25th EFSA colloquium

'A coordinated approach to assess the human health risks of micro- and nanoplastics in food'

6-7 May 2021

Online event

BOOK OF ABSTRACTS

Acknowledgements: EFSA wishes to thank for the support provided to this conference: EFSA Scientific Programme Committee: Angelo Maggiore, Aleksandra Lewandowska, Hans Verhagen (till 31/10/2021), Marco Binaglia, Karen Mackay, Ana Afonso, Sandra Rainieri, Domagoj Vrbos, Giorgia Zamariola, Marta Hugas, Stef Bronzwaer (EFSA); External Advisory Committee: Peter Simpson (European Chemicals Agency), Birgit Sokull-Klüttgen (Joint Research Centre, European Commission), Uta Faure, (Directorate-General for Research and Innovation, European Commission), Veerle Vanheusden, (Directorate-General for Health and Food Safety, European Commission), Lisa Scheuermann (World Health Organization); Technical organising Committee: Vanessa Descy, Cinzia Percivaldi, Lucia Parrino, Anthony Smith (EFSA)

Disclaimer: Please note that for the abstracts of the Oral Presentations only the affiliation of the presenters are listed.

Contents

Conference

Microplastics are small particles of plastic less than 5mm in size; nanoplastics are even smaller, ranging from 1 to 100 nanometres (N.B. 1 nanometre equals one-millionth of a millimetre).

In 2016, EFSA identified the need to generate more data on their occurrence levels in food and on their potential effects on human health following exposure. Recently, international reports on microplastics and nanoplastics have been published, yet many of the food safety aspects remain unaddressed.

Public concern about this topic is high. EFSA's 2019 Eurobarometer on Food Safety in the EU found that microplastics in food was a topic of increasing concern among EU citizens.

Therefore, EFSA is holding a scientific colloquium with the following objectives:

- bringing stakeholders together,
- understanding the current state of play and ongoing research on this topic
- building possible synergies,

The colloquium is held online on 6 and 7 May 2021

Conference programme

DAY 1

OPENING SESSION | micro- and nanoplastics in food and feed: setting the scene **Chair: Helle Knutsen, former chair of CONTAM Panel, member of NDA Panel (NO)** **Co-chair: Tobin Robinson, EFSA**		
09:30-09:35	**Welcome and introduction to the event by the chair**	
09:35-09:45	**EFSA: general welcome**	Barbara Gallani (EFSA)
09:45-09:50	**Outline of the event**	Helle Knutsen (FHI, NO)
09:50-10:20	**Micro- and nanoplastics and human health** Followed by Q&A	Bart Koelmans, (WUR, NL)
10:20-10:40	**Interplay between science and society: the evolution of research, public discourse and societal concerns** Followed by Q&A	Domagoj Vrbos (EFSA) & Severine Koch (BfR, DE)
10:40-11:00	**Regulatory needs and challenges to inform fit-for-purpose research activities on micro- and nanoplastics, including outcomes of the SAM report** Followed by Q&A	Veerle Vanheusden, (DG SANTE, EC)
11:00-11:20	**Coffee/Tea break**	
SESSION 1 | Developments in analytical methods for micro- and nanoplastics		
11:20-11:50	Keynote lecture: **Definition of micro- and nanoplastics & analytical challenges** Followed by Q&A	Douglas Gilliland, (JRC, EC)
11:50-12:10	**Evaluation and optimization of extraction methods suitable for the analysis of microplastic particles occurring in the edible part of seafood** Followed by Q&A	Julia Süssmann (MRI, DE)
12:10-12:30	**Raman Tweezers for Small Microplastics and Nanoplastics Identification in liquids** Followed by Q&A	Pietro Giuseppe Gucciardi (CNR, IT)
12:30-14:00	**Lunch**	
SESSION 2 | Developments in exposure of humans to micro- and nanoplastics		
14:00-14:25	Keynote lecture: **Human exposure to micro- and nanoplastics: what drives citizens' concerns?** Followed by Q&A	Sabine Pahl, (University of Vienna, AT)
14:25-14:50	Keynote lecture: **Exposure to micro- and nanoplastics via food and feed** Followed by Q&A	Francesco Cubadda, (ISS, IT)
14:50-15:10	**Assessment of interactions of nanoplastics and wheat using Pd-doped plastics: first insights on potential uptake and impacts** Followed by Q&A	Ana Elena Pradas del Real (University Madrid, ES)

15:10-15:30	**Entry of microplastics into packed food and beverages – the example of bottled mineral water** Followed by Q&A	Jana Weisser (TUM, DE)
15:30-15:50	**Coffee/Tea break**	
SESSION 3 | Developments in hazard identification and characterisation for micro- and nanoplastics		
15:50-16:20	Keynote lecture: **Hazard identification and Risk characterization – micro and nano plastics in food and feed** Followed by Q&A	Ron Hoogenboom (WUR, NL)
16:20-16:40	**Screening & Prioritization of Nano- and Microplastic Particle Toxicity Studies for Evaluating Human Health Risks** Followed by Q&A	Robert Ellis-Hutchings (DOW, US)
16:40-17:00	**Human risks of microplastic associated chemicals** Followed by Q&A	Ruud Peters (WUR, NL)
17:00-17:10	**Introduction to the Discussion Groups**	Angelo Maggiore (EFSA)

DAY 2

SESSION 4 | Breakout sessions		
09:00-10:30	1. Developments in analytical methods for micro- and nanoplastics	Chair: Peter Simpson Rapporteur: Ana Afonso
09:00-10:30	2. Developments in analytical methods for micro- and nanoplastics	Chair: Douglas Gilliland Rapporteur: Tony Smith
09:00-10:30	3. Developments in exposure of humans to micro- and nanoplastics	Chair: Francesco Cubadda Rapporteur: Angelo Maggiore
09:00-10:30	4. Developments in exposure of humans to micro- and nanoplastics	Chair: Birgit Sokull-Klüttgen Rapporteur: Karen Mackay
09:00-10:30	5. Developments in hazard identification and characterisation for micro- and nanoplastics	Chair: Ron Hoogenboom Rapporteur: Marco Binaglia
09:00-10:30	6. Developments in hazard identification and characterisation for micro- and nanoplastics	Chair: Bart Koelmans Rapporteur: Sandra Rainieri
10:30-11:00	**Coffee/Tea break**	
11:00-11:30	**Flash reports from each breakout group (10 mins each topic)**	Breakout groups rapporteurs
11:30-12:00	**Plenary discussion**	Chair Helle Knutsen (FHI, NO)
12:00-12:45	**Panel discussion (selected speakers, breakout chair, Commission, Communication expert)** Francesco Cubadda (ISS) Douglas Gilliland (JRC) Todd Gouin (WHO) Ron Hoogenboom (WUR) Claudia Menzel (University of Koblenz-Landau) Sabine Pahl (University of Vienna) Sandra Rainieri (EFSA) Tobin Robinson (EFSA) Veerle Vanheusden (EC, DG SANTE)	Moderator Barbara Gallani (EFSA)
12:45-13:00	**Wrap-up and closure**	Chair Helle Knutsen (FHI, NO) and co-chair Tobin Robinson (EFSA)
13:00	**Adjourn**	

Oral Presentations

Opening Session: Micro- and nanoplastics in food and feed: setting the scene

Micro- and nanoplastics and human health

Koelmans B [1]

[1] *Wageningen University (NL) *Affiliation: Institution (Country code)*

Abstract: Humans are exposed to microplastic particles every day. Although it is hardly measurable so far, it is likely that this also applies to nanoplastic particles. Early reports on the occurrence of plastic particles in our diet have attracted widespread attention in the media, among scientists, policymakers and in society in general. This introductory overview provides a brief history of landmark studies and expert reports on the topic. As a start for further discussion during the colloquium, some of the most recent scientific insights and developments are briefly presented, insights which will be discussed in more detail in later presentations in the colloquium. Finally, some initial thoughts on research needs will be shared.

Interplay between science and society: the evolution of research, public discourse and societal concerns

Vrbos D [1,] Koch S [2]

[1] *European Food Safety Authority (IT),* [2] *Bundesinstitut für Risikobewertung (BfR, DE)*

Abstract: Evidence shows that scientific work in the area of microplastics has increased significantly over the past 10 years. On a smaller scale, this is also true for research on nanoplastics. Although a number of risks associated with micro- and nanoplastics still remain unknown, the topic is gaining momentum in the public discourse. Particularly the issue of microplastics, sometimes depicted as urgent or pressing, is often framed through an environmental impact lens. Increased media attention on plastics in general, together with the plastic-free policy expansions are likely to have contributed to the evolving discourse.

Consumer studies in Europe show that the discourse has trickled onto consumer perception in the area of food safety as well. Both the 2019 Eurobarometer on food safety in the EU and the BfR Consumer Monitor in Germany show microplastics in food as a topic of increasing concern among EU citizens. What remains to be explored is how these perceptions are built in absence of strong evidence for health risks and how environmental values and human health concerns are linked. We will try to delve into this through a joint research project by EFSA and BfR, based on consumer interviews and population-based surveys in two EU countries, to further our understanding of what drives citizen concerns and how these can be addressed. Such knowledge is required to inform the questions to be answered by risk assessors and policy makers.

Regulatory needs and challenges to inform fit-forpurpose research activities on micro- and nanoplastics, including outcomes of the SAM report

Vanheusden V [1]

[1] *Directorate-General for Health and Food Safety, European Commission (BE)*

Abstract: In 2019 the Scientific Advice Mechanism (SAM) of the European Commission published its report on environmental and health risks of microplastics pollution, which is based on the 2019 SAPEA report ' a scientific perspective on microplastics in nature and society'. The SAM report contains recommendations for policy regarding microplastics in nature and society amongst which several precautionary measures. Some of these measures are included in the EU strategy for plastics in a circular economy. Furthermore the report lists data gaps, which need to be filled to equip policy makers with the necessary knowledge to take preventive or risk-mitigating measures in case of scientific evidence of human and/or ecological health risks.

The EU Regulatory framework for contaminants in food foresees for the possibility to set maximum levels for specific contaminants in food under Regulation (EC) No 1881/2006, when evidence becomes available, which indicates a risk to human health. In its 2016 statement on the presence of microplastics and nanoplastics in food, EFSA concluded that toxicity and toxicokinetic data are lacking for both microplastics and nanoplastics for a human risk assessment. Therefore first the data gaps, which are listed in the EFSA statement, need to be filled, before it can be considered to regulate micro- and nanoplastics in food.

Session 1: Developments in analytical methods for micro- and nanoplastics

Definition of micro and nanoplastics & analytical challenges

Gilliland D [1]

[1] *European Commission, Joint Research Centre (JRC, IT)*

Abstract: Micro(nano)plastics are persistent pollutants whose increasing presence in land, water, food and even air may have the potential to produce harmful but poorly understood effects on the environment and human health. The sources of such materials are very varied but are generally classified into two main categories - primary microplastic which are polymeric particulates deliberately manufactured for use in products and secondary microplastics which are polymer fragments derived from the degradation of polymeric materials in use or by ageing and breakdown of bulk plastics in the environment. While the issue has been of environmental concern for many years there are continually increasing reports of micron and sub-micron sized (nanoplastics) polymer particulates directly in food products, in drinking water or released from food contact materials.

Irrespective of their source, small plastic particles are universal pollutants and research on the exposure and risks that they pose to man is ongoing but progress is highly dependent on the availability and quality of analytical methods. Micro and, above all, nanoplastic are difficult systems to analyse as their detection and quantification in real-world samples is very challenging. In fact, they combine the "classical" issues of accurate chemical detection and quantification with the need to properly measure the size (or better, the particle size distribution) of particles within a potentially very large size range from few millimetres to nanometres. In addition, they can be highly heterogeneous in terms of both chemical composition, size and even shape.

In the following presentation, consideration will firstly be given to the current understanding, both general and legislative, of what constitutes a microplastics after which an overview will be given of the current status of analytical methods for the detection and quantifications of this highly challenging class of materials.

Evaluation and optimization of extraction methods suitable for the analysis of microplastic particles occurring in the edible part of seafood

Süssmann J [1], Krause T [1], Martin D [1], Walz E [2], Greiner R [2], Rohn S [3,5], Fischer EK [4], Fritsche J [1]

[1] *Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Milk and Fish Products (DE),* [2]*Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Food Technology and Bioprocess Engineering (DE),* [3]*Universiity of Hamburg, Institute of Food Chemistry (DE),* [4] *University of Hamburg, Center for Earth System Research and Sustainability (CEN, DE),* [5] *Technische Universität Berlin, Institute of Food Technology and Food Chemistry, Department of Food Chemistry and Analysis (DE)*

Abstract: Findings of small plastic particles, called micro- (MP) or nanoplastics (NP), in the gastrointestinal tracts of aquatic organisms are reported frequently, including species used for human consumption (Hantoro et al., 2019). Due to a possible translocation of these particles from the gut into other tissues, MP might also be present in edible parts. Additionally, leaching and consecutive accumulation of additives or persistent organic pollutants adsorbed to the particle in the environment might occur. Therefore, an influence of MP on seafood and the human health might exist (Smith et al. 2018). However, for risk assessment besides assessment of the toxicity of MP, the occurrence of MP in the edible parts of seafood is an important prerequisite. Besides the low number of studies

analysing MP in the edible part of seafood no validated method for the assessment of MP in the edible parts of seafood currently exists. Therefore, this study was conducted to evaluate different approaches for extracting MP Ø ≥ 1 µm from the edible part of seafood considering for example digestion efficiency, sample preparation time, effort and costs in order to identify methods suitable for routine analysis. The preparation should be suitable for the most commonly used identification methods, including fluorescence microscopy, microspectroscopy (Infrared- and Raman-spectroscopy) and thermoanalytical methods (pyrolysis gas chromatography mass spectrometry (py-GC/MS)).

A literature research was conducted to determine the most promising extraction protocols for the isolation of MP from biota. The investigated protocols were selected depending on the overall time and effort of the method, the digestion efficiency and the corrosiveness towards plastics. Protocols using acidic, alkaline, oxidative, enzymatic as well as combinations thereof were taken into consideration. The protocols were applied with 10 g homogenized seafood matrix (fish fillet, the soft tissue of molluscs and crustaceans) to verify whether the filtration was possible with filters with a pore size of 1 µm. The polymer integrity of the most suitable and efficient protocol was tested by determining the plastic particle recovery (change in weight & size). In addition, the polymer identification was performed with py-GC/MS, FTIR- and Raman-spectroscopy before and after the digestion. Furthermore, the protocol was further optimized regarding digestion time and temperature, choice of filter material and post-digestive filter treatment. After optimization of the extraction protocol, precision and recovery were determined with seafood samples spiked with known amounts of fluorescent Nylon 12 (PA12)-particles (Ø approx. 20 µm). A qualitative detection of a mixture of polymers was tested with py-GC/MS.

The most suitable and efficient protocols were those based on the alkaline digestion with KOH according to Dehaut et al. (2016). By combining the alkaline digestion with a prior Pepsin digestion, the digestion temperature could be lowered to 37 °C, while the overall time for the complete extraction could be reduced to 6 h for most seafood sample matrices except for fish fillets with a fat content > 20 %. After reducing the digestion temperature for the alkaline treatment from 60 °C to 37 °C no adverse effects on all tested, commercially relevant polymers were examined with the exception of polyacrylonitrile. Particle integrity was analysed by FTIR- & Raman-spectroscopy as well as py-GC/MS and was not affected by the applied extraction procedure. In order to reduce analysis time with microscopic or spectroscopic methods, the application of smaller filter diameters was necessary. For the analysis of the complete filter with py-GC/MS, a smaller filter was required as well. Pre-filtration with polycarbonate (PC)-filters was the most applicable approach. However, contamination of the sample with PC from the filter occurred. A recovery of 88 % to 95 % of Ø 20 µm-PA12-particles was achieved for the lowest concentration level (15 – 20 particles per sample) with a reproducibility of 18 % to 29 %. As the particle number of the spiking solution itself could only be determined with a reproducibility of 17 % to 27 %, the reproducibility of the complete extraction procedure was deemed acceptable.

The evaluation of extraction protocols suitable for microplastic analysis in the edible part of seafood confirmed the applicability of proposals reported by Bessa et al. (2019) dedicated for the extraction of MP from biota. Introducing Pepsin into the digestion procedure lead to a quick breakup of the overall seafood sample structure. To avoid expensive enzymatic treatment costs fatty tissues were destroyed with KOH instead of a broad range of specific enzymes. Consequently, in contrast to the sole alkaline digestion described by Karami et al. (2017), the optimized seafood protocol developed in this study was able to digest 10 g to 100 g of a broad range of seafood species within 6 h instead of 72 h at a temperature of 37°C to 40°C while not affecting the integrity of most polymers. However, the seafood protocol is only of limited suitability for analysing polyacrylonitrile, which mostly derives from textile fibres. That polymer is significantly altered even at room temperature within the first 10 min of the alkaline treatment.

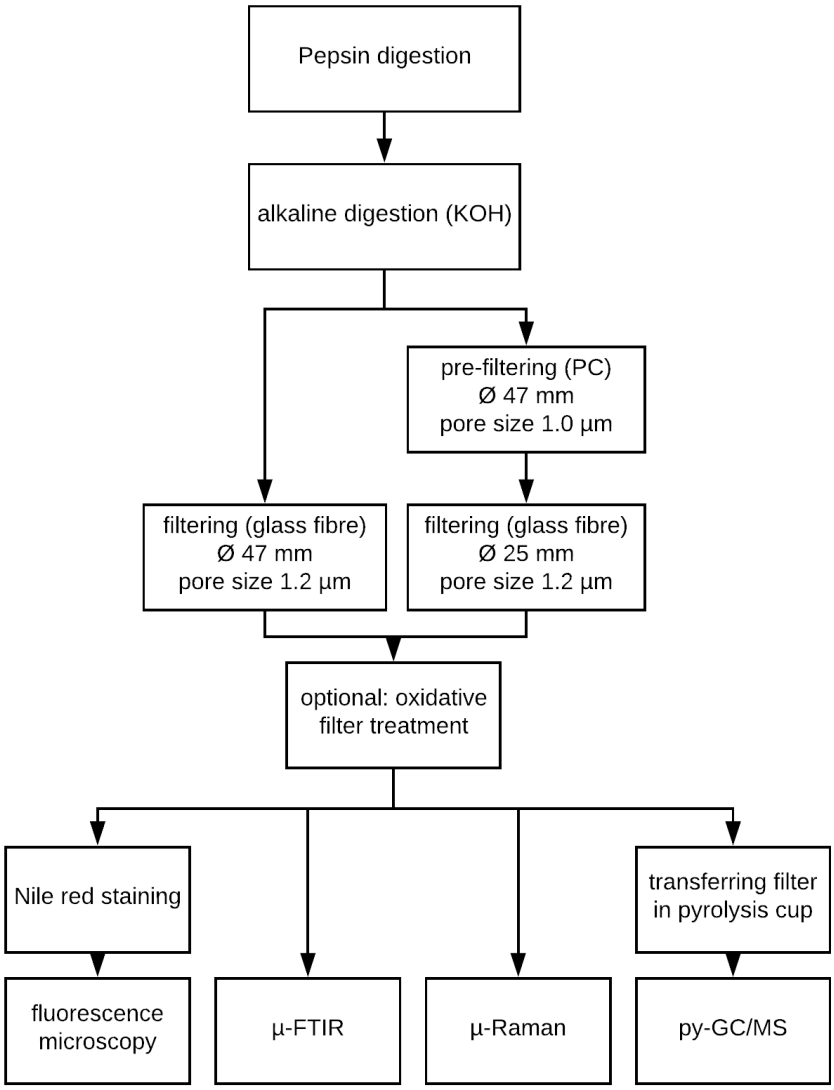


Figure 1: Overview of sample preparation for the analysis of MP in seafood with different analytical procedures.

Bibliography

Bessa et al. 2019. Harmonized protocol for monitoring microplastics in biota. JPI-Oceans BASEMAN project.

Dehaut A, Cassone AL, Frère L, Hermabessiere L, Himber C, Rinnert E, Rivière G, Lambert C, Soudant P, Huvet A, Duflos G, Paul-Pont I. 2016, Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ Pollut. 215:223-233.

Hantoro I, Löhr AJ, Van Belleghem FGAJ, Widianarko B, Ragas AMJ. 2019. Microplastics in coastal areas and seafood: implications for food safety. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 36(5):674-711.

Karami A, Golieskardi A, Choo CK, Romano N, Ho YB, Salamatinia B. 2017. A high-performance protocol for extraction of microplastics in fish. Sci Total Environ. 578:485-494.

Smith M, Love DC, Rochman CM, Neff RA. 2018. Microplastics in Seafood and the Implications for Human Health. Curr Environ Health Rep. 5(3):375-386.

Raman Tweezers for Small Microplastics and Nanoplastics Identification in liquids

Gillibert R [1], Balakrishnan G [2], Quentin Deshoules Q [3], Tardivel M [3], Magazzu A [4], Donato MG [1], Marago OM [1], de La Chapelle ML [2], Colas F [3], Lagarde F [2], Gucciardi PG [1]

[1] CNR – IPCF, Istituto per i Processi Chimico-Fisici (IT), [2] Institut des Molécules et Matériaux du Mans (FR), [3] Ifremer LDCM (FR), [4] Department of Physics, University of Gothenburg (SE)

Abstract: Pollution by micro- and nano- plastics in sea waters is receiving scientific, media and societal attention because of the potential threats to the ecosystem and, definitively, to the human health. Micrometric particles are small enough to be ingested by a wide range of organisms and, at the nanometric scale, may cross the biological barriers increasing their toxicity. Our understanding of the fate and ecological impact of these pollutants is, however, limited by the intrinsic difficulties of conventional analytical techniques (light scattering, FT-IR, Raman, optical and electron microscopies) in the detection, quantification and chemical identification of small particles in liquid.

Here we propose the use of optical tweezers, a technique recently awarded with the Nobel prize, as an analytical tool for the study of micro- and nano- plastics in liquid dispersions. In particular, we exploit the combination of optical tweezers with Raman spectroscopy (Raman Tweezers, RTs) to optically trap plastic particles and aggregates of sub-20 μm sizes, down to the 50 nm range and unambiguously reveal their chemical composition via Raman spectroscopy

Applications are shown on both model particles and naturally aged environmental samples, made of common plastic pollutants, including polyethylene, polypropylene, nylon, and polystyrene, also in the presence of a thin eco- corona. Moreover, we demonstrate that RTs, operating at the single particle level, allow us to unambiguously distinguish plastic particles from marine microorganisms and minerals typically found in sea water, overcoming the capacities of standard Raman spectroscopy in liquid, limited to ensemble measurements. We show the capability to distinguish PP from nylon fibers in artificial samples dispersed in seawater. Finally we apply RT to trap and identify Tire and Road Wear Particles with dimensions from few microns down to the 500 nm range.

RTs are a spectroscopic tool capable to study the fate of micro and nanoplastics in marine environments and to determine the effect of aging and fragmentation on plastic materials. Future experimental developments should be directed toward quanti- tative analysis, through the implementation of RT for liquid flow operation in suitable microfluidic cells, artificial intelligence routines to spot, count, and analyze the nature particles, and adopt big data analysis tools to treat the thousands of spectra required to provide reliable particles size distributions of different polymeric materials.

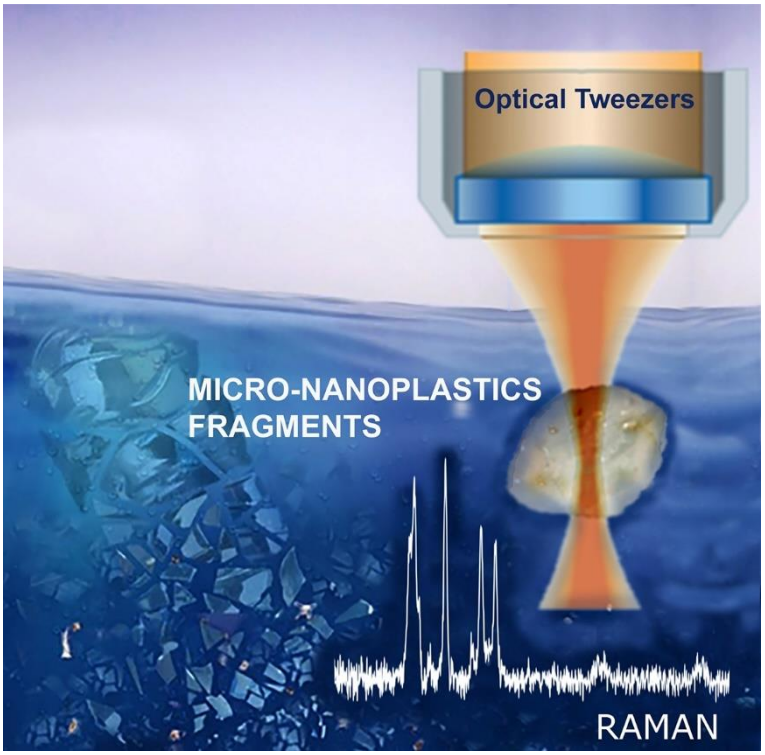


Figure 2: Raman identification of optically trapped micro and nanoplastics.

Session 2: Developments in exposure of humans to micro- and nanoplastics

Human exposure to micro- and (nano)plastics: What drives citizens' concern?

Pahl S [1]

[1] *University of Plymouth (UK)*

Abstract: Perceptions about risk and harm drive public discourse and policy decisions about hazards, whether these are driven by objective 'technical' assessments or more subjective factors such as worries, feelings, values. Different groups in society may not agree in their assessments, which can lead to conflict. The present talk will summarise recent empirical data on concern about micro- and macroplastics and their potential impacts on human health, in public and expert respondents. The talk will draw on psychological processes (e.g., cognition, motivation, emotion) and theories of risk perception to analyse what drives citizens' concern. Social and behavioural science insights can help explain 'hotspots' of public debate and attention, inform and test strategies of assessing and communicating emerging risks, including the communication of uncertain and absent evidence.

