which caused 25% mortality in isolated *B. terrestris* workers, four days after a single exposure to field-realistic doses (Dewaele et al., 2025). In addition, a comparable short-term exposure experiment demonstrated that environmental concentrations of DEHP and DnBP can impair antennal responses to floral volatile compounds in the same species, suggesting that phthalates may affect behaviours involved in resource detection and pollination (Javal et al., 2026). These approaches followed OECD guidelines for testing chemical effects on *B. terrestris* (short-term tests, i.e. 72 h, on isolated workers; OECD, 2017), but lacks ecological realism when applied to social insects. Indeed, these methods do not allow the assessment of repeated or chronic exposure under normal social conditions (i.e. multiple workers interacting within the same nest). Moreover, experiments performed on isolated workers restrict the evaluation of sublethal effects to non-reproductive individuals and overlook potential effects on alloparental care and offspring production, which may alter colony development and fitness (Klinger et al., 2019). Phthalates could alter a wide range of traits through disruption of key hormonal pathways, as observed in solitary insect species, with cascading consequences for social organisation, development, reproduction and foraging behaviour. Therefore, queenless microcolonies were used to assess the effects of phthalate exposure on worker and offspring health under controlled conditions. During this experiment, we monitored (i) worker mortality, (ii) adult abdominal lipid content, (iii) resource collection (i.e. syrup and pollen), and (iv) offspring production (i.e. number and mass). To reflect realistic exposure scenarios, workers within microcolonies were exposed weekly via their cuticle to phthalate doses measured in urban areas. These doses were selected based on previous field measurements and short-term toxicity results (Dewaele et al., 2025), thereby linking laboratory findings with real-world exposure.

2. Material and methods

2.1. Model species

B. terrestris colonies are characterised by a reproductive division of labour, with the queen suppressing worker reproduction. However, when queen influence weakens, workers can develop their ovaries and produce unfertilised male offspring (Klinger et al., 2019). This plasticity makes *B. terrestris* a valuable model for assessing how pollutants may disrupt social organisation and offspring production in this species. For this study, five queenright colonies of *B. terrestris* (*ca.* 80–120 workers) were purchased from Biobest NV (Westerlo, Belgium). Upon receipt, colonies were kept in a dark room under controlled temperature and humidity (25°C, 60% relative humidity) and used within one week to establish the microcolonies.

2.2. Phthalate treatments

We evaluated the sublethal effects of phthalate exposure using nominal field-realistic doses, following Dewaele et al. (2025): DEHP at 120 ng/g of body weight (b.w.) and DnBP at 60 ng/g b.w. A mixture treatment combining both compounds at these doses was also tested because an antagonistic effect on isolated worker mortality had previously been reported (Dewaele et al., 2025). In addition, we investigated DnBP exposure at 600 ng/g b.w., as potential metabolic disruption had been suggested in isolated workers (Dewaele et al., 2025). Finally, because DEHP doses ranging from 12 ng/g to 12 μg/g did not affect worker mortality in Dewaele et al. (2025), we selected the lowest dose previously used, i.e. 12 ng/g, to further investigate potential low-dose effects.

DnBP (CAS No. 84–74–2) and DEHP (CAS No. 117–81–7) were purchased from Sigma-Aldrich as liquid solutions (both >99%). Dilutions were prepared using an average *B. terrestris* worker fresh weight of 0.25 g. Initial dilutions were made in methanol (CAS No. 67–56–1, VWR) to ensure good phthalate solubility, whereas subsequent dilutions were prepared with mineral water to minimise the final methanol concentration (0.07%). For the mixture treatment, single-molecule stock solutions were prepared using the same procedure and combined during the final dilution step. The volume was then adjusted with mineral water to obtain concentrations of DEHP at 120 ng/g and DnBP at 60 ng/g. Two control groups were included: one containing 0.07% methanol in

mineral water and one containing mineral water only, to account for potential solvent effects.

2.3. Preventing uncontrolled PAE contamination

Phthalates are present in laboratory environments due to widespread use of plastic materials and can also be found in in both tap and plastic-bottled water. To prevent unintended contamination of treatment and feeding solutions, we used glass-bottled water (SPA Reine, SPA NV, Bruxelles, Belgium). All solutions were prepared and handled exclusively using glass and metal materials to minimise phthalate contamination and adsorption, as plastics can both release and adsorb phthalates (Fankhauser-Noti and Grob, 2007). Glass and metal materials were decontaminated with hexane, acetone and, where possible, calcination. When the use of plastic was unavoidable, hard plastic materials were prioritised (Cao et al., 2022).

2.4. Experimental design

2.4.1. Microcolony preparation

Five queenless microcolonies were established for seven treatments (i.e., 35 microcolonies in total) in boxes (10 ×16 ×16 cm), each containing 10 workers randomly sampled from one of the five commercial colonies. Each treatment included one microcolony originating from each commercial colony. Microcolonies were maintained in constant darkness at 25°C and 60% relative humidity for 35 days, with *ad libitum* access to a syrup solution consisting of 50% (w/w) sucrose in mineral water (SPA Reine, SPA NV, Bruxelles, Belgium), and pollen candi (crushed pollen mixed with syrup at 0.15 mL/g pollen). Organic frozen honeybee-collected pollen (Rosaceae: *Malus/Pyrus* sp. 40%; Salicaceae: *Salix* 27%) was used (Abeille Heureuse, Strasbourg, France; Barraud et al., 2022).

Every other day, syrup and pollen were renewed and weighed, and dead workers were counted and weighed. An empty control (syrup and pollen but no workers) was maintained to account for evaporation. At the end of the experiment, all surviving workers were weighed. Syrup and pollen collection were standardised by the total mass of live workers in each microcolony at the time of recording and then cumulated over the duration of the experiment. Pollen dilution (i.e., cumulated pollen: syrup ratio) and pollen efficacy (i.e., total offspring mass:cumulated pollen ratio) were calculated as indicators of stress response (e.g., Barraud et al., 2020).

2.4.2. Exposure protocol

Once a week, all workers were exposed to their assigned treatment following a protocol adapted from OECD guideline No. 246 (OECD, 2017) and previously used in Dewaele et al. (2025). For each microcolony, all workers were simultaneously placed inside glass jars in groups of two. The glass jars were plunged into ice for anaesthesia by chilling (maximum ten minutes). Then, each worker was exposed by applying a 2 μL droplet of either control or treatment solution to the hairless dorsal part of the thorax. After recovery at room temperature, workers were returned to their respective microcolonies. Exposures began three days post-microcolony setup and were repeated weekly over 35 days (five sessions total).

2.5. Offspring monitoring

Thirty-two days after the first exposure, microcolonies were dissected to count eggs and larvae at each developmental stage (i.e., non-isolated, pre-defecation, post-defecation, pupae, and non-emerged drones; see Fig. 1). All hatched larvae were weighed individually, except for non-isolated larvae, which were weighed collectively per microcolony (both total and per-larva masses were analysed). Male emergence was recorded daily, and emerged drones were weighed and stored at −20°C.

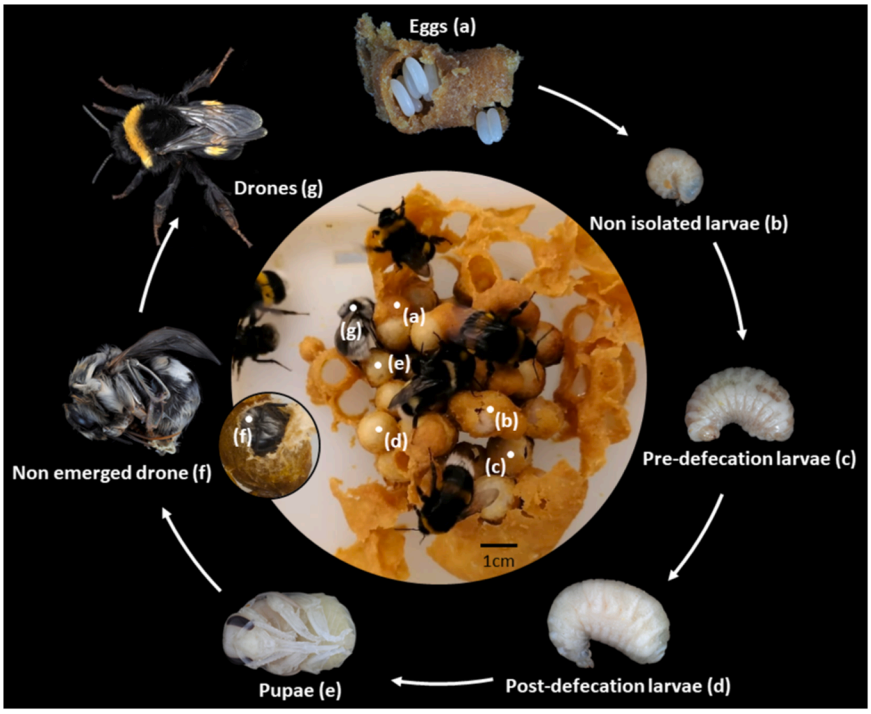

Fig. 1. Drone developmental stages monitored in the microcolonies, with associated locations in the brood. Brood cells containing several eggs (a) are located on top of the brood. Non-isolated larvae (b) develop together in brood cells. Pre-defecation larvae (c) are isolated in individual brood cells. Brood chambers containing non-isolated and pre-defecation larvae bear small feeding holes. Post-defecation larvae (d), pupae (e) and non-emerged drones (f) develop in closed, isolated brood cells, located under the brood. Newly emerged drones (g) are primarily white, and their colours develop in the first 24 h (Picture credits: individual larvae and drones by Paolo Rosa; brood and emerging male by Charlotte Terzo).

2.6. Worker and drone abdominal lipid ratio

The abdominal lipid ratio, i.e. fat storage, of adults was estimated following Ellers (1995). Fresh abdomens were excised, weighed, dried for three days at 70°C, and weighed again. Lipids were extracted by placing the abdomens in 2 mL of diethyl ether for 24 h, after which the ether was discarded and the abdomens were dried again at 70°C for seven days. The abdominal lipid ratio was calculated as the difference between pre- and post-extraction dry abdomen masses, divided by fresh abdomen mass. Two workers per microcolony and all emerged drones were analysed.

