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Ecotoxicological risk assessment of nano- and microplastics

Summary

Nano- and microplastics (NMPs) are ubiquitous, persistent, and, as is now widely recognised, a global problem for humans and the environment. Because of the many different types of plastic from which NMPs are derived, conducting a general risk assessment is challenging. In addition, many plastics contain additives such as UV stabilisers or plasticisers that have hormonal effects – so-called endocrine disruptors (EDs) – and can easily be released from the plastics. Furthermore, some endocrine disruptors can strongly adhere to the surface of NMP particles and spread with them throughout the environment.

The effects of endocrine disruptors on humans and the environment remain partially unclear, as they can be very diverse and species-specific, making monitoring difficult. However, a causal link between exposure to endocrine disruptors and various human diseases has now been established. Adverse effects have also been observed in other organisms following exposure to endocrine disruptors, particularly in relation to reproduction.

Even if greener alternatives (e.g. bioplastics) could replace conventional plastics in the future, a significant influx of NMPs into the environment and the use of hormonally active substances can still be expected in the coming decades. Therefore, it is of utmost importance to establish additional test systems to protect human health and the environment, especially at the level of organisms that may facilitate the trophic transfer of EDs into the food chain.

This dossier explores general issues related to NMPs, EDs, and ecotoxicological risk assessment using the aquatic snail *Biomphalaria glabrata*.

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Nano- and microplastics

Nano- and microplastics (NMPs) are tiny plastic particles that are of ecotoxicological concern and can now be detected in all ecosystems (hydrosphere, pedosphere, and atmosphere). The first reports of NMP contamination and its dangers focussed on seas and oceans.[1] Shortly afterwards, NMPs were also detected in soils, with the urban environment identified as a particular hotspot for NMP contamination.[2] Meanwhile, NMPs have been found in the world's most remote regions, such as the ice sheets in Antarctica[3] and the Himalayas[4], contributing to the realisation that NMPs are a global problem.

Plastic has been produced on a large industrial scale since the 1950s. It is a very versatile material with many advantages. Plastics are easy to mould, extremely durable, lightweight, corrosion-resistant, and thermally and electrically insulating. The material also offers a wide range of mechanical and multifunctional properties and is very cost-efficient to produce. As a result, global annual plastic production has increased dramatically, from 1.7 million tonnes in 1950 to more than 390 million tonnes in 2021.[5] The mass production of plastic marks the beginning of a new era, which experts refer to as the Anthropocene, replacing the Holocene.[6] Plastic, along with other man-made (anthropogenic) materials, such as concrete and cement, now collectively outweigh all biomass.[7]

Life without plastic is no longer conceivable in the 21st century, yet more and more problems have recently come to light. On the one hand, conventional plastics are made from non-renewable raw materials (crude oil). On the other, they are not biodegradable and persist in the environment for a very long time.[8] Because of weathering processes, plastic breaks down into smaller fragments known as microplastics, which then degrade further into nanoplastics. These nanoplastics are no longer visible to the human eye but accumulate in the environment because of their slow degradability.[9]

According to the definition of the European Chemicals Agency (ECHA), microplastics are particles with a size of 5mm to 0.1µm, whilst particles smaller than 0.1µm are called nanoplastics.[10,11] There are numerous sources of NMPs entering the environment, whilst dividing NMPs into secondary and primary NMPs.[12] Secondary NMPs are produced through the decomposition or abrasion of plastics, primarily entering the environment as a result of human misbehaviour, such as the improper disposal of plastic bottles and packaging (littering).[13]

Primary NMPs are classified into "type A" and "type B" NMPs. Type A refers to intentionally produced micro- or nanoparticles, such as polyethene (PE), polypropylene (PP), and polystyrene (PS), which are subsequently processed into other products. They are deliberately added to products to achieve specific properties, such as in cosmetics, medical products, and cleaning agents. Because of the harmful effects of NMPs on the environment, EU member states in the Committee for the Regulation of Chemicals (REACH) recently agreed to a comprehensive ban on intentionally added NMPs in products such as cosmetics, paints, and medicines.[14] However, it is estimated that only about 2% of primary NMPs are released via cosmetic products. The main sources of primary NMPs are abrasion from paints and varnishes, microfibres from textiles, and tyres (type B).[15] The binders in most paints and varnishes are made of plastics and are released into the environment in vast quantities through improper washing and abrasion (e.g. antifouling paints from ships or road markings).[16] Approximately 700,000 synthetic fibres are released per wash cycle (approx. 6 kg of laundry), which then enter sewage treatment plants via wastewater. [13] In addition, approximately 1 kg of tyre abrasion produced per person per year is dispersed into the environment by wind and water.[17] As a result, NMPs can be found in all environmental compartments (atmosphere, pedosphere, and hydrosphere) (Figure 1).