Assessment of interactions of nanoplastics and wheat using Pd-doped plastics: first insights on potential uptake and impacts

Ana Elena Pradas del Real AE [1,2], Denise M. Mitrano DM [3], Castillo Michel H[2], Reyes Herrera J[2], Wazne M [2,4], Bortel E [5], Hesse B [2,5], Sarret G [4]

[1] *IMIDRA (Madrid Institute for Agroenvironmental Research, ES),* [2] *ESRF, The European Synchrotron (FR),* [3] *Process Engineering Department, Eawag (Swiss Federal Institute of Aquatic Science and Technology, CH),* [4] *ISTerre (Institut des Sciences de la Terre), Univ. Grenoble Alpes, (FR)* [5]*, Xploraytion GmbH (DE)*

Abstract: Research addressing the trophic transfer of micro and nano plastics has recently been moving from marine to terrestrial ecosystems as recent studies suggest that soils are larger plastics reservoirs than the global ocean (Hurley and Nizzetto, 2018). Plastic fragments arrive to agricultural soils through two main pathways; the use of sewage sludge and bio waste digestate as fertilizers and the use of plastic mulch films. Either directly in those soil amendments or through fragmentation of larger plastic items, nanoplastic (i.e. plastic residues < 1µm in size) have the potential to interact with plants. Whether these plastics can be taken up by crops and transferred to food chain is still an open question, as available methods do not allow for quantitative detection of plastic particles in plant tissues at trace levels. Due to their smaller size, nanoplastics are more likely to cross plant tissues than other plastic residues. Recent imaging studies have shown the internalization of fluorescence synthetic nanoplastics in watercress (Bosker et al., 2019), broad bean (Jiang et al., 2019) and wheat (Lian et al., 2020). However, while these studies provide evidence of nanoplastic internalization, they do not provide quantitative information in terms of particle mass or number, which hinders the risk assessment of plastic intake by humans through crop consumption. In the present study, wheat plants were exposed to Pd-doped nanoplastics, which allowed for easier detection through the metal as a proxy for the plastic. Nanoplastic distribution in plant roots and phytotoxicity were also investigated.

The nanoplastics used in this study were synthesized via in-situ polymerization and consisted of a core/shell structure (polyacrylonitrile/polystyrene), which contained approximately 0.5% w/w Pd. Two morphologies were developed, smooth (SNPs) and raspberry (RNPs), with average diameters between 120 and 160 nm (Mitrano et al., 2019). Wheat plants were exposed in hydroponics to: i) 3 mg·l-1 RNPs; ii) 30 mg·l-1 RNPs; iii) 3 mg·l-1 SNPs; iv) 30 mg·l-1 SNPs; v) no plastic addition (control); vi) filtrate of 30 mg·l-1 RNPs (to assess impacts of e.g. residual surfactants from synthesis). Each treatment consisted of four independent replicates with 10 plants. Plants were harvested after three weeks of exposure. Nanoplastics accumulation in plant tissues was determined from Pd concentrations measured by inductively coupled plasma mass spectrometry (ICP-MS). The impact on plant physiology was evaluated by determining: biomass, lipid peroxidation and protein and chlorophyll contents. Biochemical changes in plant tissues were studied by Fourier transform infrared spectroscopy using attenuated total reflectance (FTIR-ATR). Nanoplastic distribution on the root surface was evaluated by

scanning electron microscopy (SEM). The Pd signal was used to further investigate nanoplastics distribution within roots by synchrotron micro X-ray fluorescence (µXRF). X-ray micro computed tomography (µCT) was used to assess anatomical changes in exposed roots. The aggregation state of nanoplastics in the nutrient solution, the zeta potential and the pH were also monitored. At the end of the experiment, total organic carbon and organic acids were determined in the nutrient solution as a proxy of root exudation.

Nanoplastics were absorbed by plant roots and translocated to the shoots. The concentration in plant tissues was dose-dependent, but no differences were found between plastic morphologies. By using the Pd signal, XRF showed the accumulation of nanoplastics in the root tips and in the epidermis of the maturation zone of roots. The presence of biofilm filaments on the root surface for all treatments was shown by SEM. In exposed roots, nanoplastics were agglomerated and entangled with the filaments. The exposure to nanoplastics did not induce negative effects on plant growth in terms of total biomass, oxidative stress or chlorophyll and protein contents. However, FTIR-ATR revealed changes in the molecular environment of proteins and cell wall components in both roots and shoots of exposed plants. Changes in root architecture (cell wall density/thickness, cell cytoplasm volume) are being evaluated from µCT data. The nanoplastics aggregated in the nutrient solution after four days in contact with plants roots, which was accompanied by a shift of the particle zeta potential to less negative values, and an increase in the pH of the nutrient solution. An increase in total organic carbon was found in the growing solution of plants exposed to nanoplastics compared to controls. On the other hand, the concentration of oxalic, citric, formic, succinic, and acetic acids in the nutrient solution also changed in nanoplastics treatments compared to the control.

To overcome difficulties in nanoplastics detection, in the present study we used an innovative approach based on the use of Pd-labelled nanoplastics. Results have shown that these nanoplastics were taken up by wheat plants from the growth media and were translocated to shoots. This may be an indication of subsequent risks associated with plastics entering the food chain. Three main mechanisms related with uptake can be drawn from these results: i) alterations in the cell wall matrix of root epidermal cells, ii) association of nanoplastics with root biofilm and iii) influence of root exudation on nanoplastics aggregation and, in consequence, in the effective exposition dose. These findings stress the active influence of plant rhizosphere on the fate and availability of nanoplastics. Observed alterations in plant biomacromolecules and root exudation patterns were indicators of physiological imbalances in exposed plants. The use of Pd-doped nanoplastics in this study allowed us to bring unique information about the interaction and impacts of nanoplastics on a model crop species, which constitutes a first step to evaluate crop consumption as an exposure route for nanoplastics. This study also highlights the need to evaluate whether the presence of nanoplastics in agricultural soils can hinder the ability of crops to ensure a sufficient and high quality food supply. Future research will focus on how these findings can be extrapolated when plants are grown in soil for their entire life cycle and on the potential transport of nanoplastics from shoots to grains.

Bibliography

Bosker T, Bouwman LJ, Brun NR, Behrens P, Vijver MG. 2019. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant Lepidium sativum. Chemosphere 226:774-781.

Hurley RR, Nizzetto L. 2018. Fate and occurrence of micro(nano)plastics in soils: Knowledge gaps and possible risks. Curr Opin Environ Sci Health 1:6-11.

Jiang X, Chen H, Liao Y, Ye Z, Li M, Klobučar G. 2019. Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. Environ Pollut. 250:831-838.

Lian J, Wu J, Xiong H, Zeb A, Yang T, Su X, Su L, Liu W. 2020. Impact of polystyrene nanoplastics (PSNPs) on seed germination and seedling growth of wheat (Triticum aestivum L.). J Hazard Mater. 385:121620.

Mitrano DM, Beltzung A, Frehland S, Schmiedgruber M, Cingolani A, Schmidt F. 2019. Synthesis of metal-doped nanoplastics and their utility to investigate fate and behaviour in complex environmental systems. Nat Nanotechnol. 14(4):362-368.

Entry of microplastics into packed food and beverages – the example of bottled mineral water

WeisserJ [1], Beer I [2], Ivleva NP [2], Hofmann T [1], Glas K [1]

[1] Chair of Food Chemistry and Molecular Sensory Science, Technical University of Munich (DE), [2] Institute of Hydrochemistry, Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich

Abstract: The occurrence of microplastics (MP) in various environments is currently studied extensively, as well as their impact on living creatures. Therefore, it was inevitable that scientists and the public became concerned whether MP as a new environmental pollutant might find its way into our sustenance. This applies to various contaminants and MP do not pose an exception as they have already been found in seafood, salt, beer, and drinking water from the tap and bottled (Oßmann et al., 2018; Schymanski et al., 2018). In the food and beverage sector, consumers' concern about the ingestion of MP has been extensively fueled by mass media, even though it remains unknown until today, whether MP indeed have any negative effects on human health. Intake rates of MP can currently only be estimated very roughly due to the lack of reliable data on MP occurrence in food items and beverages. In addition, the entry paths of MP into food products throughout the production and packaging processes are unknown. In the case of mineral water, the cleaning process for re-usable bottles was previously suspected to be the main entry path for MP (Oßmann et al., 2018; Schymanski et al., 2018). Thus, we investigated the process of mineral water sourcing, bottle washing and filling applying Fourier Transform Infrared (FTIR)-Imaging and a Random Decision Forest model (RDF) (Weisser et al., submitted).

Samples were taken at four mineral water bottlers (A-D) at five points along the bottling process: ground water, treated drinking water, cleaned glass bottles, caustic bottle cleaning solution, and glass bottles filled with mineral water and capped. Additionally, filled, uncapped glass-bottles were sampled at one company. Similar to other studies, approximately 1000 L of ground and treated water were filtered in-situ through stainless steel cartridges (Mintenig et al., 2019). Precipitates were removed in a 24 h citric acid treatment (0.66 M) and, when necessary, a density separation for 24 h in a zinc chloride solution ($ZnCl_2$, 1.7 g cm^{-3}). Bottled water (0.75 L, n=3) was filtered through aluminum oxide filters (Anodisc). Bottles were flushed with a 0.02% sodiumdodecylsulfate solution and 30% ethanol. Caustic solution was treated using Fenton's reagent (20 mL H_2O_2, 2.5 mL 0.05 M $FeSO_4$, 20-30°C) and cellulase. Procedural blank samples were created during sample treatment and used to calculate the limit of detection (LOD) for each sample type. MP reference particles (63-125 µm) were tested for their persistence towards all chemicals used. Treated samples were deposited on Anodisc filters for FTIR imaging (Agilent Cary 620/670, 128x128 Focal Plane Array detector). FTIR images were taken with a projected pixel size of 5.5 µm and a spectral resolution of 8 cm^{-1}. Data were analyzed for nine plastic types, cellulose and proteinaceous contaminants using a self-designed RDF in EPINA ImageLab. Only particles ≥ 2 pixels, i.e. ≥ 11 µm, were taken into account. The model was evaluated applying Monte-Carlo-Cross-Validation.

Testing the MP reference particles for their persistence towards the chemicals used for sample treatment, no chemical alterations of the particle surfaces could be observed using FTIR. However, $ZnCl_2$-treated polyamide particles aggregated strongly and ethylene vinyl alcohol particles melted and agglomerated. Consequently, particle sizes and numbers may have been altered during sample preparation. The RDF's accuracy, i.e. the amount of correct classifications over all classifications on a test data set, was calculated to be 95.45% and therefore was found to be adequate for data evaluation. In all samples of raw and treated mineral water, MP concentration was below 1 particle L^{-1}. Proceeding in the filling process, all cleaned glass bottles were found to be below LOD (40 MP) and are therefore considered free of MP. Caustic bottle cleaning solutions, however, contained 1,826 ± 1,199 MP L^{-1}. Their MP concentrations may have been underestimated as even after the multi-step digestion process, cellulose fibers were present, potentially overlaying MP particles. All un-capped, filled bottles were < LOD, as well. However, a sharp increase of MP concentrations (≥ 11 µm) to 317 ± 257 MP L^{-1} was observed after bottle capping. This observation was made in samples from all water bottlers A-D. On average, 81% of MP in capped bottles consisted of polyethylene (PE). Particle length (larger Feret diameter) ranged from 11 to 530 µm, with 63% of MP in the size range of 11 to 50 µm.

Assumptions made earlier, that the cleaning process for re-usable bottles was responsible for MP in bottled water (Oßmann et al., 2018; Schymanski et al., 2018), were not proven to be true in our study. Cleaned bottles were free of MP ≥ 11 µm, even though the cleansing solutions bore high MP loads. This shows that carryover of particles was successfully mitigated in the cleansing process. Rather, our results indicate that MP particles are produced during the process of bottle closing and

likely also through opening during sampling. This result is in accordance to Winkler et al. (2019), who found that repeated opening and closing of PET bottles released particles from the bottle caps. We can further support this hypothesis, as 81% of the MP particle spectra were similar to the PE-based cap sealing material. MP concentrations found in capped glass bottles are higher than reported by Schymanski et al. (2018) and Oßmann et al. (2018): considering only particles > 10 µm in mineral water from glass bottles, they found 28 ± 29, resp. 212 ± 175 MP L^{-1} with polyester, polyethylene and polypropylene being the most frequent polymers. Exposure to MP through bottled beverages may be higher than thought. Yet, the high deviation in all studies emphasizes that MP concentrations vary widely and comparability of results is limited. When aiming to reduce MP entry into bottled water, it should be conceived to choose abrasion-resistant sealing materials in bottle caps. Cap rinsing or aspiration before bottle capping may also effectively hinder MP from entering the bottles.

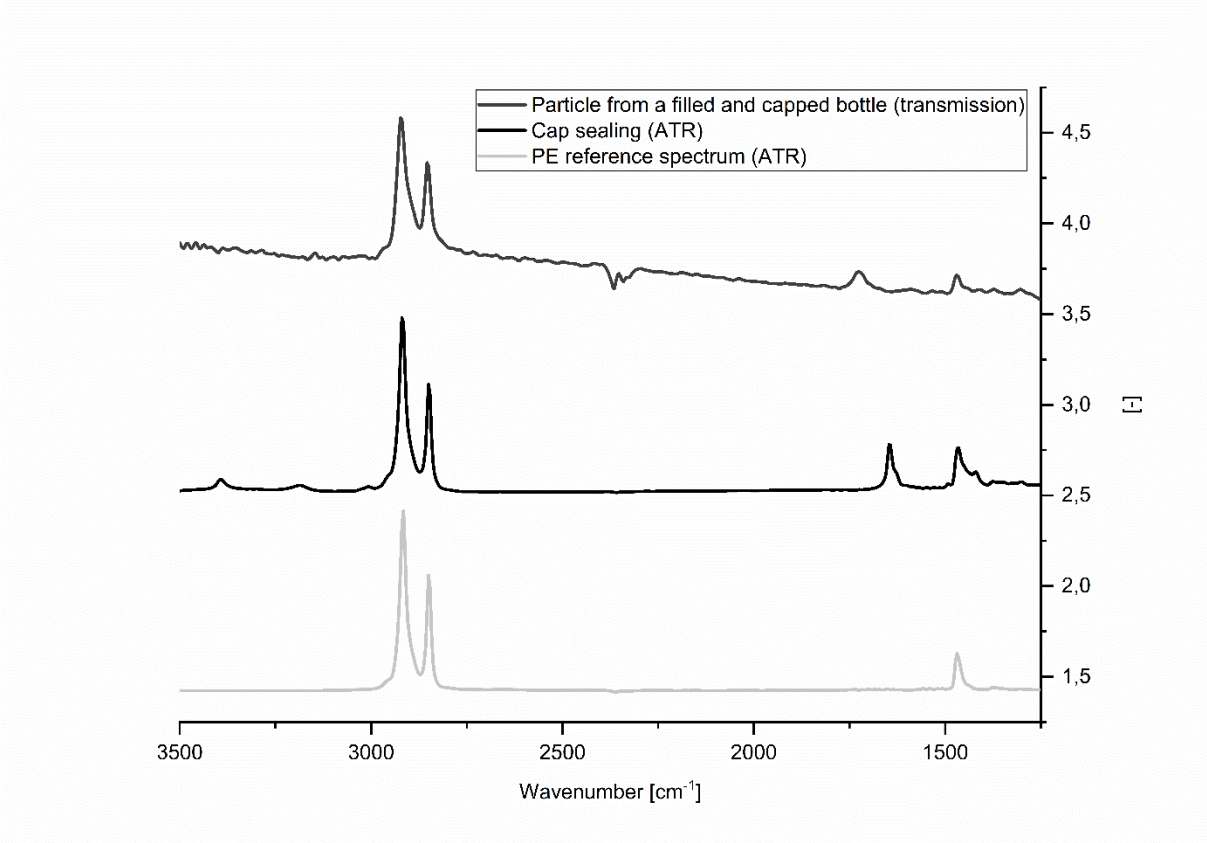


Figure 3: Example of transmission spectrum of a PE-like particle from a filled and capped water bottle compared to ATR spectra of cap sealing and PE reference material.

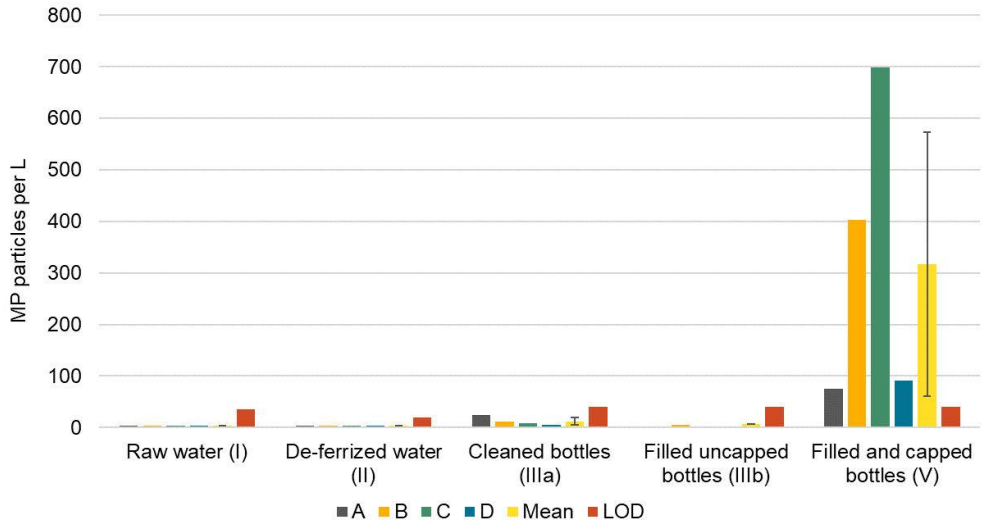


Figure 4: MP concentrations per L mineral water along the bottling process of four bottling sites (A–D), corresponding mean (error bars indicate standard deviation) and LODs for each sample type

Bibliography

Mintenig SM, Löder, MGJ, Primpke S, Gerdts G. 2019. Low numbers of microplastics detected in drinking water from ground water sources. Sci Total Environ, 648: 631-635.

Oßmann BE, Sarau G, Holtmannspotter H, Pischetsrieder M, Christiansen SH, Dicke W. 2018. Small-sized microplastics and pigmented particles in bottled mineral water. Water Res, 141: 307-316.

Schymanski D, Goldbeck C, Humpf HU, Fürst P. 2018. Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water. Water Res, 129: 154-162.

Weisser J, Beer I, Hufnag, B, Hofmann T, Lohninger H, Ivleva NP, Glas, K. submitted. From the well to the bottle: Identifying Sources of Microplastics in Mineral Water.

Winkler A, Santo N, Ortenzi MA, Bolzoni E, Bacchetta R, Tremolada P. 2019. Does mechanical stress cause microplastic release from plastic water bottles? Water Research, 166: 115082.

Exposure to micro- and nanoplastics via food and feed

Cubadda F[1]

[1] *Istituto Superiore di Sanità*

Abstract: A significant proportion of the plastic produced is not disposed of properly and persists in the environment, especially the marine environment. The volume of plastic litter in the oceans and the potential for degradation to microplastics and finally nanoplastics has attracted attention towards marine food chains in the perspective of human dietary exposure to plastic particles. Microplastics can be ingested by both marine invertebrates and vertebrates, which are exposed either directly or via lower trophic levels. As most of the larger-sized microplastics will remain confined within the GI tract, gutting is expected to decrease human dietary exposure compared to eating whole fish. However, this does not apply to shellfish and certain species of small fish, and might be less applicable to smaller-sized particles. Especially mussels, as suspension feeders, are prone to particle bioaccumulation and their consumption appears to represent a worst case for human exposure to plastic particles.

Very little is known about terrestrial food chains. Data exist indicating the occurrence of microplastics in, e.g., sugar, salt, and honey. Fish meal has some use in poultry production and pig rearing, hence, microplastics might end up in non-marine foods. Besides the influence of long-range environmental pollution on primary production, specific plastics find extensive use in a number of applications across food value chains, including vegetable farming (e.g. mulching). There is a gap of knowledge about the potential uptake and deposition of plastic particles in vegetable and animal food and any associated human exposure. Food processing might also contribute to the occurrence of plastic particles in food items and resulting human exposure. Plastic is extensively used in food and beverage packaging too.

Release of plastic particles from food contact materials depends on the processing conditions and might increase upon recycling or reuse. Finally, existing data show that drinking water, especially bottled water but also tap water, might represent a substantial source of exposure to microplastics.

Experience with particle and fibre toxicology indicates that any potential adverse effect of micro and nanoplastics on human health will be dictated by their physicochemical properties. After oral exposure, the size, morphology, surface properties, chemical composition (i.e. constituting polymers) of plastic particles will determine their ability to be taken up in the gut and cause any systemic toxicity. Chemicals contained in or adsorbed onto the particles might be other determinants of toxicity. Due to the broad definition of microplastics, encompassing a wide particle size range unrelated to potential human health effects, and the fact that smaller plastic particles are likely to originate, to a large extent, from fragmentation of larger particles, environmental micro and nanoplastics appear to occur along an extensive size continuum, portions of which have been investigated by the relatively few studies focusing on food matrices. However, the different particle size distributions targeted by each study were not associated to any established toxicological potential and their relevance for dietary exposure assessment in the perspective of food safety risk assessment is doubtful. In particular, existing analytical methods are only capable to detect relatively large microplastics. On the other hand, the plastic particles of relevance for human health following oral exposure are likely to be limited to the smaller-sized microplastics and the nanoplastics, which have the greatest likelihood of being absorbed in the gastrointestinal tract. In addition, other shortcomings affect available data, such as the lack of harmonisation in analytical techniques, the limited availability of chemically-specific data (particle composition) and the generally poor (or absent) analytical quality control, indeed an essential requirement for analytes that are in most cases ubiquitous. This means that, at best, existing data can be considered as signals and indicators that micro- and nano-plastics of potential human health relevance might be present in food-chains, relying on the notion of formation of smaller particles from fragmentation of environmental microplastics.

Any development in exposure assessment of micro- and nanoplastics via food and feed critically relies on (i) progresses in toxicokinetics and toxicology – especially effects of long-term exposure and research on the degradation of microplastics and potential formation of nanoplastics in the human GI tract – with the consequent identification of particulate plastic agents of relevance for human or animal health, and (ii) the development of advanced analytical methods capable to measure these particles at the expected low background levels in food. For nanoplastics, the 'EFSA Guidance on risk assessment of nanomaterials to be applied in the food and feed chain' provides indications for an appropriate toxicity testing and risk assessment strategy.

Session 3: Developments in hazard identification and characterisation for micro- and nanoplastics

Hazard identification and Risk characterization – micro and nano plastics in food and feed

Hoogenboom R [1]

[1] *Wageningen University & Research (NL)*

Abstract: Micro- and nanoplastics are considered an emerging issue as environmental pollutants and a potential threat to human health. In 2016 EFSA published a statement on potential health risks of microplastics and nanoplastics. It was concluded that more information is needed on both the exposure and potential adverse effects in consumers. Raamsdonk et al. (2020) reviewed existing literature on this topic. Considering exposure, there are some data on concentrations in seafood (fish, bivalves and shrimps), water, sugar, salt and honey that confirm that consumers are exposed to these particles. There are also some recent studies on the uptake and effects of fluorescent polystyrene micro- and nanoplastics in mice and zebrafish with short- or medium-term exposure (up to 42 days), to relatively high numbers of particles. Where some studies suggest absorption of polystyrene particles with µm or nm size in the GI-tract, others could not confirm this. Similar was the case for potential effects. Some of the studies reported diverse effects on gut microbiota, lipid metabolism and oxidative stress. A thorough review and additional studies are required to further investigate these issues, including their relevance for current human exposure.

Bibliography
van Raamsdonk LWD, van der Zande M, Koelmans AA, Hoogenboom RLAP, Peters RJB, Groot MJ, Peijnenburg AACM, Weesepoel YJA. 2020. Current Insights into Monitoring, Bioaccumulation, and Potential Health Effects of Microplastics Present in the Food Chain. Foods. 9(1):72.

Screening & Prioritization of Nano- and Microplastic Particle Toxicity Studies for Evaluation of Human Health Risks

Ellis-Hutchings R [1]

[1] *Dow, Inc. (US)*

Abstract: While microplastics ecotoxicology research has been underway for some time, human health toxicology studies have only recent initiated. The limited available microplastic human health hazard studies lack consistent or standard methods resulting in uncertain scientific quality. Due to this, definitive conclusions regarding hazard identification, hazard characterization, or risk assessment are not yet possible for any human health exposure pathway. The criticality of this issue necessitates that we use systematic review approaches when evaluating microplastics studies for human health risk. To catalyze quality evaluations of existing studies, and aid in highlighting critical study design aspects for new research, assessment criteria have been developed for human health in vitro and in vivo microplastic testing. Categories include particle characterization, experimental design, and applicability for risk assessment; with critical and non-critical criteria organized to allow screening and prioritization of studies towards informing human health risk assessment. The presentation will summarize results obtained from the evaluation of studies relevant for human health risk assessment and propose a tiered assessment strategy that couples the application of the Tier 1 toxicity screening assessment tool for nano- and microplastic particles (TSAT-NMP) with Tier 2 expert evaluations. Such expert judgement remains an essential element to the evaluation process, enabling appropriate technical scrutiny of the data reported from a study. The proposed Tiered-approach aims at identifying studies that are fit-for-purpose towards informing a robust risk assessment, while highlighting critical study design aspects requiring careful consideration to aid in generation of fit-for-purpose data.

Potential human health risks of microplastic associated chemicals

Peters R[1], Bouwmeester H[2]

[1] *Wageningen Food Safety Research, Wageningen University & Research, (NL)* [2] *Department of Agrotechnology and Food Sciences, chair group of Toxicology, Wageningen University & Research (NL)*

Abstract: The global production of plastic currently exceeds 300 million tonnes per year. The extensive use of plastics and bad waste management has resulted in the presence of micro- and nanoplastics at different levels in the food production chain. In addition, micro- and nanoplastics in food products may arise from food packaging materials. All together this results in an exposure to consumers and so far studies have reported the presence of microplastics in drinking water, seafood, sugar, honey, and beer. From a chemical perspective, these microplastics are complex mixtures that contain multiple additives, such as plasticizers, flame retardants, stabilizers and pigments. In addition to these additives, other chemicals can be present in micro- and nanoplastics, including unreacted monomers, starting substances, and non-intentionally added substances (NIAS, impurities and side or breakdown products). Finally, the microplastics may have adsorbed environmental contaminants such as PCB's and PAHs. As most of the chemicals and the contaminants are not covalently bound to the polymer, they can be released upon oral ingestion of the micro- and nanoplastics by consumers.

In our study we have used several types of microplastics, either form grinded beach litter or from frequently used food packing materials. We quantified the chemical and metal release from these microplastics in worst case and realistic scenarios. For the latter we used an *in vitro* model of the human digestion and analysed samples with sensitive ICP-MS and GC-MS methods. In addition we used monolayers of co-cultures of Caco-2 and mucus producing HT-29 MTX cells as a model for the intestinal epithelium to assess the transport of these chemicals across the human intestinal epithelium (see Figure 5). Lastly, we have used a CALUX bioassays to screen for estrogenic activity of the chemical leachates, both before and after in vitro digestion.

So far we found clear indications of the presences of ranges of persistent organic pollutants in both the worst case as well as biological relevant incubated microplastics. This includes metals like barium, cadmium and strontium and chemicals like phthalates and phosphorous flame retardants. The environmentally relevant microplastics (from beach litter) also contained environmental contaminants, especially polycyclic aromatic hydrocarbons. Our results indicate that the type and origin of the MP matter. Samples that were incubated using the in vitro model for the human digestion are currently evaluated. On the average 10% (ranging from 0.5 to 50%) of the metals detected in the microplastics are found in the in vitro digest and may therefore be available for uptake. For the organic compounds this ranges from <5% for the less water-soluble compounds (such as the polycyclic aromatic hydrocarbons in the environmentally relevant microplastics) to >90% for the more water soluble phosphorous flame retardants.