2.7. Statistical analyses

All analyses were performed in R v4.3.0 (R Core Team, 2023), and all graphs were generated using the "ggplot2" package v 3.5.1 (Wickham, 2016).

Survival was analysed using a mixed-effects Cox regression, with the solvent control as reference group and the initial queenright colony as a random factor, using the coxme package v2.2–22 (Therneau, 2022). Hazard ratios (HR) relative to the solvent control are reported.

Treatment effects on the different metrics were analysed using generalized linear mixed models (GLMM) with the glmmTMB package v1.1.11 (Brooks et al., 2025), considering the initial colony as a random effect and treatment as the explanatory variable. Distribution families were selected according on the metric and validated by visual inspection of model diagnostics using the DHARMa package v0.4.7 (Hartig, 2020). Beta family (logit link) was used for abdominal lipid ratio and pollen dilution (ratio values between 0 and 1). Gaussian family (identity link) was applied to pollen and syrup collection, pollen efficacy, and larval and drone masses when data were normally distributed. Gamma family (inverse link) was used for right-skewed mass data. Larval and drone numbers were analysed using Poisson family (log link), or negative binomial (log link) when overdispersion was detected (Brooks et al., 2025). To determine whether potential effects on larval and drone numbers resulted from direct phthalate toxicity or indirectly from a nurse effect associated with worker survival, similar GLMMs were fitted to the same data using the number of surviving workers after 35 days as either the main explanatory variable or a covariate of the treatment variable.

As solvent and mineral water controls did not differ in any analysis (Table S1), the solvent group was retained as the control. Custom contrasts were performed using the emmeans package v1.10.5 (Lenth, 2023), with the solvent group as the reference and Benjamini-Hochberg's p-value adjustment method (i.e., "fdr") for *post-hoc* analyses.

3. Results

3.1. Impact on worker mortality

Exposure to phthalates significantly affected worker survival (Mixed Cox regression: Chisq. = 18.30, d.f. = 6, p-value = 0.006). At the end of the experiment, survival of workers exposed to DEHP at 120 ng/g was half that of the control group (for DEHP at 120 ng/g, HR = 2.25, 95% CI = [1.15, 4.41], z = 2.37, p-value = 0.018; Fig. 2). A similar but marginally significant effect was observed in workers exposed to the mixture treatment (HR = 1.99, 95% CI = [1.00, 3.94], z = 1.98, p-value = 0.048; Fig. 2). In both groups, survival began to decline after the midpoint of the experiment (Fig. 2).

3.2. Impact on worker resource collection

A significant treatment effect was detected for cumulative syrup collection (GLMM, family gaussian: Chisq. = 11.61, d.f. = 5, p-value = 0.0405), with the highest mean quantity observed in microcolonies exposed to DEHP at 120 ng/g b.w. (105.3 ± 7.8 g) and the lowest in those exposed to DEHP at 12 ng/g b.w. (84.8 ± 6.6 g; Fig. 3a). However, none of the treatments differed significantly from the control group (95.8 ± 2.2 g; p-values > 0.05). Cumulative pollen collection was not affected by phthalate exposure (GLMM, family gaussian: Chisq. = 8.29, d.f. = 5, p-value = 0.1407), although a non-significant decrease was observed in the microcolonies exposed to the mixture (p-value > 0.05; Fig. 3b).

Exposure to the mixture significantly decreased pollen dilution (p-

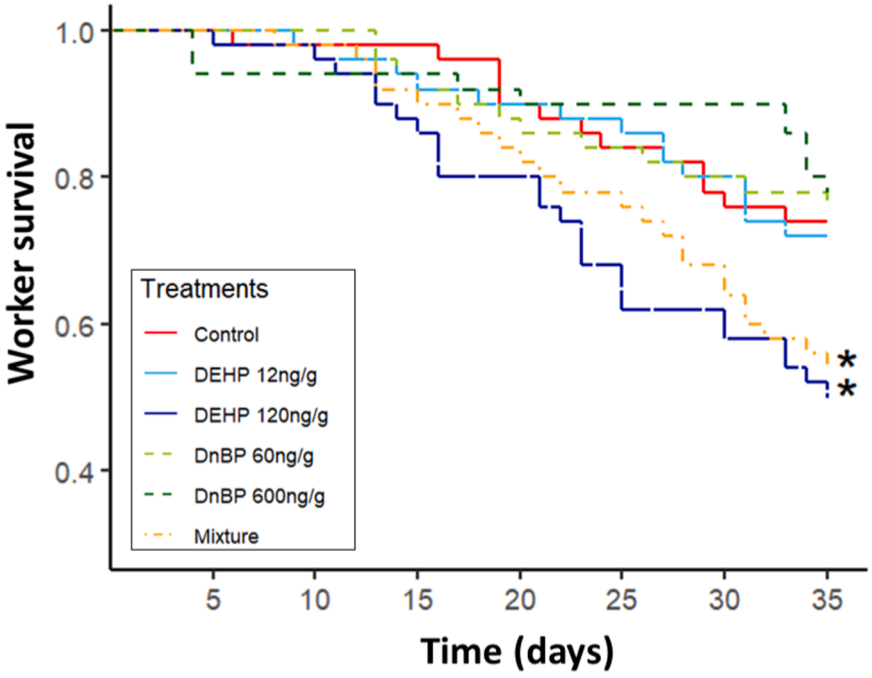

Fig. 2. Kaplan-Meier curves showing the survival in the microcolonies with the proportion of alive workers reported in the control (red solid curve) and five phthalate exposure treatment groups (N = 50 workers per treatment) over the 35 days of experiment. An asterisk "*" indicates a significant difference from the control group at $p < 0.05$, according to a Mixed Cox regression followed by a emmeans post-hoc analysis.

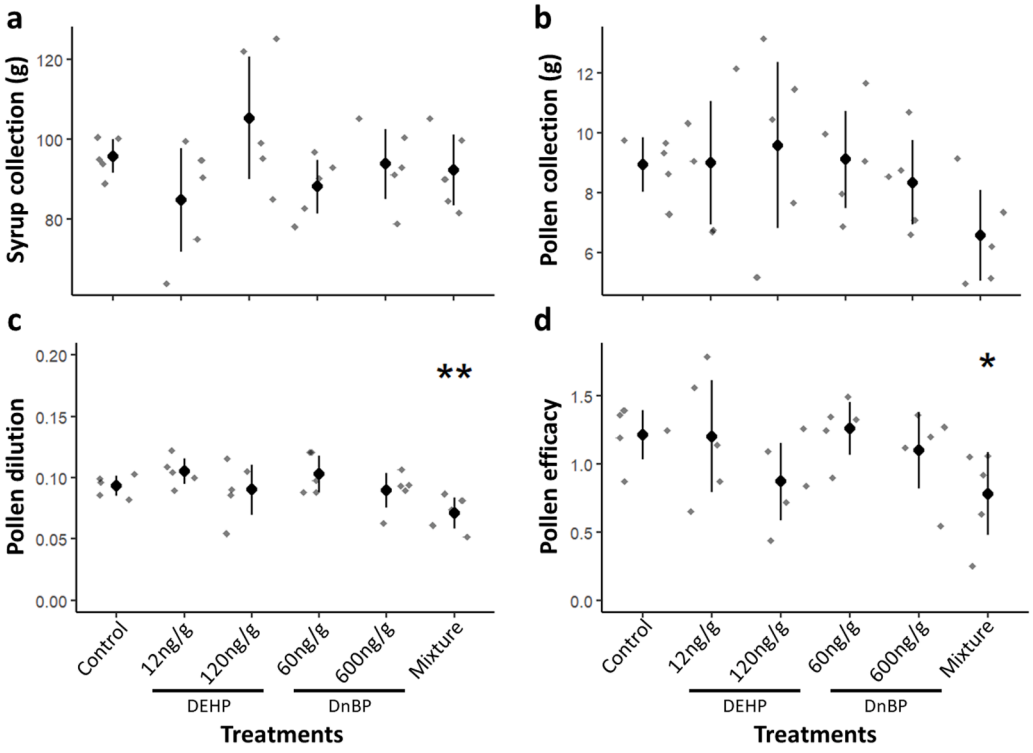

Fig. 3. Resource collection by workers and resource-use metrics in microcolonies exposed to DnBP, DEHP, or their mixture compared with the control group. a) Cumulative standardised syrup collection in grams (g), reflecting total syrup collection over the experiment, considering together consumption and storage by workers; b) cumulative standardised pollen collection (g), reflecting total pollen collection over the experiment, considering together storage and brood feeding; c) pollen dilution (g/g; ratio of collected pollen to syrup), highlighting potential stress response mechanisms; and d) pollen efficacy (g/g; ratio of total offspring mass to cumulative pollen collection), representing how collected pollen was converted into offspring biomass. Grey dots represent individual microcolonies (N = 5 per treatment). Black points show treatment means and error bars indicate 95% confidence intervals. "*" and "**" indicate significant differences from the control group at $p < 0.05$ and $p < 0.01$, according to GLMM, with family gaussian followed by emmeans post-hoc analyses.

$value_{vs.control} = 0.0072$) and pollen efficacy ($p\text{-value}_{vs.control} = 0.0240$), compared to the control (Fig. 3c,d).

3.3. Impact on offspring number and mass

Pre-defecation larval production was negatively affected by phthalate exposure, although the overall treatment effect was only borderline significant (GLMM, family negative binomial: Chisq. = 11.397, d.f. = 5, p-value = 0.0441), with significantly fewer pre-defecation larvae produced in microcolonies exposed to the mixture than in the control group (p-value = 0.0002; Fig. 4a). Additionally, non-emerged drone production differed between treatments (GLMM, family Poisson: Chisq. = 14.2, d.f. = 5, p-value = 0.0142), with the lowest mean number observed in microcolonies exposed to DnBP at 60 ng/g and the highest in those exposed to DnBP at 600 ng/g (Fig. 4a). However, none of the treatments differed significantly from the control group (p-values > 0.05; Fig. 4a).

The first drones emerged on day 25 from microcolonies exposed to DnBP at 60 and 600 ng/g, while in control microcolonies, the first drones emerged on the following day (Fig. 4b). Final drone numbers differed among treatments (GLMM, family Poisson: Chisq. = 26.1, d.f. = 5, p-value < 0.001). Significantly fewer drones emerged from microcolonies exposed to the mixture than from control microcolonies (p-value = 0.0001), while exposure to DnBP at 600 ng/g resulted in a borderline significant decrease in drone emergence compared to the control group (p-value = 0.0411; Fig. 4b).

