Investigations of micro- and nanoplastic toxicity using a model aquatic invertebrate and a model insect

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Plastic pollution and its potential impacts on human and environmental health are of growing global concern. The degradation of bulk plastics leads to the formation of microplastics (MPs, 0.1 μm to 5 mm in size) that can contaminate drinking water sources, agricultural fields, and food supplies (e.g., fish and other seafood) [1-3]. Although MP pollution in marine environments has received considerable attention from scientists and the media, there is limited to no information on the loads, types, and toxicity of MPs in freshwaters, agricultural soils, and air. Moreover, weathering of a single MP particle can yield billions of NPs [4] and NP pollution is expected to be ubiquitous in the environment [4-6]. NPs are potentially more hazardous than MPs because they can cross biological membranes; yet, there is little data on NP toxicity, especially to terrestrial organisms.

In this study, we examined the toxicity of different micro- and nanoplastics using a model freshwater invertebrate (*Daphnia magna*) and a model terrestrial insect (*Drosophila melanogaster*). *Daphnia* is an ideal representative of zooplankton because of the ease of culture and observation, and also because of its key position in the food webs of freshwater ecosystems. *Daphnia* has been long used as a model toxicity species for harmful chemicals and recommended as a standard method by multiple official agencies, such as the USEPA and the OECD. In this study, we quantified the impacts of different plastic pollutants, including leachate of rubber particles from recycled tires, and leachate of weathered plastic debris on the mortality, physiology, and swimming behavior of *Daphnia*. *Drosophila* is an ideal model terrestrial organism to study genotoxicity, cytotoxicity, and neurotoxicity effects due to its rapid offspring turnover, and considerable genetic homologies with vertebrates, including humans. In this study, the impacts of plastic debris from plastic packaging were assessed by feeding *Drosophila*. Plastic packaging (Nylon or PET) was aged in water to produce a 100% leachate suspension. Different dilutions of this concentrated leachate were used in toxicity assessments.

Results:

The water leachate of 20-day-weathered plastic debris (20 g/L) that mainly contained plastic water bottles, fruit netting, plastic bags, packaging, and candy wrappers did not cause any mortality after 24 h and 48 h exposures (Fig. 1a). In contrast, concentration-dependent mortality was found after exposure to water leachate of rubber particles. The 25% leachate of rubber particles (10 g/L) and above concentrations showed significantly higher mortality than controls (Fig. 1b). Analytical and image analyses identified a large number of micro-sized plastic particles (Fig. 1c) in the water leachate of weathered plastic debris, with polyethylene being the most abundant followed by polypropylene, polyethylene terephthalate, and nylon (Fig. 1d), as well as hazardous compounds such as bisphenol A and phthalates (data not shown). *Daphnia* treated with 100% plastic leachate grew larger and reproduced more neonates than controls over chronic exposure (10 days) (Figs. 1e,f). This could be due to the presence of hormone-mimicking compounds bisphenol A and phthalates, which stimulate the growth and reproduction of *Daphnia* at low or favorable concentrations [7,8]. Additionally, plastic leachate significantly lowered the curling rate of the thoracic appendages possibly due to the accumulated microplastics on the appendages (Fig. 1g). The reduced curling rate may have implications on the feeding rate especially when food resources become scarce. No effect on the heartbeat rate and total swimming distance was evident.

Leachate from Nylon food packaging causes higher mortality of *Drosophila* than that of polyethylene terephthalate (PET) packaging leachate. The survival of male flies exposed to 20% Nylon leachate is significantly lower than the controls (Figs. 2a,b). However, 20% PET leachate shows no difference in the survival of male flies from controls. Survival of females following exposure to 20% Nylon or PET leachate is significantly lower than controls (Figs. 2c,d). When exposed to the 100% Nylon and PET leachates, the survival of female flies (22-31%) is significantly lower than that of male flies (45-48%), suggesting that female flies are more susceptible than male flies to leachate toxicants. Significantly fewer flies climb up to 10 cm when exposed to 100% PET and Nylon leachates compared to controls (Figs. 2e,f). The particles present in plastic food packaging leachates adhere to the external surfaces of the eyes and legs of flies (Figs. 2h,j), potentially affecting fly behavior. The observed chronic effects of packaging leachates are possibly due to the co-presence of micro- and nanoplastics as well as metal(loid)s (Al, As, Cr, and Pb) in the leachates.