Currently there is a lot of uncertainty on the impact for consumers of the potential release of plastic additives and persistent organic pollutants adsorbed to micro- and nanoplastics. It is known that the environmental contaminants and some of the plastic additives are persistent organic pollutants and endocrine disrupting chemicals. However, the contribution of chemicals released from micro- and nanoplastics to the total burden is yet unknown. To begin building an evidence base, we performe a study to identify possible health threats and the underlying biological mechanisms related to oral exposure to small plastic particles. First results show that chemicals in the microplastics become available in the *in vitro* digestion and also show that some of these chemical can pass the intestinal epithelium *in vitro*.

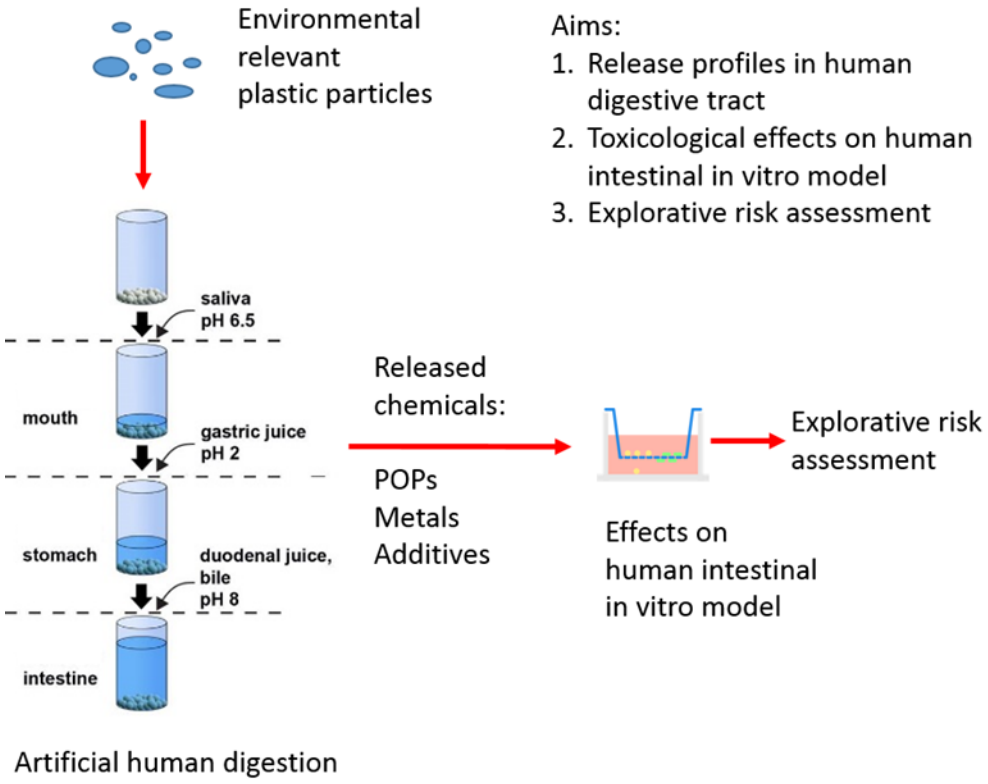


Figure 5: *In vitro* model of the human digestion and monolayers of cells as a model for the intestinal epithelium to assess the uptake of MP related chemicals.

Pitches

Session 1: Developments in analytical methods for micro- and nanoplastics

Developments in sample preparation and identification of microplastics in food and beverages

Prata JC [1], Silva Fernandes AJ [2], Mendes da Costa F [2], Duarte AC [1], Rocha-Santos T [1]

[1] Centre for Environmental and Marine Studies (CESAM) & Department of Chemistry, University of Aveiro, (PT), [2] I3N & Physics Department, University of Aveiro, (PT)

Abstract: Plastics have a widespread use in daily life, and it is not surprising that fragmentation of such polymeric materials are producing small fragments < 5 mm known as microplastics, which are now considered ubiquitous contaminants. Contamination of foodstuff and beverages for human consumption with microplastics is almost inevitable. The ingestion of foreign particles, especially of synthetic origin, causes a strong negative reaction from consumers. Indeed, ingestion is one of the main routes of human exposure to microplastics, along with inhalation, and dermal contact to a lesser extent. Human health effects from microplastics are only expected at very high concentrations, and current knowledge indicates that present environmental concentrations may not yet be harmful to humans or organisms. However, little is known about risks due to the lack of information on human exposure, especially when considering the ingestion pathway due to the variety of products comprising human nutrition. One cause of this information scarcity is the difficulty in identifying small microplastics in complex matrices, such as foodstuff and beverages, due to the presence of other particles and abundant organic matter. Considering these challenges, the objective of this work was to explore new strategies and developments in microplastic sample preparation and identification in food and beverages.

Water samples were prepared by filtration of tap water (6 L) or bottled water (3 L) in glass fiber filters (Prat DUMAS) followed by staining. White wine samples were filtered (0.75 L) in glass fiber filters (GF/F Whatman), with and without staining, and with and without adding 0.75 L of 15 % H_2O_2 (Labkem) at room temperature (20˚C) for 30 min. Animal tissues (e.g., 1-2 g of shrimp muscle, fish digestive tissues, clams) were subjected to digestion with 30 - 50 mL of 10% KOH (Labchem) at 60˚C for 24h, followed by filtration in glass fiber filters (GF/F Whatman), with additional treatment in the filtration system by filtering 50 – 100 mL of boiling water, leaving 10 mL of acetone for 10 min (Fischer Scientific, UK) followed by addition of another 10 mL of acetone for filtration, and finally staining. Staining was conducted by adding 0.5 – 1 mL of 0.01 mg mL^{-1} Nile Red (Sigma-Aldrich, USA) staining for 5 minutes followed by washing with ultrapure water. Filters dried inside glass Petri dishes. Fluorescent stained microplastics were observed under the optical microscope (10x, Olympus BX41) using an external 470 nm excitation light source (SPEX Forensic) and orange filter (Standard ProMaster® Orange Filter) (Prata et al. 2019). Micro-Raman spectroscopy (Horiba, Jobin-Yvon) was conducted using the 442 nm (He:Cd laser) and 663 nm (He:Ne laser) line, acquiring spectra of 150 – 3300 cm^{-1} with a manual multipoint baseline correction (for fluorescent background subtraction). Strict control measures were taken during sample preparation to avoid external contamination.

For food and beverage, sample preparation is an important step which allows further identification of microplastics. For less complex samples, such as drinking water, concentrations can be determined with little preparation. Using Nile Red staining, concentrations of 10 microplastics L^{-1} were found in tap water (6 L) and 0.5 microplastics L^{-1} in bottled water (3 L). For samples with higher complexity, such as white wines, sample preparation may be required due to numerous and stainable biogenic particles. H_2O_2 with Fe(II) is recommended for plant matter, depending on cellulose and lignin content (Prata et al., 2020a). However, the use of H_2O_2 in white wines produces a sticky coating of particles likely due to caramelization or Maillard reactions between sugars and amino acids or proteins (Prata et al., 2020b). Thus, specific samples may require adaptation of sample preparation protocols, with a filtration of lower volumes (0.35-0.40 L) being recommended for nOM rich beverages. Similarly, typical preparation of animal tissues (e.g., fish digestive system) in KOH leaves unremoved fats, soaps, and gelatinous matrices that are stainable, hinder identification, and therefore must be removed by

additional preparation steps with boiling water and acetone. These samples put in evidence how methods must be adapted depending on sample composition. Lack of proper sample preparation drastically increases efforts during identification, either from the presence of numerous particles or stainable debris, from which microplastics are to be identified.

Estimation of human exposure to microplastic has yet to be assessed stemming from difficulties in identifying small microplastics in complex matrices, with ingestion being the principal route worrying consumers. Removal of natural organic matter is required to extract and clean microplastics and reduce the number of confounding particles. The diversity of matrices in food and beverage requires different sample preparation methods, preceding the choice of sample identification. Visual identification is simple but least reliable. Fluorescence staining provides a compromise between an objective criterion and low equipment requirements but relies on proper removal of stainable natural organic matter. Spectroscopy is the most reliable, allowing for morphological and chemical characterization (i.e., polymer identification), but at a lower throughput and higher equipment sophistication. Micro-Raman spectroscopy is effective in the characterization of microplastics in food and beverage, such as white wines (Figure 6), but impractical for quantification. The solution relies on the combination of techniques: quantification based on fluorescence staining with Nile Red, followed by characterization of a given fraction of particles (e.g., 50%) by micro-Raman spectroscopy. Synergistic advantages between these techniques are obvious, as both rely on optical identification of fluorescent stained microplastics that can be excited by the 442 nm laser in the micro-Raman setup, thus improving sample throughput without interfering with spectra collection. Hence, further developments must be made on sample preparation for diverse matrices in food and beverages, allowing the application of this techniques after proper removal of natural organic matter.

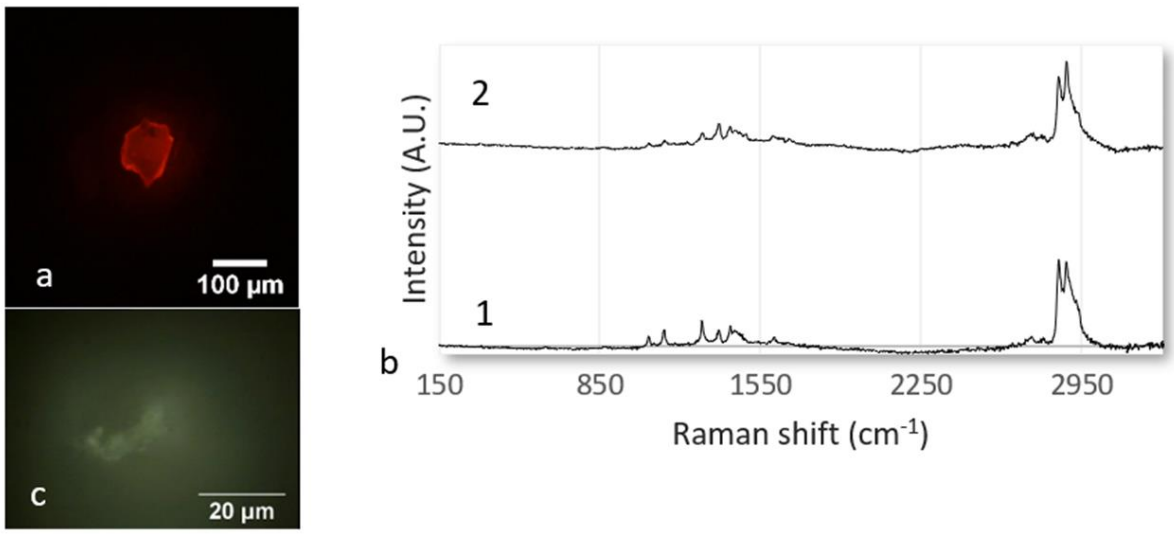


Figure 6: Nile Red stained microplastic found in a fish kidney under the 470 nm excitation light and orange filter (a). Raman spectra (b) and microscopic image (c) of a microplastic found in white wine. Polyethylene reference (1) and fragment spectra (2), with the following assignment of bands: 1061 and 1127 cm^{-1} as stretching C-C, 1448 cm^{-1} as symmetric deformation C-H, 2845 and 2886 cm^{-1} as symmetric stretching C-H (Gall and Hendra 1972; Käppler et al. 2016).

Bibliography

Prata JC, da Costa JP, Girão AV, Lopes I, Duarte AC, Rocha-Santos T. 2019. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. Sci Total Environ. 686:131-139.

Prata JC, Reis V, Matos JTV, da Costa JP, Duarte AC, Rocha-Santos T. 2019. A new approach for routine quantification of microplastics using Nile Red and automated software (MP-VAT). Sci Total Environ. 690:1277-1283.

Prata JC, Paço A, Reis V, da Costa JP, Fernandes AJS, da Costa FM, Duarte AC, Rocha-Santos T. 2020. Identification of microplastics in white wines capped with polyethylene stoppers using micro-Raman spectroscopy. Food Chem. 331:127323.

Gall MJ, Hendra PJ, Peacock OJ, Cudby MEA, Willis HA. 1972. The laser-Raman spectrum of polyethylene: The assignment of the spectrum to fundamental modes of vibration. Spectrochim. Acta, Part A 28(8): 1485-1496

Käppler A, Fischer D, Oberbeckmann S, Schernewski G, Labrenz M, Eichhorn KJ, Voit B. 2016. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? Anal Bioanal Chem. 408(29):8377-8391.

Protocol validation for microplastics detection in complex organic and inorganic matrices

Benetti F [1], Tedesco E [1], Perini A [1], Ruffato D [1], Mantovan ED [1]

[1] *ECSIN-European Center for the Sustainable Impact of Nanotechnology, ECAMRICERT SRL (IT)*

Abstract: According to a recently published document of the European Commission, microplastics are solid synthetic-polymer-containing particles of no more than five millimiters in their longest dimension, and which may contain additives or other substances. Microplastics can be classified either as primary microplastics if industrially manufactured and intentionally used or secondary microplastics if originated by chemical and physical ageing and degradation of plastic products such as plastic bags, plastic bottle, etc. Independently from their origin, microplastics pose a potential threat for both environmental and public health, due to their large diffusions (e.g. seawater, fresh-water, soil, marketed products) and (bio)persistence. Considering the vast array of different plastic types available on today's consumer market, the quantitative or qualitative analysis of microplastics is extremely challenging and no officially recognized or standardized methods are available. Based on long standing experience of ECSIN Laboratory in studying and developing procedures to detect nano- and microparticles in complex matrices, ECSIN Laboratory has developed and validated specific procedures for qualitative and quantitative analysis of microplastics in simple and complex matrices such as drinking water, soft drinks, detergents, cosmetics, infant formulas, beverages, mineral salts, fish products, beer, tea and coffee, as well as environmental matrices.

In this contribution, the developed and validated approach will be presented with the most important results.

Microplastics are characterized by a huge heterogeneity in terms of size and chemical composition. As such, many analytical methods have been applied to detect microplatics in different matrices. Independently from the applied analytical method, procedures to analyse microplastics should consider the following three steps: (i) sample preparation; (ii) filtration; (iii) microplastics detection and identification. Sample preparation concerns solubilization of powdered products and matrix degradation. Several reagents are currently used to degrade matrices, such as acid solvents, alkali solvents, or oxidation. For biological matrices, an enzymatic digestion can be also applied.

In our validated methods, we tested the best approach to degrade both inorganic and organic matrices, taking into consideration that sample preparation must not have an impact on microplastics. This approach considers that microplastics can be localized everywhere in the sample, and no visual sorting or alternative selection methods should be applied to avoid artifacts or bias. Therefore, the entire sample is always considered in sample preparation. Therefore, each sample/matrix has been degraded in the presence of microplastics standards to verify the effect of sample preparation on microplastics. Following sample preparation, microplastics are collected on filters. Filtration is performed in a ISO 7 class cleanroom to avoid any possible particle contamination. Qualitative and quantitative analysis of microplastics in terms of size and chemical composition is performed by using µFTIR in a chemical imaging mode. This approach permitted us to establish method recovery, and the ability of sample preparation to degraded matrices without altering plastic materials. Particles smaller than 6 µm can be analysed by using both TEM-EDX and SEM-EDX.

Based on the aforementioned three-steps approach, ECSIN Laboratory developed and validated a method to detect microplastics in several matrices and products. As shown in Figure 7, developed sample preparation recipe permits to completely degrade matrix.

In order to test the stability of microplastics during sample preparation, a known number of microplastics standards with well-defined size, shape and chemical composition has been added to the matrix and exposed to sample preparation. Recovery in terms of number, size and chemical identity has been then evaluated by using µFTIR/chemical imaging. Recovered number of microplastics ranges

from 80 % to 114 %, and size distribution analysis pre- and post-digestion confirmed that no alterations occur to microplastics during sample preparation (Figure 8).

In light of these results, ECSIN Laboratory applied the procedure to detect microplastics in several foodstuff such as table salt, water, soft drinks, mollusks, and collected preliminary information on microplastics typology as a function of geographical area.

Figure 7: Examples of digestion of shrimps, clams, and powdered milk with the developed sample preparation.

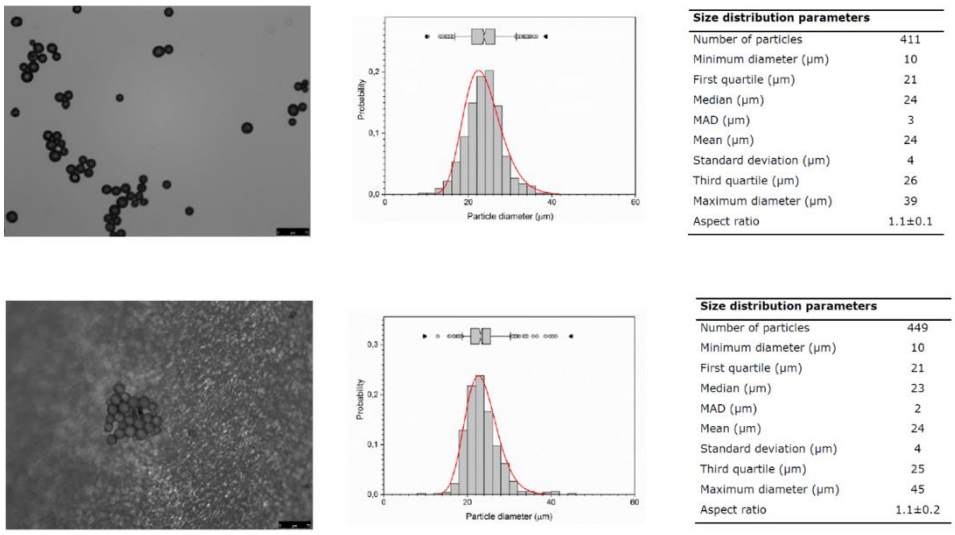


Figure 8: Comparison of size distribution analysis of microplastics before and after sample digestion.

Primary (intentionally added) and secondary (unintentionally generated) microplastics pose a potential threat for both environmental and public health. To have a coordinated approach to assess the human health risks of micro- and nanoplastics in food, the development of validated analytical methods to monitor the real microplastics exposure of humans is essential. These methods should permit to investigate the content of microplastics in consumers' goods, which are characterized by complex organic and inorganic matrices. During these last years, ECSIN Laboratory developed and validated analytical methods to detect microplastics in several foods, such as drinking water, soft drinks, infant formulas, beverages, mineral salts, fish products, beer, tea and coffee. These validated methods foresees the analysis of the entire sample in order to get representative data and information. With this approach ECSIN Laboratory gained experience on microplastics detection in different foodstuffs, and it can be take into consideration for discussion and development of standardized protocols.

Bibliography

European Chemicals Agency. 2019. Annex XV Restriction Report. Proposal for a restriction: intentionally added microplastics.

Lusher AL, Hollman PCH, Mendoza-Hill JJ. 2017. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fisheries and Aquaculture Technical Paper. No. 615. Rome, Italy.

A rapid method for the identification and classification of microplastics based on Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS)

Di Lonardo S [1]; Bonetti A [1], Pantani OL [2], D'Acqui LP [1]

[1] Research Institute on Terrestrial Ecosystems-Italian National Research Council (IRET-CNR, IT), [2] DISTA – Università degli Studi di Firenze (IT),

Abstract: Nowadays, the detection of microplastics (MPs) is an emerging critical issue in environmental and analytical science. Thus far, there is no standardized method for the identification and quantification of MPs (Nguyen et al., 2019). One method can be the visual identification without further chemical identification, which has a high potential of false counts. Therefore, chemical identification is indispensable to monitor. To determine the mass of plastic within the sample, mass spectrometry could be combined with pyrolysis gas chromatography or thermal extraction desorption gas chromatography. Both methods allow the chemical identification of the polymer types as well as the determination of mass of MPs in the sample; nonetheless, through these processes, the sample is destroyed and particle sizes and numbers cannot be calculated. In addition, these methods are time- and reagent-consuming analytical procedures because they require concentration or separation steps that present also a limiting factor in terms of sample load. Currently, Fourier Transform Infrared Spectroscopy (FTIR) is widely recognized as a reliable tool for nondestructive analysis and it is a rapidly expanding research area. In particular, Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) has been applied in many fields, such as soil physical and chemical properties analysis (Xing et al., 2016), identification of plant diseases (Andrade et al., 2008), gas monitoring (Wang and Wang, 2016), and food safety (Yang and Irudayaraj, 2001). However, the scope of FTIR-PAS to identify MPs nature remains unexplored.

To set up a general spectral database, different plastic samples from different suppliers were measured with a FTIR Perkin-Elmer Spectrum GX2000 spectrometer (running under Spectrum 5.0 software) with a MTEC 300 detector (MTEC, Ames, IA). Samples were placed in the photoacoustic cup for direct determination in the infrared photoacoustic spectrum. Before the determination was made, helium was purged for 5 min (at a slow flow rate of 5 cm^3 sec^{-1} to prevent fine particles spreading) to reduce the infrared absorption interference from carbon dioxide and water in the air. All spectra were collected in the wavenumber range of 4000 to 400 cm^{-1} at 4 cm^{-1} resolution. Each sample was scanned 256 times in succession: the final spectra was the merging version resulting from all these scans. All spectra were made compatible so they contain the same number of wavenumber datapoints in the considered spectral range (x axis). Spectra with a low signal-to-noise ratio were excluded afterwards. An initial charcoal blank spectrum was run to test the spectrometer performance and as a reference for calculating the sample spectra in photoacoustic units.

At the end of the analysis, sample spectra were processed using The Unscrambler 10.4 software to detect differences, if present, among different type of polymers.

After the collection of all database spectra, it was possible to distinguish among samples and all materials could be well separated by cluster analysis. The statistical hierarchical cluster analysis well separated spectra of different polymers. A dendrogram was obtained when normalized spectra from the FTIR-PAS database were subjected to a hierarchical cluster analysis. A number was assigned to each spectra and a library with database entries was created.

From the results of the analysis, FTIR-PAS has proven to be a versatile, bias-free tool to succeed at the prefixed task. It could overcome the disadvantage of time-costing (measurements take only about 20-30 min/sample totally; as a comparison, the fastest thermal degradation methods take approximately 2-3 h per sample) and rapidly assess chemical composition of microplastics without any chemical pretreatment. Due to differences in their chemical structure and composition, polymers exposed to Fourier transform infrared photoacoustic radiation displayed characteristic absorption

patterns which allow the distinction. In this work, through statistical analysis and clustering of spectra, the basis for an adaptable reference database for the analysis of MPs has be provided. Moreover, the database can be expanded with new spectra in the future, by allowing the implementation.

Finally, as FTIR-PAS has been successfully used for the identification of many components of complex matrices, such as bulk sediment samples using calibration databases between spectra of the sediment and known values of the detrital component, in the next future similar models for the detection of microplastics in food could be established.

Bibliography

Yang H, Irudaraj J. 2001. Characterization of Beef and Pork using Fourier-Transform Infrared Photoacoustic Spectroscopy. LWT Food Sci Technol 34(6): 402-409.

Andrade L, Freitas P, Mantovani B, Figueiredo M, Lima R, Lima S, Rangel M, Mussury R. 2008. Detection of soybean rust contamination in soy leaves by FTIR photoacoustic spectroscopy. Eur Phys J Spec Top 153,539–541.

Wang J, Wang H. 2016. Ammonia, carbon dioxide and water vapor detection based on tunable fiber laser photoacoustic spectroscopy. Optik 127(2): 942-945.

Xing Z, Du C, Tian K, Ma F, Shen Y, Zhou J. 2016. Application of FTIR-PAS and Raman spectroscopies for the determination of organic matter in farmland soils. Talanta. 158:262-269.

Nguyen B, Claveau-Mallet D, Hernandez LM, Xu EG, Farner JM, Tufenkji N. 2019. Separation and Analysis of Microplastics and Nanoplastics in Complex Environmental Samples. Acc Chem Res. 52(4):858-866.

Session 2: Developments in exposure of humans to micro- and nanoplastics

Microplastics in our diet: complementary in vitro gut and epithelium moderls to understand their fate in the human digestive tract

Fournier E [1], Etienne-Mesmin [1], Mercier-Bonin M [2], Blanquet-Diot S [1]

[1] *Université Clermont Auvergne, INRAE, MEDIS (Microbiology, Digestive Environment and Health, FR),*
[2] *Toxalim (Research Center in Food Toxicology), Université de Toulouse, INRAE, ENVT, (FR)*

Abstract: The last half-century has witnessed a steady increase of global plastic production with a significant proportion of the plastic produced persisting in the environment. Plastic products can be degraded into smaller secondary pieces (known as microplastics) that can enter the food chain and be ultimately found in the human gastro-intestinal tract (GIT). Up to date, their fate and effects on the human digestive sphere are largely unknown, especially in the child who are an at-risk population.

Innovative in vitro approaches

The aim of this work is to evaluate for the first time the fate of microplastics in the human digestive tract (both under adult and children conditions) by using complementary in vitro models of the gut lumen and intestinal epithelium : (i) the TNO Gastrointestinal model (TIM) which is currently the most complete simulator of the upper human GIT and reproduces the main physicochemical parameters of the stomach and small intestine (pH, temperature, transit time, digestive secretions and passive absorption), (ii) the Artificial Colon (ARCOL) which integrates the main physicochemical but also microbial (luminal and mucus-associated microbiota) parameters of the human colon and (iii) co-culture of Caco-2 and HT29-MTX (mucus-secreting) cells as a model of human intestinal epithelial cells.

Expected results

By using the TIM system, we will study the kinetics of physicochemical degradation of microplastics in the human stomach and the three parts of the small intestine using different analytical methods (Scanning Electron Microscopy, IR and Raman spectroscopies, Nile Red dye coupled with an automated counting software (MP-VAT)). In the lower GIT simulated by ARCOL, the impact of a chronic exposure of microplastics on the human mucus-associated and luminal gut microbiota will be

investigated by following both microbiota composition by 16S Illumina sequencing and its metabolic activity by short chain fatty acid and gas measurement. Microplastics degradation by resident microbiota will also be characterized using the same methods as for the TIM system. TIM and ARCOL experiments will be performed under adult and child conditions. Samples containing digested microplastics will be applied to co-culture cell models to monitor the effect of such exposure on host responses, including cytotoxicity, intestinal barrier permeability, mucus secretion and inflammation.

Conclusion

These data will allow a better understanding of microplastic interactions with physicochemical and microbial parameters of the human GIT, and will anticipate key outcomes for better human health risk assessment, especially in child who is an at-risk population. Next steps will be dedicated to the study of microplastics as a potential reservoir to the gut of organic contaminants and enteric pathogens.