When analysed as main explanatory variable, worker survival had a positive but non-significant effect on pre-defecation larval production (GLMM, family negative binomial: Chisq. = 2.9, d.f. = 1, p-value = 0.0871). When worker survival was included as a covariate in the treatment model, the previously borderline significant treatment effect was no longer significant (GLMM, family negative binomial: for treatment effect, Chisq. = 7.4, d.f. = 5, p-value = 0.1920; for worker survival effects, Chisq. = 3.7, d.f. = 1, p-value = 0.0531). Nevertheless, the estimated effect of the mixture treatment remained large and consistent, corresponding to an 86% reduction in pre-defecation larval number in the model without worker survival and an 80% reduction when worker survival was included. Similarly, worker survival had a positive but non-significant effect on both non-emerged and emerged drone production, whether analysed as the main explanatory variable or as a covariate, whereas the treatment effect remained significant (see Table S3 for statistical details).

Phthalate exposure did not significantly affect the number of eggs, non-isolated larvae, post-defecation larvae, and pupae in the microcolonies, nor the total number of hatched individuals (i.e., all post-egg stages) produced by each microcolony, at the end of the experiment (see Table S2 and S4 for statistical details and values).

Phthalate exposure affected the mass of individual non-isolated larvae (GLMM, family gaussian: Chisq. = 15.6, d.f. = 5, p-value =0.0080), while the total mass of non-isolated larvae only tended to differ (GLMM, family gaussian: Chisq. = 10.8, d.f. = 5, p-value = 0.0547). The highest mean mass was found in microcolonies exposed to DnBP at 60 ng/g b.w. (0.042 ± 0.005 g) and the lowest in those exposed to the mixture (0.013 ± 0.006 g), but none of the treatments differed significantly from the control group (0.032 ± 0.009 g; p-values > 0.05; Fig. S1b).

Regarding the isolated larval stages, post-defecation larval mass differed between treatments (GLMM, family gaussian: Chisq. = 11.3, d.f. = 5, p-value = 0.04595), with a borderline significant increase for DnBP treatment at 600 ng/g compared to control (p-value = 0.0464; Fig. 4c). Pupal mass also differed between treatments (GLMM, family gaussian:

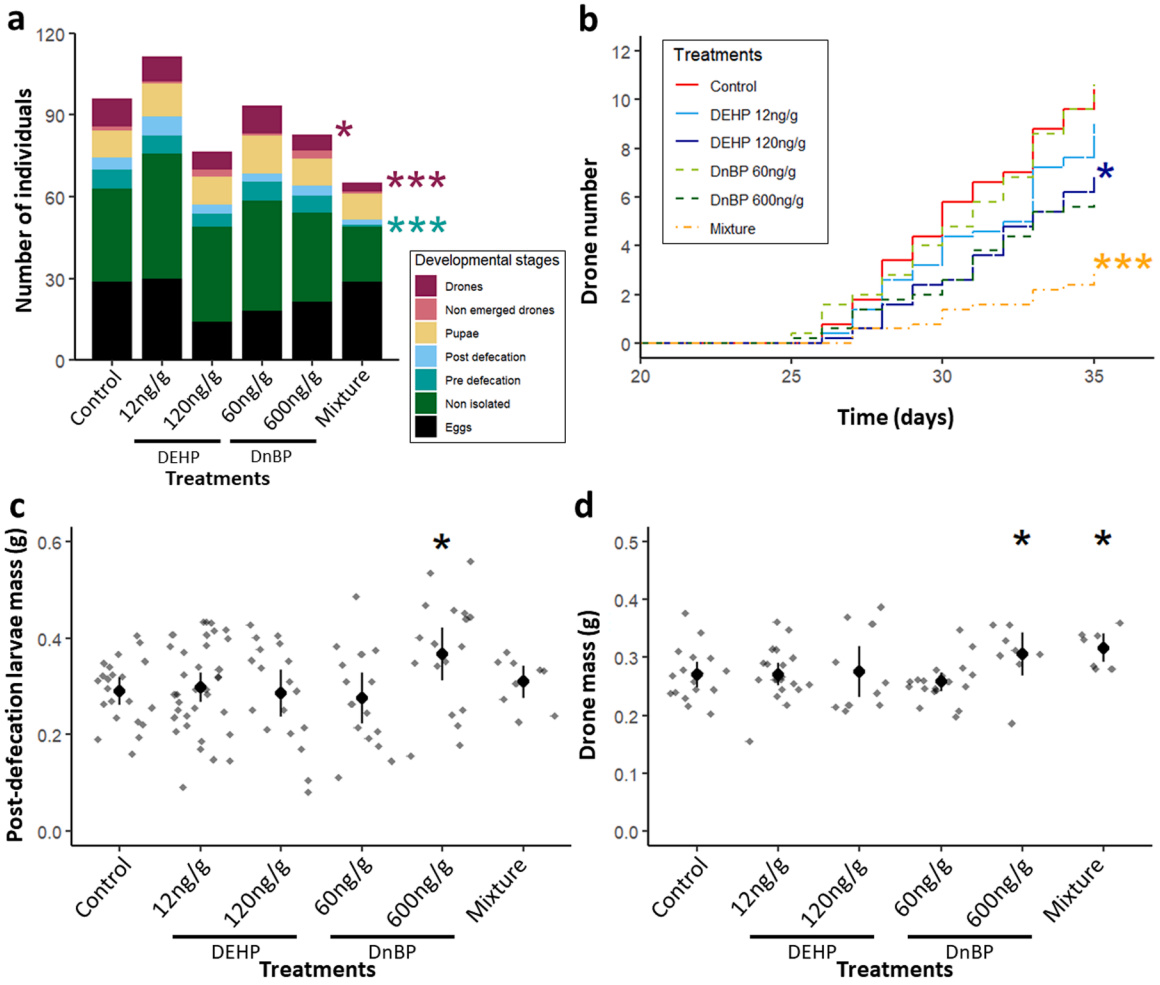

Fig. 4. Effects of phthalate exposure on offspring production and morphology in bumblebee microcolonies. a) Mean number of offspring in each developmental stage (eggs, larvae, pupae, and drones) for each treatment (N = 5 microcolonies per treatment), showing brood production in terms of number distribution among stages at the end of the experiment. b) Dynamics of drone emergence over the 35-day experiment, showing the timing and rate of adult male progeny production. Lines represent treatment means (N = 5 microcolonies per treatment) with the solid red line representing the control group. c) Individual body mass for post-defecation larvae collected at the end of the experiment from the microcolonies exposed to DEHP at 12 ng/g (N = 35) or 120 ng/g (N = 18), DnBP at 60 ng/g (N = 15) or 600 ng/g (N = 18), or the mixture (N = 9) compared to the control group (N = 22). d) Individual body mass for drones emerged during the last two days, from the microcolonies exposed to DEHP at 12 ng/g (N = 20) or 120 ng/g (N = 10), DnBP at 60 ng/g (N = 19) or 600 ng/g (N = 8), or the mixture (N = 7) compared to the control group (N = 17). Black points represent means and error bars indicate 95% confidence intervals; grey dots represent individual measurements. Single and double asterisks "*" and "***" indicate significant differences from the control group at $p < 0.05$ and $p < 0.001$, respectively, according to GLMM, families negative binomial and poisson (count data of panel a and b) and family gaussian and gamma (mass data of panel c and d), and followed by emmeans post-hoc analyses.

Chisq. = 17.8, d.f. = 5, p-value = 0.0032), with the highest mean observed for pupae produced by microcolonies exposed to DnBP at 600 ng/g b.w. (0.339 ± 0.010) and the lowest in those exposed to DEHP at 120 ng/g b.w. (0.295 ± 0.010). However, these values did not differ significantly from the control (0.308 ± 0.010; p-value $_{\text{vs. DnBP 600 ng/g}}$ = 0.1362 and p-value $_{\text{vs. DEHP 120 ng/g}}$ = 0.2489), and we found no effect of phthalate exposure on pre-defecation larval mass (see Fig. S1c, e and Table S2 for statistical details).

Drone mass did not differ among treatments when all emerged drones were considered (see Table S2 for statistical details). However, the mass of drones emerging during the final two days of the experiment (i.e., from eggs laid by workers exposed more than once) differed significantly between treatments (GLMM, family gamma: Chisq. = 20.1, d.f. = 5, p-value = 0.0012). A significant increase in lastly-emerged drone mass was observed in microcolonies exposed to DnBP at 600 ng/g b.w. and to the mixture compared to the control group (p-values = 0.0150; Fig. 4d).

Overall, total hatched offspring mass showed a non-significant tendency to differ among treatments (GLMM, family gaussian: Chisq. = 10.2, d.f. = 5, p-value = 0.0696), with the lowest mass found in microcolonies exposed to the mixture (Fig. S1f).

3.4. Worker and drone abdominal lipid ratio

Phthalate exposure significantly affected worker abdominal lipid content (GLMM, family beta: Chisq. = 18.65, d.f. = 5, p-value = 0.002). Compared with the control group, workers exposed to DnBP at 600 ng/g b.w. showed a significant 4.8% increase in abdominal lipid content (p-value = 0.0012; Fig. 5a).

No effect of worker phthalate exposure was observed on drone abdominal lipid content, whether considering all emerged individuals (Fig. 5b) or only those that emerged during the final two days of the experiment (see Table S2 for statistical details).

