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Risk assessment and toxicological research on micro- and nanoplastics after oral exposure via food products

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Abstract

Plastics are used ubiquitously and have become part of our everyday life. The global production of plastics is rising, which in consequence is leading to increasing amounts of plastics being released into the environment. Recently, the issue of human exposure to micro- and nanoplastic particles and potentially resulting toxicological consequences has been broached, triggered by the discovery of microplastics in foodstuff. In addition to dietary exposure via contaminated food and beverages, other exposure paths such as via air and cosmetics, have to be considered. Currently there is no legislation for microplastics and nanoplastics as contaminants in food. Substantial data gaps with respect to exposure as well as toxicity of such particles impede the risk assessment. Within this EU-FORA fellowship project, a comprehensive data mining approach was followed, focusing on up-to-date knowledge on the occurrence and possible toxic effects associated with micro- and nanoplastics after oral exposure, especially via food products and beverages, in order to provide a basis for risk assessment and to identify important research gaps. The fellowship project was further complemented by practical work aimed at the determination of *in vitro* toxicity of micro-sized polylactic acid particles.

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Keywords: microplastics, nanoplastics, food, occurrence, toxicity, risk assessment

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1. Introduction

The present work dedicated to the risk assessment of micro- and nanoplastics after oral exposure via food products was performed in the context of the EFSA EU-FORA fellowship programme. This programme is aimed at early- to mid-career scientists from EU authorities or other Article 36 organisations, to increase their knowledge and experience in food safety risk assessment by practical training (Bronzwaer et al., 2016). The fellow was hosted by the German Federal Institute for Risk Assessment (BfR), Department of Food Safety, jointly by the unit 'Effect-based Analytics and Toxicogenomics' and the Junior Research Group Nanotoxicology.

Plastic material is ubiquitously used in human daily life and is therefore also released into the biosphere. Due to its material properties, in particular its chemical stability, low reactivity and poor degradability, it can accumulate in the environment and reach the animal and human food chain (Bouwmeester et al., 2015). During presence in the environment, various factors can contribute to the decomposition of larger plastic fragments into smaller pieces, the so-called micro- and nanoplastics. The definition of microplastics varies depending on the source. For this reason, in this report microplastics will be referred to plastic particles of different materials and shape, namely fragments, fibres, spheroids, granules, pellets, flakes or beads, in the range of 0.1–5,000 μm , in accordance to the EFSA opinion on the presence of microplastics and nanoplastics in food, with particular focus on seafood, microplastics (EFSA, 2016). A distinction can be made between primary (intentionally produced) and secondary microplastics. According to the European Food Safety Authority (EFSA) and the European Commission's recommendation on nanomaterials definition, nanoplastics can be defined as a material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale (0.001–0.1 μm) (EFSA, 2016, European Commission, 2011). In general, there is very little information available on the environmental occurrence and toxicity of nanoplastics.

Recent publications suggest that different types of food and beverages are contaminated with microplastic particles, underlining the relevance of human exposure. Especially smaller particles, which are in a size range of several micro- or millimetre, gained public attention. Up to now, little is known about the risk for human health caused by exposure to micro- and nanoplastics via the diet. With the focus on risk assessment, there are still many data gaps which need to be addressed. First, the detection and quantification of microplastics is still very challenging and available analytical methods are not suitable for all size ranges and food matrices. Detection methods need to be established and validated. Second, microplastics contain a broad mixture of materials, surface properties and material characteristics. Therefore, general statements about 'microplastics' need to be specified in detail to different particle types and cases. Third, little is known about possible effects which can be caused by the oral consumption of microplastic particles. No systematic human exposure studies are available, and data from *in vitro* studies or *in vivo* animal experiments are scarce.

2. Description of work programme

2.1. Aims

The aims of the programme were as follows: first, the fellow should gain experience in performing risk assessment, with a primary focus on micro- and nanoplastics found in food products or beverages. For this purpose, comprehensive literature mining was performed to collect published information and identify data gaps (see Section 2.2.1). Second, the fellow should gain knowledge about practical methods used for the physicochemical characterisation of micro- and nanoplastic particles, as well as for the investigation of their toxicity. In addition, the programme aimed at introducing the fellow to a broader spectrum of risk assessment activities by a combination of in-house and external education activities. The expected scientific outcome of the project was to make a substantial contribution to the risk assessment of micro- and nanoplastics at BfR.

