Plastic is on the Table: Can We Manage to Reduce Micro- and Nanoplastics in Aquaculture Products?

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Some of the twentieth century's revolutions have greatly contributed to reducing the specter of world hunger. Among them are the industrial process for producing ammonia, which is indispensable for fertilizing agriculture fields and resulted in two Nobel Prize awards for chemistry (Fritz Haber in 1918 and Carl Bosch in 1931), and Norman Borlaug's studies on the genetics of cereals (Nobel Peace Prize in 1970).

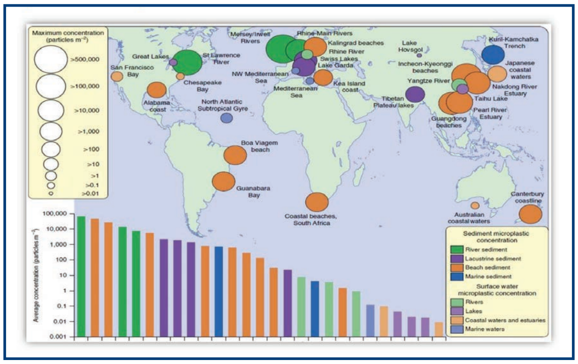

FIGURE 1. *The magnitude of microplastic pollution around the world (Source: Hurley* et al. *2018).*

Then, a number of scientific findings and technological solutions, mainly after the Kyoto FAO Conference in 1975, promoted the exponential development of aquaculture all around the world. Nevertheless, a new problem has arisen. The food produced after the "Green Revolution" as well as the food generated by the subsequent "Blue Revolution" might be threatened by the industrial products developed after another Nobel Prize for chemistry awarded to Giulio Natta and Karl Ziegler in 1963 for their study on polymers.

Being artificially created by humans, no microorganisms have been naturally developed to digest it. Therefore, the presence of micro- and nanoplastics in apparently any water source and agricultural food product, as well as in food products from fisheries and aquaculture, potentially represents one of the main food problems that science and technology should tackle in the twenty-first century.

Plastic materials are released from different sources (e.g. industrial and personal care products) into the aquatic environment (Fig. 1). The action of UV radiation, abrasion and aquatic organisms slowly fragment macroplastics into micro- and nano-size particles (Dawson *et al*. 2018). Small plastic particles may be defined as either microplastics (0.1 µm - 5 mm) or nanoplastics (<100 nm) (micro- and nanoplastics, MNP). Plastic particles are now ubiquitous in the environment, dispersed through the action of wind, waves and water currents. Even if the release of plastics would cease immediately, it is assumed that those already present in the aquatic environment would form a greater number of smaller particles and several centuries will be required for them to decay naturally.

Therefore, there is a need to gain knowledge of how to manage, control and detect the presence of these substances in food and to define an analysis of the risk that includes a still unknown threshold for humans. This is even more important considering the need to keep the human body capable of facing other threats that come from outside, such as infectious disease pandemics. Despite improvements in the analytical methods for detecting microplastics in water and food, monitoring small plastic particles in the nanometer range still represents a problem.

Effects of Microplastics on Humans

According to recommendations of the FAO and WHO, a risk analysis should involve three steps: 1) risk assessment, 2) risk management and 3) risk communication. Risk assessments for humans are generally based on studies carried out with rodents. As such data are incomplete or lacking for plastics, a formal hazard characterization of plastic particles for human health is not yet possible (FAO 2017); therefore, more accurate studies are required.

Nevertheless, some assumptions may be made according to existing literature. MNP contamination occurs mainly through food, drinking water and respiration, but other routes are also possible in humans. For instance, Pazzaglia *et al*. (1987) showed TEM electronic microscopy images of eroded polyethylene joint prostheses, in which eroded nanoparticles had evidently interacted with macrophage cells in patients, possibly representing harm to the innate immune system. This may be an example of so-called "frustrated phagocytosis," which is the failure of macrophages to engulf their targets and remove or destroy them, leading to a prolonged inflammatory response and possible tissue damage (van Raamsdonk *et al*. 2020).

