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Microplastics and Nanoplastics

PERSPECTIVE

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Farm animals as a critical link between environmental and human health impacts of micro-and nanoplastics

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Abstract

Plastic pollution is an increasing global health concern, particularly the ever-increasing amount of tiny plastic particles commonly referred to as micro- and nanoplastics (MNPs). Most research to date on MNP exposure and hazards has focused on environmental species such as aquatic organisms and, more recently, humans, leaving impacts on farm animals largely unstudied. MNPs have been detected in all environmental compartments, including agricultural environments, farm animals and food products originating from them. The health of farm animals can be directly affected by MNPs, while humans can be affected by MNPs present in animal-derived food products. In this perspective article, we argue that MNP research should give more attention to farm animals forming a critical link between the environment and human health. Here, we summarize evidence on sources, exposure routes, levels in farm animals, and potential health effects of MNPs on farm animals, and identify knowledge gaps for future research, such as effects of MNPs on reproduction and development. In particular, the bovine embryo model is a promising model to study effects of MNPs on early development of both farm animals and humans. This perspective article signals the need for follow up studies that will increase our understanding of the transfer of MNPs between environment, farm animals, and humans, and the potential of farm animals to serve as an indicator for other animals, including humans.

Keywords Microplastics, Farm animals, One health, Agricultural environment, Reproduction

Introduction

We live in an environment where plastic materials are everywhere. In the environment, plastics do not decompose to a significant extent. Instead, they disintegrate into smaller and smaller pieces, eventually becoming microplastics (MP; 1 µm – 5000 µm) or nanoplastics (NP; < 1 µm), resulting in the current ubiquity of micro- and nanoplastics (MNPs) in global ecosystems. MNPs vary in size and shape, and one of the routes of toxicity is via physical hazard by the particle itself. However, there are other routes of toxicity as plastics contain a wide range of polymers and plastic additives, such as plasticizers, residual monomers, flame retardants, antioxidants, UV stabilizers, colorants, and pigments. Some of these plastic additives have been categorized as substances of very high concern or are legacy persistent organic pollutants that may have serious adverse health and environmental effects [1]. In addition to the above-mentioned routes, MNPs can also result in toxicity because of corona formation on their surfaces. Once in the environment, MNPs can become coated with natural matter, like biomolecules e.g., lipids and proteins, and with numerous

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potentially toxic chemical and microbiological contaminants. The composition of the corona on plastic varies throughout time, depending on age and surrounding conditions. Thus, MNPs are highly complex and diverse contaminants with multiple routes of toxicity, making them a challenging topic to research [2–4].

MNPs pollute all environment compartments, including surface water, sediment, groundwater, soil, the atmosphere, drinking water, and food chains, including the agri-food chain [5–10]. Recent estimates indicate that we will reach levels of plastic waste of 11,000 million tons by 2025 [11]. Unfortunately, recycling is limited to around 9% of plastic waste, and the remaining 91% of plastic waste enters the environment [11], so environmental levels of MNPs will inevitably rise, resulting in increased MNP exposure to plants, animals, and humans [12–14].

The bulk of MNP research thus far has been performed in aquatic and terrestrial environments, using various invertebrate and vertebrate species and, more recently, humans. To properly understand MNP hazards and to protect the health of humans, domestic animals, wildlife, and our environment, a One Health perspective is warranted. One Health is defined as an integrated, unifying approach balancing and optimizing the health of people, animals and the environment [15]. For instance, both humans and animals are exposed to increasing levels of MNPs, and the mechanisms of toxicity, such as immunomodulation, DNA damage, and oxidative stress, are frequently conserved across species. Up until now, however, farm animals have largely been overlooked in the One Health approach to MNP research. Farm animals are both consumers and distributors of MNPs via animal-derived food products like milk, meat, and eggs for human consumption. The impact of MNPs on farm animals exposed to MNPs, which is impossible to avoid given the increasing load of plastics in the environment, may also extend to adverse effects on their health, including their ability to reproduce. As such, farm animals may serve as indicators of potential health hazards of MNP exposure in humans. The aim of this concise perspective paper is to signal the importance of farm animals as a critical link between environmental and human health impacts of MNPs (see Textbox and Fig. 1). To this end, we briefly summarize emerging evidence for sources of MNPs on the farm, exposure routes and levels of MNPs and potential health effects of MNPs in farm animals. We highlight the importance of better characterizing the occurrence and distribution of plastics on farms from feed and environment to animal, and humans via animal-derived food products, emphasizing a One Health approach. Finally, based on this synthesis, we describe

Fig. 1 Farm animals form a critical link in the plastic particle cycle. Plastic debris, including MNPs, originating from human activities, inadequate waste management and environmental conditions, is accumulating in the agricultural environment and enters farm animals through the uptake of MNP contaminated food sources and water. MNP uptake forms a potential risk for the health of farm animals and may result in MNP transfer to humans via animal-derived food products, which may potentially impact human health. MNPs are in the figure represented by particles with different shapes and colours

knowledge gaps and provide recommendations for future research.

