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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)

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ABSTRACT

Since the past decades, declining trends in bee populations have been reported worldwide. Among multiple anthropogenic factors, endocrine disruptors, such as phthalates, could negatively affect bees and contribute to this decline at environmental level of exposure. Yet, no data are available on phthalate toxicity in bees. Di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DnBP), widely used as plasticisers and adjuvants, are some of the most commonly recorded phthalates in the environment, including on social bee worker cuticles. In this study, we investigated DnBP and DEHP lethal effects on B. terrestris workers after contact exposure to environmentally relevant single-molecule and mixture treatments. Interactions with cold stress and social context were additionally investigated. Firstly, we evidenced for the first time an NMDRC for DnBP toxicity in bees, with an increased mortality of 25 %, 72 h after exposure. By contrast, DEHP did not affect worker survival and even lowered DnBP toxicity when administered as a mixture. Secondly, DnBP at low environmental doses enhanced worker resistance to cold stress, while exposure to cold stress modulated DnBP toxicity, shifting the onset of adverse effects to higher concentrations. Finally, we evidenced that social contacts mitigate DnBP toxicity. Overall, this study provided the first evidence of the detrimental effects caused by these overlooked pollutants on bees, and discusses potential metabolic disruption by phthalate exposure that may affect crucial life traits in bumblebees. Finally, this study highlighted that accounting for complex realistic mixtures and the social context of the studied species seems essential for accurately assessing the toxicity of endocrine disrupting molecules.

1. Introduction

Phthalates (PAEs) are ubiquitous pollutants across environmental compartments, *i.e.* soils, atmosphere and water bodies (Bergé et al., 2013; Net et al., 2015c). Used as plasticisers to improve flexibility (CEFIC, 2021), they also serve as adjuvants to paints, cosmetics, pesticides or detergents (Koniecki et al., 2011). Due to the absence of covalent bonds with the polymer matrix, PAEs leach easily into the environment (Suhrhoff and Scholz-Böttcher, 2016). Industries use numerous PAEs, but di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DnBP) are the most frequently used in European products (Fierens et al., 2012).

Both molecules co-occur in the atmosphere and soils (Net et al., 2015a) and are identified endocrine disruptors (EDs) for mammals and aquatic invertebrates (Gore et al., 2015; Regulation (EU) 2018/2005), in which they disrupt reproductive and immune functions (Liu et al., 2024; Xu et al., 2013). Only a few studies have focused on insects, revealing disruptions of immune, metabolic and hormonal pathway-related genes, some of which contribute to shortened lifespan and larval mortality (Avilès et al., 2020; Cuvillier-Hot et al., 2014; Liu et al., 2021; Šulková

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et al., 2022; Williams et al., 2016). Since 2021, these molecules are listed in Annex XIV of the REACH regulation, which mandates chemical risk assessments for human and environmental hazards before marketing in the European Union (Regulation (EU) 2021/2045, 2021). However, risk assessment of EDs such as PAEs presents substantial challenges due to their low-dose effects, which result from complex interactions with hormonal receptors naturally triggered at low concentrations, and their non-monotonic dose-response curves (NMDRCs), i.e. unpredictable relationships between exposure dose and effects (Vandenberg et al., 2012). These properties challenge traditional toxicological tier-based assessments (Warner and Flaws, 2018), as these approaches rely on determining the dose or concentration lethal for 50 percent of tested individuals (LD50 or LC50) and extrapolate the effects for the lower doses (Adriaanse et al., 2023EFSA et al., 2023; OECD, 2018). These methods have been previously used to determine PAE toxicity on various aquatic species, but are not efficient to understand their effects on organisms. Investigating the lethal effects of exposure to various and environmentally relevant PAE doses is crucial given the current decline in biodiversity, and has not yet been thoroughly performed in insects.

In the context of insect decline (Goulson, 2019), wild bees get particular attention due to their role in pollinating flowering plants (Zattara and Aizen, 2021). Different drivers of decline were identified for bees, and chemical pollution ranks high in all studies (e.g., LeBuhn and Vargas Luna, 2021; Nieto et al., 2014). While the study of pesticide exposure and impact on non-targeted species is a growing field of research, terrestrial invertebrate sensitivity to other pollutants, including PAEs, remains overlooked (Benuszak et al., 2017). Yet, wild bees are likely exposed to PAEs via multiple routes throughout their life stages. Contamination might occur through contact between their lipidic cuticle and the contaminated atmosphere, vegetation or soil (Lenoir et al., 2012; Martín-Gómez et al., 2024). Recently, we quantified PAE contamination of wild *Bombus terrestris* workers sampled in the city of Lille, France: among 17 PAEs investigated, DEHP and DnBP were the most frequently detected on bumblebee cuticles, contaminating 53 % and 60 % of the workers, respectively (unpublished data).

Here, we investigated the impact of PAEs on the buff-tailed bumblebee, *B. terrestris*. This species is a common pollinator, well-represented in Europe (Rasmont et al., 2021) and dominant in urban and peri-urban areas (Michołap et al., 2024; Persson et al., 2020). *B. terrestris* workers navigate up to 800 m from their nest (Wolf and Moritz, 2008), making them good sentinels to assess the pollution of their close environment (Moniuszko et al., 2025). Because of its domestication, commercial hives containing hundreds of workers of this species are available year-round (Velthuis and van Doorn, 2006), with standardised rearing and exposure methods available (Adriaanse et al., 2023). *B. terrestris* is therefore used as a surrogate species to assess acute toxicity of pesticides and other pollutants on wild pollinators (Arena and Sgolastra, 2014; Azpiazu et al., 2021; Gekière et al., 2024; Linguadoca et al., 2022). Consequently, we chose to evaluate the short-term lethal effects of exposure to environmentally relevant doses of DnBP and DEHP on *B. terrestris* workers, maintained in isolation according to OECD guidelines (OECD, 2017a, 2017b). Moreover, and to complete the assessment, the toxicity of DnBP and DEHP in mixture formulations was investigated, as both molecules frequently co-occur in nature (Jarošová, 2006; Net et al., 2015c; Teil et al., 2006).

Climate change is another significant driver of bee decline, especially in bumblebees (Soroye et al., 2020). Extreme weather events, such as unpredictable cold spells and longer-lasting winters, will likely be more frequent and more intense in the future (Walsh et al., 2020; Weilnhammer et al., 2021). *Bombus* spp. are well-suited to cold conditions with crucial metabolic adaptations, such as the ability to perform endothermy (Heinrich, 2004; Maebe et al., 2021). However, PAEs are known to disrupt glucose and lipid metabolism in *Drosophila melanogaster* (Williams et al., 2016) and in other model species (Prieto-Amador et al., 2021; Seyoum and Pradhan, 2019; Zhang et al., 2021); they could thus negatively impact bumblebee cold resistance. To address this hypothesis, we combined phthalate exposure with cold stress, applied on isolated *B. terrestris* workers.

Finally, current OECD guidelines recommend testing pollutant toxicity on isolated bumblebee workers. Yet, *B. terrestris* is a eusocial species, characterised by complex hormonal interactions – that could be disrupted by PAEs – mediated by social contacts (Jedlička et al., 2016; Woodard et al., 2013). This species also benefits from social mitigation of stress (i.e. detoxification, temperature balance; Walton et al., 2024). Nevertheless, pollutant toxicity in bumblebees has never been directly compared between isolation and social rearing conditions, to our knowledge. Therefore, we considered different social contexts to evaluate the toxicity of PAEs, in order to get a broader and more biologically pertinent understanding of their effects (Camp and Lehmann, 2020; Klinger et al., 2019).

Previous pollutant toxicity assessment on bees has mainly focused on pesticide testing (Benuszak et al., 2017). Therefore, by evaluating phthalate impact on bees and their interactions with cold stress and social context, we aim to broaden the scope of ecotoxicological testing and pollinator protection framework to such underestimated pollutants.

2. Material and methods

2.1. Study species

B. terrestris is a ubiquitous pollinator in Europe, including in urban areas (Persson et al., 2020). This species is easy to keep in laboratory conditions, making it a suitable model species for toxicity studies (e.g., Gekière et al., 2024). Commercial colonies (about 80–120 workers) were purchased from Biobest NV (Westerlo, Belgium). Upon receipt, colonies were kept in darkness, and all handling was performed under red light to minimise worker stress.

2.2. Preventing PAE contamination in material and solutions

Phthalates are widespread pollutants, present indoors and particularly in laboratories (Feng et al., 2020). This contamination is mostly related to extensive plastic use (Reid et al., 2007). Therefore, we replaced plastic materials with glass and metal whenever possible. All metal and glass were additionally decontaminated with hexane and acetone, and when feasible, by calcination. Moreover, water marketed in glass bottles (SPA Reine, SPA NV, Bruxelles, Belgium) was used instead of tap or plastic-bottled water to prepare the treatments and feeding solutions (Luo et al., 2018; Net et al., 2015a).

2.3. DnBP, DEHP and control treatments

DnBP (CAS n°117-81-7) and DEHP (CAS n°84-74-2) were purchased as liquid solutions from Sigma-Aldrich. Concentrations were chosen based on data collected on wild *B. terrestris* workers during a previous field campaign in Lille, France, in June–July 2021 (unpublished data). This campaign recorded DnBP and DEHP as the most prevalent PAEs on *B. terrestris* worker cuticles at median doses around 145 ng/g for DEHP and 15 ng/g for DnBP (unpublished data). We then considered 1X treatments of DEHP at 120 ng/g of body weight (b.w.) and DnBP at 60 ng/g b.w. and three other doses: 0.1X, 10X and 100X. Workers were exposed to single-molecule or mixture treatments.

All dilutions were prepared considering an averaged *B. terrestris* worker fresh weight of 0.25 g (Dewaele et al., 2024). The initial dilution used methanol (CAS n°67-56-1, VWR) for good solubility, followed by further dilutions in mineral water to minimise the methanol concentration in the treatment solutions (0.07 % for 0.1X, 1X and 10X). For the 100X treatments, PAEs were diluted in acetone only (CAS n°67-64-1, Chem-Lab NV), due to PAE low water solubility.

As DnBP and DEHP frequently co-occur in the environment, mixture treatments were also tested. For the mixture preparation, initial and subsequent dilutions followed the same method as the 1X and 10X

single-molecule treatments. However, at the last dilution step, both single-molecule solutions were mixed, and the volume was adjusted with mineral water to obtain either a mixture of DEHP and DnBP 1X treatments, or a mixture of the DEHP 1X and DnBP 10X treatments.

Exposure to a solvent control solution was performed using either 0.07 % methanol in mineral water or 100 % acetone. An additional control solution used only mineral water to assess solvent toxicity. As mortality rates in both solvent-treated groups (methanol and acetone) and the mineral water group did not differ, we considered the pooled solvent groups as control (See Table S1).