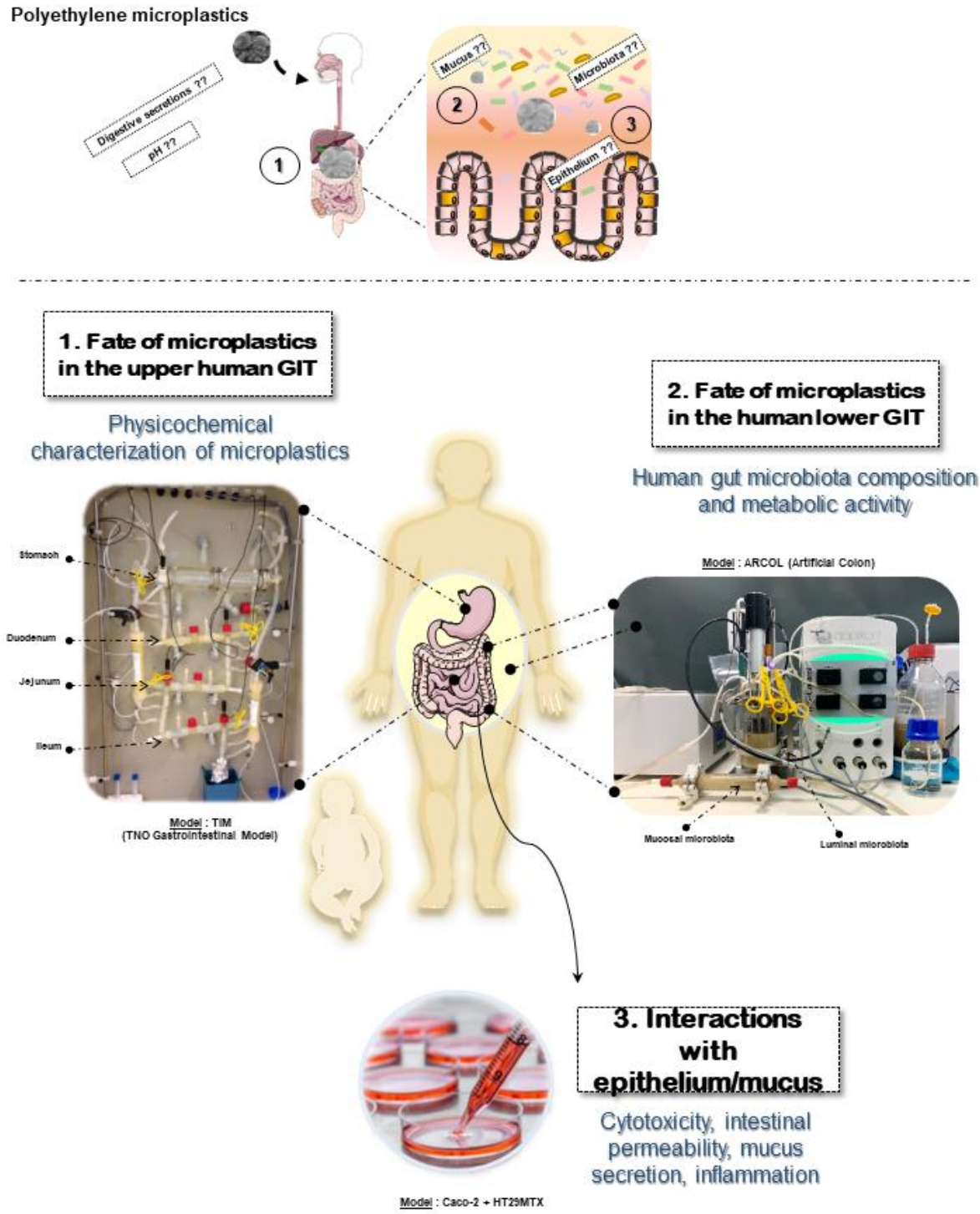


Figure 9: Gut targeted impact of microplastics: an in vitro combined approach.

Research on the impact of microplastics in the New Zealand diet on Human health

Nicolas J [1], Pantos O [2], Gaw S [3]

[1] Ministry for Primary Industries, New Zealand Food Safety (NZ), [2] Institute of Environmental Science and Research (NZ), [3] School of Physical & Chemical Sciences, University of Canterbury (NZ)

Abstract: Microplastics are an emerging area of concern with a number of studies now reporting their occurrence in different environments and matrices and this raises the question as to whether

microplastics present a food safety risk. It is expected that plastics pose a low health risk to humans because they are biologically inert. However, hazard and risk of contamination of plastics and due to manufacturing process, environmental exposure to chemical contaminants is unknown. The bioavailability of microplastics and associated contaminants in humans is unknown and internationally has been identified as a major data gap.

Recent research showed microplastic contamination of New Zealand Seafood (Webb et al., 2019; Markić et al., 2018). However, The Rethinking Plastics in Aotearoa New Zealand report (Office of the Prime Minister's Chief Science Advisor, 2019), and the risk profile on microplastics in the New Zealand diet (Pantos et al., 2019) identified that the characteristics and levels of chemical contaminants associated with microplastics in New Zealand are unknown and further research is required to assess the risk to human health. This paper discusses the New Zealand research on the potential health risks from exposure to microplastics via the food chain.

The levels of microplastics and its contaminants will be determined in a range of food categories, including mahinga kai (wild food). The harvesting and sharing of wild foods has cultural and spiritual significance for some groups in New Zealand, Māori in particular. Traditional foods, and methods of food gathering remain important to Māori and are an integral part of manaakitanga (providing for others) (King et al., 2014). The impact of cooking and food preparation on microplastics and their contaminants will be assessed and included in the research.

Microplastics collected in different food categories will be recovered and size-fractionated using USEPA and NOAA standard protocols to defined size fractions. Microplastic size fractions will be dried, transferred into plastic-free containers and stored for analysis. Isolated microplastics will be characterised visually and microscopically, and identified by FTIR analysis. Quality assurance protocols ensuring against accidental sample contamination will be implemented. Isolation protocol performance and reproducibility will be assessed using matrix-spiked recovery assessments incorporating microplastics of known number, size and polymer composition.

The key to establishing whether ingested microplastics present a toxicity concern through food is to determine their properties once consumed and whether there is appreciable systemic uptake and metabolism of plastics. In vitro bioavailability studies will assess the absorption rates of microplastics (labelled, virgin and aged-plastics, and pristine particles) and their contaminants through the human gut. Concentrations used in the in vitro study will be as close as possible to field levels determined in the food survey.

The research on the potential health risks from exposure to microplastics via the food chain has started on December 2019, preliminary results on the food survey will be available at the Scientific Colloquium in June 2020.

The toxicity of microplastics, plus the ability to leach chemical contaminants after oral exposure may potentially result in adverse health effects to humans. However, much more data is needed to characterise microplastic contamination of food. With advancing analytical methods, the detection of microplastics and smaller particles will improve the exposure risk assessment for human exposure to microplastic in food. It is important to assess whether microplastics and associated contaminants translocate across the intestinal wall and become available for systemic internal exposure, and factors will influence the absorption rate.

No information on background dietary levels of microplastic in processed food in New Zealand are available at this time. This means having an understanding of what the levels of microplastic are and potential mitigation steps would advance public health in reducing exposure through the diet. Additionally, New Zealand's food-based export industries and economy are particularly vulnerable to microplastic contamination as this could result in a food safety risk, potential yield loss, and could have potential trade implications.

This research will gain valuable knowledge that will contribute to assess the risks microplastics in food may pose to human health, tāonga (something considered to be a treasure or valuable resource), and key export industries for New Zealand, while also making a significant contribution to international microplastic research.

Bibliography

Markić A, Niemand C, Bridson JH, Mazouni-Gaertner N, Gaertner JC, Eriksen M, Bowen M. 2018. Double trouble in the South Pacific subtropical gyre: Increased plastic ingestion by fish in the oceanic accumulation zone. Mar Pollut Bull. 136:547-564. doi: 10.1016/j.marpolbul.2018.09.031. Epub 2018 Oct 6. PMID: 30509840.

Rethinking Plastics in Aotearoa New Zealand, A report from the panel convened by the Office of the Prime Minister's Chief Science Advisor, December 2019

King NJ, Lake RJ, Kerr GN. 2013. Wild foods. In Dymond JR ed. Ecosystem services in New Zealand – conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand

Pantos O, Cressey P, Nicolas J. 2019. Risk Profile: Microplastics in the diet. New Zealand Food Safety Technical Paper No: 2019/09.

Webb S, Ruffell H, Marsden I, Pantos O, S. Gaw S. 2019. Microplastics in the New Zealand green lipped mussel *Perna canaliculus*. Mar Pollut Bull 149: 110641.

Session 3: Developments in hazard identification and characterisation for micro- and nanoplastics

The *in vitro* effects of microplastics on the cytokine response of macrophages

Mengelers M [1], Yvonne Staal Y [1], Margriet Park M [1], Wim Mennes W [1], Dehaut A [2], Duflos G [2], Niemann H [3], Amaral-Zettler L [3], Beijer N [3]

[1] National Institute for Public Health and the Environment (RIVM, NL), [2] L'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES, FR), [3] Royal Netherlands Institute for Sea Research (NIOZ, NL)

Abstract: There is growing awareness of the presence of minute plastic particles found in the food we consume, raising concerns on potential adverse effects. Macrophages play an important role in scavenging of particles, which may lead to induction of an immune response. We investigated the influence of microplastic particle size and composition on the immunologic response of macrophages exposed to these particles *in vitro*.

Environmentally-weathered macroplastic samples were collected from the open-ocean South Atlantic Subtropical Gyre ("Garbage Patch") and from the French coastal environment. Plastics were cryo-milled to obtain sizes below 300 µm. We used (micro)Raman-spectrometry, FT-IR and pyrolysis-GC/MS, and phase-contrast microscopy to characterize the physico-chemical properties of the particles. Coastal plastic samples were identified as polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET) and subsequently size-fractionated to: 20-50 µm, 50-100 µm, and 100-200 µm size classes. Differentiated macrophages (THP-1 cells) were grown in 96-well plates, and exposed to sinking particles directly or were placed on the basolateral side of inserts, to expose them to floating particles. After 48 hours of exposure, we assessed cell viability and cytokine response. For the dose-response analysis we used the EFSA implementation of the RIVM developed PROAST software package.

We observed that there was some overlap in particle size distribution of the three different size classes. Macrophages responded differentially to different types and sizes of microplastic particles, reflected in different benchmark dose (BMD) levels at a critical response of 20%. PET and PS exposure produced the largest changes, including a dose-related increase in cytokine production (IL-1β, IL-6, and TNF-α). Smaller particles induced cytokine production at lower concentrations than larger particles.

We intend to use a combination of physiochemical and biological data for in-depth multi-dimensional data analysis to further explore the potency of particles properties in relation to their potential immunotoxicological effects.

This research contributes to our understanding of the potential hazards of environmentally -sourced plastics on human health.

This research was financially supported by the Netherlands Organisation for Health Research and Development (ZonMw) in the framework of the Microplastics & Health programme. This study has also been financially supported by the European Union European Regional Development Fund (ERDF), the French State, the French Region Hauts-de-France and Ifremer, in the framework of the project CPER MARCO 2015-2020.

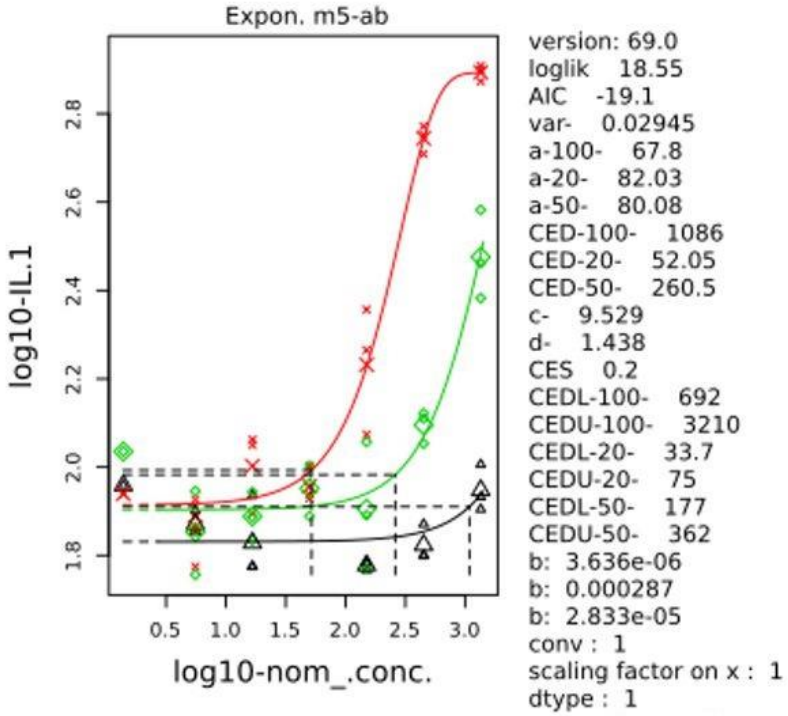


Figure 10: Cytokine (IL-1β) response after 48h of exposure of macrophages to different concentrations of polyethylene terephthalate microparticles. Average particle sizes are 20 micron (in red), 50 micron (in green) and 100 micron (in black). The horizontal dashed lines indicate the level of a 20% increase, the vertical dashed lines the associated BMD_{20}.

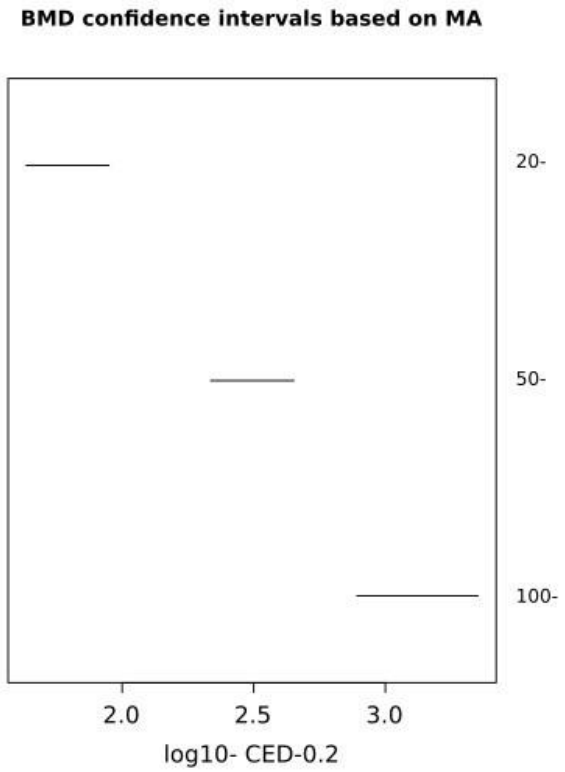


Figure 11: Confidence intervals of BMD_{20} levels (based on model averaging, MA) obtained after BMD analysis of cytokine (IL-1β) response after 48h of exposure of macrophages to different concentrations of polyethylene terephthalate particles of 20, 50 and 100 micron.

Bibliography

Boraschi D, Italiani P, Palomba R, Decuzzi P, Duschl A, Fadeel B, Moghimi SM. 2017. Nanoparticles and innate immunity: new perspectives on host defence. Semin Immunol. 34:33-51.

Kontrick AV. 2018. Microplastics and Human Health: Our Great Future to Think About Now. J Med Toxicol. 14(2):117-119.

Shah A, Dobrovolskaia MA. 2018. Immunological effects of iron oxide nanoparticles and iron-based complex drug formulations: Therapeutic benefits, toxicity, mechanistic insights, and translational considerations. Nanomedicine. 14(3):977-990.

Wright SL, Kelly FJ. 2017. Plastic and Human Health: A Micro Issue? Environ Sci Technol. 51(12):6634-6647.

A novel 3D intestine barrier model to study the immune response upon exposure to microplastics

Lehner R [1], Wohlleben W [2], Landsiedel R [3], Septiadi D [4], Petri-Fink A [4], Rothen-Rutishauser B [4]

1 Sail & Explore Association (CH), [2] BASF SE, Advanced Materials Research (DE), [3] BASF SE, Experimental Toxicology and Ecology (DE), [4] Adolphe Merkle Institute, University of Fribourg (CH)

Abstract: Plastics have shaped our society within the last centuries like no other materials. Their production has increased to more than 350 million metric tons worldwide in 2018 with around 40% of all plastics being incorporated into single use products such as plastic bags, food and drink containers or cups (PlasticsEurope, 2019). The growing production of synthetic plastic products also led to a substantial increase of poorly managed plastic waste, which results in their widespread and increasing accumulation within the environment. The plausibility of human exposure to microplastics has increased within the last years (Rochman et al., 2015). Microplastics have been found in different food types including seafood, salt, sugar and beverages (Mason et al., 2018, Liebezeit and Liebezeit, 2013). So far, human health effects of microplastics after ingestion are unknown. Herein we designed a novel, three-dimensional in vitro intestinal model consisting of the human intestinal epithelial cell lines

Caco-2 and HT29-MTX as well as human blood monocyte-derived macrophages (MDMs) and dendritic cells (MDDCs) that is suitable to assess possible effects of ingested microplastics. We used environmentally relevant microplastics representing the main sources of secondary microplastics observed in the environment, e.g. particles of polypropylene (PP), of tire dust, polyamide (PA) and harder cross-linked and a softer thermoplastic version of polyurethanes (PU).

The human colon carcinoma cell line Caco-2 and the mucus secreting cell line HT29-MTX-E12 were seeded as a co-culture on permeable membrane inserts. Cells were grown for 20 days with medium changes every other day. On day 20, the co-cultures were assembled by adding human blood monocyte derived macrophages and dendritic cells to the basolateral side of the inserts. The co-culture model characterization involved the integrity of the cell barrier, tight junction formation, spatial arrangement of the cells as well as mucus secretion.

All the microplastic particles were nebulized directly onto the cells using a dry powder insufflator connected to a nebulization chamber. The effects of the different materials on the viability of the cells from the 3D co-culture model were investigated by the release of lactate dehydrogenase (LDH) into the supernatant as a result of cell membrane rupture. The (pro-)inflammatory response of the co-cultures after exposure of the materials for 6, 24 and 48h was investigated by quantifying the release of interleukin- 8 (IL-8), interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) into the basal cell culture well.

The characterization of the 3D model by laser scanning microscopy showed monolayer formation of the enterocytes and a homogeneous distribution pattern of MDMs and MDDCs on the basolateral side of the insert. The formation of the microvilli brush boarder on the Caco-2 enterocytes was monitored by scanning electron microscopy (SEM), indicating an increase of microvilli formation with increasing time. Mucus production and identification of goblet cells within the co-culture was determined by histochemical analysis using Alcian blue. The Caco2/HT29-MTX co-cultures showed a clear, evenly distributed blue coloring, suggesting the presence of mucus over the whole cell layer. The formation of the epithelial tissue barrier by tight junction could be proven by the labelling of the protein zonula occludens 1 using fluorescently labeled antibodies. Transepithelial electrical resistance (TEER) measurements as well as permeability studies using (FITC)-dextran were conducted to assess the cell barrier integrity. Cultures composed of Caco-2/HT29-MTX seeded at a ratio of 9:1 revealed TEER values above 200 Ωcm^2 and were considered for further experiments. SEM imaging of the aerosolized microplastic particles revealed a heterogeneous deposition of the particles. No significant cytotoxicity was observed after 24h following exposure compared to untreated cells. (Pro-)inflammation response after MP exposure was assessed by measuring the cytokines IL-8, TNFα and IL-1β released into the basal medium. No significant release for all cytokines after 24 h of exposure could be detected, indicating that the particles did not induce any pro-inflammatory reactions.

In this study, to the best of our knowledge, we for the first time established and characterized a human intestinal co-culture model consisting of two epithelial cell lines and primary monocyte derived immune cells to assess possible inflammatory effects of ingested microplastics by human. We specifically focused on primary derived human monocytes as they more closely resemble interactions and innate immune responses. Relevant microplastic particles, including polypropylene, tire dust, polyamide as well as polyurethanes (on the order of 50 to 500 µm) and healing earth, were selected because they represent the main sources of secondary microplastics observed in the environment. Microplastic particles were exposed at concentrations of 823.5 - 1333.5 $\mu g/cm^2$ to the model using a dry powder insufflator system to aerosolize the particles directly on the intestinal model's surface. Cytotoxicity was investigated after 24h exposure via measuring the release of lactate dehydrogenase. Inflammatory endpoints including the cytokines IL-8, TNFα and IL-1β were additionally monitored.

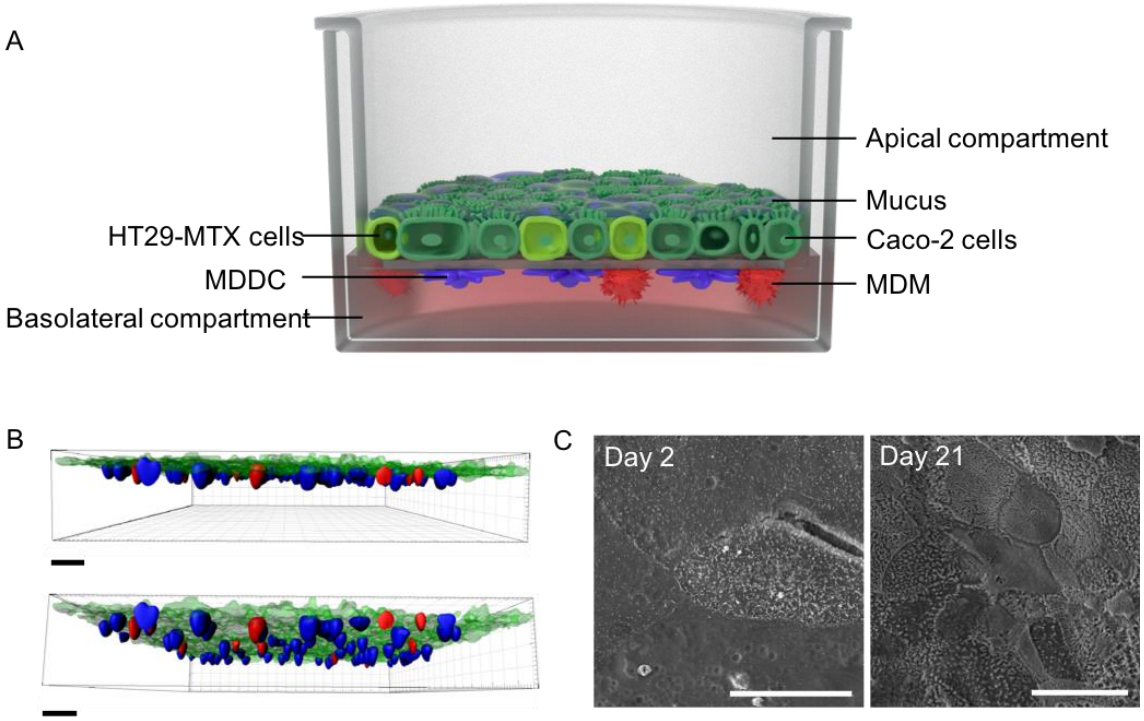


Figure 12: (A) Schematic setup of the 3D co-culture model consisting of intestinal enterocytes (Caco-2) and mucus producing HT29-MTX cells, MDMs and MDDCs. (B) 3D visualization of the co-culture model shoring Caco-2 monolayer (green), MDDCs (blue) and MDMs (red), scale bar represents 50 µm. (C) Determinatio of the microvilli brush broader formation within 21 days by SEM. Scale bar: 20 µm.

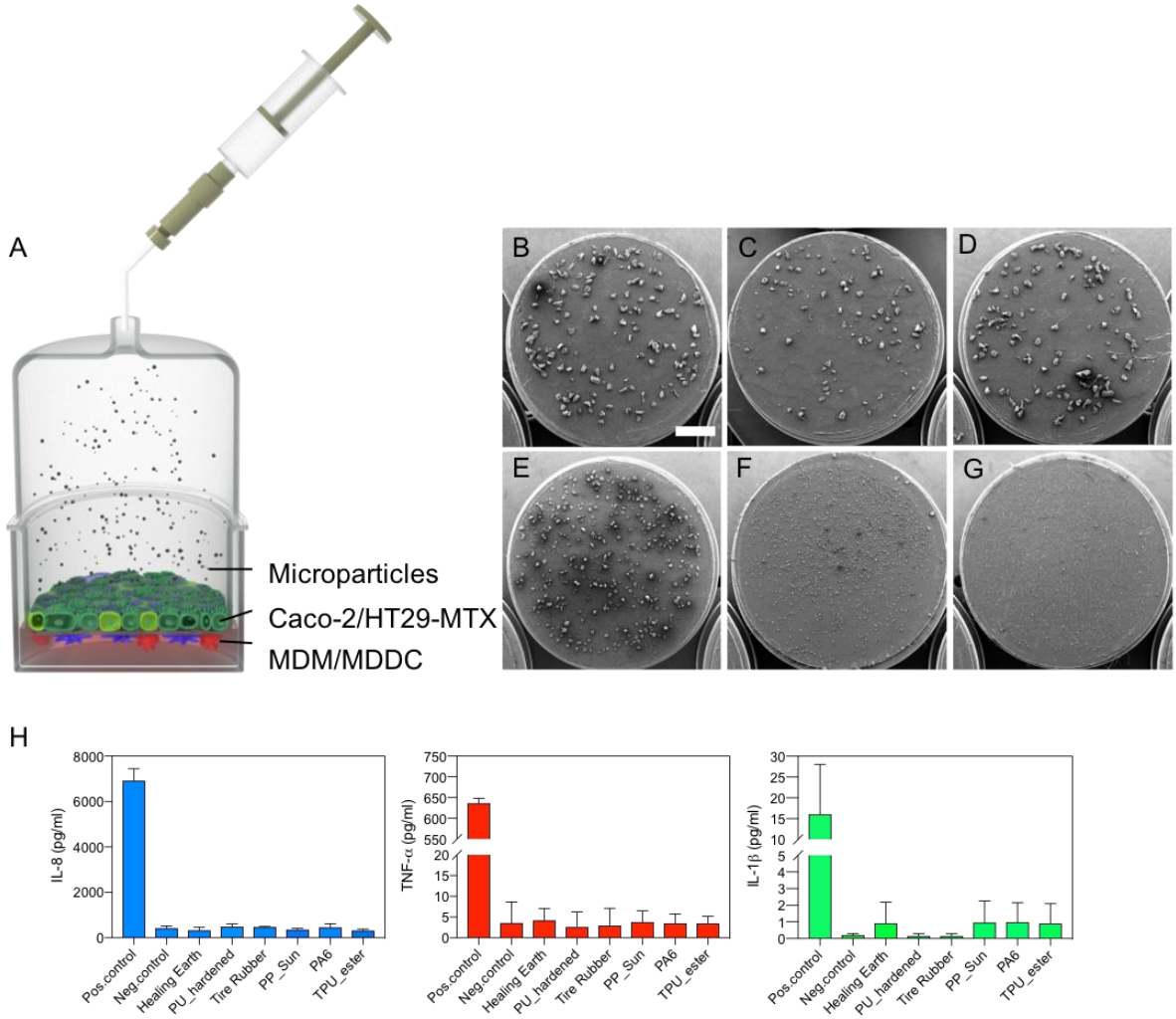


Figure 13: (A) Nebulization outline for the exposure of the different microparticles, e.g. microplastics and healing earth, to the co-culture system that included monocyte derived MDM and MDDc immune cells. (B-G) Material exposure patterns imaged by SEM for PP_Sun (B), PU_hardened (C), TPU_ester (D), Tire rubber (E), PA6 (F) and healing earth (G). Scale bar: 2 mm. (H) Pro-)inflammatory response of the intestinal model after exposure to MPs and healing earth for 24h. Release of (pro-)inflammatory cytokines IL-8, TNF-α and IL-1β using LPS (10 µg/ml) as positive control for secretion of (pro-)inflammatory markers. Data are presented as mean ± standard erron of the mean (n = 3)

Bibliography

PlasticsEurope. 2019. Plastics - the Facts 2019. Available online: https://www.plasticseurope.org/download_file/force/3183/181

Rochman CM, Tahir A, Williams SL, Baxa DV, Lam R, Miller JT, Teh FC, Werorilangi S, Teh SJ. 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci Rep. 5:14340.