2.2. Activities/methods

2.2.1. Comprehensive revision of available data

Efforts towards a risk assessment of micro- and nanoplastics, i.e. their occurrence as well as their possible health effects when taken up orally via food products, were based on publicly available data, up to and including 1 May 2020. A comprehensive literature search was performed for peer-reviewed articles referring to the presence of micro- and nanoplastics in food and beverages, as well as their

toxicity *in vivo* and *in vitro*. Scientific databases such as 'PubMed', 'Scopus', 'Google Scholar' and 'Web of Science' were searched using various keyword combinations (e.g., "polymer", "microplastics", "nanoplastics", "food", "toxicity", "*in vivo*", "*in vitro*"). Information was extracted from the literature and compiled, and the studies were classified with respect to their quality and suitability for risk assessment. Using data retrieved from the above databases, the fellow had the opportunity to work on a scientific review article jointly authored by the fellow and BfR employees, which has been submitted to a peer-reviewed toxicological journal. In addition, the fellow presented a comprehensive review of available data on the occurrence of micro- and nanoplastics in food and toxicity effects as a poster at the 5th German Pharm Tox Summit held in Leipzig, 2–5 March, 2020.

2.2.2. Practical work, research project on polylactic acid particles

To attain the goal of introduction to micro- and nanoplastics research tools, the fellow has been involved in ongoing research activities of the Department of Food Safety at BfR. The main training objectives included analysis and evaluation of data for preparing working documents, related to the assessment of micro- and nanoplastics via oral exposure, in particular the occurrence and toxicological data. The fellow had the opportunity to learn about *in vitro* models and their applicability to research on micro- and nanoplastics toxicity. To this end, practical work was performed in the laboratory. This included the cultivation of the adherent stable human intestinal cell line Caco-2 in a submerged two-dimensional model as well as the *in vitro* Transwell barrier model. The fellow performed viability testing using colorimetric assays, transepithelial resistance measurements and particle leaching experiments. From the analytical field, the fellow was introduced to dynamic light scattering, element analysis via atomic absorption spectroscopy, and flow cytometry. The fellow was introduced to working with a ball mill and a knife mill for particle grinding. In addition, as part of a visit at the German Federal Institute for Materials Research and Testing, the fellow got insight into artificial *in vitro* digestion, small-angle X-ray scattering and multi-angle light scattering. Polylactic acid (PLA) particles were chosen for experimental investigation.

2.2.3. Training in risk assessment

During the initial phase of the fellowship, the fellow obtained general information on risk assessment activities. This included three weeks of induction training in chemical risk assessment and microbiological risk assessment at the EFSA premises in Parma (2–20 September 2019). An additional 1-week training module on food and feed safety-related risk assessment organised by Austrian Agency for Health and Food Safety (AGES) took place in Vienna (25–29 November 2019). Due to the COVID-19 pandemic, further courses were held as online modules, namely a training module focusing on risk communication and crisis response in Berlin, organised by the BfR (10–14 August 2020), and an online training module focusing on emerging risks in Greece organised by the Hellenic Food Authority (EFET; 24–31 August 2020).

Additionally, at the hosting site BfR, the fellow was acquainted with the risk assessment procedures carried out at the department of food safety. Using current practical examples, the fellow was specifically introduced to the risk assessment of food and feed derived from genetically modified organisms, as well as to the risk assessment of novel foods. The fellow participated in the regular weekly meetings on the current scientific work carried out at the department of food safety at BfR. Moreover, the fellow presented and discussed the results of her project at the department seminar on 7 July, 2020.

Along with the scheduled activities, additional training opportunities were provided by the hosting institution BfR. This helped further improving the fellow's general knowledge on risk assessment. Table 1 presents the supportive training activities organised for the fellow by BfR during the EU-FORA Fellowship.