2.4. Experiment 1: Impact of contact exposure to single and mixed PAE on isolated workers

Here we considered 11 treatments: one control, four single exposure treatments per phthalate (0.1X, 1X, 10X, 100X), and two mixture treatments (DEHP 1X + DnBP 1X and DEHP 1X + DnBP 10X). The exposure protocol was based on OECD guideline n°246 (OECD, 2017a). The experiment was performed during two sessions, for control and single 0.1X, 1X, 10X exposure treatments. Mixtures and single 100X treatments were only tested during the second session. Selection of exposure conditions for mixtures were established based on the results of the single exposure treatments from the first session. We used batches of 30 workers per condition in the first session and 20 in the second. Workers were sampled randomly from three colonies per batch during the first session (i.e. 10 workers from each of the three colonies), and from two during the second session (i.e. 10 workers from each of the 2 colonies), and were evenly distributed across the treatment groups.

For both sessions, workers were isolated in decontaminated, hand-made metal cages similar in dimensions to Nicot cages® (for detailed worker numbers per session and protocol pictures, see Fig. S1a and Table S2). Caged workers remained isolated during the whole duration of the experiment (72 h). They were acclimated to the conditions for at least 8 h before exposure. They were then anaesthetised by chilling in a cold atmosphere without direct cold surface contact for 10 min. Two microlitres of treatment solution were deposited through the cage mesh on the dorsal hairless part of the thorax using a calcined 10 μL glass capillary (Drummond). Once recovered from anaesthesia at room temperature, caged workers were placed back on a metal tray. They were provided *ad libitum* 50 % w/w sucrose-water solution via a decontaminated Pasteur pipette (removed during the anaesthesia). Mortality was recorded at 16, 24, 48, and 72 h post-exposure.

In the first session, caged workers were placed on a metal tray at room temperature under natural daylight regime (50°36′31.5"N, 3°08′39.4"E; June 2022), while they were kept under constant darkness and controlled temperature and humidity (25 °C; 60 % relative humidity) in the second. In the first session, anaesthesia was performed by placing workers by treatment in a freezer for 10 min. In the second session, anaesthesia was similarly performed in a cold atmosphere by placing groups of two caged workers in glass jars in an ice bucket for 10 min (for anaesthesia parameters, see Table S3; Fig. S1c).

2.5. Experiment 2: Impact of contact exposure on isolated workers in combination with cold stress

In this experiment, we partially replicated experiment 1 using a modified anaesthesia protocol mimicking cold stress to evaluate potential interactions between phthalate and cold exposure. The data set relative to the "cold stress" group was generated using the same exposure protocol as in experiment 1. However, anaesthesia was performed by direct ice contact for a longer period of 30 min, compared to the 10-min cold atmosphere anaesthesia of experiment 1 (for anaesthesia parameters, see Table S3). Mortality was recorded 72 h post-exposure. We considered seven treatment groups of ~30 workers: a control, and three single exposure treatments per phthalate (0.1X, 1X and 10X). The data set relative to the "non-cold stress" group originates from experiment 1, from which we selected the appropriate treatments, common to both experiments.

2.6. Experiment 3: Impact of DnBP and DEHP exposure in combination with social conditions

In this experiment, we partially replicated experiment 1, but we evaluated microcolonies to assess interactions between phthalate exposure and social condition. We considered five treatments: a control, two single-molecule treatments with DnBP (1X, 10X), and two with DEHP (0.1X, 1X). The data set relative to the microcolony group was generated as follows. Ten microcolonies for each treatment, i.e. 50 in total, were established in hard plastic boxes (8 × 16 × 16 cm; Fig. S1b). Each microcolony was composed of ten workers. Workers composing a microcolony were sampled from the same colony. Five commercial colonies were used in the experiment. Microcolonies were kept under constant darkness and controlled temperature and humidity (25 °C; 60 % RH). They received *ad libitum* 50 % mass/mass sucrose-water solution. A pollen candi (1 g), i.e., a ball made of pollen mixed with the sucrose-water solution at 0.15 mL/g of pollen, was also provided to each microcolony and replaced every other day. If not fully consumed, the initial pollen candi was not replaced. Organic frozen honeybee-collected pollen (Rosaceae: *Malus/Pyrus* f. 40 %; Salicaceae: *Salix* 27 %) was used (Abeille Heureuse, Strasbourg, France; (Barraud et al., 2022). After a 24-h acclimation period, workers were anaesthetised and exposed using the same method as described in experiment 1 (second session; Fig. S1c). Once recovered at room temperature, all workers from one microcolony were returned to their box simultaneously. Mortality was recorded 72 h post-exposure. The data set relative to the isolated group originates from experiment 1, from which we selected the appropriate treatments, common to both experiments.

2.7. Statistical analyses

Data were analysed using R v 4.4.1 (R Core Team, 2023); graphs were made with 'ggplot2' package v3.5.1 (Wickham, 2016).

Mortality rates were analysed using generalised linear mixed models (GLMMs) from 'glmmTMB' package v1.1.11 (Brooks et al., 2025) with a binomial distribution family and the commercial colony as random effect. For single-molecule exposure (experiment 1), before pooling data from both sessions, we checked that the control mortality did not differ between batches and sessions (See Table S4). The dose was used as a fixed effect, and we added either a cold stress (experiment 2) or social effect (experiment 3), depending on the analyses. If one of the fixed effect levels fully explained the response variable, preventing model fitting, i.e. complete separation (Heinze and Schemper, 2002), a Bayesian GLMM was used by applying the 'bglmer' function from 'blme' package v1.0-6 (Dorie et al., 2022). Weakly informative normal priors were applied for fixed effects (fixef.prior = normal). Convergence was evaluated via optimiser diagnostics in which all models terminated without warnings (Bates et al., 2015). All model fits were further evaluated using the DHARMa package (Hartig, 2020). Contrasts using the package 'emmeans' v1.10.5 (Lenth, 2023) with the Benjamini-Hochberg's p-value adjustment method (i.e., 'fdr') were used *post-hoc*. Mortality differences between two groups exposed to different molecules were compared using two-proportion Z-tests.

For mixture exposure, a modified binomial proportion test for additivity was used to assess the potential interaction effects of environmental concentrations of DnBP and DEHP using the R code provided in Sgolastra et al. (2017), and applied similarly to Raimets et al. (2018). This test compares expected mixture mortality (based on single-molecule assays), and observed mortality (from the mixture exposure assay) by evaluating the null hypothesis of both molecules acting without interaction, *i.e.* $P_{mixture}^{observed} - P_{mixture}^{expected} = 0$. $P_{mixture}^{observed}$ is the experimentally observed mortality in the mixture-treated group, and

$P_{mixture}^{expected}$ is the expected mortality for the mixture-treated group calculated under Bliss independence criterion, *i.e.* $P_{mixture}^{expected} = P_{DEHP}^{observed} + P_{DnBP}^{observed} - P_{DEHP}^{observed} \times P_{DnBP}^{observed}$. Using a lower-tailed version of the test, a significant deviation from the null hypothesis would show an antagonistic interaction.

In experiment 3, we first analysed raw mortality rates to assess the effects of phthalates and cold stress. In case of interaction, the phthalate effect was isolated by correcting the data using Sun-Shepard's control correction formula, which allows to neglect mortality solely induced by cold stress: corrected mortality = [(% treatment survival + % change in control survival)/(100 + % change in control survival)] x 100, with "% change in control survival" = [(number of alive individual in control group - total number in control group)/total number in control group] x 100 (Sun and Shepard, 1947). Negative values obtained were set to zero.

3. Results

3.1. Mortality of isolated workers induced by DnBP and DEHP (experiment 1)

DnBP exposure significantly affected worker mortality 72 h post-exposure compared to control (Fig. 1a; bayesian GLMM, family binomial: chisq. = 14.855, df = 4, p = 0.005012). The peak mortality was found in the DnBP 1X-exposed group, which showed a significant fourfold increase in mortality compared to the control group (Fig. 1a; $p_{DnBP\ 1X\ vs.\ solvent} = 0.0045$) and a significant sevenfold increase compared to the DnBP 0.1X-exposed group (Fig. 1a; $p_{DnBP\ 1X\ vs.\ DnBP\ 0.1X} = 0.0467$). However, mortality rates observed in DnBP 0.1X, 10X, and 100X-exposed groups were not significantly different from the control group, resulting in a bell-shaped curve (Fig. 1a; for detailed mortality and statistical values, see Table S5). Moreover, most DnBP 1X-induced mortality occurred between 48 h and 72 h post-exposure (Fig. 1b).

Regarding DEHP single-molecule exposure, no significant effect on worker mortality was recorded 72 h post-exposure to any of the doses