Mason SA, Welch VG, Neratko J. 2018. Synthetic Polymer Contamination in Bottled Water. Front Chem. 6:407.

Liebezeit G, Liebezeit E. 2013. Non-pollen particulates in honey and sugar. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 30(12):2136-40.

A systematic approach to investigate microplastics hazards with specific consideration of the carrier hypothesis for polycyclic aromatic hydrocarbons (PAHs)

Emecheta E [1], Fischer L [1], Vogel A [1], Sieg H [1], Wolf C [2], Pfohl PM [3], Jessl S [3], Wohlleben W [3], Wiemann M [4], Hutzler C [1], Roloff A [1], Haase A [1]

[1] German Federal Institute for Risk Assessment (BfR, DE), [2] Institute of Energy and Environmental Technology (IUTA, DE), [3] BASF SE (DE), [4] IBE R&D Institute for Lung Health gGmbH (DE)

Abstract: Investigating possible human health effects of micro- and nanoscaled plastic particles (PPs) is challenging for several reasons. Micro-and nanoplastics are complex mixtures of diverse geometries, shapes and sizes that can be based on different polymer types (e.g. polyethylene (PE), polyamide (PA), polyurethane (PU), polymethyl methacrylate (PMMA)). Additionally they contain additives (e.g. antioxidants, plasticizers, pigments) and several contaminants (e.g. remaining traces of catalysts, traces of monomers and oligomers). Moreover, they may act as carriers for environmentally persistent organic pollutants (POPs) like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCB) or others (carrier hypothesis). There is no consensus yet which properties are most important for hazard assessment, or if all solid, persistent polymers should be assessed jointly. Thus, assessing possible hazards of PPs is a complex endeavor. Within the German BMBF funded project InnoMat.Life (www.innomatlife.de) we investigate a selection of different PPs, covering different polymers (e.g. PE, PA, PU) with broad size distributions. In a first step ad- and desorption of selected POPs are assessed. In parallel, we conduct in vitro-based studies using different cell models (intestinal, liver and lung cell models). These data will complement a planned in vivo study.

All materials of the study are relevant for industrial applications (e.g. for additive manufacturing), and were specifically selected or synthesized for the project. PE was chosen as a benchmark material. In addition, several types of PA (PA-6, PA-12), PU (4 variants), PMMA (2 variants) and a rubber material obtained from recycled truck tires were included. For assessing the sorption of PAHs benzo[a]pyrene was selected as a lead substance along with dibenzo[a,l]pyrene and anthracene. Firstly, we developed a universally applicable third polymer-phase partitioning (TPP) method, which enables us to quantify the sorption of POPs on various PPs without filtration. By this we analyzed the sorption of benzo[a]pyrene for 20 different PPs, including some aged polymers. Furthermore, the sorption of anthracene and dibenzo[a,l]pyrene was studied for selected PPs. The TPP method was validated using the commonly applied batch method.

In parallel, we investigated the biocompatibility of the PPs in three selected cell models, human intestinal epithelial Caco-2, human liver epithelial HepG2 and rat alveolar macrophages NR8383. In addition, the possible carrier effect of PPs was investigated for benzo[a]pyrene (BaP) and for two heavy metals in HepG2 and Caco-2 cells, by evaluating CYP1A1 expression and cytotoxicity, respectively.

Using the TPP method, we could show that the PPs strongly differed in their sorption behavior with surface area and hydrophobicity being the most influencing factors. That enabled us to rank and to categorize the PPs. All PPs investigated so far were not cytotoxic and no evidence was obtained supporting the carrier hypothesis. On the contrary, a few PPs tended to decrease the bioavailability of the selected pollutants. Of note, PPs derived from tire rubber were found to induce CYP1A1 expression in both cell lines even in the absence of pollutants.

Overall, data from this project will foster our understanding of possible human health hazards of PPs and, by this, contribute to the ongoing discussion about micro- and nanoplastics. From the material perspective, the investigations shall also provide new criteria for grouping of different PPs.

The role of Food Safety Agencies in the evaluation, communication and management of risks associated with microplastics in food

Rubio-Armendáriz C [1], Daschner Á [1], González Fandos E [1], González Muñoz MJ [1], M. Moreno-Arribas V [1], Talens Oliag P [1], Bustos García de Castro J [1]

[1] Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN)

Abstract: The environmental impact of micro- and nanoplastics has been of concern for many years but their potential to be transferred between trophic levels has only recently been identified as a global challenge. Therefore, the assessment of dietary exposure to microplastics constitutes now a primary challenge for all food safety agencies including the Spanish Agency for Food Safety and Nutrition (AESAN).

Accurate data on the presence of microplastics in food and drinking water are not available to assess the exact exposure of humans to micro- and nanoplastics through diet primarily due to the lack of standardized methods for analysis (Hartmann et al., 2019; Toussaint et al., 2019). Recent reports of

the reference Agencies of Food Safety (Science Advice for Policy by European Academies (SAPEA, 2019), Norwegian Scientific Committee for Food and Environment (VKM, 2019), Spanish Agency for Food Safety and Nutrition (AESAN) confirm this lack of quality and comparable data.

Although the lack of data to perform human exposure assessments is a major constraint, in the absence of total dietary exposure studies to plastics in humans, some estimates from some food groups identify marine foods as the main source of dietary exposure to plastics, micro-and nanoplastics that pollute the environment and that access the food supply.

The risks derived from the dietary intake of microplastics may be minimal compared to exposure through other routes like inhalation (Catarino et al., 2018). Nevertheless, there is also a lack of experimental data to perform a toxicity characterisation in humans and the potential health effects (the identification of dose-response relationships and threshold levels) of micro- and nanoplastics in consumers are still unknown and require further research.

Although micro- and nanoplastics in all food supplies and dietary exposure have not yet been assessed in Spain, the Spanish Agency for Food Safety and Nutrition (AESAN) noted a growing public and scientific concern about this topic and a mandate to its Scientific Committee was generated. Thus, the AESAN Scientific Committee considered a priority for 2019/2020 to publish a scientific report in order to improve the consumer's perceptions, reviewing information and data summarizing the status of knowledge of micro- and nanoplastics and its additives in foods. The Scientific Committee of AESAN made its own systematic literature search and 147 scientific references were reviewed from differing international sources in order to provide an overview of actual initiatives and future perspectives and opportunities in this research field.

A scientific report based on solid scientific data has been published to communicate the actual knowledge on micro- and nanoplastics and its additives in foods and to contribute to a better understanding of the dietary sources and the levels of dietary exposure to these contaminants. The report characterizes the current knowledge and highlights the gaps. This official report is intended not only to improve the consumer's perceptions of micro-nanoplastics in food but also the scientific community. Identifying the most relevant dietary sources of microplastics, the occurrence of the different molecules and polymers and their relevance in terms of exposure will help to focus future research projects, regulatory initiatives and monitoring programs at a regional, national and international level. It is the interest of the Spanish Food Safety Agency (AESAN) to promote research on microplastics in the food supply and fill the gaps where specific data are needed. Assessing the Spanish population dietary exposure to microplastics should be a challenge for our Food Safety experts and networking among the research groups should be promoted through our national food agencies and EFSA with the objective of assessing the dietary exposure to micro-and nanoplastics across the different countries and its populations.

Finally, a cross-nation diffusion of this report will be strategically designed.

Micro- and nanoplastics are emerging risks that are not yet fully understood but evidence regarding their toxicity is emerging. The lack of extensive knowledge on the toxic kinetics and toxic dynamics of these pollutants and their health effects prevents a solid risk characterisation. Evaluating the risks from micro- and nanoplastics requires knowledge of the hazard, exposure levels, and their effects. Risks associated with intake of microplastics are a function of hazard and exposure and tolerable daily intakes (TDI) for plastics and the different molecules have not yet been established.

Methods are available for identification and quantification of microplastics in food, but occurrence data are limited and a consensus on the micro-and nanoplastics definitions and descriptions, the standardisation of methods of analysis is needed for a better comparison and monitoring of the global results. For nanoplastics, no analytical methods or occurrence data in food are available and should urgently be developed, standarized and harmonized.

It is necessary to implement innovative solutions to mitigate/minimize humans' dietary exposure while regulating maximum levels of their main molecules or particles in different food sources. At present, the global commitment to reduce, reuse or recycle plastic materials keeps on being the best tool to minimize the environmental and health impact of these pollutants.

For a better understanding of the current state of play and ongoing research on this topic and to improve the knowledge and perceptions of consumers on micro-and nanoplastics in food, global

communication campaigns and informative materials should be designed considering multicultural perspectives.

Bibliography

Catarino AI, Macchia V, Sanderson WG, Thompson RC, Henry TB. 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environ Pollut. 237:675-684.

Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, Rist S, Karlsson T, Brennholt N, Cole M, Herrling MP, Hess MC, Ivleva NP, Lusher AL, Wagner M. 2019. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. Environ Sci Technol 53(3):1039-1047.

SAPEA. 2019. Science Advice for Policy by European Academies. A Scientific Perspective on Microplastics in Nature and Society. Berlin: SAPEA. https://doi.org/10.26356/microplastics.

Toussaint B, Raffael B, Angers-Loustau A, Gilliland D, Kestens V, Petrillo M, Rio-Echevarria IM, Van den Eede G. 2019. Review of micro- and nanoplastic contamination in the food chain. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 36(5):639-673.

VKM (2019). Norwegian Scientific Committee for Food and Environment. Microplastics; occurrence, levels and implications for environment and human health related to food. VKM report 2019: 16.

Developments in understanding consumer perceptions of micro- and nanoplastics in food

Understanding the public's perception of nanomaterials and how their safety is perceived in the EU – ECHA procurement procedure

Popelková S [1], Tengler M [2], Hamanová J [2], Novotný T [1], Holomek J [3], Kováčová V [2], Oliva F [2], Trávníčková J [3], Bašič D [1], Dibusz K [1]

[1] EcoMole s.r.o. (CZ), [2] SC&C s.r.o. (CZ), [3] ReachSpektrum s.r.o.(CZ)

Abstract: In 2019, the European Chemical Agency launched a study on understanding the public's perception of nanomaterials and how their safety is perceived in the EU. This study aims to examine some of these questions and provide insight into the perception of risks associated with nanomaterials for citizens so that the European Union Observatory for nanomaterials communications approach can be adapted to address these perceptions in an informed way. The main point of the study is to extensively answer the following questions:

- Are consumers aware of what nanomaterials are?
- Are consumers aware of where nanomaterials are used?
- What risks do consumers associate with nanomaterials?
- What benefits do consumers associate with nanomaterials?
- How do consumers think they are exposed to nanomaterials?
- Are consumers concerned about nanomaterials in the products they use?
- Are there specific areas that consumers are particularly interested in but feel they do not have sufficient information available?
- Who do consumers trust most for information on nanomaterials (authorities, companies, ngos, others)?
- What source do consumers use when looking for information on the safety and risks of nanomaterials?

The study consisted of an extensive literature search of reliable surveys on public perceptions of nanomaterials, which lead to the compilation of a survey questionnaire. This data collected through the survey questionnaire are analysed and conclusions are drawn.

The study found that despite manufactured nanomaterials being a common part of our everyday lives, general awareness about their nature, characteristics and properties is low. However, the level of awareness has increased compared to earlier surveys, and is expected to continue increasing in the future. The full report can be accessed here: https://echa.europa.eu/-/what-do-eu-citizens-think-about-nanomaterials- .

Various hypotheses about the public perceptions of nanomaterials will be confirmed/ disproved after the finalisation of the project, providing a comprehensive view on the public perceptions of nanomaterials.

Poster Sessions

Session 1: Developments in analytical methods for micro- and nanoplastics

A rapid method for detection and quantification of microplastics in bivalves molluscs: preliminary results

Bernardi C [1], Tirloni E [1], Simone S [1], Ottoboni M [1], Pinotti L [1], Giusti A [2], Armani A [2]

[1] Dipartimento di Scienze Veterinarie per la Salute, la produzione Animale e la Sicurezza Alimentare ,Università degli Studi di Milano (IT), [2] FishLab, Dipartimento di Scienze Veterinarie, Università di Pisa (IT)

Abstract: Microplastic (MPs) is considered a high concern topic because widespread in all environmental compartments. They are also found in many marine seafood species used for human consumption. MP, and in particular fibers, have been reported in the digestive tract of filter feeding organisms such as bivalves molluscs (BM) that are consumed whole. Therefore, BM consumption represents a potential exposure to MPs for humans. However, analytical procedures for MPs detection and quantification in BM are still standardized and a wide range of protocols is currently available. In particular, sample processing considerably differs among studies. Many researchers applied a two-step sample pretreatment (Dehaut et al;2016), consisting in an acid or alkaline digestion of BM and a density separation step followed, after decantation, by the filtering of the top layer of the solution, through a filter with a setting porosity. However, this two-step approach is time consuming and greatly increase the risk of airborne fibers contamination throughout the analysis. Therefore, in this preliminary work, based on an ongoing project, a one-step method consisting in alkaline digestion and direct filtration without any flotation step was developed and then used for detecting MPs in three species of BM (*Mytilus galloporovincialis, Chamelea gallina, Ruditapes philippinarum*) of great commercial interest on the Italian market. In addition, with the aim to validate the method, lab-made internal standard fibers were produced and used to contaminate some samples.

After preliminary trials aimed to define the best condition for the digestion of BM tissues, the following one-step protocol was used for the detection and quantification of MPs in M. galloporovincialis, C. gallina and R. philippinarum. After collection, MB shells were washed with ultrapure water to prevent contamination. Ten grams of internal tissues were digested in 100 ml of 35% potassium hydroxide solution at 40°C for 48h. The digested samples were then passed through a glass fibers filter (pore size: 1.6 µm) that was subsequently observed at stereomicroscope. Two blanks (atmospheric control and reagents control) were tested for the presence of microplastic particles/fibers. Internal control standards were also produced starting from monofilament polyamine suture for surgery. These were included in paraffine and then, the paraffine block was cut with microtome in 4 µm slices. The number of fibers in each slice was checked at microscope before addition to samples. Internal standards were then used to contaminate BM samples that were analyzed as previously described. The recovery was calculated as the percentage of the number of spiked microplastic fibers recovered after treatment. All preparation and measurement steps were conducted in a lab, where the surfaces were cleaned carefully with 70% ethanol solution. Cotton lab coats and nitrile gloves were always used. All plastic materials were replaced by metal or glassware, when possible. All materials were carefully cleaned and rinsed three times with ultrapure water (18.2 MΩ*cm, Elix5, Millipore) prior to use.

The one-step protocol developed in this study allowed to completely digest the bivalve tissues in all the species analyzed. The maximum concentration of MP g^{-1} was found in the R. philippinarum with values of 2.12 MPs g^{-1}. The lowest values were instead recorded in the *M. galloprovincialis* with a value of 0.8 MPs g^{-1}, and in *C. gallina* with 0.30 MPs g^{-1}. Overall, a high proportion of fibers was observed (95% of total microplastics), while fragments were the second most abundant morphotype (5%). In blank controls fibers were 84% of total microplastics while fragments were 16%. It is also recommended that operators should register colors of clothes worn underneath the lab coats. Regarding the color of MPs in samples, blue and green fibers were the most abundant, followed by black and red. The most frequent length range of MPs was 200-800 µm (66% of observed MPs). In the control blanks, the number of MPs observed in each blank ranged between 1 and 14 MPs $filter^{-1}$, with a mean of 5.33±4.59 MPs $filter^{-1}$. The fibers recovery was 100% and no alteration in shape and color was observed.

Based on the data produced in the present study, the digestion performed using 35% KOH at 40°C for 48h, can be considered a suitable technique for MB tissues processing. The 100% recovery of internal standard fibers confirm no effects on microplastic fibers confirming previous findings. In fact, also the recent work of Thiele et al., (2019) recommend the utilization of KOH as the most viable extraction method. In fact, it is not only representing the most economical and least time-consuming method but it also does not affect microplastics recovery. Therefore, the developed method, after further validation on a higher number of samples, could be considered as a possible standard method for BM tissue digestions in order to extract microplastics. Also in this study major issues arises from the airborne fibres contamination, as already reported by Woodall et al. (2015) and Nuelle et al. (2014). The airborne fibres contamination was in agreement with Reguera et al. (2019), who observed 4.29±4.68 MPs $filter^{-1}$ in the procedural blanks. In fact, despite the application of procedures aimed at preventing samples contamination, background concentration of airborne fibres reached a significant level with similar numbers both in microfibres from laboratory blanks both in environmental samples. To reduce or eliminate airborne contamination, analyzes should be implemented in a plastic-free clean room ISO 7.

Bibliography

Dehaut A, Cassone AL, Frère L, Hermabessiere L, Himber C, Rinnert E, Rivière G, Lambert C, Soudant P, Huvet A, Duflos G, Paul-Pont I. 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ Pollut. 215:223-233.Reguera P, Viñas L, Gago J. 2019. Microplastics in wild mussels (*Mytilus spp.*) from the north coast of Spain, Scientia Marina, 83(4):337-347.

Nuelle MT, Dekiff JH, Remy D, Fries E. 2014. A new analytical approach for monitoring microplastics in marine sediments. Environ Pollut 184:161-169.

Thiele CJ, Hudson MD, Russell AE. 2019. Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. Mar Pollut Bull. 142:384-393.

Woodall LC, Gwinnett C, Packer M, Thompson RC, Robinson LF, Paterson GL. 2015. Using a forensic science approach to minimize environmental contamination and to identify microfibres in marine sediments. Mar Pollut Bull. 95(1):40-6.

How to analyse microplastics in (sea)food: proposition of the MIMS concept (Minimal Information for the Microplastics Studies)

Duflos G [1], Dehaut A [1], Hermabessiere L [1]

[1] *ANSES – Laboratoire de Sécurité des Aliments*

Abstract: The global issue of microplastics (MP) is well known today. The increase of plastic production and carelessness regarding littering management led to the irrefutable pollution of the open Ocean by plastics, including the smallest particles called microplastics . For almost seventy years, the presence of MP in fish t has been described and for that time, the presence of these particles has also been recorded in cephalopods, crustaceans and shellfish. This rise the question on the quality of the product, food safety issues and risk assessment related to the presence of MP in such seafood for consumer's exposure.

To answer these questions, it is important to isolate and identify MP from seafood or other food matrices using reproducible and reliable methods in order to compare the different studies and accurately estimate the occurrence and levels of MP in seafood. Moreover, it is necessary to use the same analytical process. Based on a selection of scientific articles published so far, a review of the current practices of the community was carried out. Multiple aspects were addressed including sampling, extractions of MP, their quantification and identification, and the MP contamination at the laboratory scale.

This work allows to point out the limits of the analytical approaches and the main needs for improving the methods in order to be able to carry out the most reliable and reproducible analyzes. A final part of this work was dedicated to define the concept of "MIMS", Minimal Information for the Microplastics Studies, corresponding to essential and desirable points to be defined and included into studies These points have to be taken into account from project set up to data processing in order to generate reliable data and to ease the comparison of studies.

Bibliography

Dehaut A, Hermabessiere L, Duflos D. 2019. Current frontiers and recommendations for the study of microplastics in seafood. Trends Analyt Chem 116: 346-359.

Investigation of microplastics in Hungarian drinking water processing plants

Bordos G [1], Palotai Z [1]

[1] *Wessling Hungary Ltd. (HU)*

Abstract: In the last decade, environmental problems caused by microplastics (MPs) has been at the centre of scientific interest. MPs have been detected globally in a wide range of environmental elements. The number of studies in this area is rapidly increasing, especially regarding marine environments, but the focus on freshwater areas is moderate compared to oceans and seas, despite, these areas are very important in terms of drinking water production. Much less focus is given to microplastics in drinking water, but MPs have already been detected in drinking water (Mintenig et al., 2014) and bottled water (Schymanski et al., 2018) as well. Hungary lies in Central-Eastern Europe in the Carpathian-Basin. Despite its drinking water is processed in 97% from high quality underground sources (including ca. 40% bank filtration), water works are rather afraid of microplastic studies, than examine the potential contaminating effects. If any MPs would be detected and communicated, consumers would consider to drink more bottled water, because they think it's safer.
In 2018 a sampling campaign was organised by the Hungarian Water Utility Association in four water treatment works, two of them processing surface water while the other two underground water. In all plants samples were taken from the final treated water, and except one of the surface water processing plant, the raw water was sampled as well. For drinking water there are different sampling approaches. Some of them only taking very low quantities, such as Kosuth et al. (2018), where 0.5 L was collected in PE flask. The study by Strand et al. (2018) is better, as they excluded plastics during sampling and concentrated water on a filter, but only 50 litres were sampled. As MPs usually detected in drinking water in low quantities, it is very important to take representative samples, at least 1000-2000 litres. This allows a better calculation of microplastic load in a defined volume drinking water. Because of these considerations, we decided to use a method that is very similar as described by Mintenig et al. (2014) and used later also by Ball et al. (2019). Filters with 15 µm mesh size were exposed to approximately 2000 litre of water. The exact quantity was measured with a water meter with 0.1 L precision. Samples were analysed by FTIR microscopy, after a flotation in saturated sodium-chlorine solution and oxidation with 30% hydrogen-peroxide.

Two surface water processing plants were sampled. One of them was found to be microplastic free both in the raw and treated water. The other plant didn't provided sampling tap for the raw water, instead asked for sampling of two treated water taps. In these taps, MPs were detected in 5.5 and 17.4 particles per 1000 litre water. The particles were dominantly polyethylene (PE) and poly(tetrafluorethylene) (PTFE), but in one sample polyesther (PES) and in another sample phenoxy resin was detected too. In the underground water samples PE and PTFE was detected. One of the plants showed PE in the raw water, but no MPs in the treated sample. Probably this 2 particles/m3 was originating from the PE tube that was used in the well by the waterworks. At the other waterworks in the treated water both PE and PTFE were detected. These particles are potentially

released from the tubes, fittings and sealings, and not from the underground water. Results are shown on Figure 14.

Results shows that water processing plants in Hungary are affected by microplastic pollution, dominantly PE ant PTFE were found, and the total MP load ranged from 2-17.4 particles/m3. Based on these first results, that contamination probably originating from the facilities (pipes, fittings, sealings) and not from the raw water, as 97% in the country's drinking water production coming from underground sources. The few plants that are processing surface water can be more affected, but in this study the origin of MPs from the raw surface water could not be proved. It is important in the future, that drinking water analysis should be based on high volume water samples. In the future, the plants should be more open minded and organise more complex investigations in different stages of the treatment process. Also, the results should be communicated for the consumers on a way, that they don't intend to drink more bottled water instead of tap water. It is also urging, to get to know the effect of MPs in drinking water, so the results can be evaluated accordingly.

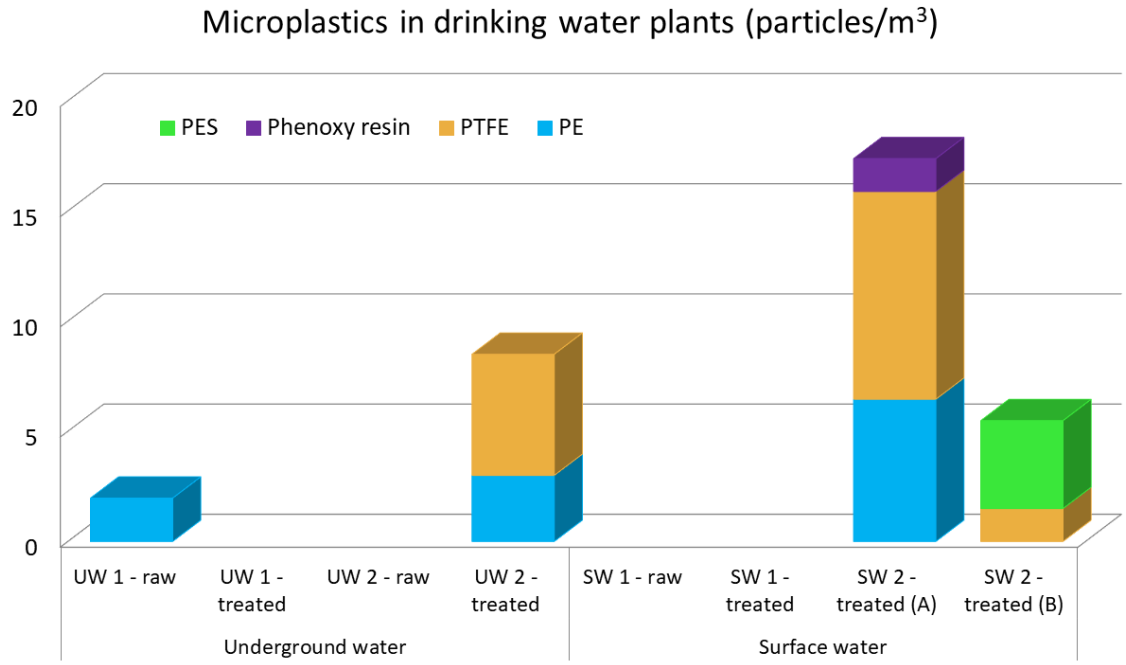


Figure 14: Microplastics in Hungarian drinking water plants with underground or surface water supply (particles/m^3).

Bibliography

Ball H, Cross R, Grove E, Horton A, Johnson A, Jürgens M, Read D, Svendsen C. 2019. Sink To River – River To Tap. A Review of Potential Risks From Nanoparticles and Microplastics. UK Water Industry Research Limited. 64 p.

Kosuth M, Mason SA, Wattenberg EV. 2018. Anthropogenic contamination of tap water, beer, and sea salt. PLoS One. 13(4):e0194970.

Mintenig S, Löder M, Gerdts G. 2014. Mikroplastik in Trinkwasser. Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung (AWI) Biologische Anstalt Helgoland. 18 p. Retrieved from http://schlicktown.stadt-media.de/wp-content/uploads/AWI_Abschlussbericht_Mikroplastik_in_Trinkwasser.pdf

Schymanski D, Goldbeck C, Humpf HU, Fürst P. 2018. Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water. Water Res. 1;129:154-162.