Reasons to involve farm animals in MNP research from a One Health perspective

The three main reasons to integrate animals, particularly farm animals, when facing the current complexity of global plastic pollution:

1) Environmental Health: MNP pollution in the environment is growing, and leads to increased exposure of farm animals via food, water, and the air.
2) Animal health: MNP exposure may impact farm animal health Farm animal models allow us to gain a deeper understanding of the potential impact of MNPs on current and future generations.
3) Human health: MNPs may enter the human food chain via food products derived from farm animals, such as milk, eggs, and meat, posing health risks for humans.

Exposure to MNPs on the farm

In general, exposure to MNPs occurs through various routes, including soil, air, and water [5–10]. Activities associated with human behavior and transportation are leading causes of plastics entering the environment. This includes the release of plastic particles from single-use plastics, packaging, food waste, car tire particles, textile fibers, and paint particles. Inadequate waste management, such as open landfills, further contributes to environmental plastic contamination, exacerbated by conditions like flooding [16]. In the agricultural setting, plastics are extensively used in various applications, such as polymer-encapsulated fertilizers, agriplastic mulching and fruit protection foams, grass silage preserved in sealed plastic, and for animal feed pellets stored in plastic bags and in silos. MNPs are also distributed via the dispersion of sewage sludge on agricultural soils and farmland [17–19].

MNPs appear to be concentrated in soils [20]. The contamination of MNPs in soil varies greatly according to local conditions ranging from 0.36 particles per kg on agricultural fields in Southern Germany to estimations up to 42,960 particles per kg in China [21]. The UN Environment Program (UNEP) recently issued a statement warning that plastic degradation in farmer's fields may endanger food security, in particular through effects on soil health [22].

While grazing, farm animals can readily come into contact with MNPs via surface freshwater or oral uptake of MNPs via grass, as the accumulation of MNPs in plants has been confirmed [23]. A study in the Southeast of Mexico, with free-range chickens foraging around plastic waste containing gardens at traditional Mayan homes, reported concentrations of plastic particles in soil, earthworm casts, and chicken feces of on average respectively 870, 14,800 and 129,800 particles per kilogram, suggesting accumulation in the food chain [24]. In South Spain, up to 2,000 particles per kg soil of agricultural fields with plastic mulch used for sheep grazing have been reported [25]. In another study in South China, a widespread distribution of plastic particles was found in feed given to chickens, pigs, and cows, with respectively 96 ± 109, 139 ± 115, and 360 ± 630 particles per kg (wet weight) feed measured, which consisted of several different plastic polymers with polyethylene (PE) being the most prominent plastic type in feed of both pigs (60%) and cows (100%). In contrast, the level of PE was lower (30%) in feed of chickens [26]. The reported levels of MNPs may vary widely between studies, potentially due to the lack of standardized and validated methods, and the challenge of measuring extremely small nanoplastic (NP) particles. Nonetheless, they provide an initial picture of MNP contamination in the agricultural environment as well as in farm animals themselves. Currently efforts are ongoing to compensate for the lack of accurate NP particle measurements by the implementation of algorithms based on the number of the larger MPs present to estimate the amount of NP particles. For example, Luo et al. published a PCA-based algorithm where Raman spectroscopy was combined with scanning electron microscopy to estimate the number of MNP particles of Teflon [27]. Ideally, the accuracy of the algorithms can soon be optimized and tested with methods that can include the measurement of smaller NP particles.