It is estimated that 39,000-52,000 microplastic particles per capita per year are ingested with food by the American population, depending on sex and age (Cox *et al*. 2019); nevertheless, more than 90 percent of microplastics entering the intestine are released (EFSA 2016). Intestinal tight junctions can stop nanoparticles >1.5 nm in size but particles 0.1-150 µm in size may be absorbed via the lymphatic system (M cells of Peyer plaques) and then found in plasma, internal organs and urine (Galloway 2015); polyvinyl chloride (PVC) particles from 5 to 110 µm have been detected in the portal vein of dogs (Volkheimer 1975). Results from in vitro trials carried out utilizing intestinal models as well as in rodents in vivo indicate that only 0.04-0.3 percent of particles 2 µm in size are retained in the system, although nanoplastics 50 nm in size account for only 0.2-7.0 percent of this total (FAO 2017).

Gut inflammatory diseases increase the percentage of microplastics assimilated, as particles 3 µm in size were present in 0.20-0.45 percent of the administered dose of drugs used in treatments (Schmidt *et al*. 2013). Even with an absorption below 0.3 percent of

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the administered dose, plastics below 150 μm in size have been found in the portal and lymphatic systems of mammals, although it is unlikely that larger particles can be absorbed. Only microplastic particles ≤20 μm have been reported in adjoining internal organs, generally resulting in kidney and liver accumulation (FAO 2017). Nevertheless, once nanoplastics have been absorbed, they may be distributed to all internal organs and it cannot be excluded that they might also cross the blood-brain barrier.

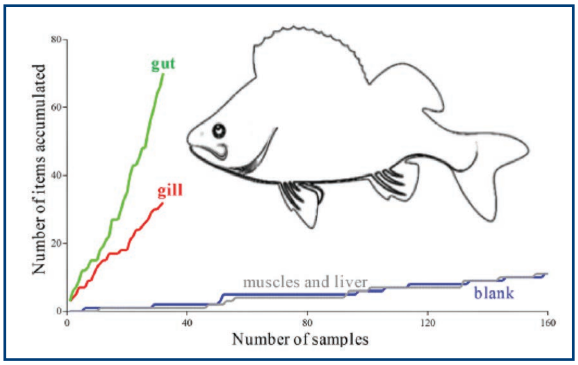

FIGURE 2. *Micro- and nanoplastics accumulate in different fish tissues and organs to varying degrees, but mainly in the gut. Fish samples represent 13 species collected from coastal estuaries of China (Source: Su* et al. *2019).*

The negative effects observed in both vertebrates and invertebrates include the immune response (Pazzaglia *et al*. 1987, Brandts *et al*. 2018) and oxidative stress in the central nervous system (Oberdörster 2004, Brown *et al*. 2001). In addition to other negative effects on human health (Galloway *et al*. 2015), a significant inverse association between exposure to 38.9-nm polystyrene nanoplastics and serum concentrations of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) was found in male rats (Amereh *et al*. 2020), thus indicating potential risk for human reproductive physiology as well.

Effects of Microplastics on Fish

Effects of MNP on fish growth, diseases and mortality have also been reported (Manabe *et al*. 2011, Pedà *et al*. 2016). In zebrafish, exposure to 1 mg/L of 47-nm polystyrene particles for 48 to 72 h was toxic, causing embryo mortality (Chenetal *et al*. 2017). Necrosis, infiltrations and liver lipid drops have been observed in zebrafish after 21 days of exposure to 2 mg/L of MNP 5 μm and 0.07 μm in size (Lu *et al*. 2016).

The effects of MNP change in relation to their size, molecular structure and interaction with plasma proteins (Waring *et al*. 2018, Cederwall *et al*. 2012). An interaction with proteins, creating a “protein corona” effect was reviewed by Nguyen and Lee (2017). MNPs may accumulate in fish tissues (Karami *et al*. 2017), affecting lipid metabolism (Cedervall *et al*. 2012) and fish feeding behavior (Mattson *et al*. 2017). Inflammatory responses have also been reported (Brown *et al*. 2001, Greven 2016). Damage in the distal intestine of 50-83 percent of European seabass *Dicentrarchus labrax* was observed after 30 days of feeding with polyvinyl chloride (PVC, 0.1 percent w/w) (Pedà *et al*. 2016). Even more serious intestinal damage occurred when PVC microplastics were collected from marine water polluted by hydrocarbon compounds.