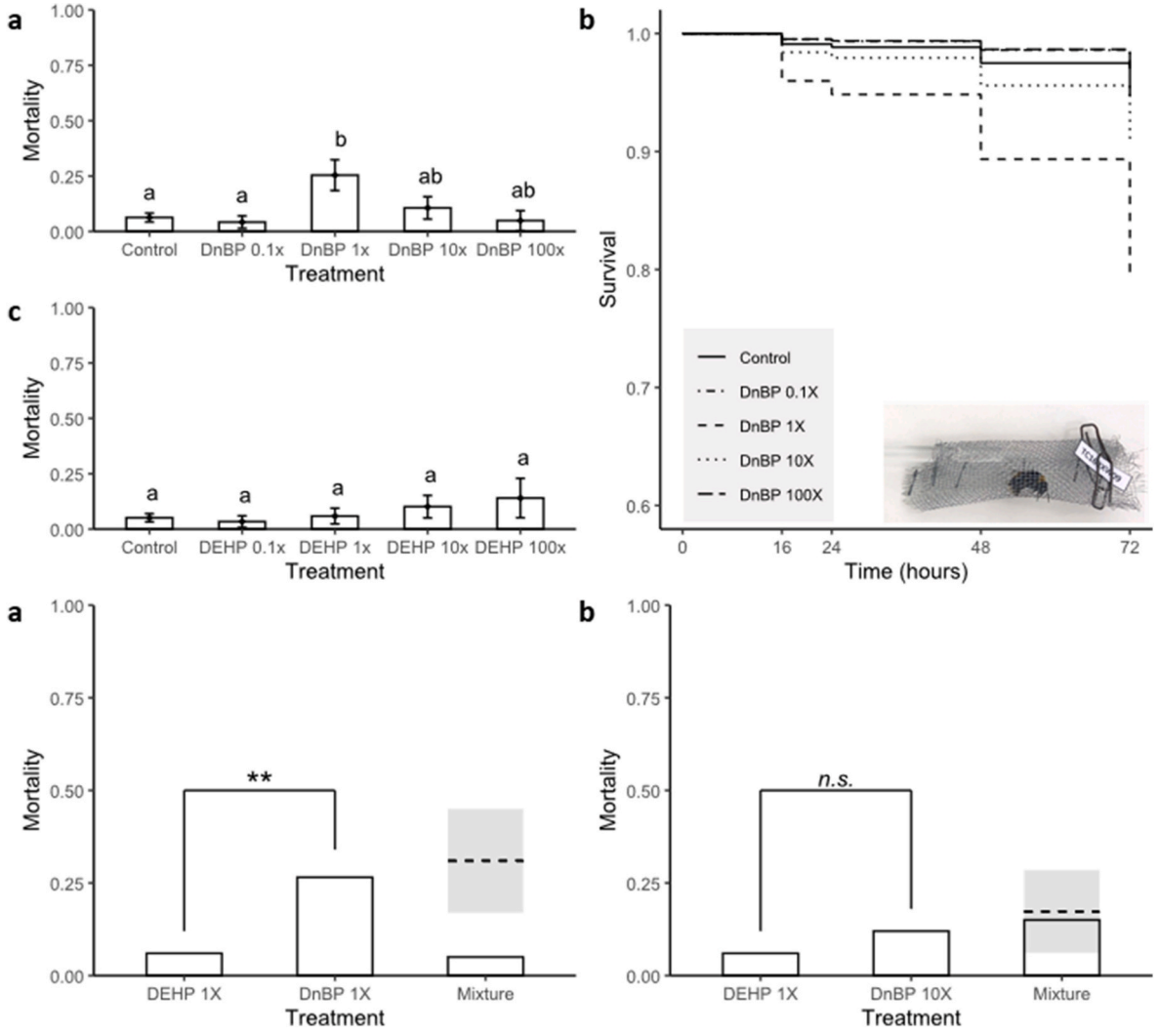

Fig. 1. Effects of single-molecule exposures to DnBP and DEHP on mortality rates of isolated workers after 72 h. a) Mortality rates in control groups (N = 170) and in groups exposed to DnBP at 0.1X (N = 50), 1X = 60 ng/g (N = 49), 10X (N = 50), or 100X (N = 20). b) Kaplan-Meier cumulative proportion of survival during the 72 h after worker exposure to DnBP 0.1X, 1X, 10X or 100X and control groups. The embedded picture shows the isolated caged setup. DnBP 0.1X and DnBP 100X curves are confounded. Please note that the Y-axis scale is cropped below 0.6. Survival curve for DEHP was not established as no treatment significantly increased mortality. c) Mortality rates in control groups (N = 188) and in groups exposed to DEHP at 0.1X (N = 49), 1X = 120 ng/g (N = 50), 10X (N = 50), or 100X (N = 20). In a) and b), columns show emmeans values with error-bars indicating standard errors, and groups with different letters significantly differ at $p < 0.05$ (GLMMs, family binomial).

(Fig. 1c; for detailed mortality and statistical values, see Table S5; GLMM, family binomial: chisq. = 2.2299, df. = 4, p = 0.6936).

Investigating the potential cocktail effects, we observed that DEHP 1X exposure induced significantly less mortality compared to DnBP 1X (Fig. 2a; for detailed mortality values, see Table S5 for DnBP and Table S6 for DEHP; Z-test: chisq. = 6.26, df. = 1, p = 0.006). The mortality observed in the group exposed to the DEHP 1X and DnBP 1X mixture was significantly lower than expected if both molecules acted independently, suggesting an antagonistic effect at these doses (Fig. 2a; for detailed mortality values, see Table S7; Lower-tailed binomial proportion test: p = 0.001).

The mortality in the DEHP 1X-treated group was not significantly different from the mortality found in the DnBP 10X-treated group (Fig. 2b; for detailed mortality values, see Table S5 for DnBP and Table S6 for DEHP; Z-test: chisq. = 0.49, df. = 1, p = 0.2423). The mortality observed in the group treated with the mixture was not significantly different from the expected mortality (Fig. 2b; for detailed mortality values, see Table S7; Lower-tailed binomial proportion test: p = 0.408).

3.2. Worker mortality induced by DnBP and DEHP in combination with cold stress (experiment 2 vs 1)

Cold stress significantly affected raw mortality rates (bayesian GLMM, family binomial: cold stress parameter, chisq. = 15.59, df. = 1, p < 0.001), but this effect varied across all dose groups, indicating a significant interaction between cold stress and DnBP exposure (interaction parameter, chisq. = 9.62, df. = 3, p = 0.022). In the DnBP 0.1X group, cold stress did not significantly change mortality compared to non-cold stress group (Fig. 3a, grey plots; p = 0.3690). However, cold stress significantly increased raw mortality rates in the control (p = 0.0002) and DnBP 10X-exposed groups (Fig. 3a, grey plots; p = 0.0044). Interestingly, the raw mortality rate of the cold-stressed DnBP 0.1X group was significantly lower than in the cold-stressed control group (p = 0.0229), while they did not differ without cold stress (p = 0.7095; Fig. 3a, grey plots; for detailed mortality values, see Table S8).

As cold stress significantly increased control raw mortality rates (p = 0.0002), we applied the Sun-Shepard's correction on the data, which enabled to adjust for mortality caused solely by cold stress, and to isolate DnBP effects. After correction, the cold stress effect on mortality was no longer significant as expected (bayesian GLMM, family binomial: for cold stress parameter, chisq. = 0.17, df. = 1, p = 0.68), but the interaction between DnBP exposure and cold treatment remained significant (bayesian GLMM, family binomial: for interaction parameter, chisq. = 13.74, df. = 2, p = 0.001). Only the corrected mortality found in the cold-stressed DnBP 10X group was significantly higher than in the non-cold stressed group (Fig. 3a, black plots; for detailed mortality values, see Table S8; p = 0.0301).

Cold stress consistently increased mortality in all DEHP-exposed groups without interaction (for statistical and results details, see Table S9 and Fig. S2a).

3.3. Mortality induced by DEHP and DnBP under different social conditions (experiment 3 vs 1)

Mortality across all DnBP dose groups was significantly higher in isolated conditions compared to the microcolony condition (bayesian GLMM, family binomial: chisq. = 41.8, df. = 1, p < 0.001), with a significant interaction between social context and DnBP exposure (chisq. = 7.81, df. = 2, p = 0.0201). In isolated workers, the DnBP 1X dose significantly increased mortality compared to the control (Fig. 3b; p = 0.0492), whereas none of the tested doses significantly affected mortality within the microcolonies (Fig. 3b; for mortality rates, see Table S10).

Regarding DEHP exposure, mortality was also significantly higher in isolation compared to microcolonies (bayesian GLMM, family binomial: chisq. = 11.6, df. = 1, p < 0.001; for mortality details, see Table S11), without significant interaction (chisq. = 1.6, df. = 2, p = 0.451). In the microcolonies, one dead worker out of the hundred exposed to DEHP 0.1X was recorded, and no mortality was recorded in the other groups (Fig. S2b; for mortality rates, see Table S10).

4. Discussion

Our study is the first to address *in vivo* PAE lethal effects on bees, specifically the social bumblebee *B. terrestris*. We report short-term mortality in workers following environmental DnBP contact exposure. We also investigated exposure to DnBP and DEHP mixtures, highlighting a dose-specific antagonistic effect. Additionally, we show that DnBP exposure modifies resistance to cold stress, notably enhancing survival at low doses. Finally, our results indicate that social conditions buffer DnBP toxicity recorded in isolated workers.

4.1. Single-exposure to DnBP and DEHP

DnBP exposure increased the mortality of *B. terrestris* workers shortly

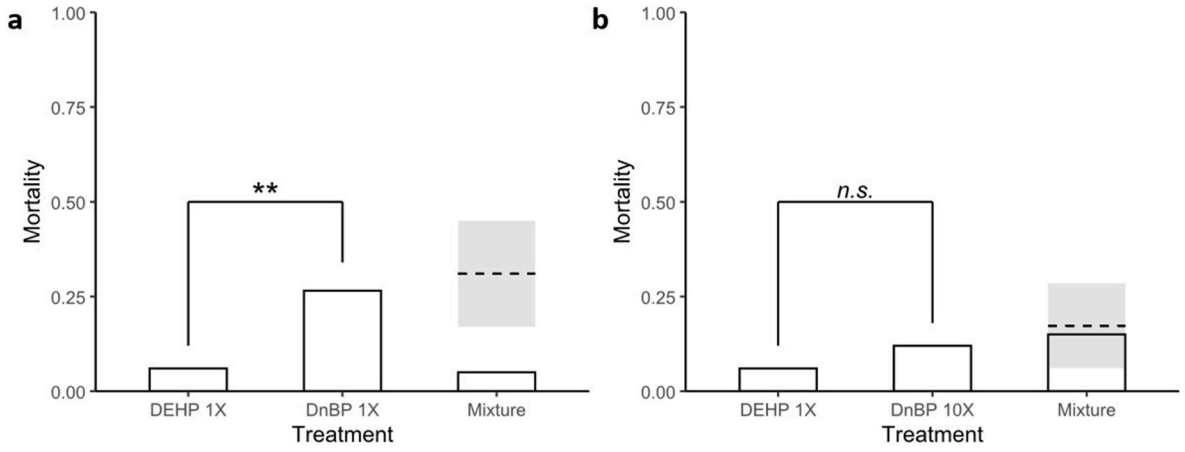

Fig. 2. Effects of mixture exposures to DnBP and DEHP on mortality rates of isolated workers after 72 h. a) Mortality rates after single exposure to DEHP 1X (N = 50), DnBP 1X (N = 49) or their mixture (N = 20). b) Mortality rates after single exposure to DEHP 1X (N = 50), or DnBP 1X (N = 50), or their mixture (N = 20). Columns indicate observed mortality. In mixture columns, the dashed line shows the expected mortality calculated using the Bliss independence criterion. If the mixture column does not overlap with the 95 %-confidence interval of the expected mortality (grey area), the expected and observed mortality differ significantly at p < 0.05 (lower-tailed binomial proportion test). **single-molecule treatments differing at p < 0.01; n.s. no difference between expected and observed mortality rates (Z-test).