4. Discussion

In this study, we investigated the impact of repeated exposure to nominal environmental doses of the phthalates DnBP and DEHP,

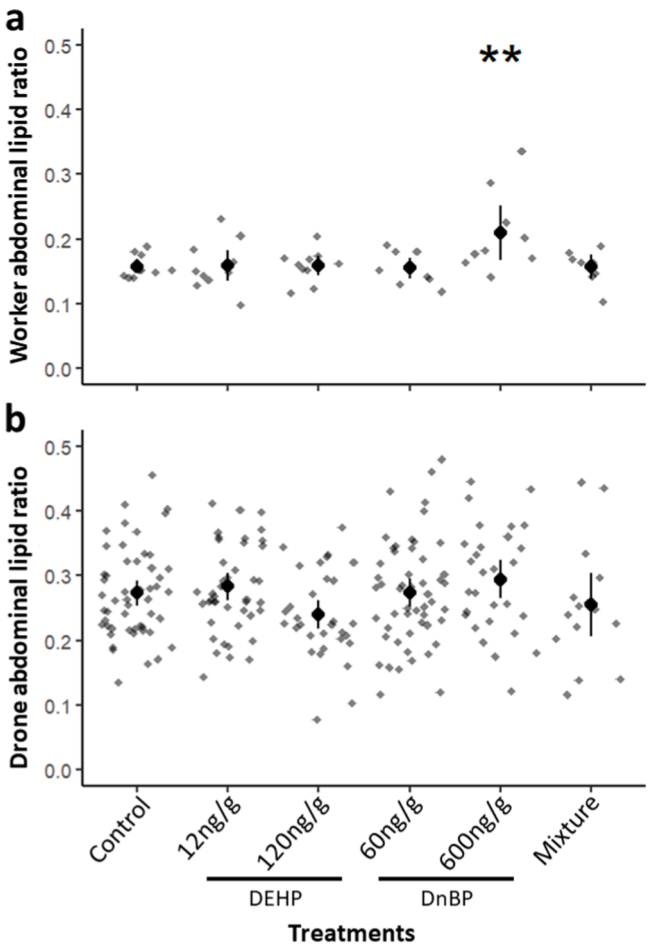

Fig. 5. Abdominal lipid ratio, a proxy to measure fat storage, in a) workers directly exposed, and b) drones produced by the workers exposed to DEHP at 12 ng/g ($N_{workers} = 10$; $N_{males} = 45$) or 120 ng/g ($N_{workers} = 10$; $N_{males} = 34$), DnBP at 60 ng/g ($N_{workers} = 9$; $N_{males} = 53$) or 600 ng/g ($N_{workers} = 9$; $N_{males} = 30$), or the mixture ($N_{workers} = 8$; $N_{males} = 15$) compared to the control group ($N_{workers} = 10$; $N_{males} = 52$). Black points show mean values with error bars indicating 95% confidence intervals. A double asterisk "**" indicates a significant difference at $p < 0.01$ compared to the control group, according to GLMM, family beta, followed by a emmeans *post-hoc* analysis.

administered either individually or in combination, on the development of *B. terrestris* microcolonies over 35 days. Our results revealed increased worker mortality following exposure to DEHP 120 ng/g, both alone and in combination with DnBP 60 ng/g. We also observed a reduction in the number of drones emerging from microcolonies exposed to the mixture, as well as from those exposed to DnBP 600 ng/g b.w. Under this latter condition, progeny had increased individual mass at the post-defecation stage and among the last drones to emerge, while exposed workers showed an elevated abdominal lipid ratio.

4.1. Increase in worker mortality

DnBP-exposed microcolonies maintained mortality rates comparable to those of the control group throughout the 35-day experiment. In contrast, isolated *B. terrestris* workers previously exhibited a fourfold increase in mortality 72 h after exposure to DnBP at a nominal environmental dose of 60 ng/g b.w. (Dewaele et al., 2025). To our knowledge, no other study has investigated the long-term effects of DnBP exposure in insects. However, similarly, in *Daphnia magna* exposed to high concentrations of DnBP (>3 μg/L) throughout a lifespan assay, no abnormal mortality was reported (Seyoum and Pradhan, 2019). In our previous study, comparing worker mortality 72 h after exposure to the same doses of DnBP but isolated and in microcolonies, we showed that sociality reduces DnBP toxicity. Several colony-level mechanisms could contribute to this buffering effect. First, workers maintained in microcolonies benefit from social thermoregulation (Livesey et al., 2019), whereas isolated workers remain closer to ambient temperature (Sepúlveda-Rodríguez et al., 2024). Because temperature can influence detoxification and excretion processes in insects, this may alter sensitivity to contaminants (Verheyen et al., 2022). Second, grooming and social contact could reduce cuticular contaminant loads and therefore limit effective exposure. While self-grooming and brood-rubbing are known to be performed by bumblebees in healthy conditions (Dronnet et al., 2005), to our knowledge, this hypothesis has never been investigated concerning pollutant transfer and removal. Third, social conditions help maintain the characteristic bee microbiota, which is increasingly recognised as an important contributor to xenobiotic tolerance and detoxification (Wu et al., 2020). Finally, queenless workers in microcolonies undergo endocrine and metabolic changes associated with reproductive competition (Amsalem et al., 2014; Bloch et al., 2000), potentially modifying their physiological response to endocrine-disrupting compounds. Although these mechanisms remain hypothetical and further research is needed to understand the social mediation of pollutant effects, our results suggest that colony-level processes may mitigate the effects of phthalate exposure. Consistent with our previous observations following acute exposure (Dewaele et al., 2025), this buffering effect appears to persist during long-term repeated DnBP exposure.

We observed an increase in worker mortality in microcolonies repeatedly exposed to DEHP 120 ng/g, both alone and in mixture with DnBP. These results contrast with the general pattern reported in invertebrates, where high molecular weight phthalates such as DEHP are typically considered less toxic than low molecular weight phthalates such as DnBP (e.g., Green-Ojo et al., 2024; Jensen et al., 2001; Seyoum and Pradhan, 2019). Similarly, Arulanandam et al. (2022) predicted a higher toxicity of DnBP than DEHP in honeybees using *in silico* toxicity models. While this prediction was supported by our previous study on acute exposure of isolated *B. terrestris* workers, the present results suggest that prediction approaches based primarily on chemical structure and acute toxicity data can overlook physiological responses occur in the case of repeated long-term exposure, as well as their interactions with the complex physiological regulatory networks underlying social interactions.

We observed decline in survival in microcolonies, only noticeable after the midpoint of the experiment. In contrast, exposure of isolated workers to DEHP doses ranging from 12 ng/g to 12 μg/g did not previously increase mortality within 72 h following exposure (Dewaele et al., 2025). Moreover, no effect on survival was reported after weekly exposure of *Lasius niger* queens to 2 ng of DEHP for 6 weeks (Cuvillier-Hot et al., 2014). However, like in our results, in female *D. melanogaster* chronically exposed to high concentrations of DEHP (>3 μg/L), mortality similar to that of controls until approximately day 50, when survival began to decline (Liu et al., 2024).

In previous microcolony studies using environmentally relevant doses of pesticides, worker survival either remained at control levels for fungicide or herbicide molecules (e.g., Nebauer et al., 2024; Straw and Brown, 2021), or declined progressively from the start of exposure in response to insecticides (e.g., Nebauer et al., 2024). Although this difference in survival profile could result from differences in the route and frequency of exposure, such delayed toxicity is also a characteristic of endocrine-disrupting compounds (reviewed in Crews et al., 2000).

Several hypotheses could explain the mortality observed in this study. First, in *D. melanogaster*, reduced lifespan following DEHP exposure has been linked to altered expression of genes involved in the AKT-FOXO pathway, resulting in accelerated ageing and reduced survival (Liu et al., 2024). We hypothesise that a similar disruption could underlie the decreased survival observed in *B. terrestris*. Further transcriptomic analyses could help clarify the mechanisms involved.

Another hypothetical explanation for the increased mortality is oxidative stress associated with DEHP detoxification. Previous work has shown that DEHP detoxification is energetically costly (Rivas et al., 2023). In rats, DEHP metabolism involves cytochrome P450 enzymes (Liu et al., 2021). In insects, these enzymes also play a central role in xenobiotic detoxification, but their activity can generate oxidative stress, leading to cellular damage and reduced survival (Murataliev et al., 2008). As DEHP is known to specifically activate xenobiotic detoxification pathways (Srivastava et al., 1976), repeated exposure might have progressively amplified oxidative stress, ultimately reducing worker survival.

It is worth noting that DEHP effects on survival have generally been reported at higher doses than those used here (e.g., Liu et al., 2024). Because of their reduced number of cytochrome P450-related genes compared to other insect groups (Claudianos et al., 2006), *B. terrestris* may be particularly sensitive to such compounds. This could also apply to other wild bees, including Megachilidae, which lack specific cytochrome P450 genes (Hayward et al., 2024), and could explain the greater sensitivity of *B. terrestris* than other invertebrate models. Furthermore, repeated exposure over 35 days may have progressively altered detoxification processes, for example through depletion of detoxification enzymes. To better understand the impact and mode of action of phthalates in bees, further physiological and molecular studies are therefore needed.

Alternatively, the increased mortality could result from disrupted social behaviour caused by the repellent properties of DEHP, as reported in ants (Cuvillier-Hot et al., 2014). In the present study, workers were exposed via their cuticle, potentially altering the chemical cues involved in nestmate recognition (van Zweden and D'Ettore, 2010). Moreover, (Javal et al. (2026) recently showed that exposure to DEHP at 1200 ng/g b.w., either alone or in mixture with DnBP (3–300 ng/g b.w.), can alter antennal responses to floral volatile compounds in *B. terrestris*, demonstrating that phthalates can impair chemosensory perception in this species. Such effects may not be restricted to floral odour detection and could also affect the perception of social chemical cues involved in nestmate recognition. In *A. mellifera*, altered cuticular odours can trigger aggression towards nestmates (Cappa et al., 2019), a mechanism that could contribute to the higher mortality observed in DEHP-exposed microcolonies. However, DnBP, which has also been reported as repellent in *A. mellifera* as well (Sahebzadeh et al., 2009), did not increase worker mortality. A thorough investigation of phthalate effects on nestmate and intraspecific recognition could therefore help clarify how these pollutants could impair bumblebee social behaviour.

Mortality in the mixture treatment was comparable to that observed in the DEHP 120 ng/g b.w. This contrasts with our previous finding that DEHP-DnBP mixture, at similar doses, reduced DnBP toxicity at 72 h after exposure in isolated workers (Dewaele et al., 2025). Here, no such antagonistic effect was observed. The discrepancy may result from differences in both exposure duration and social context. The patterns observed following individual acute exposure may primarily reflect short-term physiological responses, whereas repeated exposure over 35 days in microcolonies could lead to cumulative DEHP effects that only become apparent over time and are not mitigated by DnBP.