Table 1: Supportive training activities during the EU-FORA fellowship

	Title	Date
Training sessions	Workshop "Risk Assessment and Risk Management of Genetically Modified Organisms", BfR, Berlin, Germany	9.6.2020
	Workshop "Risk Assessment - Food contamination by plasticisers", BfR, Berlin, Germany	5.5.2020
	10th Berlin Workshop on Developmental Toxicology, BfR, Conference center Berlin Biotechpark	19–20.2.2020
	Creating characters for the BfR as a new line of communication, Berlin, Germany	10.1.2020
	Seminar and workshop "Harmonized exchange of food safety models using web-based services from RAKIP and the AGINFRA + project"	9.12.2019
	An Introduction to the Library of BfR and its Services	15.11.2019
	Testing the study appraisal methodology for the re-evaluation of BPA safety. EFSA webinar	14.11.2019
	Seminar and workshop "Big data and high-throughput driven modeling of health effects of environmental agents"	6.11.2019
Other activities	Submitted review article at Nanoscale Advances-Royal Society of Chemistry "Micro- and Nanoplastics – Current State of Knowledge with the Focus on Oral Uptake and Toxicity"	29.6.2020
	Scientific Conference: One Health EJP Annual Scientific Meeting 2020 (OHEJP ASM 2020), online event	27–29.5.2020
	Scientific Symposium (Poster): 'Risk Assessment review of micro-plastics found in food' presented at the 5th German Pharm-Tox Summit Leipzig, Germany	2–5.3.2020
	Visit at the German Federal Institute for Materials Research and Testing (BAM)	20.11.2019

3. Results and discussion

3.1. Results of the comprehensive literature review

Analysis of the available literature revealed that up to now, there is no sufficient amount of reliable information on the occurrence, composition, particle size and quantity of micro- and nanoplastic particles in food. Ultimately, microplastics can enter the environment in several ways and enter the food chain via the air, seawater, fresh or underground water. It is considered secured that there is an oral exposure to microplastic particles. Microplastics have been detected in a number food products, such as for example drinking water, beverages, honey, mussels, and table salt (see Table 2). Data on nanoplastics is barely available. According to the published data no firm conclusions can be drawn about the quantity and composition of the detected microparticles. Nevertheless, some initial studies worked on the subject of oral exposure. It is obvious that only a small fraction of the broad spectrum of food products has been investigated so far. This includes mainly aqueous and/or simple matrices such as water, beer or honey. The investigation of complex matrices like meat or dough is analytically more challenging. Only for seafood, some results are available, probably due to the high relevance of plastic contaminations in aquatic ecosystems. A comprehensive exposure assessment was not possible since there is a lack of representative data on the occurrence of microplastics in different food groups. Only some selected examples are available (Cox et al., 2019). Another obstacle was that there is no appropriate tool to quantify the dietary exposure of micro- and nanoplastics.