Uptake of MNPs in farm animals

MNPs have been measured in the manure of farm animals, including sheep, pigs, chickens, and cows, at levels ranging from 10^2 – 10^5 particles per kg [24–26], confirming the actual uptake of MNPs by farm animals. MNPs have also been found in the digestive system of sheep [28, 29]. In a recent pilot study, plastic particle pollution was also reported in the blood of pigs and cows [30]. In this preliminary study in the Netherlands, all 12 blood samples of farm animals contained polyvinyl chloride (PVC-P), PE, and polymers of styrene (Styr-P) [30]. The presence of MNPs in the blood of farm animals indicates that after oral uptake of MNPs, the particles are not simply transported via the intestines and excreted via manure, which may still be the case for most of the MNPs [31], but are able to pass critical epithelial barriers in the body. Small fractions (~1%) of MNPs may be internalized in the gut or lung or other tissues [31]. Experimental studies in rodents reveal that pristine polystyrene (PS)-MNPs can indeed accumulate in several body tissues after oral uptake, including the lung, spleen, liver, kidney, intestine, brain, and reproductive organs like the testis and ovary [32–34]. Since uptake and transport mechanisms are largely conserved between vertebrates, these studies would also suggest that MNPs can pass these barriers in other animals and may accumulate in several tissues. However, monitoring and effect studies in farm animals are needed to investigate the effect of MNPs in these animals given their specific physiological differences. Cows, for example, have ruminal microbial flora that could potentially degrade certain MNPs [35], which may affect the levels of MNP exposure. Interestingly, in the aforementioned study that analyzed polymer types in food and manure from chickens, pigs and cows, the plastic polymer type found in the food of cows was PE (100%), whereas manure of these cows contained the plastic polymer type polypropylene (PP; 100%) [26]. The only difference between PE and PP is the replacement of an H atom by methane (CH3), a substance that is excreted in large amounts by cows. This finding suggests the transformation of polymers and in this specific case from PE into PP in the cow. Furthermore, in chickens the uptake of MNPs from the air may differ from rodents due to the presence of air sacs in addition to lungs. Since in chickens the pressure build up in the air sacs moves the air via the rigid lung. This is in contrast to the lungs of mammals that expand and retract during breathing, which may affect the routing of MNPs and the exchange of particles between the respiratory tract and circulation [36].

Potential health effects of MNPs

Upon exposure and uptake, MNPs can pose chemical, physical and microbiological hazards that may act cumulatively and may impair animal and human health [37]. With regards to the chemical hazard, a recent study showed that over 10,000 compounds can leach from plastics throughout their life cycle, with 2,400 considered potentially hazardous [38]. These include endocrine-disrupting chemicals that are known to affect hormone balance, leading to disorders on reproduction, development, and metabolism [39]. MNPs have the potential to transport these plastic-associated chemicals to target tissues, inducing cellular changes or bioaccumulation [40]. However, the relative importance of MNPs as carrier of hazardous hydrophobic organic chemicals is currently estimated to be low compared to other media [41]. There is some evidence that plastic particles may transport and release toxic chemicals into lungs or other organs, but it is unclear if the concentrations released will lead to toxicity. This issue is still under debate and requires further research [2, 42].

MNPs can affect animal and human health via the physical effects of particles, which are generally manifested in inflammatory and oxidative stress responses [2]. Commonly reported cell responses after exposure to MNPs in rodent studies are increased inflammatory markers and reactive oxygen species, suggesting an inflammatory and oxidative stress response that may eventually lead to apoptosis [11]. In male mice, oral exposure to 5 µm pristine PS-MPs (1000 µg/L) via water during 6 weeks affected the intestinal barrier, resulting in a shift in the structure and metabolism of the microbial flora [43]. Furthermore, exposure to pristine PS particles also affected metabolic pathways in mice based on the levels of metabolites in serum [43]. It should be noted that most in vivo and in vitro studies to date have been conducted at high doses of commercially available pristine spherical-shaped (virgin) PS as the model MNP. Several publications have addressed this issue [44]. For example, a recent review showed that the exposure levels of pristine PS MNPs for in vivo rodent studies are significantly higher than those of plastics measured in terrestrial soils [45]. It should also be noted that the effects of weathered, environmentally relevant MNPs on animal and human health remain largely unstudied. Accordingly, a recent study in our group demonstrated an augmented response of dendritic cells after exposure to weathered PS-NPs [46]. Further toxicological research using environmentally relevant concentrations of weathered MNPs from different plastic sources is clearly necessary to improve our understanding of the health impacts of MNPs. Studies on the microbiological effects of MNPs are also emerging. The specific niche of microbial life on the corona of MNPs, referred to as the 'plastisphere' may form a vector for pathogenic microorganisms and antibiotic resistance genes (ARGs)) [47–50], which can promote horizontal gene transfer in aquatic ecosystems [51]. This raises concerns about plastic particles introducing pathogens into the body, and enhancing infection risks through antimicrobial resistance [37]. Furthermore, the micro-organisms associated with MNPs have been related with a shift in the microbiome and may affect both animal and human health [44, 52]. In particular, farm animals such as cows may be sensitive, given their highly specialized physiology, i.e. the rumen and gut which require a well-functioning microbiome for fermentation and digestion [53–55].