While the antagonism observed in the short term could result from DEHP activating DnBP detoxification pathways (Dewaele et al., 2025), our results suggest that DnBP does not reciprocally promote DEHP detoxification. The similar mortality observed in DEHP and mixture treatments further suggests that DEHP could contribute substantially to the mortality observed in the mixture. Such effects could arise through the mechanisms proposed above, including disruption of ageing-related pathways, increased oxidative stress associated with detoxification, or altered social recognition cues leading to increased worker aggression. Further research is now needed to identify the molecular targets of phthalates in bees.

4.2. Decrease in offspring production

We observed a reduction in late-stage larvae production and, more markedly, in the number of emerged drones in microcolonies exposed to the DEHP-DnBP mixture, alongside a marginally significant increase in worker mortality. In microcolonies, reduced offspring production often accompanies increased worker. For example, in *B. impatiens* microcolonies exposed to the insecticide dimethoate (10 mg/kg of food) for 42 days, worker survival declined to 20%, while drone emergence decreased to less than 10 individuals (compared to around 20 in control microcolonies; Krueger et al., 2021). Based on these results, Krueger et al. (2021) concluded that when a compound affects adult survival in microcolony assays, impacts on brood production may largely reflect impaired brood-care behaviours. In our study, attempting to link worker mortality to larval number reduction by including worker survival as a covariate had little effect on the estimated treatment effect, particularly for non-emerged and emerged drone production. However, the loss of statistical significance after inclusion of worker survival likely reflects either limited statistical power due to the small sample size or shared variance between treatment and worker survival, as treatment had a significant direct effect on worker mortality. Therefore, although worker survival may contribute to the effects observed on offspring production, our analyses could not fully disentangle direct larval toxicity from indirect nurse effects associated with a 50% reduction in worker survival.

It is worth noting that, although comparable mortality was observed in microcolonies exposed to DEHP alone, no reduction in larval production was detected, suggesting that the combined exposure may affect brood survival more strongly than exposure to DEHP alone. To our knowledge, such an additive impact has not yet been reported in invertebrates. While the mixture treatment had no clear impact on total pollen and syrup collection, it significantly reduced pollen dilution (i.e., collected pollen:syrup ratio), indicating a shift in worker behaviour. In addition, pollen collection tended to be lower than in control microcolonies, although the difference was not significant. This pattern may reflect an energetic trade-off favouring detoxification over brood care in workers. Indeed, sugars from syrup and nectar primarily serve as an energy source for adult bees (Suarez et al., 2005), whereas pollen provides the proteins and lipids required for larval development (Pereboom, 2000). Pollen efficacy (i.e., offspring mass:collected pollen ratio), used here as an indicator of microcolony performance, was also significantly reduced in the mixture treatment, consistent with the non-significant reduction in pollen collection.

Like in the mixture treatment group, microcolonies exposed to DnBP 600 ng/g produced fewer emerged drones, despite no significant change in worker mortality or resource collection. Although this effect was only borderline significant, it suggests that DnBP, both alone and in combination with DEHP, may impair offspring production at environmentally relevant doses. Both compounds have previously been shown to disrupt egg laying in insects. For example, ant queens exposed by contact to DEHP and *D. melanogaster* exposed to DnBP through food laid around 40% fewer eggs than their control group (Atlı, 2010; Cuvillier-Hot et al., 2014). In the present study, egg number did not differ among treatments. However, eggs were only counted at the end of the experiment, and monitoring egg-laying dynamics throughout the exposure period could provide further insight into potential effects on reproduction. Alternatively, exposure to DnBP 600 ng/g or to the mixture may have delayed larval development, thereby reducing drone emergence by the end of the experiment. Indeed, both phthalates have previously been reported to affect post-embryonic development in insects. In *S. littoralis*, high DEHP concentrations in food shortened larval stage duration but prolonged the pupal stage (Avilès et al., 2019), and whereas DnBP exposure extended pupation duration in *D. melanogaster* (Atlı, 2010). Direct larval exposure experiments could help clarify the effects of DEHP and DnBP, alone or in combination, on larval development in *B. terrestris*.

4.3. Increase in worker abdominal lipid ratio and offspring mass

In this study, microcolonies exposed to nominal environmental doses of DEHP showed no differences in larval and drone body mass, nor in adult abdominal lipid ratio, compared to controls. Similarly, in *S. littoralis*, high DEHP concentrations (19.5 mg/g of food) affected larval development and body mass, whereas environmentally concentrations (e.g., 1.1 ng/g of food) had no effects on these traits (Avilès et al., 2019). Likewise, effects on body mass and lipid content in invertebrates are generally reported at concentrations exceeding environmental levels, as observed in *D. magna* exposed to > 3 μg/L DEHP (Seyoum and Pradhan, 2019).

In contrast, workers exposed to DnBP at 600 ng/g showed an increased abdominal lipid ratio, despite no corresponding increase in resource collection, together with a borderline significant increase in post-defecation larval mass and a significant increase in the mass of the last-emerged drones. Obesogenic effects of DnBP have been documented in vertebrates (Dalamaga et al., 2024) and aquatic invertebrates (Lee et al., 2018). For example, in *D. magna* reared in DnBP-contaminated media, increased lipid storage was linked to impaired fatty acid catabolism (Seyoum and Pradhan, 2019). Unlike other hymenopterans, such as parasitoid wasps, in which adults lack enzymes required for *de novo* lipogenesis and accumulate lipid reserves during larval development, adult bumblebees rely on both dietary intake and *de novo* lipogenesis for lipid storage (Furse et al., 2023). We therefore hypothesise that the increase in lipid content observed without elevated ressource collection may result from reduced lipid catabolism and/or enhanced lipogenesis, indicating a potential disruption of lipid metabolism following DnBP exposure. Such effect could involve the insulin-signalling pathway, which regulates fat body cell production and lipogenesis (Skowronek et al., 2021). This pathway was shown to be disrupted by low concentrations of DnBP in *D. melanogaster*, leading to increased starvation resistance through reduced lipid mobilisation (Williams et al., 2016). However, further research is needed to link morphological and physiological changes observed at the organism and tissue levels in this study to underlying molecular mechanisms of phthalate disruption in bumblebees, for example through gene expression or hormone titration analyses.

Alongside the effects observed in workers, post-defecation larvae and the last drones to emerge from microcolonies exposed to DnBP at 600 ng/g showed higher body mass than controls. Pre-pupal stages, such as post-defecation larvae, are sensitive to juvenile hormone signalling (Rachinsky et al., 1990), which is influenced by the insulin-signalling pathway (Zhang et al., 2022) and regulates lipid metabolism and mobilisation (Shpigler et al., 2021). Consequently, disruption of this pathway could underlie the obesity-related phenotype observed for larvae, through alterations of lipid metabolism and insulin signalling similar to those hypothesised in workers. Together, these results highlight the need for a better understanding of the molecular mechanisms underlying phthalate exposure in bumblebees.

Additionally, microcolonies exposed to DnBP at 600 ng/g produced fewer drones despite no changes in resource collection or related metrics, suggesting that larvae may have received proportionally more food. As most bumblebee species are monoandrous, a shortage of males could have detrimental consequences for queen mating success and population fitness. This could directly reduce the number of new colonies founded in the following year. However, reproductive success depends not only on the number of males produced but also on their morphological and physiological characteristics, including body mass or fertility. The production of larger drones, as observed here, has previously been associated with higher mating success (Park et al., 2025). Nevertheless, a shortage of males, even if individual mating success is increased, could more subtly promote inbreeding and ultimately weaken populations (Goulson et al., 2008). Finally, exposure to DEHP, DnBP, and their mixture has been shown to affect male fertility through alterations in sperm number and function in vertebrate models (e.g., Mohanty et al., 2023), and similar effects of DnBP have been reported in *D. melanogaster* (Misra et al., 2014). To our knowledge, this effect has never been investigated in bees. Given that around 45% of European bumblebee species are considered at least “Near Threatened” to “Critically Endangered” (Michez et al., 2026), the negative impacts of phthalates on the production and health of reproductive individuals, particularly drones, may represent an overlooked factor contributing to ongoing population declines.

Additionally, as neither pollen collection nor pollen efficacy (i.e., total offspring mass:collected pollen ratio) differed significantly from the control, the increase in larval mass is unlikely to result solely from changes in larval feeding. Instead, DnBP may have altered larval development timing, as previously reported in *D. melanogaster* (Atlı, 2010). Prolonged larval development could reduce the number of drones emerging before the end of the experiment while allowing larvae more time to feed and grow, potentially explaining the unchanged pollen efficacy.

In *Bombus*, both development duration and food intake influence caste determination (Ribeiro, 1994). We therefore hypothesise that phthalate exposure could alter the balance between workers and queen production, with potentially important consequences for colony fitness in queenright colonies. To date, such effects have only been reported in *B. terrestris* colonies exposed to elevated temperatures (Guiraud et al., 2021), and not pollutants. Future studies should investigate the effects of phthalate on caste-specific development and the underlying molecular pathways in queenright colonies.

4.4. Potential transgenerational impact of phthalates

In this study, larvae were not directly exposed to phthalates. Nevertheless, post-defecation larvae and the last drones to emerge from microcolonies exposed to DnBP at 600 ng/g showed increased body mass, suggesting an effect of repeated exposure on offspring development of the mother workers. We propose three hypothetical mechanisms that could explain the observed effects in the offspring of the exposed workers.

First, larvae could have been indirectly exposed through transfer, from contaminated workers and nest structures. While phthalate transfer dynamics within social bee colonies remain largely unexplored, foragers are known to introduce environmental pollutants into nests, including plasticisers, which can accumulate in lipid-rich nest materials (Gómez-Ramos et al., 2016). Such accumulation could result in chronic larval exposure through contact with contaminated workers and nest structures, potentially explaining the effects observed here.

Second, exposure may have occurred during oogenesis in contaminated workers. In queenless microcolonies, workers develop ovaries and begin laying unfertilised eggs within the first days following isolation from the queenright colony (Klinger et al., 2019). In terrestrial invertebrates such as wolf spiders and soil mites, the entire burden of some inorganic pollutants (e.g., mercury and cadmium) can be transferred to eggs (Saxton et al., 2013). However, data regarding the transfer of organic pollutant transfer, particularly in bees, remain scarce. Notably, *Spodoptera frugiperda* individuals reared on DnBP-contaminated media (1 mg/L) produced second-generation offspring containing approximately 100 times more DnBP than their parents, suggesting transfer and bioaccumulation despite the absence of parental care (Filho et al., 2013). However, egg contamination was not assessed in that study.