Table 2: Selected results on the occurrence of micro- and nanoplastics in food

Food type	Level of microplastics contamination	Samples	Location	References
Honey	40–660 coloured fibres/kg of honey, with a mean value of 166 ± 147 /kg of honey; 0–38 fragments/kg of honey; mean 9 ± 9 /kg of honey; fibres and fragments supposed to be synthetic	19	From Germany, France, Italy, Spain and Mexico, from supermarkets (8) or producers (11)	Liebezeit and Liebezeit (2013)
	10–336 fibres/kg and 2–82 fragments/kg supposed to be synthetic	47	From German local supermarkets or beekeepers	Liebezeit and Liebezeit (2015)
	32–108 coloured fibres/kg (mostly cellulose but a minor part being PET fibres); 8–28 blue particles/kg (unknown origin)	5	From 5 locations in Switzerland	Mühlschlegel et al. (2017)
Salt	16–84 item/kg (sea salt), 8–102 item/kg (lake salt) and 9–16 item/kg (rock salt)	16	From Turkish market	Gündoğdu (2018)
	46.7 ± 0.58 – 806 ± 15.3 particles/kg (average: 212 particles/kg, all positive)	12	From US grocery stores (from North, Celtic, Sicilian, Mediterranean, Utah, Hawaiian, Atlantic, Pacific, Baja Seas and Himalaya)	Kosuth et al. (2018)
	1–10 items/kg (16 positive)	17	From a Malaysian market originating from 8 countries (Australia, France, Iran, Japan, Malaysia, New Zealand, Portugal, South Africa)	Karami et al. (2017)
	50 ± 7 – 280 ± 3 items/kg salt	21	From sea: Atlantic Ocean (Huelva, Cádiz, Lanzarote, La Palma, Galicia), Mediterranean sea (Barcelona, Gerona, Valencia, Alicante, Murcia, Menorca) From well: Alicante, Cuenca, Añana	Iñiguez et al. (2017)
	7–680 items/kg (550–681 particles/kg in sea salts, 43–364 particles/kg in lake salts, and 7–204 particles/kg in rock/well salts)	15	From supermarkets throughout China	Yang et al. (2015)
Sugar	217 ± 123 transparent and coloured fibres/kg of sugar; 32 ± 7 fragments/kg of sugar	5 commercial sugars	–	Liebezeit and Liebezeit (2013)
Fish	1–3 items/contaminated brand (4 contaminated brands – cans containing 2–30 fish [canned sardines and sprats] each)	20 brands (cans containing 2–30 fish each)	From Australian and Malaysian markets (originating from Canada, Germany, Iran, Japan, Latvia, Malaysia, Morocco, Poland, Portugal, Russia, Scotland, Thailand, Vietnam)	Karami et al. (2018)

Food type	Level of microplastics contamination	Samples	Location	References
Beer	2–79 fibres/L (RSDs = 130%), 12–109 fragments/L (RSDs = 205%), 2–66 granules/L (RSDs = 103%)	24	From German local supermarkets	Liebezeit and Liebezeit (2014)
	16 ± 15 fibres/L (Blank sample: 15 ± 9 fibres/L); 21 ± 16 fragments/L (Blank sample: 20 ± 13 fragments/L); 27 ± 10 granules/L (Blank sample: 15 ± 12 granules/L)		–	Lachenmeier et al. (2015)
	0–14.3 particles/L (average: 4.05 particles/L, all positive)	12	From breweries using water from the five Laurentian Great Lakes (US)	Kosuth et al. (2018)
Bottled water	10.4 particles (> 100 µm)/L; 325 particles (6.5–100 µm)/L; 242 bottles contaminated, all single-use plastic bottles except 1 glass bottle	259	From 19 locations in 9 countries (China, USA, Brazil, India, Indonesia, Mexico, Lebanon, Thailand, Germany) – 11 brands	Mason et al. (2018)
	118 ± 88 particles/L (returnable bottles), 14 ± 14 particles/L (single-use plastic bottles), 11 ± 8 particles/L (beverage cartons), 50 ± 52 particles/L (glass bottles)	22 bottles, 3 cartons, and 9 glass bottles	From grocery stores in Germany	Schymanski et al. (2018)
Tap water	0–61 particles/L (average: 5.45 particles/L)	159	From Cuba (1), Ecuador (24), England (3), France (1), Germany (2), India (17), Indonesia (21), Ireland (1), Italy (1), Lebanon (16), Slovakia (8), Switzerland (2), Uganda (26), US (36)	Kosuth et al. (2018)
	Particles > 100 µm: 15.6 particles/50 L (Blank sample: 13.2 particles/50 L) Limit of detection = mean blank + (1.645 × SD for blank) = 29 particles/50 L	17 + 3	Danish tap water	Strand et al. (2018)

Little is known about toxic effects of orally ingested microplastics (Bouwmeester et al., 2015). Due to its inert characteristics, direct effects of the plastic material are only likely at extraordinary high doses, which are often referred to as overload situations. Here, it is not possible to determine a precise molecular mode of action or clear dose–response relationships, since many of these effects are likely to be caused by rather unspecific stress reactions. Another problem is that the vast majority of available studies only show effects of polystyrene particles, since these particles provide the best commercial availability for researchers. Other more abundant materials such as polyethylene, polypropylene or polyvinylchloride are only available as polydisperse powders which are not so well-suitable for experimental investigations unless there are fractioning steps included. Taken together, toxicological investigations of microplastics give only an exemplary insight into possible health impact and do not yet allow for a systematic hazard assessment.