Strand J, Feld L, Murphy F, Mackevica A, Hartmann NB. 2018. Analysis of microplastic particles in Danish drinking water. Aarhus University, DCE – Danish Centre for Environment and Energy, 34 pp. Scientific Report No. 291. http://dce2.au.dk/pub/SR291.pdf

Analysis of microplastics in clean water: Minimum requirements and best practice guidelines

Oßmann BE [1], Schymanski D [2], Benismail N [3], Boukerma K [4], El Rakwe M [4], Dallmann G [5], Käppler A [5], Fischer D [6], Fischer F [6], Gilliland D [7], Lacorte S [8], Marco J [9], Weisser J [10], Glas K [10], Hofmann T [10], Witzig CS [11], Zumbülte N [11], Ivleva NP [10]

[1] Bavarian Health and Food Safety Authority (DE), [2] Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe (CVUA-MEL, DE), [3] Nestlé Waters, Nestle Quality Assurance Center (FR), [4] IFREMER, REM/RDT/LDCM (FR), [5] SGS Institut Fresenius GmbH (DE), [6] Leibniz-Institut für Polymerforschung Dresden e.V. (DE), [7] European Commission, Joint Research Centre (JRC, IT), [8] Department of Environmental Chemistry, IDAEA-CSIC (ES), [9] Danone Waters (FR), [10] Chair of Food Chemistry and Molecular Sensory Science, Technical University of Munich (DE), [11] TZW: DVGW-Technologiezentrum Wasser (DE)

Abstract: During the last years, several studies were published, investigating the abundance of microplastics in clean waters, such as ground, tap or bottled waters (Mintenig et al., 2019, Pivokonsky et al., 2018, Schymanski et al., 2018, Oßmann et al., 2018). However, there is a huge diversity in experimental setups, e.g. regarding sampling, sample preparation, particle counting and identification. Even when two labs use the same spectroscopic method for particle identification, their results cannot be compared because of too many different handlings during processing and analytics. To overcome this issue, harmonization of analytical methods is necessary. To take a step towards a methodological alignment, a group of 12 laboratories from all over Europe, all experienced in microplastic analysis, was founded. This group, representing public authorities (e.g. universities, research institutes and authorities for consumer protection) as well as industrial labs, discussed and developed a common working paper for analysis of microplastics in clean water.

First, common practices for microplastic analytics of all labs were collected and compared. Based on these experiences, regular telephone or web meetings were conducted to discuss proceedings for all of the following methodological steps: sampling, laboratory working environment, sample preparation, filtration, identification and quantification of microplastic particles by means of micro-FT-IR-spectroscopy (µ-FTIR) and Raman microspectroscopy (RM). Thereby, we addressed important issues of quality assessment such as detection limit, negative controls (laboratory and procedural blanks), positive controls (e.g. using reference materials), data processing and method validation. Every chapter was discussed thoroughly and minimum requirements and best practice guidelines were established.

Minimum requirements and best practices guidelines will be described in detail during the oral presentation. These include general recommendations for the laboratory environment, e.g. equipment, cleaning procedures and precautions to avoid sample contamination, which must be strictly adhered to. Furthermore, specific requirements for the nature of filter, adequate to the detection method (µ-FTIR or RM), measurement parameters, selection of a representative number of particles or the match acceptance criteria for database search are shown. In addition, we specify required validation steps and parameters, which have to be included when reporting results.

Expertise in analyzing microplastics of research groups from public authorities as well as from industrial labs from all over Europe was summarized and concluded to guidelines for the analysis of microplastics in clean water. These guidelines contribute to a harmonization of analytical methods and to a reliable comparability of analytical results. Moreover, it can pave the way for national and international standardization efforts.

Bibliography

Mintenig SM, Löder MGJ, Primpke S, Gerdts G. 2019. Low numbers of microplastics detected in drinking water from ground water sources. Sci Total Environ. 15;648:631-635.

Pivokonsky M, Cermakova L, Novotna K, Peer P, Cajthaml T, Janda V. 2018. Occurrence of microplastics in raw and treated drinking water. Sci Total Environ. 643:1644-1651.

Schymanski D, Goldbeck C, Humpf HU, Fürst P. 2018. Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water. Water Res. 129:154-162.

Oßmann BE, Sarau G, Holtmannspötter H, Pischetsrieder M, Christiansen SH, Dicke W. 2018. Small-sized microplastics and pigmented particles in bottled mineral water. Water Res. 141:307-316.

Preliminary study concerning the use of Nile Red staining for detecting microplastics in marine mussels

Nalbone L [1], Russell M [2], Giarratana F [1]

[1] Department of Veterinary Science, University of Messina (IT), [2] Marine Scotland Science Marine Laboratory (UK)

Abstract: The global interest on the environmental impact of plastic pollution has grown rapidly during the last decades. Although the benefits of plastic today are unquestionable, its usage and the management of its disposal have led to an accumulation in the environment. Particular attention from the scientific community has been paid to the issue of microplastics (MPs). They are ubiquitous in the marine environment and the likelihood of their bioavailability has aroused concern, especially for the interaction with marine organisms intended for human consumption (EFSA, 2016). A fast, economic and standardized protocol is necessary for a correct enumeration and qualitative evaluation of MPs polymers dispersed in the environment. Nile Red (NR) staining has been tested and proposed as alternative or complementary method for quicker, more economical and routine analysis of MPs in biological samples (Maes et al., 2017). NR is a lipophilic dye that exploits the hydrophobic properties of plastic which, once stained, may emit fluorescence if excited with certain wavelengths. A recognized problem of using NR-staining for environmental samples is the presence of organic matter that would also be stained and therefore fluoresce, possibly leading to overestimation of MPs present (Shim et al., 2016). The NR-method has been used previously for sediments and water samples, together with an organic matter digestion step and/or density separation with saline solutions (Erni-Cassola et al., 2017). The aim of the present work is to evaluate the limits of using the NR-staining method for the identification of MPs in marine mussels intended for human consumption.

An exposure experiment was performed using wild (WM) and farmed (FM) blue mussels. Each group was split into three subgroups: the blank-samples (BS) were frozen immediately after collection, the dosed-samples (DS) and the control-samples (CS) were placed in four tanks with 60L of clean and filtered seawater for 2h. Virgin white low-density polyethylene (LDPE) particles <250µm (2 grams) and 500 to 710µm (2 grams) were added to each of the DS-tanks. Three mussels of each subgroup were processed individually. Samples were digested using 10% KOH solution (40 g/ml w.v.) overnight (Dehaut et al., 2016) and filtered through 10µm pore-size translucent polycarbonate filters. NR was prepared at 1 mg/ml in ethanol. A Canon EOS-760D camera, equipped with a macro-lens and an orange filter, was mounted on a milling machine which allowed automated movement in XYZ-axes. Thirty photographs of each whole filter area were recorded under a blue-LED-light (420–470nm) and a white-light source. Autostitch software was used to generate single magnified blue- and white- filter images. Recovery and particle size detection limits were evaluated by spiking known amounts of different size particles (from 63µm to 90µm; from 91µm to 125µm; from 126µm to 180µm; from 181µm to 355µm and from 356 to 510µm) onto FM-BS. Nine individual mussels were spiked for each size of LDPE particle. The potential MPs were detected by evaluating their fluorescence, shape and appearance. Thereafter, the selected particles were analyzed under a dissection microscope and their polymer type confirmed by microscopy Fourier Transform Infrared (microFTIR) spectroscopy.

Observing the blue-light images of DS, undigested organic residues of different shapes, colors and fluorescence interfered at various levels with the identification of the tested LDPE-particles. A large number of aggregate fragments characterized by an intense yellow/green fluorescence were evident in the blue-filter images of the WM-DS (Figure 15). Visual sorting of the corresponding filters under a dissection microscope showed potential LDPE-particles mixed with organic residues. It was not possible to perform microFTIR on all of the particles therefore 20 representative particles were selected from each of the three WM – DS individuals for microFTIR, which were confirmed as being LDPE. A lower number of particles was observed in the FM-DS and all 26 -particles collected from the three FM-DS mussels were confirmed as LDPE. Empirically, shape and size influenced the ability to recognize and distinguish fluorescent particles by the blue-filter images. Organic residues were characterized by soft and pale profile against a definite and solid appearance of the plastic particles. Furthermore, as size decreased, the plastic particles were less recognizable. LDPE-particles of 180–355 µm were the smallest size tested where it was possible to distinguish plastic particles from organic residues in the spiked FM-BS (Figure 16). From these 9 mussels (each spiked with 20 LDPE

particles), a total of 169 potential LDPE-particles were selected for microFTIR. MicroFTIR confirmed 165 of the 169 particles were LDPE-particles, with recovery rates of between 80 to 100 % per mussel.

Observing the blue-light images of DS, undigested organic residues of different shapes, colors and fluorescence interfered at various levels with the identification of the tested LDPE-particles. A large number of aggregate fragments characterized by an intense yellow/green fluorescence were evident in the blue-filter images of the WM-DS (Figure 15). Visual sorting of the corresponding filters under a dissection microscope showed potential LDPE-particles mixed with organic residues. It was not possible to perform microFTIR on all of the particles therefore 20 representative particles were selected from each of the three WM – DS individuals for microFTIR, which were confirmed as being LDPE. A lower number of particles was observed in the FM-DS and all 26 -particles collected from the three FM-DS mussels were confirmed as LDPE. Empirically, shape and size influenced the ability to recognize and distinguish fluorescent particles by the blue-filter images. Organic residues were characterized by soft and pale profile against a definite and solid appearance of the plastic particles. Furthermore, as size decreased, the plastic particles were less recognizable. LDPE-particles of 180–355 µm were the smallest size tested where it was possible to distinguish plastic particles from organic residues in the spiked FM-BS (Figure 16). From these 9 mussels (each spiked with 20 LDPE particles), a total of 169 potential LDPE-particles were selected for microFTIR. MicroFTIR confirmed 165 of the 169 particles were LDPE-particles, with recovery rates of between 80 to 100 % per mussel.

The main limitation of the NR-staining application for MPs research on biota is the persistence of organic residues. LDPE-partides were easily detectable with the use of NR-staining on its own but only if the digestion resulted in a fine and uniform degradation of the mussel tissue. In this regard, the achieved results showed a high variability. Even if the 10% KOH digestion allowed easy filtration of the digested samples through a 10µm pore-size filter, the organic residues sometimes interfered with the identification of the MPs when using the NR-method only. However, using the implemented method in conjunction with studying the filter with a microscope gave increased efficiency. Observing blue-images of the processed samples, use of NR-staining on biota seems to be dependent on the plastic fragment size. A good recovery rate was obtained for LDPE-particles >180µm, when the physical characteristics of the fluorescent fragments were more obvious, i.e. it was obvious from the size and shape that they were plastic particles. Based on the fluorescence of these fragments, LDPE-particles were similar only to certain organic residues and different from others that did not emit fluorescence or appeared of another color. Other digestive protocols, testing different polymers characterized by different colors, and the use of saline solutions for density separation of plastics fragments from organic residues should be investigated. Combining the NR-method with a visual-sorting of the filter may have some positive implication on the selection of particles to be subjected to the next step of chemical identification.

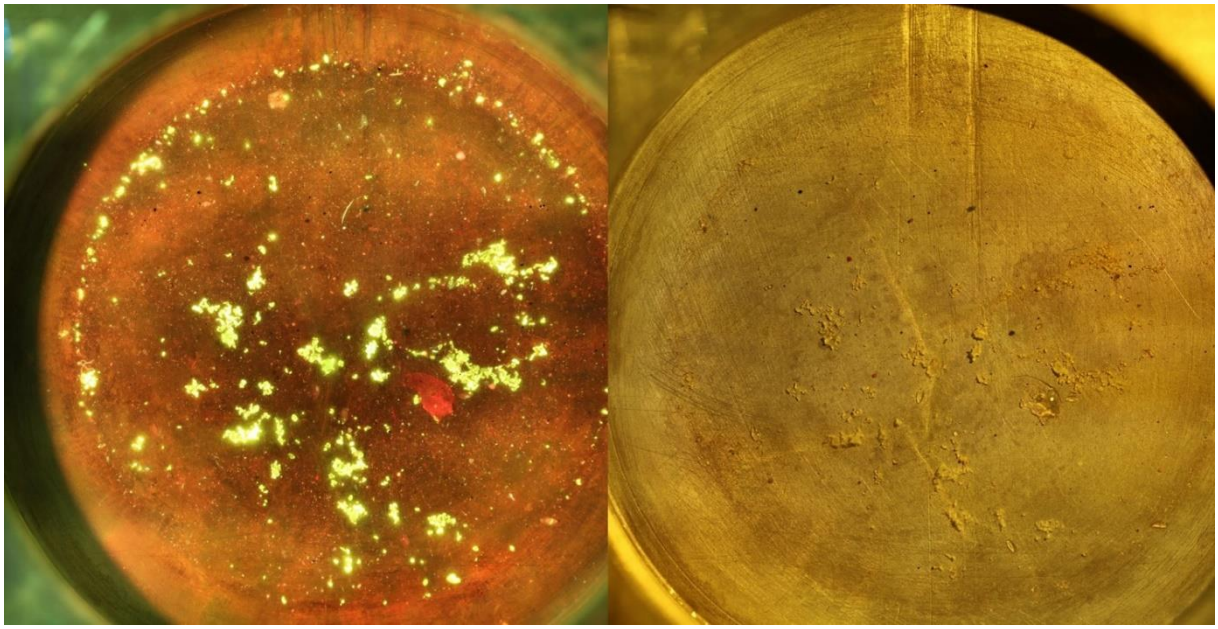


Figure 15: Blue (left) and white (right) magnified images recorded respectively under a blue LED light (420–470 nm) and a white-light source of a wild mussel experimentally exposed to white low-density polyethylene

microplastics in the size range of <250 µm and 500-710 µm, digested with 10% KOH, filtered through a translucent polycarbonate filter (10 µm pore size) and stained with Nile Red (1 mg/ml in ethanol).

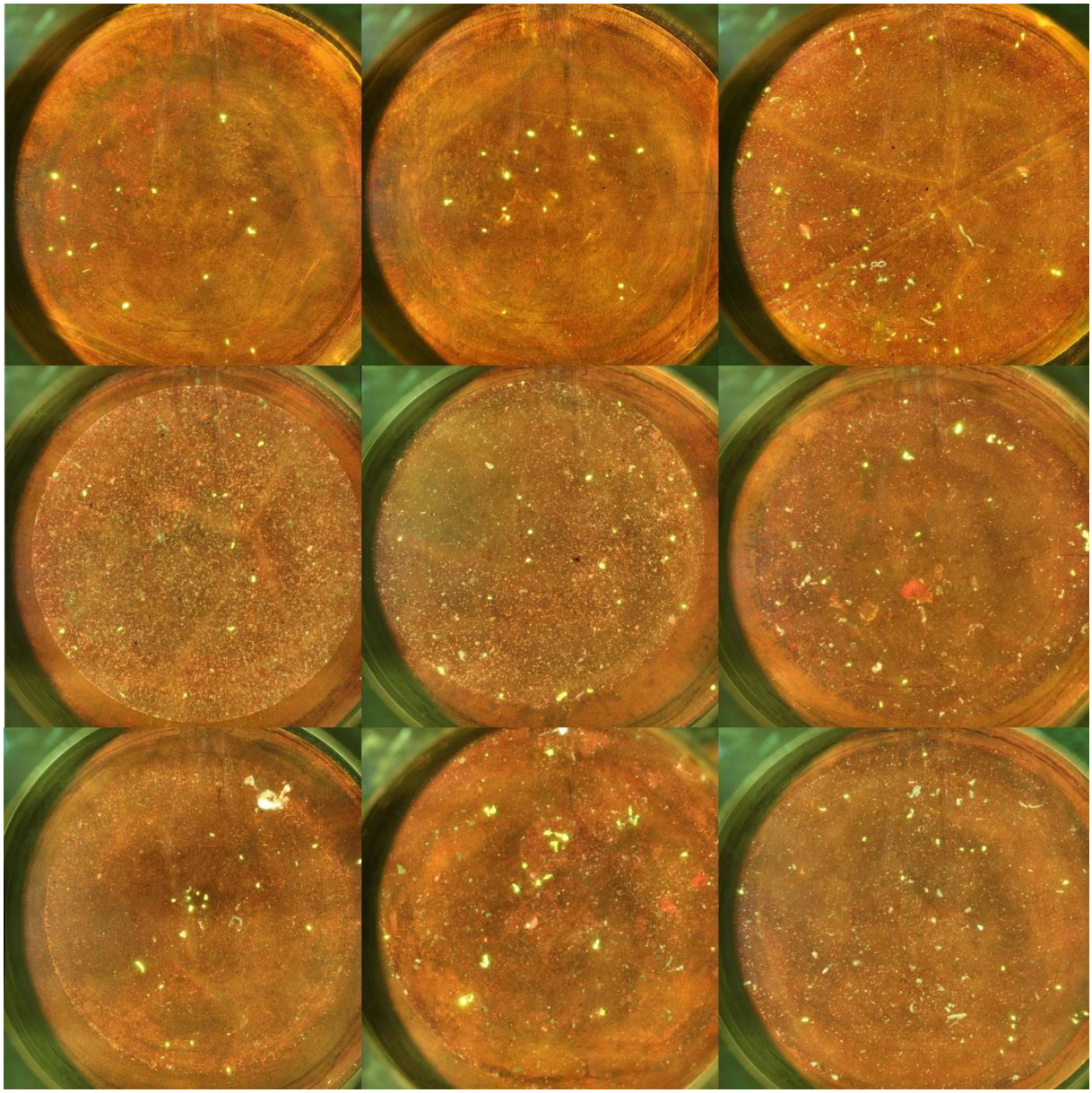


Figure 16: Blue magnified images recorded under a blue LED light (420–470 nm) of nine farmed mussels each spiked with twenty white low-density polyethylene microplastics in the size range of 180-355 µm, digested with 10% KOH, filtered through translucent polycarbonate filters (10 µm pore size) and stained with Nile Red (1 mg/ml in ethanol).

Bibliography

Hoogenboom LAP. 2016. Statement: Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal, 14(6), e04501.

Maes T, Jessop R, Wellner N, Haupt K, Mayes AG. 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci Rep. 7:44501.

Shim WJ, Song YK, Hong SH, Jang M. 2016. Identification and quantification of microplastics using Nile Red staining. Mar Pollut Bull. 113(1-2):469-476.

Erni-Cassola G, Gibson MI, Thompson RC, Christie-Oleza JA. 2017. Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 µm) in Environmental Samples. Environ Sci Technol. 51(23):13641-13648.

Dehaut A, Cassone AL, Frère L, Hermabessiere L, Himber C, Rinnert E, Rivière G, Lambert C, Soudant P, Huvet A, Duflos G, Paul-Pont I. 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ Pollut. 215:223-233.

Microplastics in a diverse food basket and exposure of Belgian consumers

Dekimpe M [1], De Witte B [1], Deloof D [1], Hostens K [1], Van Loco J [2], Andjelkovic M [2], Van Hoeck E [2], Robbens J [1]

[1] Flanders Research Institute for Agriculture, Fisheries and Food (ILVO, BE), [2] Sciensano (BE)

Abstract: Many studies have focused on the presence of microplastics in the marine environment, including seafood such as mussels, crab and fish. Data on other food matrices is scarce and limited to a few food groups. This illustrates the need for an extensive analysis of the diverse food basket, as it remains unclear to what extent different food items contribute to the microplastic exposure of consumers. In the 'Plastic in Food' project (financed by the Federal Public Service of Health, Food Chain Safety and Environment)), ILVO and Sciensano determined the occurrence of microplastics in multiple food items, in order to estimate the dietary exposure to microplastics of the Belgian population. The occurrence of microplastics in different food groups is compared and the impact of packaging and food processing is assessed. Also, the impact of contamination by air and water will be investigated.

A sampling plan was developed to select the most relevant food items on the Belgian food market. Data on the market share were gathered, literature on microplastic occurrence in food was consulted, and data on the Belgian consumption habits were considered. A total of 213 food items from 15 different groups (FOODex2 classification) were selected, based on four weighted criteria: (1) relative contribution of the food group in Belgian consumption; (2) probability of containing microplastics; (3) type of packaging material; and (4) variability of food items within every food group.

The edible part of each food item was destructed, followed by active filtration on a cellulose filter and the amount of microplastics were counted through a stereomicroscope. The microplastic chemical composition was determined by µFTIR. Through validation with spiked samples, it was proven that destruction at 60°C during 24h with 10% KOH resulted in a high performance for microplastics analyses in mussel tissue, i.e. an efficient matrix degradation with limited impact on the polymer structure. Colorless polyethylene and red polystyrene beads of 106 to 125 µm and 500 to 600 µm size classes were dosed to the sample, and accuracy and precision were calculated. Also, the limit of quantification (LOQ) and robustness were determined. Color, category and size were recorded for all microplastics. Procedure blanks and positive control samples ensured the quality of the method. For a number of food matrices, the KOH protocol did not suffice, and a series of modifications and alternative digestion solvents were tested.

The KOH method revealed high recoveries for red and colorless beads of 500-600 µm. For smaller beads, only the colored ones showed high recoveries. The accuracy for these types of microplastics was well within predefined performance criteria (80-120 % for 500-600 µm particles, 70-130% for 106-125 µm particles). For small colorless beads the average accuracy was only 41%. Also for precision, the criteria were reached for the bigger red and colorless beads, but not for small colorless beads.

The KOH analytical protocol proved suitable for a variety of food matrices. However, for matrices like fruit and vegetables, tissue destruction was insufficient and filtration impeded. Subsequently, H_2O_2 oxidation was used to digest sugar, candy and salt, while food items of vegetable origin were successfully digested by the Fenton reagent. Water based juices and soft drinks were filtered without applying any tissue destruction method. Microplastics were found in 38% of the analyzed foods, ranging from 3 to 209 microplastics per kg or litre food spread across multiple food-groups. In water-based beverages, drinking water and eggs, no significant amounts of microplastics were detected. No significant impact was found from packaging or degree of processing. Almost 40 % of the 50 representative microplastics analyzed through µFTIR were semi-synthetic fibers such as viscose. The synthetic particles were mainly Polyamide (PA), Polyester (PES) and Polyurethane (PUR).

Since the colorless beads of 106-125 µm did not meet the predefined performance criteria, we concluded that there is a different method cut-off size for colored and colorless beads. Colored beads can be easily detected down to 100 µm or even 50 µm, which is not the case for colorless beads. Additional tests with 200 µm colorless beads are planned to define the exact cut-off for colorless

beads. Detection methods with fluorescent staining will be developed in the future, and may offer a solution for the small colorless beads.

As degree of processing or type of packaging have no significant influence on the amount of microplastics in food items, the microplastic content seems to be determined by other sources. For seafood and salt, contamination of the aquatic environment is a proven source (EFSA, 2016). Air exposure, e.g. by clothing, or exposure to industrial equipment such as conveyor, fabric and felt belts or potential other sources of microplastics. The most commonly used plastics for these belts are polyvinylchloride (PVC), PU, PES and PA.

The assessment of the exposure of Belgian consumers to microplastics is still ongoing. Different exposure scenarios (upper, medium and lower bound) will be assessed and the contribution of each food group to the total microplastics ingestion through food will be determined.

Bibliography

Hoogenboom LAP. 2016. Statement: Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal, 14(6), e04501.

Recovery rate as a measure of quality assurance in microplastic research

Dimante-Deimantovica I [1], Suhareva N [1], Barone M [1], Putna-Nimane I [1], Prokopovica A [1], Svipsta S [1], Aigars J [1]

[1] *Latvian Institute of Aquatic Ecology (LIAE, LV)*

Abstract: Recovery rate control samples are important in standardized microplastic research protocol development in order to ensure quality. Here we investigated how much material might be lost due to samples treatment and how harmful samples processment methods are on plastic partides.

We used standardized micro particles based on polystyrene, size of 100 µm that were treated following different samples processment protocols (for water and sediments). The treatment depending on protocol consisted of sample pre-oxidation with hydrogen peroxide, sonication, filtration, drying, homogenization, density separation/floatation, SDS-treatment, enzymes treatment and oxidation by Fenton reaction. After every treatment step microparticles were counted and particles shape was examined.

Our results illustrate that existing protocols for microplastic isolation from different matrices can deform and change particles shape. Particles can even be destroyed to smaller pieces and be lost due to filtration in-between every treatment step. Hence, significant part of the material can be lost during the treatment. However, it depends on choosen protocol, since the number of sample processing steps has a direct impact on recovery rate and is more significant compared to possible chemical impact. Sample extraction method can increase recovery rate from less than 40% to more than 80%. Relation between sample volume and separating funnel is crucial.

Based on the findings, we provided a table containing threshold values to assess the quality of microplastics studies. It shows the dependence of the overall recoverability on the total number of processing stages in a selected method.