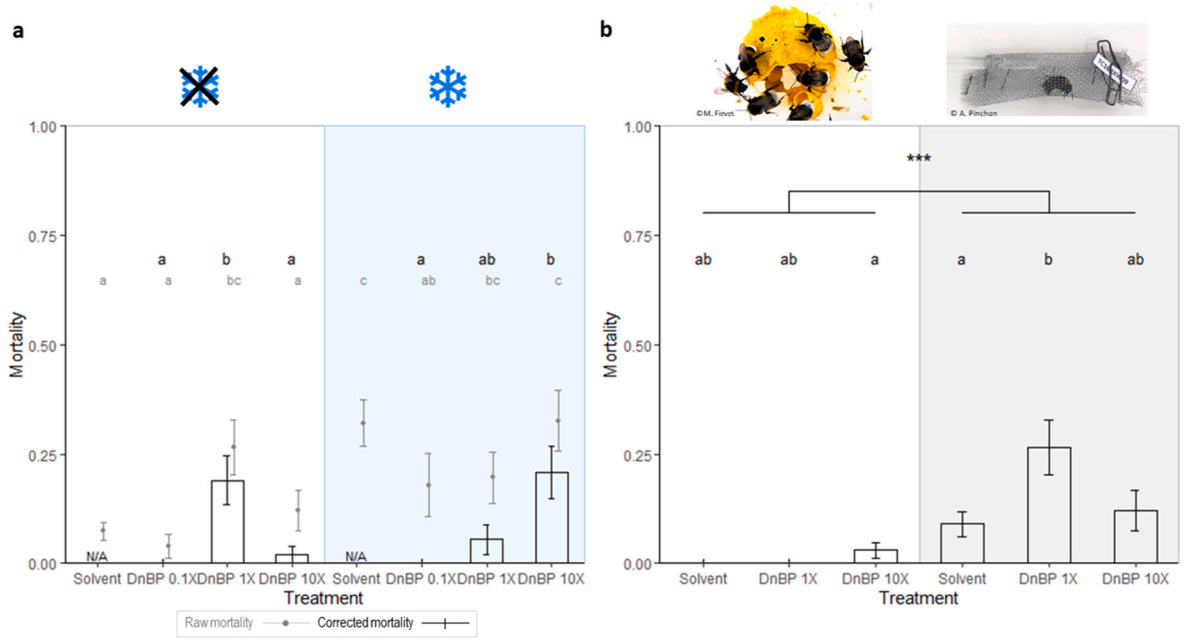

Fig. 3. a) Effects of DnBP 0.1X, 1X (=60 ng/g) and 10X on mortality in non-cold stressed workers (white panel; $N_{control} = 150$, $N_{DnBP\ 0.1X} = 50$, $N_{DnBP\ 1X} = 49$ and $N_{DnBP\ 10X} = 50$) and cold-stressed workers (blue panel; $N_{control} = 78$, $N_{DnBP\ 0.1X} = 28$, $N_{DnBP\ 1X} = 46$ and $N_{DnBP\ 10X} = 46$). Grey points, error bars and letters show results for raw mortality rates. Black bars, error bars and letters show results for corrected mortality. No bars indicate no mortality, and N/A indicates no mortality data available for the control groups after correction. Groups that show different letters are significantly different at $p < 0.05$ (bayesian GLMM, family binomial). b) Effect of DnBP 1X and 10X on mortality in microcolony (white panel; N = 100 per treatment) and in isolated workers (grey panel; $N_{control} = 150$, $N_{DnBP\ 1X} = 49$ and $N_{DnBP\ 10X} = 50$). Bars show mortality values with error bars indicating standard errors. The absence of bars indicates no mortality. Groups that show different letters are significantly different at $p < 0.05$ for the social and treatment interaction effect and *** indicates significant effect of social conditions at $p < 0.001$ (bayesian GLMM, family binomial).

after contact exposure to environmental doses, an effect not observed with DEHP. The few acute toxicity tests performed on invertebrates highlighted that low molecular weight PAE, like DnBP, show higher toxicity, compared to high molecular weight PAEs, like DEHP (Adams et al., 1995; Staples et al., 1997). For instance, Cuvillier-Hot et al. (2014) did not report any mortality in the ant *Lasius niger* queens after exposure to DEHP environmental doses, using a protocol similar to ours (*i.e.*, drop deposition of the molecule diluted in methanol). Similarly, in the aquatic larvae of the insect *Chironomus riparius*, no mortality occurred 24 h post-exposure to DEHP, while the toxicity of butyl benzyl phthalate (BBP), a low molecular weight PAE, increased with dose (Planelló et al., 2011). In addition, PAE chronic exposure studies in other invertebrates align with our findings. For example, DnBP chronic exposure was highly toxic to adults and juveniles of the collembolan *Folsomia fimetaria*, while DEHP exposure did not increase mortality (Jensen et al., 2001). Overall, exposing isolated *B. terrestris* workers to PAEs provided comparable results to other models. The observed absence of DEHP effects and mortality increase caused by DnBP further confirm a toxicity pattern shared across invertebrate taxa and tested life stages, similarly in long- and short-term mortality assessments (e.g., Andreyeva et al., 2023; Green-Ojo et al., 2024; Hobson et al., 1984; Jensen et al., 2001; Li, 2020; Pradhan et al., 2018; Seyoum and Pradhan, 2019).

Interestingly, Arulanandam and colleagues (2022) approached this question *in silico* on the honey bee (*A. mellifera*) using toxicological modelling based on molecular sequences SMILES ("Simplified Molecular Input Line Entry System") and existing toxicity data (Arulanandam et al., 2022). Their results suggested strong toxicity for DnBP and low toxicity for DEHP (Arulanandam et al., 2022), which aligned with our *in vivo* results. Yet, only limited ecotoxicological data on endocrine disruptors (EDs) are available for bees, and there is a need for complementary *in vivo* testing, especially to consider NMDRCs and low-dose effects in PAE risk assessments (Crane et al., 2022). In our study, exposure to four ecologically relevant DnBP doses revealed a bell-shaped toxicity profile, with peak mortality reached at 60 ng/g b.w., and negligible mortality at lower and higher tested doses. NMDRCs associated with DnBP-related mortality have not been reported so far, probably because previous studies investigated PAE toxicity in invertebrates using LD50/LC50 determination (Adams et al., 1995; Staples et al., 1997). These methods are based on linear dose-response relationship and high-to-low dose extrapolation, which overlooks NMDRC and low-dose effects (Futran Fuhrman et al., 2015; Lagarde et al., 2015; Vandenberg, 2014). Most invertebrate risk assessment guidelines, including for bees, still rely on LD50 or LC50s (Adriaanse et al., 2023). In contrast, approaches testing environmental and low doses allow for revealing non-linear profiles, as previously shown for other re-evaluated xenobiotics (e.g. Baines et al., 2017) and here with DnBP. Indeed, we used the OECD guideline n°246 for toxicity testing of chemicals in *B. terrestris* (OECD, 2017a),normally used for LD50 determination, and rather tested four environmental doses, which allowed to highlight the DnBP-associated NMDRC. Therefore, these findings underline the need to include NMDRC frameworks in ecotoxicological risk assessment procedures.

This study demonstrated the lethal effect of phthalate exposure, specifically DnBP, on *B. terrestris* workers, providing the first evidence of phthalate toxic effects on bees. A more comprehensive assessment, however, requires investigation on queens and drones, which differ from workers in their sensitivity to xenobiotics (Gekière et al., 2024; Linguadoca et al., 2022), and could also differ in their response to phthalates. In addition, protocols to test pollutant toxicity in *B. terrestris* larval stages are emerging in bee ecotoxicology (Kato et al., 2022). Such studies are particularly relevant because larvae develop within lipid-rich nest material, which can accumulate lipophilic contaminants like phthalates, as previously observed in honeybee wax combs (Gómez-Ramos et al., 2016). Nevertheless, the suitability of this species as a surrogate for wild bees in ecotoxicological assessments is debated (e.g., Dewaele et al., 2024; Ghisbain, 2021; Linguadoca et al., 2022),

since morphological traits (e.g. hairiness, cuticle thickness, body mass, surface-to-volume ratio) and physiological characteristics (e.g. haemolymph pH, detoxification pathways) vary considerably among wild bees and strongly influence pollutant sensitivity (Balabanidou et al., 2018; Dewaele et al., 2024; Linguadoca et al., 2022).

Nonetheless, our findings provide a first step towards integrating underexplored pollutants into pollinator risk assessment frameworks. They also highlight the importance of extending research to other taxa, particularly emerging solitary bee models such as Osmia spp. (Megachilidae), for which standardised exposure protocols are currently under development (Azpiazu et al., 2023). Finally, current phthalate regulations insufficiently address environmental contamination, focusing mainly on direct human exposure (Billings et al., 2021; Chapon et al., 2023; Gunaalan et al., 2020; Regulation (EU) 2018/2005). By showing that DnBP exposure reduces bee survival, this study contributes to growing evidence of phthalate impacts on terrestrial insects and underlines the urgent need to strengthen regulation of plastic leachate in the environment (Crane et al., 2022), especially in agricultural and urban landscapes where bee populations are already weakened by multiple stressors (Goulson et al., 2015).

4.2. Exposure to DnBP and DEHP in mixture

We exposed workers of *B. terrestris* to mixtures of DnBP and DEHP at environmental doses to go beyond the single-molecule exposure scheme. Indeed, organisms, including bees, are simultaneously exposed in their environment to a combination of pollutants, e.g. heavy metals, pesticides, or plastic leachates (Gómez-Ramos et al., 2016), and thus to ED mixtures (Cuvillier-Hot and Lenoir, 2020). Surprisingly, we found antagonistic effects between DEHP 120 ng/g of bee and DnBP 60 ng/g of bee, *i.e.*, the most toxic dose when tested in single molecule solution. Similarly, in a previous study, single-molecule exposure to cadmium or DEHP caused around 15 % mortality in *Spodoptera littoralis* adults that had developed on contaminated food, while mortality dropped to less than 5 % in the mixture treatment (Humann-Guilleminot et al., 2024). These congruent results suggest that DEHP acts on detoxification pathways. Cadmium is another endocrine disruptor in several species groups (Akhavan et al., 2016), that has been shown to affect ecdysteroid signalling and stress response in insects (Planelló et al., 2010). Recent LD50s calculated for cadmium in *B. terrestris* workers and males were lower than the field concentrations (Gekière et al., 2024), revealing potential toxicity of this compound in bees. Similarly to results found in *S. littoralis*, mixture with DEHP could modify its toxicity in bees. Moreover, in rats, DEHP was shown to act on xenobiotic detoxification processes, as its mix with parathion lowered the latter's deleterious effects by disrupting the pesticide breakdown into toxic metabolites (Srivastava et al., 1976). In this study, DEHP could have similarly lowered DnBP toxicity, as PAE metabolites are often more toxic at lower doses and more potent than their parent compounds (Erkekoglu et al., 2010; Saillenfait et al., 2001). Similar results to ours were found when testing a mixture of polystyrene microparticles (MPs) and DnBP on the copepod *Tigriopus japonicus* mortality (Li et al., 2020). DnBP toxicity was lowered when mixed with MPs, and the authors hypothesised that the antagonism was caused by a lower bioavailability of the DnBP molecules adsorbed onto MPs (Li et al., 2020). Alternatively, polystyrene may have released DEHP, a common polystyrene additive (Coffin et al., 2019), which may have lowered DnBP toxicity by acting on detoxification pathways and associated enzymes. For example, exposure to DEHP has been shown to upregulate detoxification enzymes of the CYP450 family in the liver tissues of quails and rats, and in *Caenorhabditis elegans* (Eveillard et al., 2009; Yen et al., 2024; Zhang et al., 2018). Since these enzymes are involved in phthalate detoxification (Zheng et al., 2024), upregulation of the corresponding genes could enhance DnBP detoxification efficiency, thereby reducing its toxicity. In addition, this would be consistent with evidence that DnBP metabolites could be less harmful than the parent compound (reviewed in Sicińska, 2018). Bees are simultaneously exposed to diverse chemical pollutants (Gómez-Ramos et al., 2016). For example, in agricultural environments where pesticides are used, sewage sludge application directly contributes to environmental contamination with phthalates and heavy metals, such as cadmium (Billings et al., 2021; Knoll and Cappai, 2024). Our results underline the need to investigate more deeply the effects of DEHP on detoxification-related enzymatic activity and gene expression, and to integrate mixture exposure into ecological risk assessment. Mixture of phthalates with other endocrine disrupting compounds such as cadmium (Planelló et al., 2010), or neonicotinoids (Baines et al., 2017), already known to co-occur and interact in humans (Guo et al., 2025; Lecomte et al., 2017), should especially be investigated.