Effects on different trophic levels in aquatic invertebrates suggest that 55-110 nm MNPs cause acute toxicity at 0.4-416.5 μg/mL (Casado *et al*. 2013). Survival and reproduction are reduced in *Daphnia galeata* after 5 days of exposure to 52-nm polystyrene particles (Cui *et al*. 2017).

Safe Levels of MNPs: Complexity and Uncertainty

Our knowledge is still in its infancy, although some of the recent literature may help to address the problem and suggest conditions potentially harmful for some aquatic animal species. Therefore, we clearly cannot afford not to manage the risk. No rules and policies regulating safety levels of MNP in food exist, whereas in feeds for animals, a contamination level up to 0.15 percent of plastics is generally tolerated, more for opportunistic reasons than a decision based on science.

Although a number of challenges on utilizing standardized MNPs were recently reported in the literature, this is not sufficient for a risk management approach. Indeed, a risk threshold for standardized MNPs that is reasonable is needed. Different risks of harm may be due to the chemical nature, shape and size as well as from interactions with other pollutants. The fact that additives such as bisphenol, phthalates and heavy metals are found in commercial plastics, allied with their ability to adsorb hydrophobic contaminant pollutants, can no longer be ignored.

Therefore, defining safe/tolerable levels to establish a reference target for a risk management approach, developing a database of fish responses to the MNPs present in the environment, and conducting experiments on rodents and collecting observations in humans could represent first steps in this process. It is necessary to find appropriate bioindicators and identify target organs and physiological interferences to develop accurate monitoring and management protocols; fish species may also represent good animal models for such studies. Nevertheless, accurately standardized experimental protocols should be developed, also allowing the comparison of the results produced by different research groups.

Microplastics as Food Contaminants

Today, no legislation exists for microplastics and nanoplastics as contaminants in food. Following a request from the German Federal Institute for Risk Assessment (BfR), the Panel for Contaminants in the Food Chain of the European Food Safety Authority (EFSA) delivered a statement on the presence of microplastics and nanoplastics in food, with a particular focus on seafood. Nevertheless, this report (EFSA 2016) does not include recommendations for a possible risk threshold because toxicity and toxicokinetic data are lacking for both microplastics and nanoplastics for a human risk assessment. At the same time, it was recommended that an analytical method be further developed for microplastics and nanoplastics to assess their presence and to identify and quantify the amounts in food. Furthermore, a database should be generated documenting their occurrence in food, in particular for smaller-sized particles (<150 μm).

Limited data are available concerning microplastics in food, including agriculture foods, seafood species such as fish, shrimp and bivalves, as well as for other foods, including honey, beer, table salt and tap water. In honey, 0.166 microplastic fibers/g have been reported, in beer 0.017-0.033 particles/g, and in table salt 0.007-0.680 particles/g.

In seafood, microplastics in marine species are concentrated mainly in the digestive tract (Fig. 2). The average number of particles was 1-7/g in fish, 0.75 particles/g in shrimp and 0.2-4/g in bivalves. Although fish are eaten after removing the gut, bivalves are eaten without removing the digestive tract. Therefore, by consuming 225 g portion of mussels, up to 900 pieces of microplastic are consumed. Small microplastics and nanoplastics are reported to pass the fish intestinal barrier and to be transported in the muscle; nevertheless, because of the difficulties in assessing nanoplastics, little data is available about their presence in fish fillets, and in some cases, a revision of the same data is envisaged.

Touissaint *et al*. (2019), in a review that analyzes peer-reviewed publications since 2010, documented the presence of MNPs in the human food chain. Along with Hantoro *et al*. (2019), they concluded that it is still not possible to assess human exposure to MNPs through food consumption due to a scarce availability of data and a lack of standardization of methodologies.

