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Original article

Wild bees and ubiquitous anthropogenic pollutants: Contamination rates and multilevel effects

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1. Introduction

Wild bees are a crucial link of terrestrial biodiversity [1]. They also provide essential ecosystem services, delivering significant and quantifiable benefits to humanity by pollinating over 70% of cultivated plant species [2]. Nevertheless, bee populations, along with insect populations in general, are experiencing a decline [3]. The last IPBES global assessment report on biodiversity [4] identified pollution as one of the five direct drivers of biodiversity loss. While pesticides are recognized as major threats to bee populations [5], other common pollutants, such as phthalates, may also play a role. Phthalates are semi-volatile anthropogenic pollutants that now contaminate nearly every ecosystem and environmental compartment (air, water, soil) [6]. Despite limited research on the impact of phthalates on insect health, they appear particularly vulnerable due to their cuticle, which acts as a trap for such contaminants [7]. Moreover, these organic molecules can accumulate in the organic matrices that make up bee nests (e.g., wax, nectar, pollen) [8,9]. As a result, phthalates may contribute to the decline in pollinator populations.

Phthalates are a family of synthetic organic compounds extensively used in the plastic industry to enhance the ductility and flexibility of plastic products. These plasticizers are non-covalently bound to plastic matrices, making them readily released into the environment. Due to the widespread use of plastics globally, phthalates have become ubiquitous contaminants. Consequently, they represent significant concerns for both human and wildlife health, as they can be encountered through all possible exposure routes, including inhalation, direct contact, and ingestion. From a toxicological perspective, phthalates are associated with to numerous adverse effects in humans such as testicular dysfunction, impaired neurodevelopment, asthma, diabetes and obesity [10]; they also exhibit reprotoxic and developmental impacts in various animal species [11,12]. Some phthalates are identified as endocrine disruptors (EDs) in vertebrates, including human, based on studies made on rodents and fish [13,14]; studies on invertebrates suggest similar effects [11]. As with other EDs, the effects of phthalates are characterized by non-monotonic dose-response curves and toxicity thresholds (NOEC, LOEC) virtually impossible to determine using conventional toxicological approaches (i.e., low or very low dose effects cannot be predicted by observed high dose effects) [15]. Therefore, studying phthalate toxicity presents significant challenges for toxicologists, combining complex environmental mixtures, multiple exposure routes, various affected physiological endpoints, and non-monotonic dose-response relationships.

In this general context, expanding the range of species studied for the effects of phthalates to include a well-established pollinator model, the buff-tailed bumblebee *Bombus terrestris* [16], makes sense, and for several reasons. First, it would provide valuable data on the exposure to and impacts of phthalates on terrestrial insects, a group for which such information is currently limited. The potential role of these contaminants in the decline of insects, particularly pollinators, is especially pertinent in light of the ongoing biodiversity crisis. Second, *Bombus terrestris* offers the opportunity to study the effects of phthalate exposure across multiple levels of biological organization. The molecular and cellular levels are easily accessible, as well as the individual level, in this commercially available species, for which genetically standardized colonies of 100–200 highly related individuals can be obtained. Moreover, the high social plasticity of *B. terrestris* allows to split a single colony into multiple units of 5–10 individuals, called microcolonies. When maintained in a queenless environment, each microcolony establishes a new small nest, where a dominant worker quickly shifts to reproduction, mimicking a natural social structure (except that only male brood is produced, due to the haplodiploid system of sex determination; workers do not mate, only queens do). By maintaining multiple microcolonies simultaneously, a large number of units with similar genetic backgrounds can be compared, ensuring substantial sample sizes and high replication capacity [17]. Additionally, this invertebrate model allows extending the endpoints investigated for phthalate exposure to social and communal behaviors. Social interactions with brood and between nestmates, as well as sensory and cognitive aspects related to plant pollination (e.g., floral scent sensitivity and recognition, flight control, memorization), present novel endpoints to explore in relation to phthalate exposure. Finally, since many of the effects of phthalates identified

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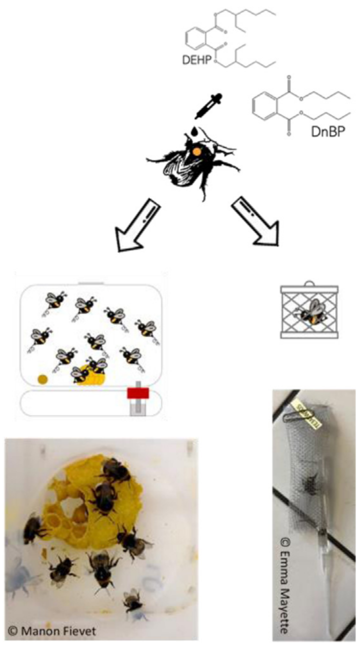

Figure 1. Overview of *B. terrestris* phthalate contamination in the present study.

thus far are associated with processes that are highly conserved across the tree of life, particularly lipid and glucose metabolism, the knowledge gained from studying molecular and cellular disruptions in *B. terrestris* could offer valuable insights into the broader mechanisms of phthalate toxicity.

Following this rationale, the present study aimed to: (1) assess phthalate exposure levels in natural populations of *B. terrestris* from various sites within the Métropole européenne de Lille (MEL); and (2) evaluate the effects of phthalate contamination on *B. terrestris* maintained either in isolation or in microcolonies, and exposed under controlled conditions to phthalate levels similar to those observed in the studied natural populations. Several endpoints were examined across the different levels of biological organization described above, from molecular to communal level.

2. Materiel and methods

B. terrestris workers were captured at various sites in Lille and the contaminants adsorbed onto their cuticle were extracted. The types and concentrations of phthalates were determined, offering insight into the environmental doses to which wild bees are exposed.

Commercial bumblebees were exposed to the phthalates identified as major contaminants in natural populations, at various concentrations based on measured environmental doses. Molecular, cellular and individual effects were monitored. By comparing the exposure of workers maintained either in isolation or in microcolonies (Fig. 1), the study explored the influence of sociality on the expression of phthalate toxicity.

3. Results

The natural populations of *B. terrestris* sampled showed significant contamination with phthalates. We observed both lethal and sublethal effects of phthalate exposure in bumblebees exposed to environmental concentrations. Gene expression analyses confirmed the endocrine-disrupting activities of the tested phthalates on bees. The social context had a significant impact on the outcomes of phthalate exposure. Finally, the results suggest that bumblebee interactions with plant partners may be disrupted as a response to phthalate contamination.

4. Conclusion

Phthalates may contribute to the decline of wild bee populations in large western cities. *B. terrestris* would serve as a highly relevant and sensitive model for investigating the toxicity of phthalates.

Disclosure of interest

The authors declare that they have no competing interest.

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