4.3. Interaction between cold stress and DnBP or DEHP exposure

In this study, we demonstrated that DnBP, but not DEHP, exposure interacted with the effects of cold stress. Bumblebees are well-adapted to cold and temperate climates (Heinrich, 2004; Rasmont et al., 2021). They can exit cold torpor states using non-flight thermogenesis, which combines muscle shivering and metabolic mechanisms (Esch et al., 1991; Surholt et al., 1991). In the context of climate change, studies considering thermal stresses in addition to pollutant toxicity evaluation are emerging, but as of now, mainly focus on heat stress and pesticides (Albacete et al., 2023; Nebauer et al., 2024). Yet, acute cold stress is known to negatively affect workers (Owen et al., 2013; Poissonnier et al., 2015; Potts et al., 2018), as shown here as the cold stress increased mortality, in most treatments, including in control groups. Moreover, bee resistance mechanisms to cold can be disrupted by pollutants (Potts et al., 2018; Verheyen et al., 2022). We highlighted that low doses of DnBP counteracted the cold stress effect as worker survival was enhanced. Similarly, exposure to low doses of DnBP was found to increase survival in starved *D. melanogaster* flies. This rescueing effect was associated with a decrease in the expression of insulin-like protein related genes (Williams et al., 2016). DnBP at low doses could enhance cold stress resistance by acting on similar endocrine and metabolic targets in *B. terrestris*. This highly conserved insulin-like signalling pathway regulates the availability of energetic resources by directly acting on glucose and lipid metabolism. It also notably modulates the juvenile hormone biosynthesis in insects (Tatar et al., 2001), a metabolic rate accelerator in *B. terrestris* which regulates the switch between energy storage and expenditure (Shpigler et al., 2021). By modifying energetic resources availability through these metabolic pathways, DnBP could therefore enhance thermogenesis ability at low dose and benefit workers by increasing cold stress resistance.

However, when neutralising the mortality solely induced by cold stress, we observed a shift of the toxicity peak from the intermediate to the highest DnBP dose. Testing higher DnBP doses combined with cold stress could provide valuable information on temperature interactions with chemical stress in bees. Stress interaction effects can depend on chemical and temperature stress types, as well as species-specific physiology (Verheyen et al., 2022). The DnBP toxicity curve under cold stress could therefore either become dose-dependent or maintained as a higher dose bell-shaped profile.

The toxicity profile observed here, compared to the results without cold stress, might highlight a hormesis effect of DnBP on thermogenesis. The hormesis phenomenon is characterised by beneficial effects at low doses that become adverse at higher doses (Cutler et al., 2022). A similar effect was previously observed after exposure of *B. terrestris* workers to the neonicotinoid imidacloprid (Potts et al., 2018). Indeed, while low imidacloprid doses enhanced cold stress survival in *B. terrestris*, higher doses increased mortality after cold stress (Potts et al., 2018). The authors hypothesised that the neonicotinoid neurotoxic effects enhanced shivering at low doses and became deleterious with increasing doses (Potts et al., 2018). Yet, Erban and colleagues (2019) highlighted a disruption of *B. terrestris* lipid metabolism by imidacloprid, which could also explain the observed hormesis. Here, we observed a similar effect of

DnBP on cold resistance, but no increased shivering has yet been reported linked to PAE neurotoxic effects (e.g. Chen et al., 2018; Ran et al., 2012; Wu et al., 2024). Therefore, the hormesis effect found here might rather be caused by a metabolism disruption resulting from DnBP exposure, already observed in other invertebrates species (Jordão et al., 2016; Liu et al., 2021; Prieto-Amador et al., 2021; Seyoum and Pradhan, 2019; Williams et al., 2016). In the context of cold resistance, the same metabolic pathways activated by DnBP at low doses helping with thermogenesis, could be overstimulated at high doses and become deleterious. Yet, DnBP effects on insect metabolism have not been thoroughly investigated. Future research should now aim to confirm the molecular pathways disrupted by DnBP in bees, notably by investigating potential metabolic rate or metabolic-related gene expression modification in workers exposed to DnBP. While phthalates are known to disrupt metabolism in other insect models, such as *S. littoralis* (Rivas et al., 2023) and D. *melanogaster* (Williams et al., 2016), no data are yet available explaining the molecular mode of action of DnBP in bees. Moreover, DnBP disruption of molecular mechanisms specifically related to cold stress resistance in workers should be further investigated, through for example metabolic rate measurements (Bretzlaff et al., 2023) or analyses of the expression of heatshock protein, metabolism and insulin-related genes, crucial for thermal stress resistance in workers (Kim et al., 2024). Finally, during their hibernation, Bumblebee queens could be exposed to phthalate though soil contamination (Net et al., 2015b). Therefore, the impact of long-term DnBP exposure on hibernating queens should be tested, as cold resistance is a crucial characteristic of this caste (Shi et al., 2023).

4.4. Influence of sociality on the impact of DnBP and DEHP exposure

Worker mortality in the microcolonies was lower than the mortality observed in isolated workers, which highlights potential mitigation effects of social conditions. *B. terrestris* is a eusocial species (Rasmont et al., 2008). Workers benefit from social mechanisms, such as social thermoregulation, hygienic and allogrooming behaviour, as well as social modulation of metabolic and immune pathways. These are well-studied in the context of social immunity against pathogen and parasite infections (Cremer et al., 2007; Gaubert et al., 2023). However, social mitigation of xenobiotic exposure and sensitivity is less known.

First, microcolonies thermoregulate to maintain brood temperature at higher temperature (28–32 °C; Livesey et al., 2019). In contrast, isolated worker body temperature equals surrounding temperatures at rest (20–25 °C in the present study; Sepúlveda-Rodríguez et al., 2024). In insects, lower body temperatures can thus lower chemical detoxification and excretion rates (Verheyen et al., 2022), and could similarly strengthen DnBP effects.

Moreover, social grooming effects on xenobiotic exposure and sensitivity have not yet been investigated, but the removal of parasites by intensified allogrooming is an efficient mechanism against *Varroa* mite infections in *Apis* species (Mondet et al., 2020). This behaviour could modify cuticle contaminant quantities and lower their impact on workers, as we observed. Though *B. terrestris* perform less allogrooming behaviour than honeybee, self-grooming and brood rubbing are part of the behavioural array performed by workers in healthy colonies (Dronnet et al., 2005). Investigating the effects of grooming on cuticle chemical contamination could help understand contamination dynamics in social systems.

The bee microbiota could also play a crucial role in xenobiotic detoxification. It was shown in *A. mellifera* that gut microbiota depletion increases sensitivity to neonicotinoid insecticides (Wu et al., 2020). In social bees, the microbiota is maintained by grooming and use of shared honeypots (Motta and Moran, 2024). A quick drop in relative abundance of several bacterial groups, of which *Lactobacillus*, was highlighted after isolation of adult *B. terrestris* workers from their colony, with a timescale comparable to ours (72 h; Billiet et al., 2017). Interestingly, *Lactobacillus* were also shown to mitigate DEHP and DnBP reprotoxic effects in rodents (Chen et al., 2022; Shi et al., 2020; Tian et al., 2019). The increased vulnerability of isolated workers to DnBP observed here could result from a modification of microbiota composition following social isolation, but this hypothesis would benefit from further investigation.

Finally, when kept in queenless microcolonies, workers undergo deep endocrine changes. In the first days after microcolony installation, reproductive competition, with aggressive behaviour and ovary development, occurs among workers (van Doorn, 1989). In this context, multiple hormonal and metabolic pathways are activated and represent as many new targets for potential ED-induced sub-lethal effects. Notably, in *B. terrestris*, variation in ecdysteroid titres in the haemolymph and vitellogenin gene expression were found to influence aggressivity, dominance and oviposition behaviour at queen-less microcolony establishment (Amsalem et al., 2014; Bloch et al., 2000). Therefore, PAE effects on microcolony and isolated workers might differ. While mortality was buffered in the microcolony setup, DnBP could differentially affect individuals depending on reproductive and metabolic status, especially as DnBP and DEHP were shown to affect the ecdysteroid pathway in *S. littoralis* (Avilès et al., 2020) and vitellogenin gene expression in *L. niger* and *Daphnia magna* (Cuvillier-Hot et al., 2014; Seyoum and Pradhan, 2019). Investigating the effects of PAEs on the fitness and health of *B. terrestris* in microcolonies would provide a better understanding of their effects on hormonal pathways and the consequences of such disruptions.

4.5. Perspectives

The current bee population decline is the result of multiple anthropogenic factors probably acting in synergy. Characterising the effects of PAE alone and in combination with other stressors on bee fitness seems important as these pollutants are ubiquitous and will continue to contaminate environments for long (Chapon et al., 2023). In this context, *B. terrestris* would be a relevant and useful model for PAE toxicity evaluation. Commercial colonies readily available give access to all life stages, castes and sexes (e.g., Gekière et al., 2024; Kato et al., 2022; Linguadoca et al., 2022), and would allow a comprehensive evaluation of PAE effects on several levels of biological complexity, from molecular pathway to social behaviour disruption. Given the ED characteristics of PAEs (e.g. NMDRCs and low-dose sub-lethal effects), research must focus on evaluating the risk PAEs pose to both bee larval development and adult health at environmental doses. Finally, while laboratory experiments overly simplify exposure schemes, they allow control over non-investigated external stressors (e.g., floral resources shortage, bad weather, pollutant combined exposure, etc.), which can be detrimental to bee populations in field studies (Straw and Stanley, 2025). Yet, further field study would also provide complementary insights on phthalate risk.

Aligning with the current shift towards a multiple stressor paradigm in bee ecotoxicology, this study provided new evidence of PAE toxic effects on bees. Adding to the existing data on bee phthalate exposure, it should motivate further multiscale investigation from the molecular assay to field and semi-field experiments that would allow a better understanding of the impact of phthalates on wild populations of bees.

CRediT authorship contribution statement

Justine Dewaele: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Marion Javal:** Writing – review & editing, Investigation. **Audrey Pinchon:** Investigation. **Dimitri Evrard:** Investigation. **Nina Hautekèete:** Writing – review & editing, Supervision. **Denis Michez:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Virginie Cuvillier:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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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. Supplementary data

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Data availability

Data are available at https://doi.org/10.5281/zenodo.15405116

References

Adams, W.J., Biddinger, G.R., Robillard, K.A., Gorsuch, J.W., 1995. A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environ. Toxicol. Chem. 14, 1569–1574. https://doi.org/10.1002/etc.5620140916.

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.

Akhavan, O., Hashemi, E., Zare, H., Shamsara, M., Taghavinia, N., Heidari, F., 2016. Influence of heavy nanocrystals on spermatozoa and fertility of mammals. Mater. Sci. Eng. C 69, 52–59. https://doi.org/10.1016/j.msec.2016.06.055.

Albacete, S., Sancho, G., Azpiazu, C., Rodrigo, A., Molowny-Horas, R., Sgolastra, F., Bosch, J., 2023. Bees exposed to climate change are more sensitive to pesticides. Glob. Change Biol. 29, 6248–6260. https://doi.org/10.1111/gcb.16928.

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.

Andreyeva, A.Yu, Lobko, V.V., Gostyukhina, O.L., Tkachuk, A.A., Murashova, A.I., Malakhova, L.V., Kladchenko, E.S., 2023. Accumulation, functional and antioxidant responses to acute exposure to Di(2-ethylhexyl)phthalate (DEHP) in Mytilus galloprovincialis. Mar. Pollut. Bull. 191, 114923. https://doi.org/10.1016/j.marpolbul.2023.114923.