Third, we hypothesise that the increased larval mass observed in DnBP-exposed microcolonies could reflect potential parental effects mediated by hormonal disruption. Similar phenomena have been reported in species lacking parental care. For example, long-term parental exposure of *D. melanogaster* to high concentrations of DEHP altered offspring body mass, with maternal exposure reducing body weight, and paternal exposure increasing it (Chen et al., 2019), highlighting the importance of parental endocrine endocrine status. In our study, only workers were exposed to DnBP, and increased body mass was observed

in late-stage larvae. Because bumblebee males are haploid, any parental effects of phthalate exposure may differ from those described in *Drosophila*. Drone traits are influenced solely by maternal factors, whereas female development is shaped by both maternal and paternal contributions. Assessing the consequences of phthalate exposure in second-generation queens and workers would therefore be important for understanding potential impacts on colony development and population health.

However, our data do not allow us to disentangle these mechanisms. Additional information is needed, including chemical analyses of exposed workers, nest structures, eggs, and drones, as well as experiments involving direct larval exposure in the absence of worker contact and nest structure. Such approaches would help determine which of the proposed mechanisms best explains the patterns observed in this study.

4.5. Limitations

Three limitations of our experimental setup should be considered when interpreting these results.

First, phthalate concentrations in the treatment and control solutions, as well as background contamination in pollen and syrup, were not quantified. In previous experiments by Avilès et al. (2019), phthalate contamination was detected in both treatment and control feeding solutions. Consequently, we cannot exclude the presence of background phthalate contamination in control solutions or pollen used here. To minimise this issue, treatment and control solutions were prepared using decontaminated glassware. In addition, feeding solutions and pollen candies were prepared using decontaminated earthenware, glass, and metal materials to limit phthalate contamination. Although the absence of analytical quantification prevents confirmation of the actual exposure concentrations and background contamination levels, the biological responses observed nevertheless indicate that exposure to the prepared solutions was associated with significant effects on worker survival and offspring production.

Second, analyses conducted at the microcolony level (e.g., larval production) were limited to five microcolonies per treatment, resulting in low statistical power. While strong effects on offspring production, such as those observed in the mixture and DnBP 600 ng/g treatments, could be detected, more subtle effects may have gone unnoticed. Moreover, given both the limited sample size and the number of analysed endpoints, we acknowledge an increased probability to detect false positive effects. However, several responses were consistent across related reproductive and physiological endpoints, supporting the overall biological interpretation of our findings.

Third, workers were exposed through localised contact exposure, i.e., deposition of a droplet on the thorax. However, because of the ubiquity of phthalates in the environment (Billings et al., 2021), bees are likely exposed through multiple routes, including soil, in which *B. terrestris* nests, contaminated nest material and atmosphere, as well as atmosphere during foraging activities. Although cuticular contamination is likely to represent an important exposure route in bees (Lenoir et al., 2012), nectar and pollen may also contribute. While phthalate contamination of floral resources remains poorly documented, some plants, such as corn and alfalfa, have been shown to accumulate phthalates in their vegetative parts (Fan et al., 2022; Sun et al., 2015), and honeybee-collected pollen has been reported to contain phthalates (Martín-Gómez et al., 2024). However, repellent effects of phthalates have been reported in insects, including bees (e.g., Sahebzadeh et al., 2009), suggesting that oral exposure could potentially be partially limited by avoidance behaviours. Taken together, these observations may reinforce the importance of contact exposure through contaminated plant surfaces and nest materials, potentially accompanied by lower levels of oral exposure during pollen handling and consumption. Therefore, our results should be interpreted in the context of a single, but likely, exposure route.

Despite these limitations, this study is, to our knowledge, the first to investigate and demonstrate effects of repeated phthalate exposure in bees.

5. Conclusion

Overall, our findings suggest that environmental exposure to the ubiquitous phthalates DEHP and DnBP can negatively affect *B. terrestris* worker survival and offspring development. The contrast between the present long-term study and previous short-term evaluations highlights the importance of assessing chronic and repeated exposure in chemical risk evaluation. Current frameworks, which rely heavily on short-term toxicity tests (EFSA et al., 2023), may fail to detect delayed effects that are characteristic of endocrine disruptors (Futran Fuhrman et al., 2015). Such overlooked effects could have long-term consequences at both colony and population levels, potentially contributing to bumblebee declines.

CRediT authorship contribution statement

Justine Dewaele: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Denis Michez:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Virginie Cuvillier:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Nina Hautekèete:** Writing – review & editing, Supervision. **Laetitia Verdy:** Writing – review & editing, Investigation. **Sébastien Duterne:** Writing – review & editing, Investigation. **Charlotte Terzo:** Writing – review & editing, Investigation.

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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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2026.120648.

Data availability

Data are available at https://doi.org/10.5281/zenodo.18486280.

References

Amsalem, E., Malka, O., Grozinger, C., Hefetz, A., 2014. Exploring the role of juvenile hormone and vitellogenin in reproduction and social behavior in bumble bees. BMC Evol. Biol. 14, 45. https://doi.org/10.1186/1471-2148-14-45.

Antonova, Y., Arik, A.J., Moore, W., Riehle, M.A., Brown, M.R., 2012. Insulin-Like Peptides. in: Insect Endocrinology. Elsevier, pp. 63–92. https://doi.org/10.1016/B978-0-12-384749-2.10002-0.

Arulanandam, C.D., Hwang, J.-S., Rathinam, A.J., Dahms, H.-U., 2022. Evaluating different web applications to assess the toxicity of plasticizers. Sci. Rep. 12, 19684. https://doi.org/10.1038/s41598-022-18327-0.

Atlı, E., 2010. The effects of dibutyl phthalate (DBP) on the development and fecundity of *Drosophila melanogaster*. Drosoph. Inf. Serv. 164, 171.

Avilès, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D., 2019. Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth *Spodoptera littoralis*. Chemosphere 215, 725–738. https://doi.org/10.1016/j.chemosphere.2018.10.102.

Barraud, A., Barascou, L., Lefebvre, V., Sene, D., Le Conte, Y., Alaux, C., Grillenzoni, F.-V., Corvucci, F., Serra, G., Costa, C., Vanderplanck, M., Michez, D., 2022. Variations in Nutritional Requirements Across Bee Species. Front. Sustain. Food Syst. 6, 824750. https://doi.org/10.3389/fsufs.2022.824750.

Barraud, A., Vanderplanck, M., Nadarajah, S., Michez, D., 2020. The impact of pollen quality on the sensitivity of bumblebees to pesticides. Acta Oecologica 105, 103552. https://doi.org/10.1016/j.actao.2020.103552.

Billings, A., Jones, K.C., Pereira, M.G., Spurgeon, D.J., 2021. Plasticisers in the terrestrial environment: sources, occurrence and fate. Environ. Chem. 18, 111. https://doi.org/10.1071/EN21033.

Bloch, G., Hefetz, A., Hartfelder, K., 2000. Ecdysteroid titer, ovary status, and dominance in adult worker and queen bumble bees (*Bombus terrestris*). J. Insect Physiol. 46, 1033–1040. https://doi.org/10.1016/S0022-1910(99)00214-0.

Bonneton, F., Laudet, V., 2012. Evolution of Nuclear Receptors in Insects. in: Insect Endocrinology. Elsevier, pp. 219–252. https://doi.org/10.1016/b978-0-12-384749-2.10006-8.

Brooks, M., Bolker, B., Kristensen, K., Maechler, M., Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Van Bentham, K., 2025. Package 'glmmTMB.'

Cao, Y., Lin, H., Zhang, K., Xu, S., Yan, M., Leung, K.M.Y., Lam, P.K.S., 2022. Microplastics: A major source of phthalate esters in aquatic environments. J. Hazard. Mater. 432, 128731. https://doi.org/10.1016/j.jhazmat.2022.128731.

Cappa, F., Petrocelli, I., Dani, F.R., Dapporto, L., Giovannini, M., Silva-Castellari, J., Turillazzi, S., Cervo, R., 2019. Natural biocide disrupts nestmate recognition in honeybees. Sci. Rep. 9, 3171. https://doi.org/10.1038/s41598-019-38963-3.

Chen, M.-Y., Liu, H.-P., Cheng, J., Chiang, S.-Y., Liao, W.-P., Lin, W.-Y., 2019. Transgenerational impact of DEHP on body weight of *Drosophila*. Chemosphere 221, 493–499. https://doi.org/10.1016/j.chemosphere.2018.12.193.

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.

R. Core Team, 2023. R: A language and environment for statistical computing.

Crews, D., Willingham, E., Skipper, J.K., 2000. Endocrine Disruptors: Present Issues, Future Directions. Q. Rev. Biol. 75, 243–260. https://doi.org/10.1086/393498.

Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A., 2014. Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant *Lasius niger*. Environ. Res. 131, 104–110. https://doi.org/10.1016/j.envres.2014.03.016.

Dalamaga, M., Kounatidis, D., Tsilingiris, D., Vallianou, N.G., Karampela, I., Psallida, S., Papavassiliou, A.G., 2024. The Role of Endocrine Disruptors Bisphenols and Phthalates in Obesity: Current Evidence, Perspectives and Controversies. IJMS 25, 675. https://doi.org/10.3390/ijms25010675.

Dewaele, J., Javal, M., Pinchon, A., Evrard, D., Hautekèete, N., Michez, D., Cuvillier, V., 2025. Acute exposure to environmental doses of di-n-butyl phthalate but not di-2-ethylhexyl phthalate induces mortality in isolated and cold-stressed workers of *Bombus terrestris* L. (Hymenoptera: Apidae). Environ. Res. 286, 122836. https://doi.org/10.1016/j.envres.2025.122836.

Dronnet, S., Simon, X., Verhaeghe, J.-C., Rasmont, P., Errard, C., 2005. Bumblebee inquilinism in Bombus (Fernaldaepsithyrus) sylvestris (Hymenoptera, Apidae): behavioural and chemical analyses of host-parasite interactions. Apidologie 36, 59–70. https://doi.org/10.1051/apido:2004070.