NMP particles, which vary in shape and size, enter rivers via wastewater and are subsequently transported into seas and oceans. The high-profile CleanDanube project, initiated by Andreas Fath, Professor of Chemistry at Furtwangen University, highlighted the severe pollution of the Danube, which carries 4 tonnes of plastic into the Black Sea every day (https://www.cleandanube.org/projekt/).

Oceans are widely recognised as the ultimate sink for microplastics. Approximately 14 million tonnes of plastic end up in the ocean each year, accounting for 80% of all marine litter, found on surface waters right down to deep-sea sediments.[13] Even at the ocean's deepest point, the Mariana Trench, microplastic particles have been found in the hindgut of amphipods.[18] Lighter plastics, such as PE and PP, float on the surface first, whilst denser and heavier plastics, such as PVC, sink, accumulate, and remain in sediments.[13] However, as biofilm forms on lighter plastics, these particles become heavier and can also sink as a result.

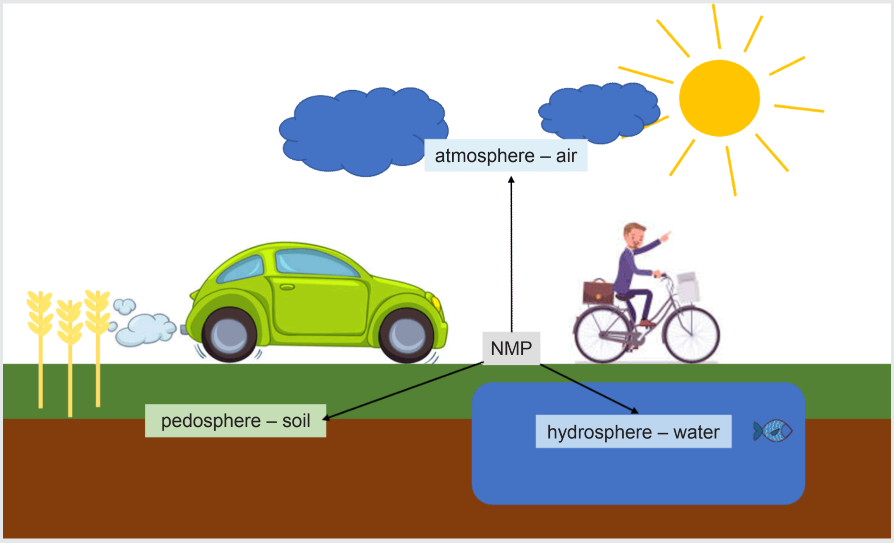

Figure 1: *Transfer of NMPs to all compartments. NMPs, in this case tyre abrasion, enters the atmosphere, the hydrosphere, and pedosphere.*

Filtering organisms (e.g. mussels) can mistake NMPs for food (e.g. plankton).[13] Over time, NMPs accumulate in microorganisms (bioaccumulation) and thus also enter the food chain, resulting in trophic transfer to higher organisms/animals, leading to bioaccumulation and biomagnification along the food chain.[13] The ingestion of NMPs can also impair energy reserves in many animals by disrupting their sense of satiety, whilst their shape (sharp-edged, pointed, etc.) can cause physical injuries.

The entry of NMPs into terrestrial ecosystems is mainly due to agriculture. On the one hand, this occurs through spreading of sewage sludge on fields as fertiliser; on the other, it is done through mulch films.[13] Here, too, NMPs are absorbed by soil organisms and enter the food chain.[19] Moreover, NMPs have already been identified in rice, amongst other things.[20] It has also been shown that substances of concern that can be released from NMPs, such as tyre abrasion, enter plants (e.g. lettuce) via the soil and, in turn, enter the food chain.[21]