Arena, M., Sgolastra, F., 2014. A meta-analysis comparing the sensitivity of bees to pesticides. Ecotoxicology 23, 324–334. https://doi.org/10.1007/s10646-014-1190-1.

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.

Avilès, A., Cordeiro, A., Maria, A., Bozzolan, F., Boulogne, I., Dacher, M., Goutte, A., Alliot, F., Maibeche, M., Massot, M., Siaussat, D., 2020. Effects of DEHP on the ecdysteroid pathway, sexual behavior and offspring of the moth Spodoptera littoralis. Horm. Behav. 125, 104808. https://doi.org/10.1016/j.yhbeh.2020.104808.

Azpiazu, C., Bosch, J., Bortolotti, L., Medrzycki, P., Teper, D., Molowny-Horas, R., Sgolastra, F., 2021. Toxicity of the insecticide sulfoxaflor alone and in combination with the fungicide fluxapyroxad in three bee species. Sci. Rep. 11, 6821. https://doi.org/10.1038/s41598-021-86036-1.

Azpiazu, C., Hinarejos, S., Sancho, G., Albacete, S., Sgolastra, F., Martins, C.A.H., Domene, X., Benrezkallah, J., Rodrigo, A., Arnan, X., Bosch, J., 2023. Description and validation of an improved method to feed solitary bees (Osmia spp.) known amounts of pesticides. Ecotoxicol. Environ. Saf. 264, 115398. https://doi.org/10.1016/j.ecoenv.2023.115398.

Baines, D., Wilton, E., Pawluk, A., De Gorter, M., Chomistek, N., 2017. Neonicotinoids act like endocrine disrupting chemicals in newly-emerged bees and winter bees. Sci. Rep. 7, 10979. https://doi.org/10.1038/s41598-017-10489-6.

Balabanidou, V., Grigoraki, L., Vontas, J., 2018. Insect cuticle: a critical determinant of insecticide resistance. Current Opinion in Insect Science 27, 68–74. https://doi.org/10.1016/j.cois.2018.03.001.

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.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67. https://doi.org/10.18637/jss.v067.i01.

Benuszak, J., Laurent, M., Chauzat, M.-P., 2017. The exposure of honey bees (Apis mellifera ; Hymenoptera: apidae) to pesticides: room for improvement in research. Sci. Total Environ. 587–588, 423–438. https://doi.org/10.1016/j.scitotenv.2017.02.062.

Bergé, A., Cladière, M., Gasperi, J., Coursimault, A., Tassin, B., Moilleron, R., 2013. Meta-analysis of environmental contamination by phthalates. Environ. Sci. Pollut. Res. 20, 8057–8076. https://doi.org/10.1007/s11356-013-1982-5.

Billiet, A., Meeus, I., Van Nieuwerburgh, F., Deforce, D., Wäckers, F., Smagghe, G., 2017. Colony contact contributes to the diversity of gut bacteria in bumblebees (*Bombus terrestris*). Insect Sci. 24, 270–277. https://doi.org/10.1111/1744-7917.12284.

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.

Bretzlaff, T., Kerr, J.T., Darveau, C.-A., 2023. High temperature sensitivity of bumblebee castes and the colony-level costs of thermoregulation in Bombus impatiens. J. Therm. Biol. 117, 103710. https://doi.org/10.1016/j.jtherbio.2023.103710.

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

Camp, A.A., Lehmann, D.M., 2020. Impacts of neonicotinoids on the Bumble bees *Bombus terrestris* and *Bombus impatiens* examined through the lens of an adverse outcome pathway framework. Environ. Toxicol. Chem. 40, 309–322. https://doi.org/10.1002/etc.4939.

CEFIC, 2021. Plasticisers Fact Sheet.

Chapon, V., Brignon, J.-M., Gasperi, J., 2023. Non-persistent chemicals in polymer and non-polymer products can cause persistent environmental contamination: evidence with DEHP in Europe. Environ. Sci. Pollut. Res. 30, 44952–44962. https://doi.org/10.1007/s11356-023-25405-x.

Chen, M.-Y., Liu, H.-P., Liu, C.-H., Cheng, J., Chang, M.-S., Chiang, S.-Y., Liao, W.-P., Lin, W.-Y., 2018. DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environ. Pollut. 243, 1558–1567. https://doi.org/10.1016/j.envpol.2018.09.063.

Chen, Q., Kong, Q., Tian, P., He, Y., Zhao, J., Zhang, H., Wang, G., Chen, W., 2022. Lactic acid bacteria alleviate di-(2-ethylhexyl) phthalate-induced liver and testis toxicity via their bio-binding capacity, antioxidant capacity and regulation of the gut microbiota. Environ. Pollut. 305, 119197. https://doi.org/10.1016/j.envpol.2022.119197.

Coffin, S., Lee, I., Gan, J., Schlenk, D., 2019. Simulated digestion of polystyrene foam enhances desorption of diethylhexyl phthalate (DEHP) and in vitro estrogenic activity in a size-dependent manner. Environ. Pollut. 246, 452–462. https://doi.org/10.1016/j.envpol.2018.12.011.

Crane, M., Dungey, S., Lillicrap, A., Thompson, H., Weltje, L., Wheeler, J.R., Lagadic, L., 2022. Commentary: assessing the endocrine disrupting effects of chemicals on invertebrates in the European Union. Environ. Sci. Eur. 34, 36. https://doi.org/10.1186/s12302-022-00613-3.

Cremer, S., Armitage, S.A.O., Schmid-Hempel, P., 2007. Social immunity. Curr. Biol. 17, R693–R702. https://doi.org/10.1016/j.cub.2007.06.008.

Cutler, G.C., Amichot, M., Benelli, G., Guedes, R.N.C., Qu, Y., Rix, R.R., Ullah, F., Desneux, N., 2022. Hormesis and insects: effects and interactions in agroecosystems. Sci. Total Environ. 825, 153899. https://doi.org/10.1016/j.scitotenv.2022.153899.

Cuvillier-Hot, V., Lenoir, A., 2020. Invertebrates facing environmental contamination by endocrine disruptors: novel evidences and recent insights. Mol. Cell. Endocrinol. 504, 110712. https://doi.org/10.1016/j.mce.2020.110712.

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.

Dewaele, J., Barraud, A., Hellström, S., Paxton, R.J., Michez, D., 2024. A new exposure protocol adapted for wild bees reveals species-specific impacts of the sulfoximine insecticide sulfoxaflor. Ecotoxicology 33, 546–559. https://doi.org/10.1007/s10646-024-02750-2.

Dorie, V., Bates, Douglas, Maechler, Martin, Bolker, Ben, Walker, Steven, 2022. Package 'Blme' : Bayesian Linear Mixed-Effects Models.

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.

Erban, T., Sopko, B., Talacko, P., Harant, K., Kadlikova, K., Halesova, T., Riddellova, K., Pekas, A., 2019. Chronic exposure of bumblebees to neonicotinoid imidacloprid suppresses the entire mevalonate pathway and fatty acid synthesis. J. Proteonomics 196, 69–80. https://doi.org/10.1016/j.jprot.2018.12.022.

Erkekoglu, P., Rachidi, W., Yuzugullu, O.G., Giray, B., Favier, A., Ozturk, M., Hincal, F., 2010. Evaluation of cytotoxicity and oxidative DNA damaging effects of di(2-ethylhexyl)-phthalate (DEHP) and mono(2-ethylhexyl)-phthalate (MEHP) on MA-10 Leydig cells and protection by selenium. Toxicol. Appl. Pharmacol. 248, 52–62. https://doi.org/10.1016/j.taap.2010.07.016.

Esch, H., Goller, F., Heinrich, B., 1991. How do bees shiver? Sci. Nat. 78, 325–328. https://doi.org/10.1007/BF01221422.

Eveillard, A., Lasserre, F., De Tayrac, M., Polizzi, A., Claus, S., Canlet, C., Mselli-Lakhal, L., Gotardi, G., Paris, A., Guillou, H., 2009. Identification of potential mechanisms of toxicity after di-(2-ethylhexyl)-phthalate (DEHP) adult exposure in the liver using a systems biology approach. Toxicol. Appl. Pharmacol. 236, 282–292. https://doi.org/10.1016/j.taap.2009.02.008.

Feng, Y.-X., Feng, N.-X., Zeng, L.-J., Chen, X., Xiang, L., Li, Y.-W., Cai, Q.-Y., Mo, C.-H., 2020. Occurrence and human health risks of phthalates in indoor air of laboratories. Sci. Total Environ. 707, 135609. https://doi.org/10.1016/j.scitotenv.2019.135609.

Fierens, T., Servaes, K., Van Holderbeke, M., Geerts, L., De Henauw, S., Sioen, I., Vanermen, G., 2012. Analysis of phthalates in food products and packaging materials sold on the Belgian market. Food Chem. Toxicol. 50, 2575–2583. https://doi.org/10.1016/j.fct.2012.04.029.

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.

Gaubert, J., Giovenazzo, P., Derome, N., 2023. Individual and social defenses in Apis mellifera: a playground to fight against synergistic stressor interactions. Front. Physiol. 14, 1172859. https://doi.org/10.3389/fphys.2023.1172859.

Gekière, A., Breuer, L., Dorio, L., Vanderplanck, M., Michez, D., 2024. Lethal effects and sex-specific tolerance of copper and cadmium in the buff-tailed Bumble bee. Environ. Toxicol. Pharmacol. 110, 104546. https://doi.org/10.1016/j.etap.2024.104546.

Ghisbain, G., 2021. Are bumblebees relevant models for understanding wild bee decline? Front. Conserv. Sci. 2, 752213. https://doi.org/10.3389/fcosc.2021.752213.

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.

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., 2019. The insect apocalypse, and why it matters. Curr. Biol. 29, R967–R971. https://doi.org/10.1016/j.cub.2019.06.069.

Goulson, D., Nicholls, E., Botias, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347, 1255957. https://doi.org/10.1126/science.1255957.

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.

Gunaalan, K., Fabbri, E., Capolupo, M., 2020. The hidden threat of plastic leachates: a critical review on their impacts on aquatic organisms. Water Res. 184, 116170. https://doi.org/10.1016/j.watres.2020.116170.

Guo, L.-C., Zhu, P., Gui, C., Deng, J., Gao, Y., Long, C., Zhang, H., Lv, Z., Yu, S., 2025. Disrupting effects of neonicotinoids and their interaction with metals on thyroid hormone, an evidence of children in a rural area, South China. Ecotoxicol. Environ. Saf. 290, 117788. https://doi.org/10.1016/j.ecoenv.2025.117788.

Hartig, F., 2020. Package 'Dharma.

Heinrich, B., 2004. Bumblebee Economics, New ed. Harvard University Press, Cambridge, Mass. London.

Heinze, G., Schemper, M., 2002. A solution to the problem of separation in logistic regression. Stat. Med. 21, 2409–2419. https://doi.org/10.1002/sim.1047.

Hobson, J.F., Carter, D.E., Lightner, D.V., 1984. Toxicity of a phthalate ester in the diet of a penaied shrimp. J. Toxicol. Environ. Health 13, 959–968. https://doi.org/10.1080/15287398409530553.