EFSA, Adriaanse, P., Arce, A., Focks, A., Ingels, B., Jölli, D., Lambin, S., Rundlöf, M., Süßenbach, D., Del Aguila, M., Ercolano, V., Ferilli, F., Ippolito, A., Szentes, C., Neri, F.M., Padovani, L., Rortais, A., Wassenberg, J., Auteri, D., 2023. Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFS2 21. https://doi.org/10.2903/j.efsa.2023.7989.

Ellers, J., 1995. Fat and Eggs: an Alternative Method To Measure the Trade-Off Between Survival and Reproduction in Insect Parasitoids. Neth. J. Zool. 46, 227–235. https://doi.org/10.1163/156854295X00186.

Fan, Y., Zeng, Y., Huang, Y.-Q., Guan, Y.-F., Sun, Y.-X., Chen, S.-J., Mai, B.-X., 2022. Accumulation and translocation of traditional and novel organophosphate esters and phthalic acid esters in plants during the whole life cycle. Chemosphere 307, 135670. https://doi.org/10.1016/j.chemosphere.2022.135670.

Fankhauser-Noti, A., Grob, K., 2007. Blank problems in trace analysis of diethylhexyl and dibutyl phthalate: Investigation of the sources, tips and tricks. Anal. Chim. Acta 582, 353–360. https://doi.org/10.1016/j.aca.2006.09.012.

Filho, I.D.N., Vieceli, N.C., Cardoso, E.M., Lovatel, E.R., Gonzatti, C.F., Marzotto, J.A., Montezano, D.G., Specht, A., 2013. Two Generations of Fall Armyworm (Lepidoptera: Noctuidae) Contamination by Di- n -Butylphthalate. J. Toxicol. Environ. Health Part. A 76, 973–977. https://doi.org/10.1080/15287394.2013.827996.

Furse, S., Koch, H., Wright, G.A., Stevenson, P.C., 2023. Sterol and lipid metabolism in bees. Metabolomics 19, 78. https://doi.org/10.1007/s11306-023-02039-1.

Futran Fuhrman, V., Tal, A., Arnon, S., 2015. Why endocrine disrupting chemicals (EDCs) challenge traditional risk assessment and how to respond. J. Hazard. Mater. 286, 589–611. https://doi.org/10.1016/j.jhazmat.2014.12.012.

Geddes, L.H., McQuillan, H.J., Aiken, A., Vergoz, V., Mercer, A.R., 2013. Steroid hormone (20-hydroxyecdysone) modulates the acquisition of aversive olfactory memories in pollen forager honeybees. Learn. Mem. 20, 399–409. https://doi.org/10.1101/lm.030825.113.

Gómez-Ramos, M.M., García-Valcárcel, A.I., Tadeo, J.L., Fernández-Alba, A.R., Hernando, M.D., 2016. Screening of environmental contaminants in honey bee wax comb using gas chromatography–high-resolution time-of-flight mass spectrometry. Environ. Sci. Pollut. Res. 23, 4609–4620. https://doi.org/10.1007/s11356-015-5667-0.

Gómez-Ramos, M.M., Ucles, S., Ferrer, C., Fernández-Alba, A.R., Hernando, M.D., 2019. Exploration of environmental contaminants in honeybees using GC-TOF-MS and GC-Orbitrap-MS. Sci. Total. Environ. 647, 232–244. https://doi.org/10.1016/j.scitotenv.2018.08.009.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. Executive Summary to EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr. Rev. 36, 593–602. https://doi.org/10.1210/er.2015-1093.

Goulson, D., Lye, G.C., Darvill, B., 2008. Decline and Conservation of Bumble Bees. Annu. Rev. Entomol. 53, 191–208. https://doi.org/10.1146/annurev.ento.53.103106.093454.

Green-Ojo, B., Tan, H., Botelho, M.T., Obanya, H., Grinsted, L., Parker, M.O., Ford, A.T., 2024. The effects of plastic additives on swimming activity and startle response in marine amphipod *Echinogammarus marinus*. Sci. Total. Environ. 918, 170793. https://doi.org/10.1016/j.scitotenv.2024.170793.

Guiraud, M., Cariou, B., Henrion, M., Baird, E., Gérard, M., 2021. Higher developmental temperature increases queen production and decreases worker body size in the bumblebee *Bombus terrestris*. JHR 88, 39–49. https://doi.org/10.3897/jhr.88.73532.

Hartig, F., 2020. Package 'DHARMa.'

Hayward, A., Hunt, B.J., Haas, J., Bushnell-Crowther, E., Troczka, B.J., Pym, A., Beadle, K., Field, J., Nelson, D.R., Nauen, R., Bass, C., 2024. A cytochrome P450 insecticide detoxification mechanism is not conserved across the Megachilidae family of bees. Evolut. Appl. 17, e13625. https://doi.org/10.1111/eva.13625.

Javal, M., Vanderplanck, M., Bachelet, T., Dolfi, M., Fernandes, J., Lapeyre, B., Roux, L., Cuvillier, V., Proffit, M., 2026. Effects of ubiquitous plasticizers on olfactory perception in the buff-tailed bumble bee. Ecotoxicology 35, 88. https://doi.org/10.1007/s10646-026-03050-7.

Jedlička, P., Ernst, U.R., Votavová, A., Hanus, R., Valterová, I., 2016. Gene Expression Dynamics in Major Endocrine Regulatory Pathways along the Transition from Solitary to Social Life in a Bumblebee, *Bombus terrestris*. Front. Physiol. 7. https://doi.org/10.3389/fphys.2016.00574.

Jensen, J., Van Langevelde, J., Pritzl, G., Krogh, P.H., 2001. Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan *Folsomia fimetaria*. Enviro Toxic. Chem. 20, 1085–1091. https://doi.org/10.1002/etc.5620200520.

Kickham, P., Otton, S.V., Moore, M.M., Ikonomou, M.G., Gobas, F.A.P.C., 2012. Relationship between biodegradation and sorption of phthalate esters and their metabolites in natural sediments. Environ. Toxicol. Chem. 31, 1730–1737. https://doi.org/10.1002/etc.1903.

Klinger, E.G., Camp, A.A., Strange, J.P., Cox-Foster, D., Lehmann, D.M., 2019. *Bombus* (Hymenoptera: Apidae) Microcolonies as a Tool for Biological Understanding and Pesticide Risk Assessment. Environ. Entomol. 48, 1249–1259. https://doi.org/10.1093/ee/nvz117.

Krueger, A.J., Early, T.M., Ripperger, R.J., Cabrera, A.R., Schmehl, D.R., 2021. The Utility of a Bumble Bee (*Bombus* spp. [Hymenoptera: Apidae]) Brood Test for Evaluating the Effects of Pesticides. Environ. Entomol. 50, 1105–1117. https://doi.org/10.1093/ee/nvab072.

Lee, M.-C., Park, J.C., Lee, J.-S., 2018. Effects of environmental stressors on lipid metabolism in aquatic invertebrates. Aquat. Toxicol. 200, 83–92. https://doi.org/10.1016/j.aquatox.2018.04.016.

Lenoir, A., Boulay, R., Dejean, A., Touchard, A., Cuvillier-Hot, V., 2016. Phthalate pollution in an Amazonian rainforest. Environ. Sci. Pollut. Res. 23, 16865–16872. https://doi.org/10.1007/s11356-016-7141-z.

Lenoir, A., Cuvillier-Hot, V., Devers, S., Christidès, J.-P., Montigny, F., 2012. Ant cuticles: A trap for atmospheric phthalate contaminants. Sci. Total. Environ. 441, 209–212. https://doi.org/10.1016/j.scitotenv.2012.10.003.

Lenoir, A., Touchard, A., Devers, S., Christidès, J.-P., Boulay, R., Cuvillier-Hot, V., 2014. Ant cuticular response to phthalate pollution. Environ. Sci. Pollut. Res. 21, 13446–13451. https://doi.org/10.1007/s11356-014-3272-2.

Lenth, R.V., 2023. Package 'emmeans': Estimated Marginal Means, aka Least-Squares Means.

Liu, Xudong, Gao, L., Li, X., Liu, Y., Lou, X., Yang, M., Wu, W., Liu, Xiaomeng, 2024. DEHP and DINP accelerate aging effects in male and female of *Drosophila melanogaster* depend on AKT/FOXO pathway. Toxicol. Vitr. 95, 105742. https://doi.org/10.1016/j.tiv.2023.105742.

Liu, H., Zhou, X., Huang, S., Yang, J., Liu, R., Liu, C., 2021. *Lycium barbarum* Polysaccharides and Wolfberry Juice Prevent DEHP-Induced Hepatotoxicity via PXR-Regulated Detoxification Pathway. Molecules 26, 859. https://doi.org/10.3390/molecules26040859.

Livesey, J.S., Constable, C., Rawlinson, W.G., Robotham, A.M., Wright, C., Hampshire, A. E., Klark, E.G., Borrows, W.A., Horsell, D., Cresswell, J.E., 2019. The power and efficiency of brood incubation in queenless microcolonies of bumble bees (*Bombus terrestris* L. Ecol. Entomol. 44, 601–609. https://doi.org/10.1111/een.12736.

Martín-Gómez, B., Valverde, S., Bernal, J., Ares, A.M., 2024. Development and validation of a new analytical method for the determination of plasticizers in bee pollen. Microchem. J. 205, 111404. https://doi.org/10.1016/j.microc.2024.111404.