Human exposure to NMPs occurs through oral intake (primarily via food), inhalation (e.g. fibres from clothing and building materials, tyre abrasion, and particulate matter), and via the skin (e.g. textiles and use of personal care products containing NMPs).[22,23,24] However, the main route of exposure is through food, on the one hand through trophic transfer and accumulation of NMPs in food, and on the other hand through migration of NMPs and additives from plastic packaging into food.[25] Especially when food is heated in microwavable plastic containers, unintentionally introduced substances ("non-intentionally added substances" or NIAS), such as derivatives, degradation products, external contaminants, and NMPs, can migrate into the food.[26] Microplastics were first detected in human faeces in 2019.[27] It is now assumed that humans ingest an average of 5 g of plastic per week (roughly the size of a credit card), mainly through food.[28] However, NMPs have been detected not only in the digestive tract but also circulating in the blood, reaching other organs and tissues.[29] Because of their small size and lipophilic properties, NMPs can cross the body's protective barriers, such as the blood-brain barrier and the blood-placenta barrier, and have therefore already been detected in both the brain and placenta.[30,31]

The effects of NMPs at different trophic levels (from exposed individuals to entire ecosystems) have not yet been fully understood. Particularly concerning are chemicals that are released by NMPs or can adhere to them. Because of the significant increase in surface area of NMPs (with the same mass) and their lipophilic properties, problematic and toxic substances such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and other persistent organic pollutants (POPs) can adsorb to NMPs.[13] In addition, additives incorporated during production to achieve specific properties (e.g. elasticity) leach from NMPs. Different additives are used depending on the type of plastic, such as plasticisers, stabilisers, flame retardants, dyes, etc.[32] Many of these substances have adverse effects. Substances with hormonal effects – known as endocrine disruptors – are particularly concerning, as they impair many biological processes controlled by the body's hormone system.

Endocrine disruptors

Endocrine disruptors (EDs) are substances that interact with an organism's endocrine (hormonal) system and can adversely affect health. Their effects are diverse and vary between individuals and species, and include developmental and reproductive disorders, hormone-dependent tumour formation, and neurological and metabolic defects.[33,34,35]

EDs are present in many everyday consumer products. It is estimated that more than 1000 authorised chemicals in circulation have endocrine effects.[53] These include substances found in pesticides, biocides, industrial chemicals, plasticisers, pharmaceuticals, and cosmetics. Additionally, other EDs can also be found in nature (e.g. phytoestrogens and heavy metals) (Figure 2).[36]

Plastics and NMPs play a unique role in the spread of EDs in the environment, as these EDs are present in the form of additives, such as plasticisers (bisphenols and phthalates) and brominated flame retardants, or adsorb onto their surface.[37] Persistent organic pollutants are particularly concerning, as they have a broad spectrum of (harmful) effects (carcinogenic, neurotoxic, hepatotoxic, nephrotoxic, immunotoxic, and also endocrine-disrupting).[36] Furthermore, POPs are easily dispersed in the environment by binding to NMPs (Figure 2).[13]

Because of their chemical properties, many EDs are persistent and remain in the environment for long periods.[53] Furthermore, these substances are primarily lipophilic, allowing them to penetrate cell membranes and enter cells and tissue, where they accumulate primarily in fatty tissue. Along the food chain and with the increasing age of exposed organisms, this results in the accumulation of EDs (biomagnification). Although many substances, such as the industrial chemical PCB and the pesticide dichlorodiphenyltrichloroethane (DDT), have been banned for some time under the Stockholm Convention, they remain global contaminants and can still be detected in organisms.[38]

The molecular mechanisms of action of EDs are very diverse. The best-known and most researched mechanism of action is the interaction of a substance with a hormone receptor (e.g. oestrogen receptor (ER) and androgen receptor (AR)) because of structural similarities to endogenous hormones, whereby the receptor is either activated (agonist) or inhibited (antagonist).[39] Other known mechanisms of action include the impairment of the organism's hormone synthesis and degradation, as well as the transport and distribution of endogenous hormones within the organism. It is also known that EDs can alter the epigenome, which plays a significant role in gene regulation and reacts dynamically to environmental influences.[40,41]

The extent of the effects following exposure to EDs is highly dependent on the organism's genetics and the life or developmental phase it is in at the time of exposure.[33] Exposure during the foetal and infant stages, for example, causes more significant damage to health than exposure in adulthood. and in the case of pregnant women, exposure can affect the unborn offspring and future grandchildren (due to the exposure of the germ cells of the developing foetus), meaning that effects can occur across generations.[42]