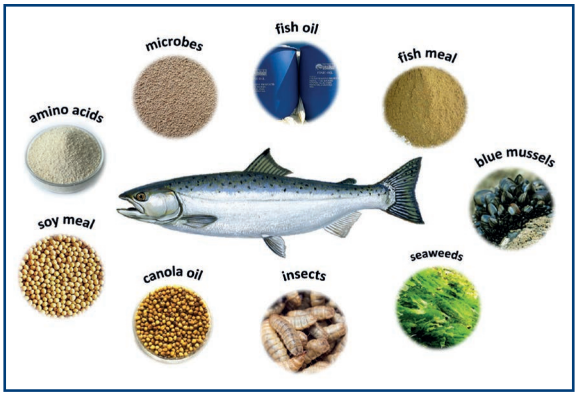

FIGURE 3. *Various protein and oil components of fish feed may potentially carry micro- and nanoplastics (Source: https://analyticalscience.wiley.com/do/10.1002/gitlab.16837/full/#media-1).*

Microplastics in Food and Feed of Farmed Seafood

Being aware of the existing difficulties, a question that arises when risk management in fish and shellfish is carried out would be: Can we tackle the control of micro- and nanoplastics, at least in farmed seafood? Fish may assimilate MNP from feed and the water (Su *et al*. 2019). Separating micro- and nanoplastics from water and feed may represent an economically insurmountable problem with existing technologies. Roch *et al*. (2020) compared wild-feeding fish and farmed fish when feeding on the environment. They also considered fish feeding behavior by comparing visual foraging and chemosensory foraging fish. Among farmed fish, a developed sense of taste limits the unintentional ingestion of microplastic particles. The same authors reported experimental data indicating that fish actively foraged on microplastics when no food was available.

A huge difference in the content of microplastics has been reported in water from different world regions, ranging from 10^{-6} to 10^4 microplastic particles/m^3 (Shim *et al*. 2018). Moreover, along the coast, placement of net pens in areas with major accumulation of MNPs due to the tidal currents should be avoided. Adequate feeding strategies that avoid loss of feed pellets should also be applied. Although few data are available on the micro- and nanoplastics content in fishmeal, important differences may be expected in fishmeals of different oceans of origin (Fig. 3). Moreover, a compromise between the nutritional value and the micro- and nanoplastics content in other protein sources, such as cereal grain meals, insect meals, poultry meals and in lipid sources, could possibly lead to the production of a feed in which the content of micro- and nanoplastics is reduced to a minimum.

Depuration

While waiting for a threshold of risk to be developed for food, a precautionary approach would suggest reducing insofar as possible the amount of plastics in the food we consume. Seafood from different world regions may be contaminated by MNPs to different degrees due to different levels of local contamination in the water and food chain (Shim *et al*. 2018). In contrast, the objective to exert some control over farmed fish and shellfish does sound reasonable, at least to some extent.

Therefore, any tentative plan to depurate seafood that assimilated micro- and nanoplastics from water or from the natural food chain, or removing MNPs from the flow water used in aquaculture and from the ocean where pen cages are immersed, sounds idealistic. Nevertheless, the development of technologies to depurate mussels in a closed system such as a depuration basin and intensive water recirculation systems (RAS) for fish farming might be feasible after adequate effort is invested in research: here, preliminary results are already available from our laboratory (M. Saroglia *et al*., unpublished data).

The Need for a Precautionary Approach

The main reviews reporting communication of the risk for humans are EFSA (2016), which reported "no evidence of risk," and FAO (2017). The adverse effects of microplastic ingestion underscored by the FAO have only been observed in aquatic organisms under laboratory conditions, while in wild aquatic organisms microplastics have only been observed within the gastrointestinal tract, which at least in finfish is removed before eating. Plastics found in terrestrial and marine environments, nanoplastics in particular, can enter the human body, either by inhalation or ingestion, particularly of shellfish and crustaceans (Waring *et al*. 2018). Even if absorption across the gastrointestinal tract is relatively low, nanoplastics that are more readily absorbed may accumulate in the brain, liver and other tissues in aquatic species and other animals, suggesting that toxicity could potentially affect the central nervous and reproductive systems.

Although EFSA (2016) reported "no evidence of risk," we should bear in mind that it is different from "evidence of no risk." Thus, together with the recommendation to dedicate financial resources to studies oriented toward the management of the MNPs risk, a precautionary approach should be applied to reduce the consumption of micro- and nanoplastics with food as much as possible.

Notes

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