Michez, D., Boustani, M., Sentil, A., Benrezkallah, J., de Manincor, N., Wood, T.J., Álvarez Fidalgo, P., Aubert, M., Bellotto, V., Biella, P., Bogusch, P., Bosch, J., Brau, T., Browne, K., Carion, F., Castro, L., Cederberg, B., Clay, J., Debont, T., Bila Dubaić, J., Dall'Olio, R., Dellicour, S., Devalez, J., Devorsine, R., Dufrêne, E., De la Rúa, P., De Tandt, B., Evrard, D., Flaminio, S., Fiordaliso, W., Fontana, P., Gadoum, S., Galbany Casals, M., Gaspar, H., Gekière, A., Gérard, M., Geslin, B., Kasparek, M., Kierat, J., Kohl, P.L., Kuhlmann, M., Kryger, P., Leclercq, N., Le Divelec, R., Lemaire, S., Lescot, S., Lhomme, P., Litman, J., Marshall, L., McCormack, G.P., Moro, A., Mudri Stojnić, S., Ockermueller, E., Oleksa, A., Ortiz-Sánchez, F.J., Patiny, S., Paukkunen, J., Perrard, A., Petanidou, T., Pinto, M.A., Potts, S.G., Praz, C., Put, S.C.L., Radchenko, V.G., Rasmont, P., Requier, F., Reverté, S., Risch, S., Rogenstein, S., Rosa, P., Ruiz, C., Rutschmann, B., Santerre, R., Smit, J., Straka, J., Thulier, J., Tourbez, C., Trottet, A., Vereecken, N.J., Williams, P. H., Winter, E., Zimmermann, D., Ghisbain, G., 2026. Measuring the pulse of European biodiversity - European Red List of Bees. Publications Office of the European Union, LU. https://doi.org/10.2779/521877.

Misra, S., Singh, A., C.H, R., Sharma, V., Reddy Mudiam, M.K., Ram, K.R., 2014. Identification of Drosophila-Based Endpoints for the Assessment and Understanding of Xenobiotic-Mediated Male Reproductive Adversities. Toxicol. Sci. 141, 278–291. https://doi.org/10.1093/toxsci/kfu125.

Mohanty, G., Tourzani, D.A., Gervasi, M.G., Houle, E., Oluwayiose, O., Suvorov, A., Pilsner, J.R., Visconti, P.E., 2023. Effects of preconception exposure to phthalates on mouse sperm capacitation parameters. Andrology 11, 1484–1494. https://doi.org/10.1111/andr.13423.

Murataliev, M., Guzov, V., Walker, F., Feyereisen, R., 2008. P450 reductase and cytochrome b5 interactions with cytochrome P450: Effects on house fly CYP6A1 catalysis. Insect Biochem. Mol. Biol. 38, 1008–1015. https://doi.org/10.1016/j.ibmb.2008.08.007.

Nebauer, C.A., Prucker, P., Ruedenauer, F.A., Kollmann, J., Leonhardt, S.D., 2024. Bumblebees under stress: Interacting effects of pesticides and heatwaves on colony development and longevity. iScience 27, 111050. https://doi.org/10.1016/j.isci.2024.111050.

OECD, 2017. Test No. 246: Bumblebee, Acute Contact Toxicity Test. Section 2 OECD Guidelines for the Testing of Chemicals. OECD. https://doi.org/10.1787/9789264284104-en.

Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? Oikos 120, 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x.

Pandey, A., Bloch, G., 2015. Juvenile hormone and ecdysteroids as major regulators of brain and behavior in bees. Curr. Opin. Insect Sci. 12, 26–37. https://doi.org/10.1016/j.cois.2015.09.006.

Park, M.S., Woo, J.H., Yoon, H.J., Kim, B.Y., Lee, K.Y., Trewick, S.A., Lee, K.S., Jin, B.R., 2025. Body mass and mate choice in bumblebees (*Bombus terrestris*) under climate heating. J. Therm. Biol. 131, 104210. https://doi.org/10.1016/j.jtherbio.2025.104210.

Pereboom, J.J.M., 2000. The composition of larval food and the significance of exocrine secretions in the bumblebee *Bombus terrestris*. Insectes Sociaux 47, 11–20. https://doi.org/10.1007/s000400050003.

Pinto, L.Z., Hartfelder, K., Bitondi, M.M.G., Simões, Z.L.P., 2002. Ecdysteroid titers in pupae of highly social bees relate to distinct modes of caste development. J. Insect Physiol. 48, 783–790. https://doi.org/10.1016/S0022-1910(02)00103-8.

Rachinsky, A., Strambi, C., Strambi, A., Hartfelder, K., 1990. Caste and metamorphosis: Hemolymph titers of juvenile hormone and ecdysteroids in last instar honeybee larvae. General. Comp. Endocrinol. 79, 31–38. https://doi.org/10.1016/0016-6480(90)90085-Z.

Rasmont, P., Ghisbain, G., Terzo, M., 2021. Bumblebees of Europe: and neighbouring regions, Hymenoptera of Europe. NAP éditions VerrièRes. -Le. -Buisson.

Ribeiro, M.F., 1994. Growth in bumble bee larvae: relation between development time, mass, and amount of pollen ingested. Can. J. Zool. 72, 1978–1985. https://doi.org/10.1139/z94-270.

Rivas, J., Fuentes, A., Maria, A., Bergerot, B., Siaussat, D., Renault, D., 2023. Effects of phthalate and bisphenol plasticizers on the activity of glycolytic enzymes of the moth *Spodoptera littoralis*. J. Insect Physiol. 149, 104533. https://doi.org/10.1016/j.jinsphys.2023.104533.

Sahebzadeh, N., Ebadi, R., Khajehali, J., 2009. Effect of selected repellent chemicals on honey bees in canola and alfalfa fields. J. Apic. Res. 48, 29–33. https://doi.org/10.3896/IBRA.1.48.1.07.

Saxton, H.J., Goodman, J.R., Collins, J.N., Black, F.J., 2013. Maternal transfer of inorganic mercury and methylmercury in aquatic and terrestrial arthropods. Environ. Toxicol. Chem. 32, 2630–2636. https://doi.org/10.1002/etc.2350.

Sepúlveda-Rodríguez, G., Roberts, K.T., Araújo, P., Lehmann, P., Baird, E., 2024. Bumblebee thermoregulation at increasing temperatures is affected by behavioral state. J. Therm. Biol. 121, 103830. https://doi.org/10.1016/j.jtherbio.2024.103830.

Seyoum, A., Pradhan, A., 2019. Effect of phthalates on development, reproduction, fat metabolism and lifespan in *Daphnia magna*. Sci. Total. Environ. 654, 969–977. https://doi.org/10.1016/j.scitotenv.2018.11.158.

Shpigler, H., Amsalem, E., Huang, Z.Y., Cohen, M., Siegel, A.J., Hefetz, A., Bloch, G., 2014. Gonadotropic and Physiological Functions of Juvenile Hormone in Bumblebee (*Bombus terrestris*) Workers. PLoS. ONE 9, e100650. https://doi.org/10.1371/journal.pone.0100650.

Shpigler, H., Magory Cohen, T., Ben-Shimol, E., Ben-Betzalel, R., Levin, E., 2021. Juvenile hormone functions as a metabolic rate accelerator in bumble bees (*Bombus terrestris*). Horm. Behav. 136, 105073. https://doi.org/10.1016/j.yhbeh.2021.105073.

Skowronek, P., Wójcik, Ł., Strachecka, A., 2021. Fat Body—Multifunctional Insect Tissue. Insects 12, 547. https://doi.org/10.3390/insects12060547.

Škrbić, B.D., Ji, Y., Đurišić-Mladenović, N., Zhao, J., 2016. Occurence of the phthalate esters in soil and street dust samples from the Novi Sad city area, Serbia, and the influence on the children's and adults' exposure. J. Hazard. Mater. 312, 272–279. https://doi.org/10.1016/j.jhazmat.2016.03.045.

Srivastava, S.P., Agarwal, D.K., Mushtaq, M., Seth, P.K., 1976. Interaction of DI-2-ethylhexyl phthalate (DEHP) with parathion in rats. Chemosphere 5, 177–181. https://doi.org/10.1016/0045-6535(76)90072-2.

Straw, E.A., Brown, M.J.F., 2021. No evidence of effects or interaction between the widely used herbicide, glyphosate, and a common parasite in bumble bees. PeerJ 9, e12486. https://doi.org/10.7717/peerj.12486.

Suarez, R.K., Darveau, C.-A., Welch, K.C., O'Brien, D.M., Roubik, D.W., Hochachka, P.W., 2005. Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. J. Exp. Biol. 208, 3573–3579. https://doi.org/10.1242/jeb.01775.

Sun, J., Wu, X., Gan, J., 2015. Uptake and Metabolism of Phthalate Esters by Edible Plants. Environ. Sci. Technol. 49, 8471–8478. https://doi.org/10.1021/acs.est.5b01233.

Therneau, T.M., 2022. Package 'coxme': Mixed Effects Cox Models.

Tran, B.C., Teil, M.-J., Blanchard, M., Alliot, F., Chevreuil, M., 2015. Fate of phthalates and BPA in agricultural and non-agricultural soils of the Paris area (France). Environ. Sci. Pollut. Res. 22, 11118–11126. https://doi.org/10.1007/s11356-015-4178-3.

Verheyen, J., Delnat, V., Theys, C., 2022. Daily temperature fluctuations can magnify the toxicity of pesticides. Curr. Opin. Insect Sci. 51, 100919. https://doi.org/10.1016/j.cois.2022.100919.

Wagner, D.L., Grames, E.M., Forister, M.L., Berenbaum, M.R., Stopak, D., 2021. Insect decline in the Anthropocene: Death by a thousand cuts. Proc. Natl. Acad. Sci. U. S. A. 118, e2023989118. https://doi.org/10.1073/pnas.2023989118.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, 2nd ed. Use R! Springer, Cham. https://doi.org/10.1007/978-3-319-24277-4.

Williams, M.J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L.V., Schiöth, H.B., 2016. Dibutyl Phthalate Exposure Disrupts Evolutionarily Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157, 2309–2321. https://doi.org/10.1210/en.2015-2006.

Wu, Y., Zheng, Y., Chen, Yanan, Wang, S., Chen, Yanping, Hu, F., Zheng, H., 2020. Honey bee (*Apis mellifera*) gut microbiota promotes host endogenous detoxification capability via regulation of P450 gene expression in the digestive tract. Microb. Biotechnol. 13, 1201–1212. https://doi.org/10.1111/1751-7915.13579.

Zhang, X., Li, S., Liu, S., 2022. Juvenile Hormone Studies in *Drosophila melanogaster*. Front. Physiol. 12, 785320. https://doi.org/10.3389/fphys.2021.785320.

van Zweden, J., D'Ettore, P., 2010. Nestmate recognition in social insects and the role of hydrocarbons. In: Blomquist, G., Bagneres, A.G. (Eds.), Insect Hydrocarbons. Cambridge University Press, Cambridge.