Our results show the *Daphnia* 48 h test provides a fast screening evaluation on the acute toxicity of plastic pollutants of different forms, suggesting low toxicity of plastic debris and higher toxicity of rubber leachates. Microplastics as well as harmful chemical substances can leach from weathered plastic debris and lead to chronic behavioral effects in both *Daphnia* and *Drosophila*. Finally, it can be concluded that the invertebrate bioassay offers an effective tool for assessing the toxicity of plastic pollutants in different forms.

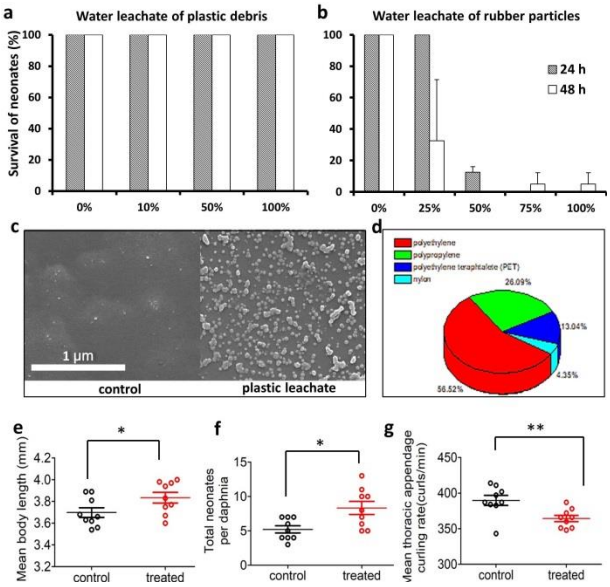

Fig. 1 Toxic effect of plastic pollutants in *Daphnia magna*. Survival of *Daphnia* neonates after 24 h and 48 h exposure to water leachate of plastic debris (a), and water leachate of rubber particles (b). SEM images of control and water leachate showing the presence of micro-sized particles (c). Polymer composition of particles in the water leachate of plastic debris (d). Body length (e), total neonates produced per *Daphnia* (f), and thoracic appendage curling rate (g) of *Daphnia* exposed to plastic leachate and control (* p-value < 0.05, ** p-value <0.01; n = 10).

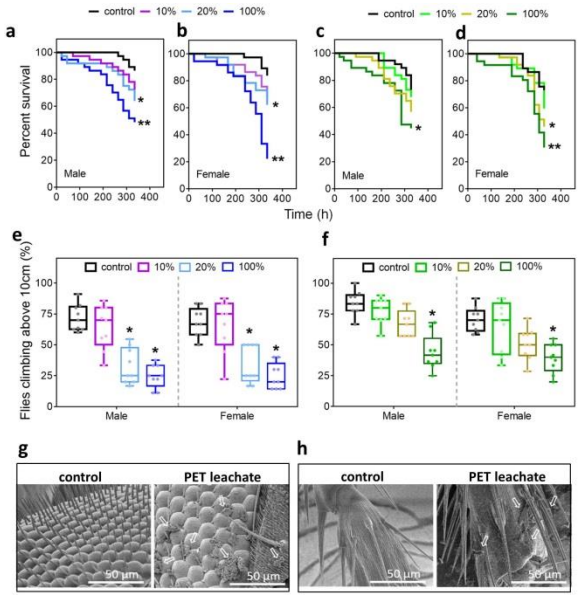

Fig. 2 Toxic effects of plastic food packaging leachate on *Drosophila melanogaster*. Survival of male and female adult *Drosophila* after 14 day exposure to Nylon packaging leachate (a,c) and PET packaging leachate (b,d) (* p-value <0.05, ** p-value <0.001,n =36). Percentage of *Drosophila* climbing above 10 cm after exposure to Nylon leachate (e) and PET leachate (f) (* p-value <0.05; n = 9). Leachate micro-sized particles on eye (g) and leg (h) of *Drosophila* indicated with white arrows.

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