Another issue with EDs is the latency period between the actual exposure and the first appearance of effects. Consequently, adverse health effects resulting from foetal exposure may not manifest until adulthood. It should also be noted that exposure to EDs typically occurs in combination with other chemicals or substances, which can also lead to synergistic effects. Furthermore, EDs do not follow typical dose-response curves but instead exhibit non-monotonic dose-response curves, making it impossible to predict the effects at higher or lower concentrations.[43]

The consequences of exposure to EDs can, therefore, vary significantly and depend on many parameters (e.g. the substance, the time and duration of exposure, the dose, etc.). In humans, a causal relationship with EDs has already been established for various metabolic, reproductive, and degenerative diseases, as well as certain forms of cancer.[33,44] Many EDs interfere with the action of sex steroid hormones, leading to reduced fertility (e.g. lower sperm count and abnormal sperm morphology in men), altered sexual behaviour, changes in age of puberty onset, and the development of hormone-dependent tumours (e.g. breast and prostate cancer).[33,45,46,47] Some EDs also affect the functions of the thyroid and pituitary glands (hypothalamus), which can lead to metabolic disorders such as obesity and type II diabetes, subsequently increasing the risk of cardiovascular diseases and neurological conditions such as autism and a lower IQ.[48,49,50,51]

In addition to their adverse effects on human health, EDs are also harmful to animals and the environment. EDs enter the environment mainly through wastewater disposal into bodies of water or atmospheric transport. Surface water ecosystems are the most heavily polluted. Most studies on the effects of EDs in animal populations have focussed on vertebrates.[52,53] Research on harbour seals, for example, has shown that bioaccumulation of, e.g., PCBs in fatty tissue has led to impaired reproduction and immune function.[54,55,56,57] Further studies have demonstrat-