Session 2: Developments in exposure of humans to micro- and nanoplastics

Microplastics and Bisphenol A in Mussels along Italian and Croatian Coast of the Adriatic Sea

Bogdanović T [1], Di Renzo L [2], Petričević S [1], Mascilongo G [2], Brkljača M [3], Cito F [4], Calistri P [4], Notarstefano V [5], Gioacchini G [5], Giorgini E [5], Giansante C [2], Berti M [2], Sokolić D [6], Ferri N [2], Listeš E [1], Di Giacinto F [2]

[1] *Croatian Veterinary Institute (CR),* [2] *Water Biology Unit, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM, IT),* [3] *Faculty of Food Technology and Biotechnology, (CR),* [4] *National Reference Centre for Veterinary Epidemiology, Programming, Information and Risk Analysis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM, IT),* [5]

Department of Life and Environmental Sciences, Università Politecnica delle Marche (IT), [6] Croatian Agency for Agriculture and Food (CR)

Abstract: This study examined the overall bisphenol A (BPA) and microplastics (MPs) contamination status in mussels (Mytilus galloprovincialis) collected from aquaculture farms and one natural bed (Croatia) from all Italian and Croatian regions of the Adriatic Sea. Quantitative and qualitative assessments of MPs with a minimum size of 1 µm in the mussels were performed by stereomicroscope observation and Raman Microspectroscopy (RMS). In addition, an analytical quantification of polyethylene terephthalate (PET), polycarbonate (PC), para phthalic acid (PTA) and BPA has been conducted in mussel soft tissue by LC MS/MS method. The MP contamination, detected in the soft tissues of all investigated mussels from the semi-enclosed basin of the Adriatic Sea, varied from 0.3 items/g wet weight to 4.8 items/g wet weight. Out of total items, the most abundant was fragment morphotype, with a percentage of 65%. Preliminary results of chemical characterisation detected 7 different polymers and 5 pigments, mostly represented by polypropylene and Pigment Black 9 (C.I. Constitution 77267). The highest Mussels (Mytilus galloprovincialis) were sampled from 16 farms and 1 natural bed of Italian and Croatian Adriatic coast. Analytical quantification of polyethylene terephthalate (PET), polycarbonate (PC), para phthalic acid (PTA) and bisphenol A (BPA) has been conducted in mussel soft tissue by LC MS/MS method in gradient mode. 1 g of mussels dried tissue were depolymerized via hydrolysis within 30 min in 1-pentanol solution with potassium hydroxide heating at 135°C and extracted HPLC-grade water according to Zhang et al. 2019. The chromatographic separations were performed in gradient mode on Poroshell 120 EC-C18 column (100×2.1mm internal diameter, 2.7 µm particle size) and Polaris C18-Ether column (100x3.0 mm internal diameter, 3.0 µm particle size. The extraction protocol of MPs from mussels was arranged into several steps: (1) basic digestion with KOH 10% w/w for 24 hour at 60 °C and 300 rpm; (2) density separation with separating fuel with KI 10% w/w (Phuong et al., 2017); (3) filtration with a vacuum system using nitrate cellulose filter at 1 µm of porosity; to clean the filter, 10 ml of 30% H_2O_2 for 5 minutes were added on the top of the paper; as soon as the final washing procedure was concluded with 10 ml of milliq water for 5 minutes, the filter was immediately put in a glass petri dish. All paper filters were observed under stereomicroscope at 40x magnification (Leica MZ6) shooting pictures with digital 156 camera (model JVC TKC1381). Micropartides that were assumed by eye to be MPs were registered according to number, shape and colour. Potential environmental contamination was evaluated arranging a blank sample for each extraction phase. The vibrational characterization of microparticles extracted as previously described was performed by Raman MicroSpectroscopy by using a XploRA Nano Microspectrometer (Horiba Scientific) directly on the filters, which were inspected by visible light using a ×10 objective (Olympus MPLAN10x/0.25). of BPA (53.33 ng/g wet weight) was found in mussels sampled at one sampling point along the Italian coastline. A significant spatial distribution trend was observed based on LC MS/MS method data. The group of sites from Italy showed higher values of BPA and MPs, despite, based on PCA results for LC MS/MS method, the Croatian and Italian groups of site overlapped due to polymer types content being low. The obtained results highlight the need of more research on mussel contamination by MPs and associated chemicals and allow a preliminary assessment of the possibile effects on human health.

Mussels (Mytilus galloprovincialis) were sampled from 16 farms and 1 natural bed of Italian and Croatian Adriatic coast. Analytical quantification of polyethylene terephthalate (PET), polycarbonate (PC), para phthalic acid (PTA) and bisphenol A (BPA) has been conducted in mussel soft tissue by LC MS/MS method in gradient mode. 1 g of mussels dried tissue were depolymerized via hydrolysis within 30 min in 1-pentanol solution with potassium hydroxide heating at 135°C and extracted HPLC-grade water according to Zhang et al. 2019. The chromatographic separations were performed in gradient mode on Poroshell 120 EC-C18 column (100×2.1mm internal diameter, 2.7 µm particle size) and Polaris C18-Ether column (100x3.0 mm internal diameter, 3.0 µm particle size. The extraction protocol of MPs from mussels was arranged into several steps: (1) basic digestion with KOH 10% w/w for 24 hour at 60 °C and 300 rpm; (2) density separation with separating fuel with KI 10% w/w (Phuong et al., 2017); (3) filtration with a vacuum system using nitrate cellulose filter at 1 µm of porosity; to clean the filter, 10 ml of 30% H_2O_2 for 5 minutes were added on the top of the paper; as soon as the final washing procedure was concluded with 10 ml of milliq water for 5 minutes, the filter was immediately put in a glass petri dish. All paper filters were observed under stereomicroscope at 40x magnification (Leica MZ6) shooting pictures with digital 156 camera (model JVC TKC1381). Microparticles that were assumed by eye to be MPs were registered according to number, shape and colour. Potential environmental contamination was evaluated arranging a blank sample for each

extraction phase. The vibrational characterization of microparticles extracted as previously described was performed by Raman MicroSpectroscopy by using a XploRA Nano Microspectrometer (Horiba Scientific) directly on the filters, which were inspected by visible light using a ×10 objective (Olympus MPLAN10x/0.25).

The MP contamination, detected in the soft tissues of the investigated mussels from the first twelve sampling points of semi-enclosed basin of the Adriatic Sea, varied from 0.3 items/g wet weight to 4.8 items/g wet weight (Table 1). Out of total items, the most abundant was fragment morphotype, with a percentage of 68% (Figure 17). Preliminary results of chemical characterisation detected 7 different polymers and 5 pigments (Figure 18), mostly represented by polypropylene and Pigment Black 9 (C.I. Constitution 77267). PCA results of LC MS/MS analyses indicate that 4 variables representing 4 polymer types described the spatial distribution of sampling sites in two-dimensional space. Figure 3. shows the PCA biplot of the 4 variables in mussels tissue at 12 sampling sites with PC1 and PC2 explaining 83.83% of the total variance. PC1, explaining 58.97% of the total variance, is contributed moderately positive by free BPA, and moderate negatively by PTA free, PC and PET. PC2, explaining 24.86% of the total variance, is strongly positive influenced by PTA free and moderate negatively by PET, and the contribution of BPA free and PC was weak. Based on the position in the PC1-PC2 plot, among the four initial variables, the PC and PET show the positive correlation, while the variables PC and BPA free show negative correlation. Regarding spatial distribution of sampling sites, Figure 3. shows the grouping of the sampling sites from two geographical regions of Italy and Croatia. The sites from Italy were closely grouped in the space with moderate to high positive scores in PC1 and low to moderate negative scores in PC2 and tend to have greater values of BPA free variable. The sites from Croatia were distributed over the larger space in PC1-PC2 plot. One site had high positive score in PC2 with high negative score in PC1 and tend to have high value of PTA free. Three sites had high negative score in PC2 tending to have higher values in PET, with one site having also high score in PC1 tending to have high value of PC. One site with moderate negative score in PC1 tending to have low microplastic abundance. The two groups of samples overlap due to high polymer content variability in samples from Croatia.

This study examined the overall bisphenol A (BPA) and MP contamination status in mussels (Mytilus galloprovincialis) collected from aquaculture farms and one natural bed (Croatia) located along the Italian and Croatian coasts of the Adriatic Sea and implications for human health. Quantitative and qualitative assessment of MPs until 0,45 µm in the mussel has been evaluated by stereomicroscope observation and characterized by Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Raman Microspectroscopy (RMS). In addition, an analytical quantification of PET, PC, para phthalic acid (PTA) and BPA has been conducted in mussel soft tissue by LC MS/MS method. The highest concentration of BPA (53.33 ng/g wet weight)) was found in mussels sampled at VR-V-L-2 point along the Italian coastline. A significant spatial distribution trend was observed based on LC MS/MS method data. The group of sites from Italy tend to have greater values of BPA, however based on PCA results for LC MS/MS method the Croatian and Italian groups of site overlap due to polymer types content being low.

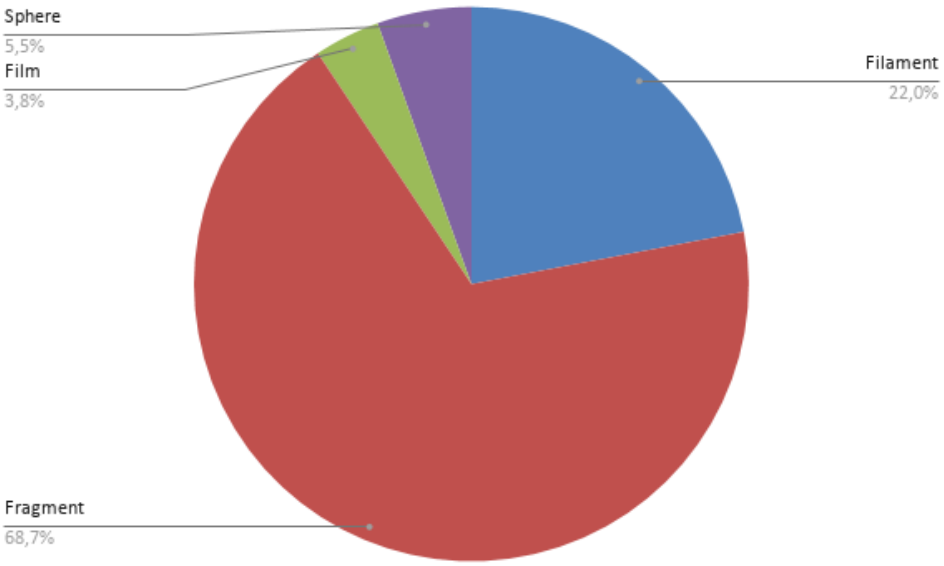


Figure 17: Morphotype of MPs extracted from Italian and Croatian mussels

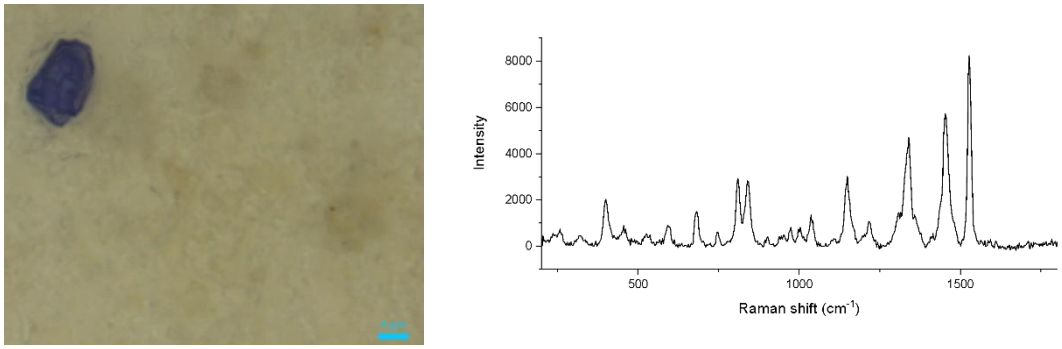


Figure 18: Chemical characterization by Raman Microspectroscopy (RMS) from Croatian mussel: polypropylene + Pigment Blue 16 (C.I. Constitution 74100)

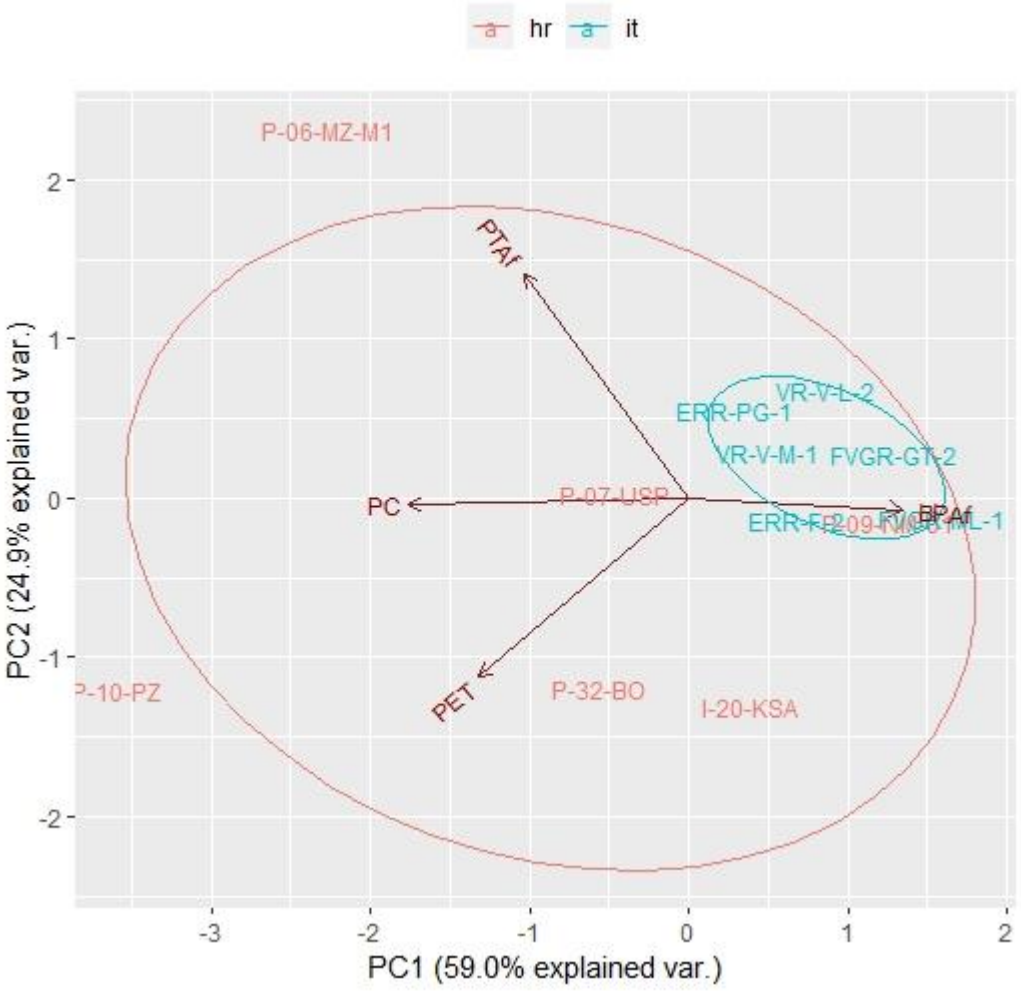


Figure 19: Principal component analysis for the mussels of the 12 sites. PC1, principal component 1, PC2, Principal component 2. Location identifiers for sites within the biplot are provided in Table 1° and 1b, arrows represent the initial variables free bisphenol A (BPAf), polythylene therephtalate (PTAf), polycarbonate (PC) and polyethylene (PET) on PC1 vs. PC2 plane. The two ellipses encircle samples locations groups, Croatian (hr) and Italian (it).

Table 1: Number of items/g wet weight

Sampling point	Italian mussels	Croatian mussels
1	1	0,5
2	1,7	2,7
3	1	0,3
4	1,8	0,1
5	1,5	4,8
6	0,3	2,8

Bibliography

e.g Denancé N, M Briand, R Gaborieau, S Gaillard, M-A Jacques. 2019. Identification of genetic relationships and subspecies signatures in *Xylella fastidiosa*. BMC Genomics 20:239.

Phuong NN, Zalouk-Vergnoux A, Kamari A, Mouneyrac C, Amiard F, Poirier L, Lagarde F. 2018. Quantification and characterization of microplastics in blue mussels (Mytilus edulis): protocol setup and preliminary data on the contamination of the French Atlantic coast. Environ Sci Pollut Res Int. 25(7):6135-6144.

Wang L, Zhang J, Hou S, Sun H. 2017. A simple method for quantifying polycarbonate and polyethylene terephthalate microplastics in environmental samples by liquid chromatography-tandem mass spectrometry Environ. Sci. Tech. Let., 4: 530-534.

Detection of microplastics in sports drinks and bottled water by fluorescence microscopy and Nile Red dye

Guevara LV [1], Diong LPE [1], Quiles Romagosa M [1]

[1] *Sheffield Hallam University (UK)*

Abstract: Recent research has reported the presence of micro plastics in tap water and bottled water (BW) but excluded samples of plastic bottled water. The aim of this study was to determine if microplastics are present in sports drinks (SD) and BW using fluorescence microscopy and Nile Red dye. The increasing usage of plastics to support modern lifestyles has drawn concerns about its abundance and the risks to human health. The lightweight and durable nature of plastic makes it ideal to accommodate a wide range of products for food packaging. Plastics left in the environment as waste form microplastics upon disintegration into smaller fragments through physical abrasion, weathering and microbial degradation. Microplastics are defined by most authors as plastic particles with longest diameter <5mm. There have been some suggestions to redefine the size range for the term microplastics to be particles which are <1mm, but there is no official lower limit. Microplastics are highly mobile after being discarded into the environment while ecosystems heavily contaminated with microplastics have been identified in convergence areas, such as ocean gyres, or near heavy human activities, such as industrial ports. Microplastics have also been identified in remote environments such as Arctic and alpine snow. Microplastics last in the environment for a very long time, up to thousands of years, as microplastics are highly stable, durable and non-biodegradable. Inhalation, ingestion and skin perfusions have been suggested to be the main entry route for microplastics into humans, though its impact on health is as yet unknown.

Nile Red, a hydrophobic metachromatic dye, was used to label microplastics with fluorescence and viewed under fluorescence microscopy. This relatively novel and inexpensive method uses readily available equipment. We first tested Nile Red on two commercial polymers dispersed in deionised water to examine the efficacy in staining these with our Nile red solution. Six different brands of BW were analysed. All water samples were bottled in the EU. The body of all the bottles were made of Polyethylene terephthalate (PET). Eight different brands of SD were analysed. The SD were all manufactured in the EU. The body of all the bottles were made of PET. Considerations were conducted to ensure the open and closing mechanism of the bottles were similar. Samples were vacuum filtered through polycarbonate track-etched filter membranes, after filtration Nile red solution was added. The membrane was then transferred onto microscopic glass slides and examined under fluorescence microscopy Images were captured using Micropix Cytocam Software (v 1.4.0.4), to evaluate the morphology of the particle for comparison. The number of fluorescent particles observed was counted and recorded. The concentration of fluorescent particles was calculated based on microscopic observation using the formula as shown: Concentration (particles/L)=Number of particles per 100 mL x 10 The pH of each sample was determined using a portable pH meter (Jenway, 370 pH meter, EU).

The commercial synthetic polymer PE, fluoresced in orange (but appeared yellow upon image capturing) while the PVC fluoresced in yellow after staining in Nile red (Figure 20A – B), which provides evidence of the ability of Nile red to stain microplastics. This finding was consistent with published literature on the use of NR to identify microplastics. Numerous particles were identified with fluorescence in both BW and SD. These were of multiple shapes and fluoresced in yellow and orange, hence visual quantification was possible using a light microscope. The number of particles identified was 350 – 1560 particles/L in BW samples, and 500 – 4330 particles/L in SD samples. Different water

brands showed no significant difference ($p = 0.097$). There is a statistically significant difference for the mean concentration of particles between SD samples (S1 – S8) ($p = 0.05$) (Figure 21). The mean concentration of particles was higher in SD (1599 ± 1104 particles/L) compared to BW (1019 ± 352 particles/L). This was not statistically significant ($p= 0.05$). There is a statistically significant difference for mean pH between BW and SD ($p = 0.001$). The mean pH values were higher in BW (7.03 ± 0.55) compared to SD (3.36 ± 0.24). There was a statistically significant inverse relationship between the concentration of particles and pH value ($r = -0.355$, $p = 0.039$). The coefficient of determination suggests that pH level explains 12.6% of the variation in abundance of particles in the samples ($R^2 = 0.126$).

While a limitation of analysis of micro plastics with Nile Red is co-staining of natural organic material all samples used in our study was processed BW or SD made to EU standards. We felt that the likelihood of significant organic material was small compared to environmental samples. Therefore the microparticles we identified most likely represent microplastics. All samples were found to contain microparticles. To date, this study is the first to suggest the presence of microplastics in SD. Our results demonstrated an inverse correlation between number of micro particles and pH. The low pH value of the beverages contained in the bottles may be an influencing factor on the concentration of microplastics being detected. The microplastics detected were >10 microns due to the size of the PCTE filter membrane pores. The pore size of the filter may therefore result in underestimation on the concentrations of particles <10 microns. Previous reports on microplastic concentration in BW samples has varied from 14 plus or minus 1.4 particles/L to 325 particles/L. This variation may represent the difficulty in accurately assessing concentration or variation in manufacture and water source. Tracing the source of microplastics in the beverages is deemed to be an almost impossible task due to the multiple entry points for contamination to occur. The abundance of microplastics observed in BW and SD could be a potential public health issue. Until a robust method for determining and detecting the risk of microplastics, its impact towards human wellbeing being remains uncertain.

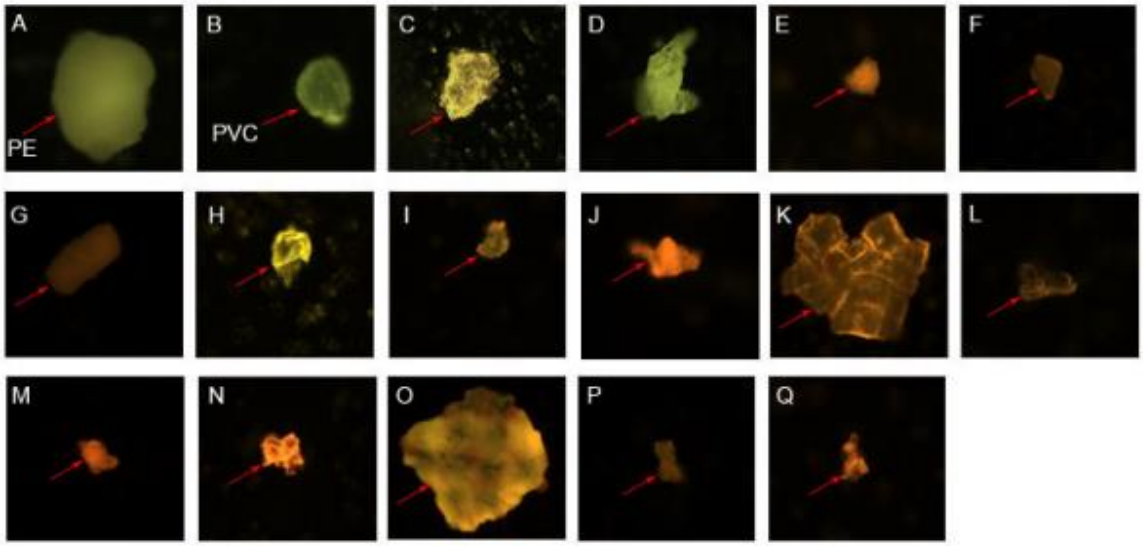


Figure 20: Images under a fluorescence microscope for Nile Red stained commercial synthetic polymers (A – B), and stained polymers in tap water (C), bottled water W1 – W6 (D – I) and sports drinks S1 – S8 (J – Q).

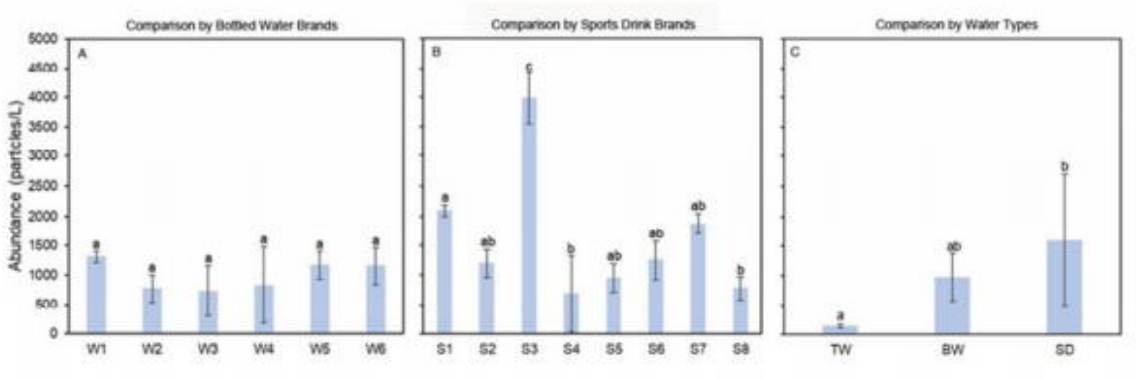


Figure 21: Comparison of the concentration of microplastics (particles /L) in samples of bottled water and sports drinks. (A–C). Each value represents the mean ± standard deviation. The letters above the bars indicates significant differences ($p < 0.05$). Similar letters indicate that values are not significantly different. The abbreviations on the X-axis are as follows: TW, tap water; BW, bottled water; SD, sports drinks.

Bibliography

Erni-Cassola G, Gibson MI, Thompson RC, Christie-Oleza JA. 2017. Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 µm) in Environmental Samples. Environ Sci Technol. 51(23):13641-13648.

Maes T, Jessop R, Wellner N, Haupt K, Mayes AG. 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci Rep. 7:44501.

Mason SA, Welch VG, Neratko J. 2018. Synthetic Polymer Contamination in Bottled Water. Front Chem. 6:407.

Shim WJ, Song YK, Hong SH, Jang M. 2016. Identification and quantification of microplastics using Nile Red staining. Mar Pollut Bull. 113(1-2):469-476

Zuccarello P, Ferrante M, Cristaldi A, Copat C, Grasso A, Sangregorio D, Fiore M, Oliveri Conti G. (2019) Exposure to microplastics (<10 µm) associated to plastic bottles mineral water consumption: The first quantitative study. Water Res. 157:365-371.

Microplastics in bivalve molluscs: literature revision to support the exposure assessment

Giusti A [1], Susini F [2], Diano Marco [1], Ricci E [2], Guardone L [1], Armani A [1]

[1] FishLab, Department of Veterinary Sciences, University of Pisa (IT), [2] Experimental Zooprophylactic Institute of Lazio and Tuscany (IT)

Abstract: Microplastics (MP) presence in the marine environment is one of the most concerning contemporary pollution problems. MP are defined as heterogeneous mixture of differently shaped materials in the range of 0.1–5,000 µm and they are worldwide distributed as result of the fragmentation of larger plastic debris or may be introduced into the water already as micro-sized. Because of their small size and widespread occurrence, MP are now available to marine species throughout the food web. Bivalve molluscs (BM), which are filter-feeding organisms, are particularly subjected to the phenomenon of MP accumulation and, in addition, they are usually consumed as whole. Even though the risk of MP ingestion via BM consumption, such as mussels, was proved to be minimal respect to other exposure via, a correct human exposure assessment cannot disregard a detailed collection data analysis on MP level in BM categories of commercial interest. In general, data gaps in microplastic research lead to an insufficient information to assess the true amount of microplastics humans may be exposed to via food. In a context when mass-media often cause excessive alarm by leveraging on the current high citizens sensibility towards environment and health issues, a scientifically validated consumers information is needed to reduce the potential damage against the seafood sector. In fact, awareness about MP in BM could lead consumers to reduce their consumption. In this work the data resulting from a preliminary analysis of data obtained by reviewing the scientific literature dealing with the accumulation of MP, are presented.

To collect data on MP bivalve categories of major commercial appeal, a systematic literature review was conducted. Search terms were employed to explore published literature in the bibliographic databases Web of Science, Scopus and PubMed. The following search string was used to maximise the return of relevant literature sources: Microplastic* AND (bivalve* OR mussel* OR clam* OR oyster* OR scallop*). The following information was established as inclusion criteria for retaining the study in the analysis: 1) bivalve type (species, genus or family) 2) geographical area of the investigation; 3) MP quantification (reported as number of MP/specimen or number of MP/weight). The year of publication and the subject area of the journals in which the selected papers were published were even considered and discussed. When available, also information on the microplastics characterization (size, colour, type, material) and analytical procedures used for MP detection were considered. The data were organized in an excel sheet for further analysis.