Humann-Guilleminot, S., Fuentes, A., Maria, A., Couzi, P., Siaussat, D., 2024. Cadmium and phthalate impacts developmental growth and mortality of Spodoptera littoralis, but not reproductive success. Ecotoxicol. Environ. Saf. 281, 116605. https://doi.org/10.1016/j.ecoenv.2024.116605.

Jarošová, A., 2006. Phthalic acid esters (PAEs) in the food chain. Czech J. Food Sci. 24, 223–231. https://doi.org/10.17221/3318-CJFS.

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*. Environ. Toxicol. Chem. 20, 1085–1091. https://doi.org/10.1002/etc.5620200520.

Jordão, R., Garreta, E., Campos, B., Lemos, M.F.L., Soares, A.M.V.M., Tauler, R., Barata, C., 2016. Compounds altering fat storage in Daphnia magna. Sci. Total Environ. 545–546, 127–136. https://doi.org/10.1016/j.scitotenv.2015.12.097.

Kato, Y., Kikuta, S., Barribeau, S.M., Inoue, M.N., 2022. In vitro larval rearing method of eusocial bumblebee Bombus terrestris for toxicity test. Sci. Rep. 12, 15783. https://doi.org/10.1038/s41598-022-19965-0.

Kim, Y.H., Kim, B.Y., Kim, H.S., Kim, J.M., Qiu, W., Yoon, H.J., Lee, K.S., Jin, B.R., 2024. Elevated developmental temperature affects gene expression and oxidative stress in bumblebee (Bombus terrestris) workers. J. Asia Pac. Entomol. 27, 102311. https://doi.org/10.1016/j.aspen.2024.102311.

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.

Knoll, S., Cappai, M.G., 2024. Foraging Activity of honey bees (Apis mellifera L., 1758) and exposure to cadmium: a review. Biol. Trace Elem. Res. 202, 5733–5742. https://doi.org/10.1007/s12011-024-04118-3.

Koniecki, D., Wang, R., Moody, R.P., Zhu, J., 2011. Phthalates in cosmetic and personal care products: concentrations and possible dermal exposure. Environ. Res. 111, 329–336. https://doi.org/10.1016/j.envres.2011.01.013.

Lagarde, F., Beausoleil, C., Belcher, S.M., Belzunces, L.P., Emond, C., Guerbet, M., Rousselle, C., 2015. Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment. Environ. Health 14, 13. https://doi.org/10.1186/1476-069X-14-13.

LeBuhn, G., Vargas Luna, J., 2021. Pollinator decline: what do we know about the drivers of solitary bee declines? Current Opinion in Insect Science 46, 106–111. https://doi.org/10.1016/j.cois.2021.05.004.

Lecomte, S., Habauzit, D., Charlier, T., Pakdel, F., 2017. Emerging estrogenic pollutants in the aquatic environment and breast cancer. Genes 8, 229. https://doi.org/10.3390/genes8090229.

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.

Lenth, R.V., 2023. Package 'Emmeans' : Estimated Marginal Means, Aka Least-Squares Means.

Li, M.-H., 2020. Enzymatic biomarker responses of freshwater planarians exposed to endocrine disrupting compounds. Fresenius Environ. Bull. 29.

Li, Z., Zhou, H., Liu, Y., Zhan, J., Li, W., Yang, K., Yi, X., 2020. Acute and chronic combined effect of polystyrene microplastics and dibutyl phthalate on the marine copepod Tigriopus japonicus. Chemosphere 261, 127711. https://doi.org/10.1016/j.chemosphere.2020.127711.

Linguadoca, A., Jürison, M., Hellström, S., Straw, E.A., Šima, P., Karise, R., Costa, C., Serra, G., Colombo, R., Paxton, R.J., Mänd, M., Brown, M.J.F., 2022. Intra-specific variation in sensitivity of *Bombus terrestris* and *Osmia bicornis* to three pesticides. Sci. Rep. 12, 17311. https://doi.org/10.1038/s41598-022-22239-4.

Liu, W., Cao, H., Liao, S., Kudłak, B., Williams, M.J., Schiöth, H.B., 2021. Dibutyl phthalate disrupts conserved circadian rhythm in Drosophila and human cells. Sci. Total Environ. 783, 147038. https://doi.org/10.1016/j.scitotenv.2021.147038.

Liu, L.-L., Yue, J.-Z., Lu, Z.-Y., Deng, R.-Y., Li, C.-C., Yu, Y.-N., Zhou, W.-J., Lin, M., Gao, H.-T., Liu, J., Xia, L.-Z., 2024. Long-term exposure to the mixture of phthalates induced male reproductive toxicity in rats and the alleviative effects of quercetin. Toxicol. Appl. Pharmacol. 483, 116816. https://doi.org/10.1016/j.taap.2024.116816.

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.

Luo, Q., Liu, Z., Yin, H., Dang, Z., Wu, P., Zhu, N., Lin, Z., Liu, Y., 2018. Migration and potential risk of trace phthalates in bottled water: a global situation. Water Res. 147, 362–372. https://doi.org/10.1016/j.watres.2018.10.002.

Maebe, K., Hart, A.F., Marshall, L., Vandamme, P., Vereecken, N.J., Michez, D., Smagghe, G., 2021. Bumblebee resilience to climate change, through plastic and adaptive responses. Glob. Change Biol. 27, 4223–4237. https://doi.org/10.1111/gcb.15751.

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.

Michołap, P., Sikora, A., Sikora, M., Zając, K., Kadej, M., 2024. Long-term changes in composition and distribution of bumblebees in urban areas. J. Apicult. Sci. 68, 167–182. https://doi.org/10.2478/jas-2024-0009.

Mondet, F., Beaurepaire, A., McAfee, A., Locke, B., Alaux, C., Blanchard, S., Danka, B., Le Conte, Y., 2020. Honey bee survival mechanisms against the parasite Varroa destructor: a systematic review of phenotypic and genomic research efforts. Int. J. Parasitol. 50, 433–447. https://doi.org/10.1016/j.ijpara.2020.03.005.

Moniuszko, H., Przybysz, A., Borański, M., Splitt, A., Jachuła, J., Popek, R., 2025. Buff-tailed bumblebee, an underrated indicator of air pollution: a comparison of particulate matter accumulation by *Bombus terrestris* L. and *Apis mellifera* L. Environ. Toxicol. Chem. 44, 282–293. https://doi.org/10.1093/etojnl/vgae020.

Motta, E.V.S., Moran, N.A., 2024. The honeybee microbiota and its impact on health and disease. Nat. Rev. Microbiol. 22, 122–137. https://doi.org/10.1038/s41579-023-00990-3.

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.

Net, S., Delmont, A., Sempéré, R., Paluselli, A., Ouddane, B., 2015a. Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): a review. Sci. Total Environ. 515–516, 162–180. https://doi.org/10.1016/j.scitotenv.2015.02.013.

Net, S., Rabodonirina, S., Sghaier, R.B., Dumoulin, D., Chbib, C., Tlili, I., Ouddane, B., 2015b. Distribution of phthalates, pesticides and drug residues in the dissolved, particulate and sedimentary phases from transboundary rivers (France–Belgium).