The impact of MNPs on farm animal development and reproduction

MNPs may affect the susceptible period of reproduction and early life development, and the health of future generations [56]. The current information on the potential impact of MNPs on reproduction is predominantly based on aquatic species, soil fauna and, more recently, some studies in rodents [57]. MNPs can pass the blood-testis and blood-follicle barrier, giving them access to the immediate environment of sperm cells and oocytes, as demonstrated with rodent models [33, 34, 58]. In addition to the accumulation of PS-MNPs in the ovary and uterus, these studies have shown a negative impact on fertility after maternal exposure to MNPs, and a potential carry-over effect of maternal PS-MNPs exposure during gestation and lactation on the next generation, resulting in aberrant physiologic behaviour in progeny and a higher risk for metabolic disorders [32, 33, 59, 60]. Furthermore, depending on type and concentration, MNPs can impair the quality of sperm cells and oocytes in mice and increase granulosa cell apoptosis in rats [57]. Plastic particles are also able to pass the blood-placenta barrier, as demonstrated by the presence of MNPs at the fetal side of the human placenta [61] and suggested by the presence of plastic- associated chemicals in human amniotic fluid [62]. The demonstration of MNPs passing genital barriers indicates that we are surrounded by plastics from early life onwards, the gametes and embryo, until the end of life. However, much more information is needed to understand the effects of MNPs on early life.

Farm animals are not commonly used for studying reproductive and developmental health effects that originate from exposures during the early phase of development. The rodent model is much more commonly used as an animal model due to the easy access of large numbers of comparable animals from a defined strain, the smaller size, and the short generation time. However, farm animals, and, particularly cows, have great potential to be an appropriate model for in vitro early life studies. The bovine embryo model, which is based on the culture of oocytes derived from slaughtered cows' ovaries (rest material) and embryos derived after in vitro fertilisation (IVF), is a powerful model to study the periconception phase of humans. This has been used to investigate the effects of environmental stressors, for example, maternal metabolic stress in relation to oocyte developmental competence [63, 64]. The bovine embryo model is attractive due to the considerable similarities between bovine and human reproduction during oocyte and embryo development. In contrast to rodents, cows are mono-ovulatory (single oocyte ovulated), and their reproductive cycle length, follicular development, oocyte maturation length, oocyte size, and the timing of embryonic genome activation are comparable to humans [65]. Preliminary studies in our laboratory with the bovine embryo model demonstrated the uptake of pristine PS-NPs of 200 nm by the cumulus-oocyte-complex (Fig. 2; [66]) and indicate delayed oocyte maturation following exposure to 50 nm PS-NPs (unpublished data).

The above-mentioned studies indicate that MNPs could negatively interfere in the crucial process of oocyte nuclear maturation and development [66]. Due to the high sensitivity of oocytes and embryos to environmental perturbations, it is important to understand the impact of developmental exposure to MNPs, including the potential of MNPs to induce epigenetic modifications that may affect long-term health. By using the rest materials, from slaughtered animals, we gain a deeper understanding of the potential impact of MNPs on oocytes and embryos, knowledge which is difficult to obtain from humans because of ethical concerns. In addition, the use of rest materials from slaughterhouses reduces the use of experimental animals.

Exposure of humans to MNPs via animal-derived food products

In addition to potential health effects of MNPs in farm animals, MNPs in animal-derived food products form a source of exposure to these contaminants in humans. MNPs have been detected in processed, liquid and powdered dairy milk products [67–69]. The MNPs found in milk can be a consequence of the intake of plastic by