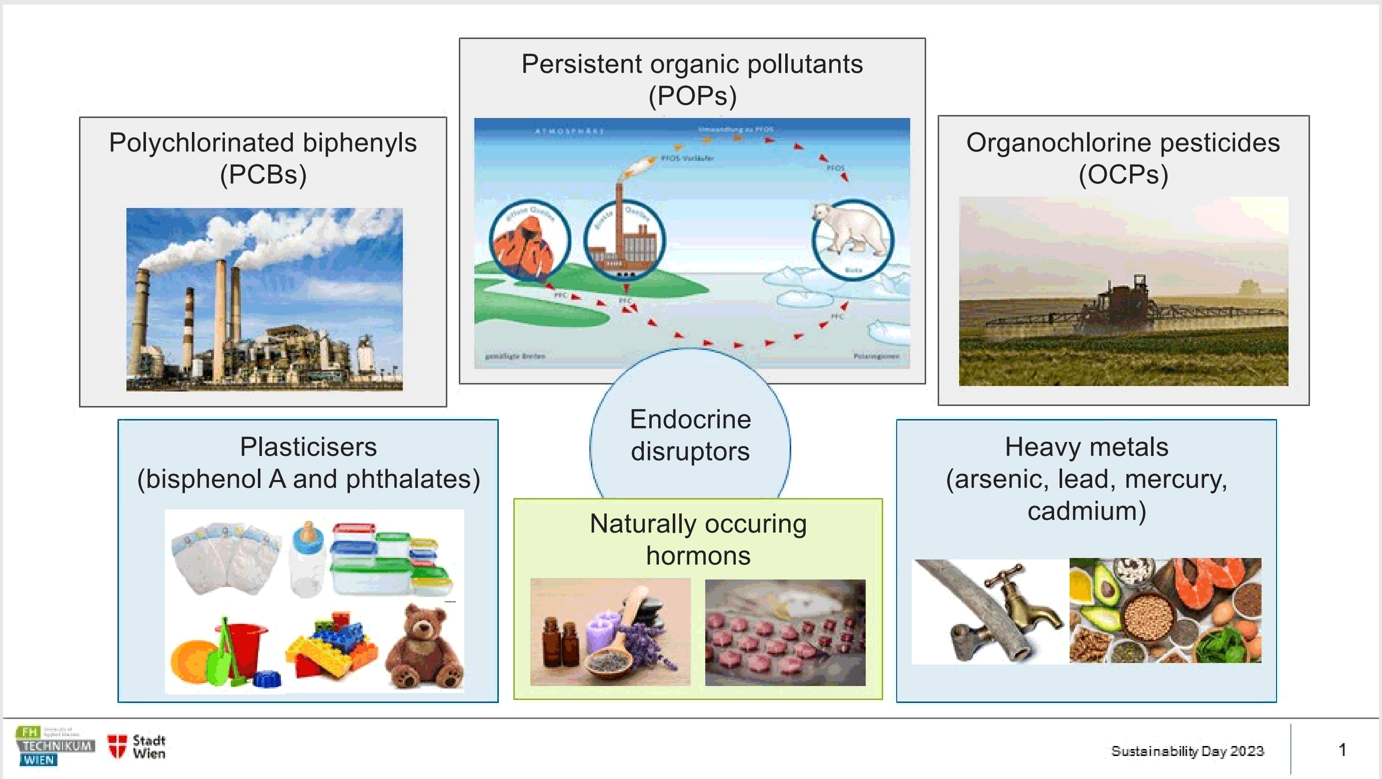

Figure 2: *Endocrine disruptors. From industrial chemicals such as PCBs and plasticisers to pesticides and heavy metals, a variety of endocrine disruptors are present in the environment to which humans are exposed to daily. (Images sourced from Pixabay, except for persistent organic pollutants (*https://worldoceanreview.com/de/wor-1/verschmutzung/organische-schadstoffe/*) and lead pipes in heavy metals (*https://www.wassertest-online.de/blog/bleirohre-erkennen/*)).*

ed a clear link between ED contamination and reproductive and developmental effects in fish, amphibians, and reptiles. Field observations on fish, for instance, have shown that exposure to oestrogenic substances leads to feminisation or increased vitellogenin content in the plasma of male animals.[57]

To date, very little research has been conducted on the effects of EDs in invertebrates, despite their vital and relevant role in global ecosystems. One reason for this is the limited understanding of the function of the hormone systems and the associated species-specific differences in sensitivity, as well as the resulting lack of internationally recognised and validated test methods.[58]

A well-documented example and strong evidence that EDs can influence invertebrate populations is the imposex phenomenon in female molluscs (e.g. snails and mussels) following exposure to tributyltin (TBT), a substance used to prevent the growth of mussels on ships. Female molluscs developed male reproductive organs as a result of contact with the endocrine substance, although these organs are non-functional in terms of reproducing. This has led to a significant decline in certain mollusc populations.[53,59]

To better assess the potential danger posed by EDs and NMPs in the future, suitable systems to conduct risk assessments are needed. These include *in vivo* test systems, as they account for the toxicokinetics and metabolism of the organism.[60] However, the standardised test methods recognised by the EU for evaluating EDs currently only include test methods for testing vertebrate animals.

Biomphalaria glabrata as an *in vivo* model for testing endocrine disruptors

Biomphalaria glabrata is an invertebrate belonging to the phylum of freshwater molluscs (Figure 3). *B. glabrata* is best known for its important role in medicine, as it serves as the intermediate host of the parasite *Schistosoma mansoni*, which infects humans and can cause schistosomiasis (also known as bilharzia).[61] In addition to its significance in healthcare, *B. glabrata* has also become increasingly important in environmental biomonitoring, as well as in ecological and ecotoxicological studies.[62,63] One of the main reasons for its growing popularity as a model organism in environmental research is its short generation time of approximately 1–2 months and its high reproduction rate.[62,64]

In general, invertebrates, particularly aquatic invertebrates, play a crucial role in ecotoxicological studies, as this group makes up approximately 90% of all animal species. Molluscs have also proven to be valuable test organisms, e.g. in studies on heavy metal pollution and nanomaterials.[64,65,66] Molluscs are also known to be susceptible to endocrine-active substances. A key example of this is the aforementioned well-documented effect of TBT on the masculinisation of female aquatic snails and the subsequent population decline, which impacts the entire ecosystem.[59] However, research into the effects of EDs on the group of aquatic molluscs remains limited. As existing studies have already demonstrated, exposure to EDs can have devastating effects on molluscs. Consequently, it is essential to develop test systems for invertebrates that allow identification and risk assessment of EDs in this group.[57,67]

For this reason, the University of Applied Sciences Technikum Wien is researching the environmental impact of NMPs as part of a project funded by the City of Vienna (https://www.technikum-wien.at/forschungsprojekte/mikroplastik/). The ongoing research aims to develop an *in vivo* test system for the freshwater snail *B. glabrata* to screen for EDs. For this purpose, a reproductive toxicity test was conducted, and the establishment of genetic biomarkers for identifying endocrine-active substances and EDs in freshwater snails was investigated. Initial results indicate that the aquatic snail is sensitive to and affected by oestrogenic substances such as 17β-oestradiol. In addition, the observed effects suggest a non-monotonic dose-response curve characteristic of EDs, namely, a higher sensitivity to lower concentrations. Furthermore, the expression of specific genes is influenced by oestrogens. Vitellogenin has already been identified as an endocrine biomarker in other organisms[57] and could serve the same function in the aquatic snail under investigation.