A selection of available studies which investigated possible toxicological effects are shown in Table 3. Many of these studies have been performed with invertebrates, mussels or fish, which means that the results are difficult to compare with the situation in humans. Only a few studies were performed with mice, and no human data is available. The variety of possible effects ranges from inflammatory responses, disturbance of the reactive oxygen species levels and impairment of the immune system to unspecific cellular damage, like for example cell growth disturbance, disturbance of cellular transport processes of cell division, whereas other studies showed that toxicity did not occur even at high concentrations or doses of microplastic particles. Noteworthy, microplastics might also function as a carrier of environmental contaminants, which is referred to as the so-called ‘Trojan horse mechanism’. Since there are still many data gaps concerning dose–response relationships, it is not possible to estimate the risk for human consumers, which are exposed to microplastics via the diet.

Table 3: Selected toxicological effects of micro- and nanoplastics

Toxic effects	Microplastics	Model	Main findings	References
Gastrointestinal toxicity	PE	Blue mussel <i>Mytilus edulis</i> L.	Notable histological change and a strong inflammatory response	von Moos et al. (2012)
	PS	Adult male zebrafish	PS microplastics increased the expression of IL-1 α , IL-1 β and interferon in the gut; indicated microbiota dysbiosis and inflammation	Jin et al. (2018)
	PA, PE, PP, PVC and PS	Zebrafish and nematode	Villi cracking and splitting of enterocytes	Lei et al. (2018)
	PS	Male mice	Accumulation of PS microplastics in mice guts, consequently caused the reduction of intestinal mucus secretion damage of gut barrier function; metabolic disorders in mice	Jin et al. (2019)
	PS	AGS cells	Inflammatory gene expressions such as IL-6 and IL-8	Forte et al. (2016)
Liver toxicity	PS	Zebrafish	Inflammation and lipid accumulation both in 5 μ m and 70 nm; oxidative stress and alterations in their metabolic profiles; disturbance of lipid and energy metabolism	Lu et al. (2016)
	PS	<i>Eriocheir sinensis</i>	Decreased activities of AChE, CAT, and ALT in <i>Eriocheir sinensis</i> liver; antioxidants CAT, SOD, GPx and GST level decreased in the liver; expressions of the genes encoding p38 in the MAPK signalling pathway was upregulated while significantly declined in ERK, AKT and MEK	Yu et al. (2018)
Liver toxicity	PS	Mouse	TG and TCH levels decreased; decreases on key gene expressions related to lipogenesis and TG synthesis in liver indicating mouse hepatic lipid disorder	Lu et al. (2018)
	PS	Zebra mussel <i>Dreissena polymorpha</i>	Dopamine concentration increased	Magni et al. (2018)
Neurotoxicity	PS	T98G cells	Increases of ROS, oxidative stress	Schirinzi et al. (2017)
Reproductive toxicity	PS	Oysters	Oocyte number, diameter and sperm velocity decreased in oysters	Sussarellu et al. (2016)
	PS	acs-22 mutant <i>Caenorhabditis elegans</i>	Accumulation of nanopolystyrene particles in gonad, dysregulation of some oxidative stress genes	Man et al. (2018)

PA: polyamide; PE: polyethylene; PP: polypropylene; PVC: polyvinylchloride; PS: polystyrene.

3.2. Results of the Analysis of polylactic acid particles

PLA particles were obtained from a commercial provider as PLA granules in the millimetre range and were ground using a knife mill. The resulting particle size was determined using a light microscope and dynamic light scattering for the smaller fraction. The particles showed a very broad size distribution, mostly in the micro- and millimetre range.