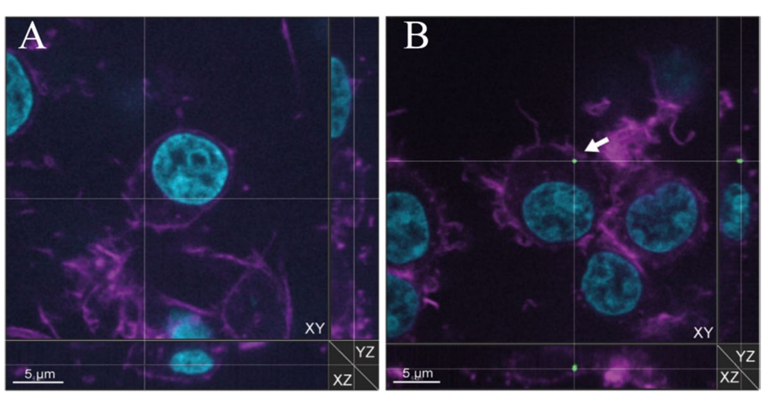

Fig. 2 Uptake of fluorescently labelled NPs by cumulus cells. Cumulus cells, nuclei in cyan and actin in magenta, support and surround the oocyte (not visible in picture) during development. Confocal microscopy images of A) control and B) NP-exposed cumulus cells. Cells were exposed to pristine 200 nm polystyrene (PS)-NPs for 23 h during maturation of the cumulus-oocyte-complex. The arrow shows one 200 nm PS nanoparticle. Scale bar is 5 µm

the cow via the air, food or water, as already discussed, or be introduced during different phases of the milking process, from the cow milking process at the farm and downstream processing, until final packaging in the milk factory [68]. A recent study showed that PE was the most prominent plastic type found in milk collected in the tank from the cows at the farm and most likely derived from the milking machines [30]. Polyethersulfone (PES) was also a highly abundant plastic type in milk, and this polymer is commonly used for membrane filters used in dairy and food processing [69, 70]. Since dairy products and substitutes constitute a significant portion of the human diet, the presence of MNPs in dairy products is of concern. For example, in The Netherlands, the mean uptake of dairy products was 333 g/day in the years 2012–2016 and was rated as the category with the highest level of daily food consumption [71]. In addition to dairy products, meat also appears to contain MNPs, as demonstrated in a study from Mexico where MNPs were found in the gizzard of poultry used for human consumption [24]. Packaged poultry meat also contained MNPs [72]. Recently, in a study performed in China, an average content of 11.6.7 ± 3.98 MP particles/egg, composed of mainly PE, has been detected in eggs from supermarkets [73]. MNPs may enter animal-derived products after uptake of MNPs by the animal, but can also be introduced during food processing. Currently, to our knowledge, there are no studies that investigate the presence of MNPs in raw milk, directly from the udder, or in eggs directly after laying, before the products are further processed for human consumption. Thus, we stress the need for further studies that investigate critical control points for the introduction of MNPs during the food process, from the animal to the consumer.

Conclusions and future research needs

The presence of MNPs in farm animals confirms that plastic particles introduced into the environment by humans are taken up by farm animals. The transfer of MNPs to the animal-derived food products that humans process and consume forms a source of human exposure to MNPs. It is also clear that MNPs have the potential to affect farm animal health through various mechanisms, and early studies with the bovine embryo model highlight the potential of farm animals to expand our understanding of the potential health effects of MNPs for the benefit of both animals and humans.

This perspective paves the way for follow up studies that focus on farm animals to achieve a better understanding of the plastic particle cycle on the farm and the transfer of MNPs between environment, farm animals, and humans. More research is needed to better characterize the distribution and abundance of different types of plastics in the agricultural environment, during the processing of animal-derived products for human consumption, and in animal-derived food products themselves, in order to pinpoint critical control points and to reduce exposure to animals and humans. This research is needed to guide the choice for plastic in livestock farming, including plastic packaging, water supply systems, and automatic milkers food processing, but also for plastics used for human consumption. It should be noted that, overall, reducing the use and waste of plastic in farm environments and during food processing seems desirable and beneficial not only for the animals but also for the human population.

Future studies are also needed to understand the impact of MNPs on the health in farm animals after exposure to MNPs, using a wider palette of polymers, weathered particles, at environmentally realistic concentrations and exposure durations. The role of farm animals as an indicator species for the health of other animals, including humans, warrants further experimental research, for example using the bovine embryo model. In addition, large-scale epidemiological approaches are needed to provide insights into environmental MNP exposures, associated health effects in farm animals, and transfer of MNPs into the human food chain, similar to studies performed in the past to trace the sources of dioxin and polychlorinated biphenyls in food [74]. With the daily expanding plastic pollution there is an urgent need to better understand the transfer of MNPs between environment, farm animals, and humans, and the potential of farm animals to serve as an indicator for other animals, including humans.

Abbreviations

IVF	In vitro Fertilisation
MP	Microplastics (1 µm – 5000 µm)
MNPs	Micro- and nano plastics
NP	Nanoplastics (< 1 µm)
PCBs	Polychlorinated biphenyls
PE	Polyethylene
PES	Polyethersulfone
POPs	Persistent organic pollutants
PS	Polystyrene
PVC-P	Polyvinyl chloride
Styr-P	Polymers of styrene
UNEP	UN Environment Program

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