These observations are of interest in the context of current discussions on the sensitivity of molluscs to steroid hormones, as it has not yet been clarified whether molluscs can synthesise steroids or respond to receptor-interacting environmental hormones.[68,69]

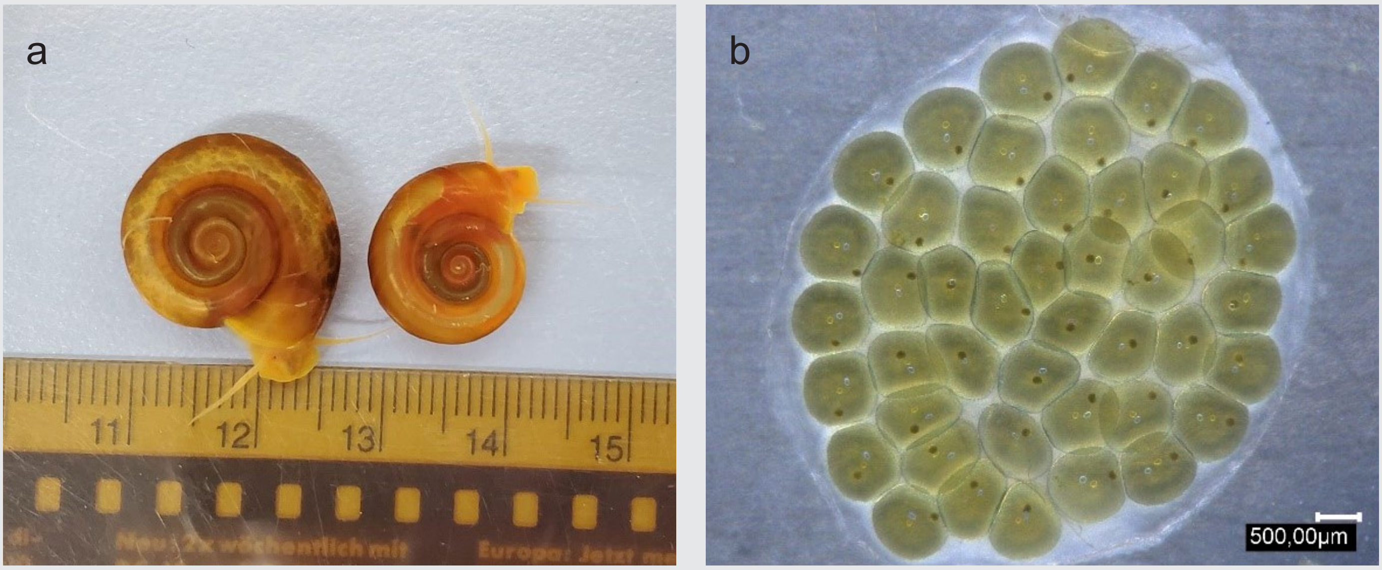

Figure 3: *Biomphalaria glabrata. a) Two adult snails of the genus B. glabrata. b) An egg clutch (clutch) with approximately 1-day-old embryos.*

Conclusion

The dangers, risks, and problems associated with nano- and microplastics (NMPs) are increasingly being discussed and recognised. The environment, animals, and humans are exposed to NMPs on a daily basis. It is not only the particles themselves that pose a significant risk, but also the substances released from or adsorbed onto the particles. One group of these substances includes endocrine disruptors (EDs). EDs have many mechanisms of action and are often difficult to detect, as they can act even at the slightest concentrations, or their effects may only become apparent in subsequent generations. Because of the many harmful aspects of EDs on health, it is crucial to conduct further tests and screenings, and to continuously improve them.

Study results show that *B. glabrata* is a promising model organism for screening EDs. Both reproduction and the expression of potential biomarkers are affected by exposure to 17β-oestradiol. The molecular mechanisms are not yet understood and require further investigation in the future.

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