Sci. Total Environ. 521–522, 152–159. https://doi.org/10.1016/j.scitotenv.2015.03.087.
Net, S., Sempéré, R., Delmont, A., Paluselli, A., Ouddane, B., 2015c. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environ. Sci. Technol. 49, 4019–4035. https://doi.org/10.1021/es505233b.
Nieto, A., Roberts, S.P.M., Kemp, J., Rasmont, P., Kuhlmann, M., Criado, M.G., Biesmeijer, J.C., Bogush, P., Dathe, H.H., De la Rúa, P., De Meulemeester, T., Dehon, M., Dewulf, A., Ortiz-Sánchez, F.J., Lhomme, P., Pauly, A., Potts, S.G., Praz, C., Quaranta, M., Radchenko, V.G., Scheuchl, E., Smit, J., Straka, J., Terzo, M., Tomozii, B., Window, J., Michez, D., 2014. European Red List of Bees. Publications Office, Luxembourg.
OECD, 2017a. Test No. 246: Bumblebee, Acute Contact Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2. OECD. https://doi.org/10.1787/9789264284104-en.
OECD, 2017b. Test No. 247: Bumblebee, Acute Oral Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2. OECD. https://doi.org/10.1787/9789264284128-en.
OECD, 2018. In: Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Series on Testing and Assessment. OECD Publishing, Paris. https://doi.org/10.1787/9789264304741-en.
Owen, E.L., Bale, J.S., Hayward, S.A.L., 2013. Can winter-active bumblebees survive the cold? Assessing the cold tolerance of Bombus terrestris audax and the effects of pollen feeding. PLoS One 8, e80061. https://doi.org/10.1371/journal.pone.0080061.
Persson, A.S., Ekroos, J., Olsson, P., Smith, H.G., 2020. Wild bees and hoverflies respond differently to urbanisation, human population density and urban form. Landsc. Urban Plann. 204, 103901. https://doi.org/10.1016/j.landurbplan.2020.103901.
Planelló, R., Martínez-Guitarte, J.L., Morcillo, G., 2010. Effect of acute exposure to cadmium on the expression of heat-shock and hormone-nuclear receptor genes in the aquatic midge Chironomus riparius. Sci. Total Environ. 408, 1598–1603. https://doi.org/10.1016/j.scitotenv.2010.01.004.
Planelló, R., Herrero, O., Martínez-Guitarte, J.L., Morcillo, G., 2011. Comparative effects of butyl benzyl phthalate (BBP) and di(2-ethylhexyl) phthalate (DEHP) on the aquatic larvae of Chironomus riparius based on gene expression assays related to the endocrine system, the stress response and ribosomes. Aquat. Toxicol. 105, 62–70. https://doi.org/10.1016/j.aquatox.2011.05.011.
Poissonnier, L.-A., Jackson, A.L., Tanner, C.J., 2015. Cold and CO2 narcosis have long-lasting and dissimilar effects on Bombus terrestris. Insectes Soc. 62, 291–298. https://doi.org/10.1007/s00040-015-0404-8.
Potts, R., Clarke, R.M., Oldfield, S.E., Wood, L.K., Hempel De Ibarra, N., Cresswell, J.E., 2018. The effect of dietary neonicotinoid pesticides on non-flight thermogenesis in worker Bumble bees (Bombus terrestris). J. Insect Physiol. 104, 33–39. https://doi.org/10.1016/j.jinsphys.2017.11.006.
Pradhan, A., Olsson, P.-E., Jass, J., 2018. Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190, 375–382. https://doi.org/10.1016/j.chemosphere.2017.09.123.
Prieto-Amador, M., Caballero, P., Martínez-Guitarte, J.-L., 2021. Analysis of the impact of three phthalates on the freshwater gastropod Physella acuta at the transcriptional level. Sci. Rep. 11, 11411. https://doi.org/10.1038/s41598-021-90934-9.
R Core Team, 2023. R: a Language and Environment for Statistical Computing.
Raimets, R., Karise, R., Mänd, M., Kaart, T., Ponting, S., Song, J., Cresswell, J.E., 2018. Synergistic interactions between a variety of insecticides and an ergosterol biosynthesis inhibitor fungicide in dietary exposures of Bumble bees (*Bombus terrestris* L.). Pest Manag. Sci. 74, 541–546. https://doi.org/10.1002/ps.4756.
Ran, D., Cai, S., Wu, H., Gu, H., 2012. Di (2-ethylhexyl) phthalate modulates cholinergic mini-presynaptic transmission of projection neurons in Drosophila antennal lobe. Food Chem. Toxicol. 50, 3291–3297. https://doi.org/10.1016/j.fct.2012.03.070.
Rasmont, P., Coppee, A., Michez, D., De Meulemeester, T., 2008. An overview of the *Bombus terrestris* (L. 1758) subspecies (Hymenoptera: apidae). Int. J. Entomol. 44, 243–250. https://doi.org/10.1080/00379271.2008.10697559.
Rasmont, P., Ghisbain, G., Terzo, M., 2021. Bumblebees of Europe: and neighbouring regions, Hymenoptera of Europe. NAP Éditions, verrières-le-buisson.
Regulation (EU) 2018/2005, 2018. Commission Regulation (EU) 2018/2005 of 17 December 2018 Amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as Regards bis(2-ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Benzyl Butyl Phthalate (BBP) and Diisobutyl Phthalate (DIBP).
Regulation (EU) 2021/2045, 2021. Commission Regulation (EU) 2021/2045 of 23 November 2021 Amending Annex XIV to Regulation (EC) No 1907/2006 of the European Parliament and of the Council Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (Text with EEA relevance).
Reid, A.M., Brougham, C.A., Fogarty, A.M., Roche, J.J., 2007. An investigation into possible sources of phthalate contamination in the environmental analytical laboratory. Int. J. Environ. Anal. Chem. 87, 125–133. https://doi.org/10.1080/03067310601071183.
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.
Saillenfait, A.M., Langonné, I., Leheup, B., 2001. Effects of mono-n-butyl phthalate on the development of rat embryos: *in vivo* and *in vitro* observations. Pharmacol. Toxicol. 89, 104–112. https://doi.org/10.1111/j.1600-0773.2001.890207.x.
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.
Sgolastra, F., Medrzycki, P., Bortolotti, L., Renzi, M.T., Tosi, S., Bogo, G., Teper, D., Porrini, C., Molowny-Horas, R., Bosch, J., 2017. Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species: synergistic interactions between pesticides in three bee species. Pest Manag. Sci. 73, 1236–1243. https://doi.org/10.1002/ps.4449.
Shi, X., Hu, C., Cai, S., Tao, X., Zhou, Y., Smidt, H., Ye, B., 2020. Protective effects of Lactobacillus plantarum strain P1 against toxicity of the environmental oestrogen di-n-butyl phthalate in rats. BM 11, 803–814. https://doi.org/10.3920/bm2019.0181.
Shi, M., Dong, K., Wu, J., Huang, J., 2023. Effects of low-temperature acclimation on nutrients of Bumble bee Bombus terrestris queens during prediapause and diapause. Insects 14, 336. https://doi.org/10.3390/insects14040336.
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.
Sicińska, P., 2018. Di-n-butyl phthalate, butylbenzyl phthalate and their metabolites induce haemolysis and eryptosis in human erythrocytes. Chemosphere 203, 44–53. https://doi.org/10.1016/j.chemosphere.2018.03.161.
Soroye, P., Newbold, T., Kerr, J., 2020. Climate change contributes to widespread declines among Bumble bees across continents. Science 367, 685–688. https://doi.org/10.1126/science.aax8591.
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.
Staples, C.A., Adams, W.J., Parkerton, T.F., Gorsuch, J.W., Biddinger, G.R., Reinert, K.H., 1997. Aquatic toxicity of eighteen phthalate esters. Environ. Toxicol. Chem. 16, 875–891. https://doi.org/10.1002/etc.5620160507.
Straw, E.A., Stanley, D.A., 2025. An introduction to bee and pesticide research. J. Appl. Ecol. 62, 19–27. https://doi.org/10.1111/1365-2664.14834.
Suhrhoff, T.J., Scholz-Böttcher, B.M., 2016. Qualitative impact of salinity, UV radiation and turbulence on leaching of organic plastic additives from four common plastics — a lab experiment. Mar. Pollut. Bull. 102, 84–94. https://doi.org/10.1016/j.marpolbul.2015.11.054.
Šulková, E., Kolena, B., Šuláková, H., Hlisníková, H., Nagyová, M., Poláčiková, Z., Babosová, R., Vondráková, M., Petrovičová, I., 2022. Effect of DEHP exposure in Calliphora vicina (Diptera: calliphoridae). J. Asia Pac. Entomol. 25, 101984. https://doi.org/10.1016/j.aspen.2022.101984.
Sun, Y.-P., Shepard, H.H., 1947. Methods of calculating and correcting the mortality of Insects1. J. Econ. Entomol. 40, 710–715. https://doi.org/10.1093/jee/40.5.710.
Surholt, B., Greive, H., Baal, T., Bertsch, A., 1991. Warm-up and substrate cycling in flight muscles of male bumblebees, Bombus terrestris. Comp. Biochem. Physiol. Physiol. 98, 299–303. https://doi.org/10.1016/0300-9629(91)90536-L.
Tatar, M., Kopelman, A., Epstein, D., Tu, M.-P., Yin, C.-M., Garofalo, R.S., 2001. A mutant *drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science 292, 107–110. https://doi.org/10.1126/science.1057987.
Teil, M.J., Blanchard, M., Chevreuil, M., 2006. Atmospheric fate of phthalate esters in an urban area (Paris-France). Sci. Total Environ. 354, 212–223. https://doi.org/10.1016/j.scitotenv.2004.12.083.
Tian, X., Yu, Z., Feng, P., Ye, Z., Li, R., Liu, J., Hu, J., Kakade, A., Liu, P., Li, X., 2019. Lactobacillus plantarum TW1-1 alleviates Diethylhexylphthalate-Induced testicular damage in mice by modulating gut microbiota and decreasing inflammation. Front. Cell. Infect. Microbiol. 9. https://doi.org/10.3389/fcimb.2019.00221.
van Doorn, A., 1989. Factors influencing dominance behaviour in queenless bumblebee workers (Bombus terrestris). Physiol. Entomol. 14, 211–221. https://doi.org/10.1111/j.1365-3032.1989.tb00954.x.
Vandenberg, L.N., 2014. Non-monotonic dose responses in studies of endocrine disrupting chemicals: bisphenol a as a case study. dose-response 12. Dose Response 13–20. https://doi.org/10.2203/dose-response.13-020.Vandenberg. Vandenberg.
Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.-H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378–455. https://doi.org/10.1210/er.2011-1050.
Velthuis, H.H.W., van Doorn, A., 2006. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie 37, 421–451. https://doi.org/10.1051/apido:2006019.
Verheyen, J., Delnat, V., Theys, C., 2022. Daily temperature fluctuations can magnify the toxicity of pesticides. Current Opinion in Insect Science 51, 100919. https://doi.org/10.1016/j.cois.2022.100919.
Walsh, J.E., Ballinger, T.J., Euskirchen, E.S., Hanna, E., Mård, J., Overland, J.E., Tangen, H., Vihma, T., 2020. Extreme weather and climate events in northern areas: a review. Earth Sci. Rev. 209, 103324. https://doi.org/10.1016/j.earscirev.2020.103324.
Walton, A., Herman, J.J., Rueppell, O., 2024. Social life results in social stress protection: a novel concept to explain individual life-history patterns in social insects. Biol. Rev. 99, 1444–1457. https://doi.org/10.1111/brv.13074.
Warner, G.R., Flaws, J.A., 2018. Bisphenol A and phthalates: how environmental chemicals are reshaping toxicology. Toxicol. Sci. 166, 246–249. https://doi.org/10.1093/toxsci/kfy232.

Weilnhammer, V., Schmid, J., Mittermeier, I., Schreiber, F., Jiang, L., Pastuhovic, V., Herr, C., Heinze, S., 2021. Extreme weather events in Europe and their health consequences – a systematic review. Int. J. Hyg Environ. Health 233, 113688. https://doi.org/10.1016/j.ijheh.2021.113688.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, second 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.

Wolf, S., Moritz, R.F.A., 2008. Foraging distance in *Bombus terrestris* L. (Hymenoptera: apidae). Apidologie 39, 419–427. https://doi.org/10.1051/apido:2008020.

Woodard, S.H., Bloch, G., Band, M.R., Robinson, G.E., 2013. Social regulation of maternal traits in nest-founding bumble bee (*Bombus terrestris*) queens. J. Exp. Biol. 216, 3474–3482. https://doi.org/10.1242/jeb.087403.

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.

Wu, F., Kong, Z., Ge, P., Sun, D., Liu, D., Dong, Z., Chen, G., 2024. Ecotoxicological evaluation and regeneration impairment of planarians by dibutyl phthalate. Environ. Res. 257, 119403. https://doi.org/10.1016/j.envres.2024.119403.

Xu, H., Shao, X., Zhang, Z., Zou, Y., Wu, X., Yang, L., 2013. Oxidative stress and immune related gene expression following exposure to di-n-butyl phthalate and diethyl phthalate in zebrafish embryos. Ecotoxicol. Environ. Saf. 93, 39–44. https://doi.org/10.1016/j.ecoenv.2013.03.038.

Yen, P.-L., Lin, T.-A., Chang, C.-H., Yu, C.-W., Kuo, Y.-H., Chang, T.-T., Liao, V.H.-C., 2024. Di(2-ethylhexyl) phthalate disrupts circadian rhythm associated with changes in metabolites and cytochrome P450 gene expression in Caenorhabditis elegans. Environ. Pollut. 363, 125062. https://doi.org/10.1016/j.envpol.2024.125062.

Zattara, E.E., Aizen, M.A., 2021. Worldwide occurrence records suggest a global decline in bee species richness. One Earth 4, 114–123. https://doi.org/10.1016/j.oneear.2020.12.005.

Zhang, Y.-Z., Zuo, Y.-Z., Du, Z.-H., Xia, J., Zhang, C., Wang, H., Li, X.-N., Li, J.-L., 2018. Di (2-ethylhexyl) phthalate (DEHP)-induced hepatotoxicity in quails (Coturnix japonica) via triggering nuclear xenobiotic receptors and modulating cytochrome P450 systems. Food Chem. Toxicol. 120, 287–293. https://doi.org/10.1016/j.fct.2018.07.019.

Zhang, W., Li, J., Wei, X., Wang, Q., Yang, J., Hou, H., Du, Z., Wu, X., 2021. Effects of dibutyl phthalate on lipid metabolism in liver and hepatocytes based on PPARα/SREBP-1c/FAS/GPAT/AMPK signal pathway. Food Chem. Toxicol. 149, 112029. https://doi.org/10.1016/j.fct.2021.112029.

Zheng, Y., Liu, C., Chen, J., Tang, J., Luo, J., Zou, D., Tang, Z., He, J., Bai, J., 2024. Integrated transcriptomic and biochemical characterization of the mechanisms governing stress responses in soil-dwelling invertebrate (Folsomia candida) upon exposure to dibutyl phthalate. J. Hazard Mater. 462, 132644. https://doi.org/10.1016/j.jhazmat.2023.132644.