Two consecutive cell viability assays were used for the determination of PLA toxicity. Cell viability was first determined by the CellTiter Blue (CTB) assay followed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to a previously developed protocol (Sieg et al., 2018). For this purpose, differentiated Caco-2 cells were incubated with 200 μ L of different concentrations of PLA microparticles for 24 h and 48 h in 96-well plates. After incubation, 100 μ L of the media was removed and 40 μ L CTB reagent was added to the cells. After 30 min, fluorescence was determined with a Tecan plate reader (Ex. 560, Em. 590 nm). Next, 10 μ L of the MTT reagent in

PBS was added for another 1 h. After that, whole media was removed and 130 μ L pre-warmed desorption solution (0.7% w/v sodium dodecylsulfate in isopropanol) was added. Plates were shaken for 30 min and absorption was measured by a plate reader (570 nm) with subtraction of the background absorption (630 nm). Results were normalised to untreated controls after subtraction of treated cell without reference wells. Triton X-100 solution (0.01%) was used as a positive control. The experimental scheme is shown in Figure 1.

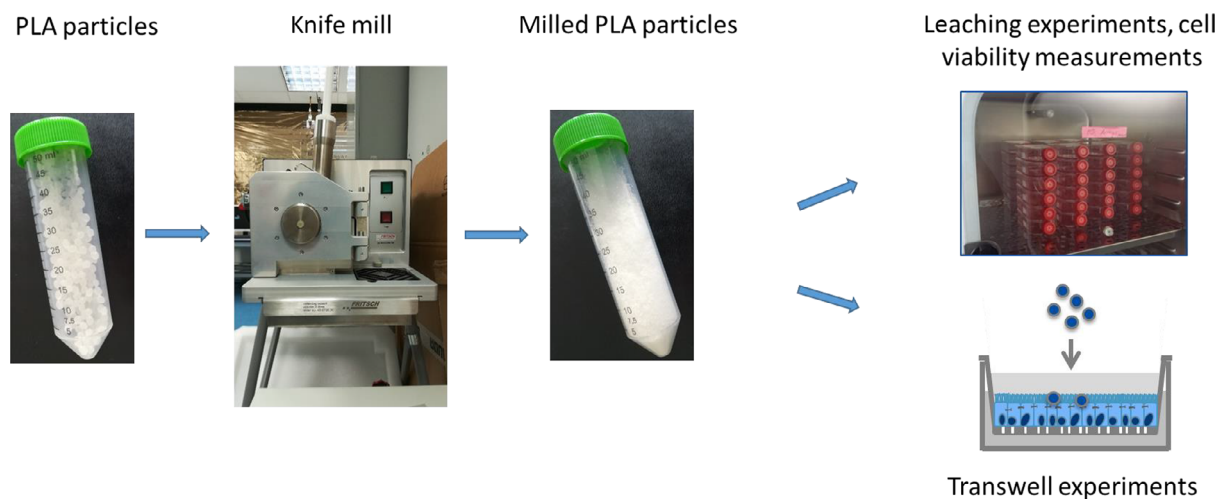


Figure 1: Experimental scheme of PLA particle generation and investigation

None of the PLA microparticles showed substantial cytotoxicity on Caco-2 cells after 24 h or 48 h of treatment. A weak toxic effect was observed with a high concentration of 500 μ g/mL only. In addition, leaching experiments in cell culture medium over a period of up to one week showed no altered cell viability on Caco-2 cells when incubated with centrifuged particle supernatant.

4. Conclusions

Overall, the work programme allowed the fellow to gain knowledge on the assessment of micro- and nanoplastics in food. On the one hand, a core area of training was on retrieving, analysing and evaluating available data, focused on the occurrence and toxicological profiles of micro- and nanoscaled plastic particles. On the other hand, the fellow gained knowledge in practical research work on micro- and nanoplastics, with respect to both, particle characterisation and *in vitro* toxicity assessment. The work on plastic particles was embedded into the overall context of food risk assessment and opportunities for scientific networking and collaboration. Results of the fellow's project were presented as a conference poster and will become part of a scientific paper on the oral uptake and toxicity of micro- and nanoplastics to be published in a peer-reviewed scientific journal.

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Abbreviations

AGES	Austrian Agency for Health and Food Safety
BAM	German Federal Institute for Materials Research and Testing
BfR	German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)
CTB	CellTiter Blue assay
EFET	Hellenic Food Authority
EU-FORA	European Union Food Risk Assessment fellowship programme
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay
PA	polyamide
PE	polyethylene
PLA	polylactic acid
PP	polypropylene
PS	polystyrene
PVC	polyvinylchloride