A total of 88 scientific papers were included. The most part of them (79.5%) were published in the three-year period of 2018-2020 (Figure 22). The papers were published in 30 different scientific journals, most of which (70%) specifically referred to the environmental sciences research area and only a minority were published on journals focusing on food science. Overall, data on MP were found for 67 species, 5 genera and 1 family; In details, 42 clams (36 species, 5 genera and 1 family), 16 mussels (15 species and 1 genus), 13 oysters and 5 scallops were investigated. Overall, mussels were the most analysed category (68 scientific papers) with Mytilus spp.as the most targeted genus. Among the other categories, the clam Ruditapes philppinarum and the oyster Crassostrea gigas were the most frequently analysed. FAO fishing area 61, 27 and 37 were the most investigated geographical areas. Sampling conducted in Asia were the most representative in term of species variability. Data on the MP quantification were given as a mean value, a range or both and reported as number of MP/gram and/or number of MP/individual. In most cases, the MP size was less than 5 mm. Blue, red and black fibres or fragments composed by polyethylene, polypropylene, polyvinylchloride and polyamide were the most representative MP type. The analytical procedures applied to detect MP, although all sharing similar basic steps, vary greatly.

The MP presence and distribution in the ecosystem has attracted great interest within scientific community in the last years. Although this study focused on an extremely specific target such as BM, the abovementioned trend was confirmed by the increase of scientific production observed in the last three-year period. However, literature especially addressed to the food inspection field is scarce. Moreover, although MP were also investigated in the most commercially relevant BM species, other studies covering all commercial species are needed, especially considering the high species variability that can be found in the context of the global market. In addition, a standardization of the MP quantification both as analytical procedures and unit of measure used for reporting data is required, as already mentioned in a recent EFSA scientific opinion on this topic. Overall, the abovementioned gaps limit the exposure assessment that, as part of the risk analysis, is stated as cornerstone of the EU legislation. This study, by highlighting the main issues related to the MP investigation in BM, may represent a valid starting point for the scientific community for improving the research in this field.

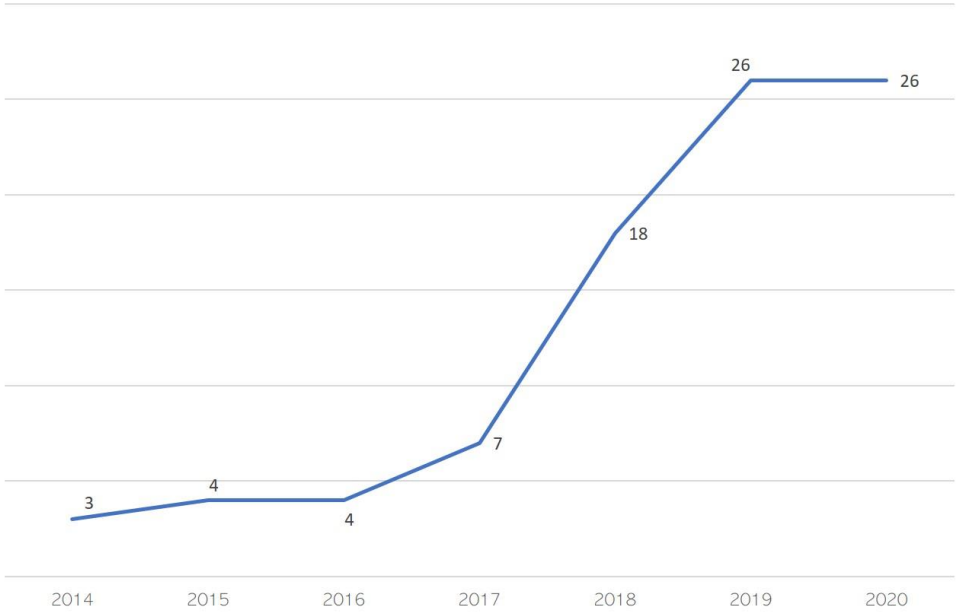


Figure 22: increasing trend in the number of scientific papers about microplastics per year.

Bibliography

Catarino AI, Macchia V, Sanderson WG, Thompson RC, Henry TB. 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environ Pollut. 237:675-684.

Hoogenboom LAP. 2016. Statement: Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal, 14(6), e04501.

Smith M, Love DC, Rochman CM, Neff RA. 2018. Microplastics in Seafood and the Implications for Human Health. Curr Environ Health Rep. 5(3):375-386.

Thiele CJ, Hudson MD, Russell AE. 2019. Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. Mar Pollut Bull. 142:384-393.

Zhang F, Man YB, Mo WY, Man KY, Wong MH. 2019. Direct and indirect effects of microplastics on bivalves, with a focus on edible species: A mini-review. Crit Rev Environ Sci Technol 1-35.

Potential trophic transfer of microplastics from marine to human food chain: preliminary study on commercial seafood from Tyrrhenian sea [Title]

Santonicola S [1], Avio CG [2], Regoli F [2], Colavita G [1], Mercogliano R [3]

[1] Department of Medicine and Health Sciences, University of Molise (IT), [2] Department of Life and Environmental Sciences (DiSVA), Università Politecnica delle Marche (IT), [3] Department of Veterinary Medicine and Animal Production, University of Naples (IT)

Abstract: The widespread distribution of microplastics in the marine environment has subsequently affected the aquatic biota including seafood for human consumption. An increasing number of reports document the ingestion of microplastics by fish species and their occurrence in the gastrointestinal tract (Efsa, 2016). Although demersal fish are usually eviscerated before consumption, both fresh and dried small fishes are often consumed as a whole (Renzi et al., 2019). This is the case of Engraulis Encrasicolus, a commercially important small pelagic fish species, which has been proposed as a small-scale indicator both of microplastic contamination in open waters and human exposure (Compa et al., 2018).

E. encrasicolus (n.20 samples from the Tyrrhenian Sea) were collected. The gastrointestinal tract was removed and analyzed applying the microplastic extraction method, according to Avio et al. (2015) and Foekema et al. (2013). Then microplastic morphological and physical classification, and

quantification analysis were carried out. Further FT-IR/RAMAN spectroscopy analysis should be carried out to identify the microplastic polymers of origin.

As preliminary data, the application of the extraction method resulted in the efficient separation of microplastic from the organic tissues. Results showed the occurrence of fibers and plastic particles in the digestive tract of some fish samples.

Seafood represents a considerable food vector for microplastic human exposure. Under the perspective of the human food chain, the microplastic trophic transfer and their bioaccumulation and biomagnification represent a serious issue to food safety (Efsa, 2016).

Microplastics impact a high proportion of the wild E. encrasicolus caught in the Mediterranean Sea and their occurrence may be found also in other tissues than stomach contents. E. Encrasicolus as selective feeders specie may ingest microplastics as prey (Compa et al., 2018). Considering that anchovies composing the main diet for pelagic predators in the Mediterranean Sea, and their relevance for human consumption, further studies targeting levels of litter and microplastics in natural stocks are essential (Renzi et al., 2019). Traceability of the fate of microplastic in contaminated seafood is essential to assess their bioaccumulation and biomagnification in the marine habitat and the potential trophic transfer from marine to the human food chain.

Bibliography

Avio CG, Gorbi S, Regoli F. 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. Mar Environ Res. 111:18-26.

Compa M, Ventero A, Iglesias M, Deudero S. 2018. Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. Mar Pollut Bull. 128:89-96.

Foekema EM, De Gruijter C, Mergia MT, van Franeker JA, Murk AJ, Koelmans AA. 2013. Plastic in north sea fish. Environ Sci Technol. 47(15):8818-24.

Hoogenboom LAP. 2016. Statement: Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal, 14(6), e04501.

Renzi M, Specchiulli A, Blašković A, Manzo C, Mancinelli G, Cilenti L. 2019. Marine litter in stomach content of small pelagic fishes from the Adriatic Sea: sardines (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*). Environ Sci Pollut Res Int. 26(3):2771-2781.

Influence of microplastics on the germination of ryegrass

Cornelsen H [1], Wrage-Mönnig N [1]

[1] *University of Rostock (DE)*

Abstract: Environmental pollution by plastic has become a growing scientific and societal concern throughout the last years. Due to its longevity and subsequent resistance to degradation, mismanaged plastic debris has led to polymers of all sizes that enter and persist in the environment. Recently, studies showed the effects of microplastics (MP) on aquatic organisms and oceans. Considerably less is known on MP acting as a potential terrestrial hazard (Horton et al., 2017, Rillig 2012). The sources of MP in soils are manifold: direct deposition can occur via aerial transport or rain (Bergmann et al., 2019). Further sources are commonly used agricultural practices like fertilizer application of compost or sewage sludge from biowaste (Nizzetto et al., 2016). Although wastewater treatment plants are very efficient in the retention of polymer particles, MP still accumulate in the sludge. Another source is irrigation from wastewater, which could potentially be contaminated with MP. As agricultural soils are the basis for the global food-web, identifying the consequences of exposure to MP is very important. Therefore, the aim of this research was to investigate the influence of different types and sizes of MP on a commonly used forage plant. We tested the hypothesis that the addition of MP to the germination environment of ryegrass would have adverse effects like reduction in germination and subsequent plant growth.

We conducted a germination experiment under laboratory conditions, using *Lolium multiflorum Lam.var. westerwoldicum* as a model plant. 50 seeds each were applied onto moistured filter papers in petri dishes. We obtained polyvinylchloride (PVC, 1-63 µm) from Pyropowders (www.pyropowders.de, Erfurt, Germany) and compared its effects with shredded tyre wear material

from MRH Mülsen (www.mrh-muelsen.de, Mülsen, Germany). The latter material was purchased in three different size ranges: W0004 (> 80-400 µm); W0610 (600-1000 µm); W2550 (> 2000-5000 µm). The tyres were originally manufactured with a granular core of vulcanized rubber compound out of Natural rubber (NR) and Styrene Butadiene Rubber (SBR) as well as tyre-typical additives. MP was applied to the seeds at five concentrations: 0 g; 0.5 g; 1 g; 1.5 g; 2 g. Additionally, we used cold extracts of the MPs for watering in further treatments. Seeds were grown for 13 days under growth lamps (Growlight Duo, Parus, Michelstadt, Germany) at an average air temperature of 21°C in the laboratory. State of germination was recorded daily. After the experiment, seedlings were classified according to the ISTA-handbook of seedling evaluation (Don, 2013) into normally, abnormally and ungerminated seeds. Afterwards, they were separated into above- and belowground biomass for analysis. Dried roots were analyzed for total root length per petri dish by scanning and following analysis using the program ImageJ and the Plugin Ridge Detection. Statistical analysis was performed using Microsoft Excel and R Studio.

For plants growing with MP in their germination environment, growth depression could be observed throughout. PVC seemed to be most disrupting for the plants, there was almost no above- and below-ground biomass at harvest, especially with the larger amounts of PVC. As a consequence, there was a high number of ungerminated seeds with all PVC treatments. Those seeds that germinated with PVC had a large percentage of abnormally germinated seeds. Large numbers of ungerminated or abnormally germinated seeds were also observed on exposure to tyre wear derived particles (W2550; W0004; W0610). Root lengths and general biomass data of ryegrass showed a tendency towards larger adverse effects of smaller particles of MP. Highly significant effects of MP were observed for the root length of ryegrass. The concentration of MP also had a significant effect on the root length. The treatments with MP-extracts that were used for irrigation were less harmful than MP itself, but growth depression and inhibition of germination could also be observed throughout.

MP had a large effect on ryegrass growth in the experiment. To assess this effect, we tested different parameters of plant growth. The hypothesized plant growth inhibitions caused by MP could clearly be seen. Purposefully, we chose a robust plant for the experiment, but could still observe decreases in germination and plant performance. Especially root growth, a parameter of plant health and stability, was clearly affected by MP particles. PVC, simulating the smallest particles used in the experiment (1-63 µm), had the largest effect on both plant growth and root length. The MP-extracts we used simulated the irrigation with MP-contaminated water. Irrigation with MP affected these plants in all treatments, showing effects due to water-soluble substances derived from MPs. Nevertheless, effects of irrigation were not as harmful as the direct exposure to MP particles. The amounts of MP used in the experiment are unlikely to occur in field conditions. Nevertheless, investigating the effect of MP on plants is one of the future challenges of MP research. Grassland species do not only serve as environmental and soil stabilizers, but also represent a basis of feed and therefore food production. To the current state of knowledge, there is no solution for the continuing accumulation of MP in agricultural soils. More knowledge is needed on the mechanisms by which plants are being affected by MP, to point out if factors of plant health might be agronomically addressed to inhibit the harm of MP for plants and consequently livestock and humans.

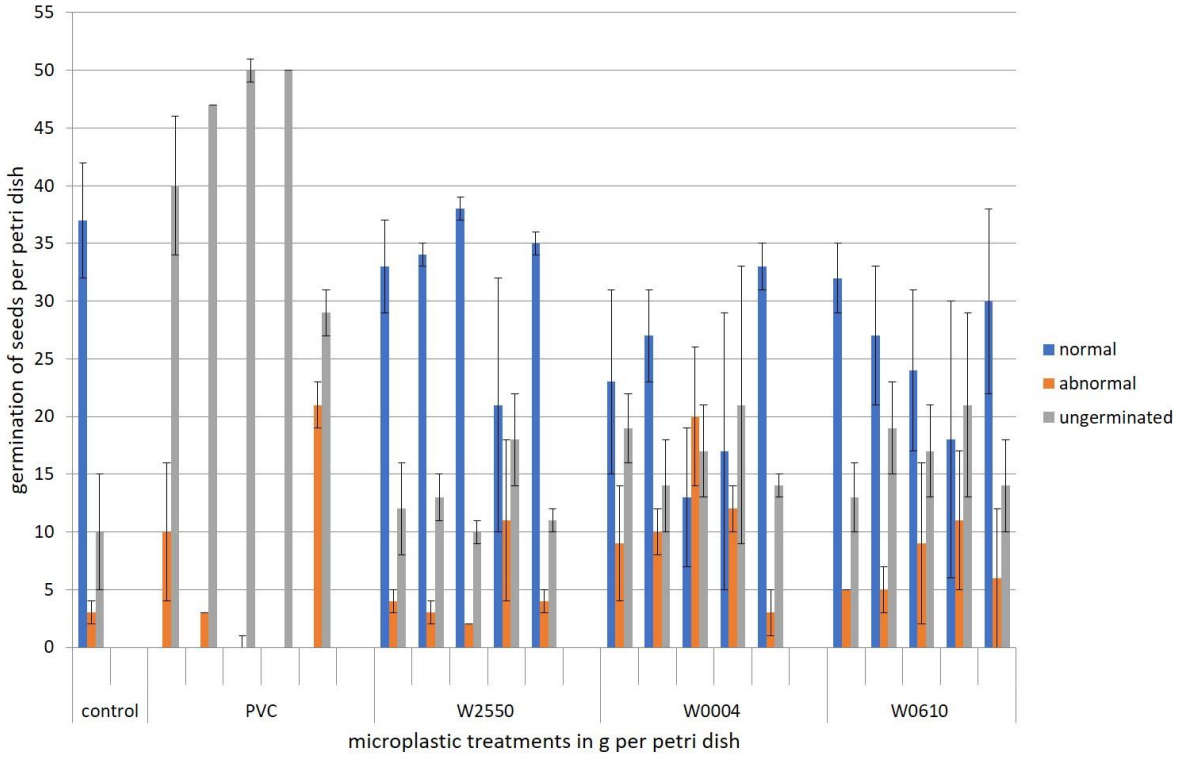


Figure 23: Total root length in cm, shown in boxplots for the different microplastic treatments (PVC, tyre wear derivatives (W0004; W0610;W2550). Extracts and concentration levels are included).

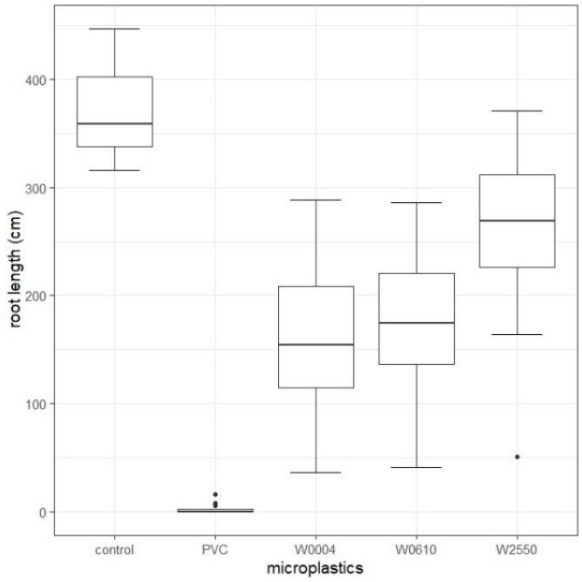


Figure 24: Germination rate of ryegrass seedlings (50 seeds = maximum), counted according to ISTA (International Seed Testing Association), differentiated into normal, abnormal and ungerminated for microplastic treatments (PVC, tyre wear derivatives (W0004; W0610; W2550), MP-extracts: E) and concentration levels (in g). Mean ± standard error are presented.

Bibliography

Bergmann M, Mützel S, Primpke P, Tekman MB, Trachsel J, Gerdts G. 2019. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. Sci Adv 5 (8): eaax1157.

Don, R. (Ed.). 2013. Ista handbook on seedling evaluation (3., rev. ed., including Amendments 2006, 2009 and 2013). International Seed Testing Association.

Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C. 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci Total Environ. 15;586:127-141.

Nizzetto L, Langaas S, Futter M. 2016. Pollution: Do microplastics spill on to farm soils? Nature. 537(7621):488.

Rillig MC. 2012. Microplastic in terrestrial ecosystems and the soil? Environ Sci Technol. 46(12): 6453-4.

Steinmetz Z, Wollmann C, Schaefer M, Buchmann C, David J, Tröger J, Muñoz K, Frör O, Schaumann GE. 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? Sci Total Environ. 15;550:690-705.

The Occurrence and the Assessment of health risks related to the presence of Microplastics in Drinking Water in Saudi Arabia

Almaiman L [1], Bineid M [1], Aljomah A [1], Alhuthiel M [1], BaAbdullah A [1], Al-arfaj R [1]

[1] *Saudi Food and Drug Authority (SA)*

Abstract: The implications and health effects of microplastics (MPs) ingestion are still unclear, yet researchers and organizations around the world are increasingly examining the levels of micro- and nanoplastics in the environment. Microplastics have been recently detected in seawater, fresh water, drinking water, food, and air around the globe. There are limited data on the concentrations of microplastics in drinking water in both tap and bottled water in the region. The current study investigates the presence and the quantity of microplastics in bottled and tap water from four regions in Saudi Arabia, and measures the intake of the Saudi population of the detected microplastics.

Thirty samples of drinking water were collected for the detection and quantification of microplastics. The majority of the samples were collected from the retail market of Saudi Arabia. Twenty four samples were PET bottled drinking water, 2 PC bottled drinking water, and 2 glass bottles. In addition, 2 samples of tap water were taken to represent desalinated seawater. The samples were analyzed at Umweltbundesamt GmbH (Environment Agency Austria) laboratory services, Vienna, Austria. Sample extraction was performed using stainless steel sieves. Identification and classification of microplastics particles using Fourier-transform infrared micro spectroscopy (FTIR microspectroscopy) followed by software assisted determination of particle number the determination was performed for the following polymers: PE, PP, PVC, PS, PET, PA, PU, PC, PMMA, and POM. The particle size ranges screened for in this study are 0.025-0.5 mm, and 0.005-0.025 mm.

The results of this study will be presented as mean concentrations for all size ranges of the micro particles. Both the mean of all positive findings as well as the mean of all the samples analyzed will be presented separately. The latter mean values are the values that will be applied for the exposure assessment. The non-detects will be set LOD.

The purpose of this sample selection is to identify the significant sources of microplastics in water in Saudi Arabia. To our knowledge, this is the first study to measure microplastics level in the region. The results of this study are considered preliminary in calculating the intake of microplastics and assessing the risk associated with the exposure of the Saudi population to microplastics from water. The brands of water bottles analyzed in this study are of the most popular in the country, most importantly, extracted from the groundwater of Saudi Arabia. Two types of plastic materials are used for drinking-water bottling in Saudi Arabia, PET and PC. Therefore, it was important to analyze these specific types for the occurrence of microplastics in drinking water.

Holistic approach to study microplastics in foodstuff and their health effects – Experiences on a pilot study

Koistinen A [1], Lahtinen P [1], Raninen K [1], Selenius M [1], Uurasjärvi E [1]

[1] *University of Eastern Finland (FI)*

Abstract: The prevalence of microplastics (MPs) have been extensively reported in aquatic environment. Further, their effects on aquatic biota and accumulation in foodwebs are also widely investigated. However, consequences of MPs to human health are still unknown. The human annual exposure to microplastics has been estimated to be 39000 - 52000 particles from food and drinks (Cox

et al., 2019), and partially food packages are a probable source. Quantities of MPs in various foods have barely been studied (Lee et al., 2019), still less relations of different food packages or digestion dynamics of ingested MPs.

In our research, a holistic approach will be taken to evaluate health effects of MPs including chemical analysis, in vitro cell studies and gastrointestinal tract models. Eventually, we will be able to conduct comprehensive chemical analysis for the MPs and associated compounds in foods and food packages. The gastric and intestinal digestion on MP particles will be simulated with the in vitro digestion models (Brodkorb et al., 2019). Further, selected plastic associated chemicals will be introduced to cell cultures and to established gut models to investigate their impact to somatic cells and colon microflora (Gómez -Gallego et al., 2014).

In the presented pilot study, we developed routine methods for determining MPs from milk products and further, for monitoring leachates of MPs originating from food packages in environment simulating gastric digestion with gas chromatography – mass spectrometry.

In this pilot study, fat-free protein standardized milk products packed in carton were acquired from local superstore for analysis. To analyze microplastic particles, they were isolated from the sample matrix after dissolution of other solids and filtration of plastics. Various chemicals were tested for dissolution, and triethanolamine was found to be the most effective in dissolving milk proteins, consisting of mainly casein. Finally, isolated plastic particles were filtered to reflective silver membrane filters and analyzed with an imaging Fourier-transform infrared (FTIR) spectroscope (Agilent Cary 620/670), equipped with a 128*128 FPA detector. Spectral image data was collected in reflectance mode with 15X objective, 5,5 µm pixel size, 3800 - 800 cm^{-1} spectral range, 8 cm^{-1} spectral resolution and number of scans 4. The spectral maps were processed with siMPle software.

Further, food packages consisting of polystyrene or polypropylene were manually grinded and the resulting microplastics (0,148 g and 0,186 g, respectively) were incubated in HCl solutions (40 ml, pH 1,3, 37°C) for 2 hours. The sample solutions were extracted to MTBE, filtered with 0,45 um filters, and analyzed using comprehensive gas chromatography – mass spectrometry (2D-GC-MS) consisting of GCMS-QP2010 Ultra and AOC-5000 Plus injection system (Shimadzu Scientific Instruments), Optic-4 multi-mode inlet (GL Science) and ZX-1 thermal modulator (Zoex Corporation). Injection was done in 300°C utilizing cryotrap (-20°C) after injection, compounds were separated with column set ZB-5HT (Phenomenex, 30m/0,25mm/0,25µm) + BPX50 (Trajan, 3m/0,15mm/0,15µm) using temperature 40-350°C and 8 s modulation time.

On the average, milk products contained 396 MPs/liter (range 197-792 MPs/liter, N=4). Majority of the MPs were common plastic materials, such as polyethylene (PE) and polypropylene (PP). The average size of the MPs was 73,0 +- 59,1 µm (range 11,0 - 607,4 µm). Estimated mass of the individual MPs was 26,0 +- 17,8 ng resulting in average mass of 10,4 +- 7,1 µm/liter, respectively. Negative controls showed very low plastic contamination rate and recovery rate was also studied using stereomicroscope.

No leachates were detected with 2D-GC-MS in the pilot trial mimicking gastric digestion of food package MPs.

From the limited set of samples, relatively high number of MPs were found in milk products. The origin of the MPs found in the study is unknown, i.e. whether they are from the package material or from the production process. However, this indicate potential of milk products being an exposure route to MPs and therefore origin of MPs should be studied with more inclusive sample sets. Further research on MP analysis methods for various food products is needed to enable quality control. In this study, milk pretreatment and analysis methods were found to be effective, reliable and easy to use.

Additionally, material degradation during lifetime of food packaging and their interaction with food proteins are other critical features. Hypothetically, the MPs and the chemicals leaching and/or migrating from the plastics after ingestion may have impacts on human gut microbiota or gastro-intestinal epithelium.

Systematic and holistic research on the impact of MPs and their chemicals is urgently needed to understand their effects on human health. With the suggested approach, we can provide a unique platform providing information that will be of great importance for the comprehensive risk assessment of health effects caused by (micro)plastics.

Bibliography

Brodkorb A, Egger L, Alminger M, Alvito P, Assunção R, Ballance S, Bohn T, Bourlieu-Lacanal C, Boutrou R, Carrière F, Clemente A, Corredig M, Dupont D, Dufour C, Edwards C, Golding M, Karakaya S, Kirkhus B, Le Feunteun S, Lesmes U, Macierzanka A, Mackie AR, Martins C, Marze S, McClements DJ, Ménard O, Minekus M, Portmann R, Santos CN, Souchon I, Singh RP, Vegarud GE, Wickham MSJ, Weitschies W, Recio I. 2019. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc. 14(4):991-1014.

Cox KD, Covernton GA, Davies HL, Dower JF, Juanes F, Dudas SE. 2019. Human Consumption of Microplastics. Environ Sci Technol. 53(12):7068-7074.

Gómez-Gallego C, Collado MC, Pérez G, Ilo T, Jaakkola UM, Bernal MJ, Periago MJ, Frias R, Ros G, Salminen S. 2014. Resembling breast milk: influence of polyamine-supplemented formula on neonatal BALB/cOlaHsd mouse microbiota. Br J Nutr. 111(6):1050-8.

Lee H, Kunz A, Shim WJ, Walther BA. 2019. Microplastic contamination of table salts from Taiwan, including a global review. Sci Rep. 9(1):10145.

Exposure Assessment to Microplastic through the Consumption of Sea Salt Collected from the Mediterranean Coastal

Bou-Mitri C [1], Dgheim N [1]

[1] *Notre Dame University-Louaize (LB)*

Abstract: The Mediterranean is a closed basin with limited water exchange. It is surrounded by 22 countries producing 10% of all plastic goods, making it the world's 4th largest plastic producer. Among those countries, three of them account for two-thirds of plastic leaked into nature including Egypt (42.5%), Turkey (18.9%), and Italy (7.5%). Sites along its shores showed the greatest densities of marine debris in the world. Studies have shown that plastics in Western Mediterranean come from Spain and in Eastern Mediterranean from Turkey. Evidences also showed that plastic littered in the Mediterranean would float all around. Shores in Cyprus represented the highest amount of microplastics after Hong Kong. Recent study conducted showed the presence of microplastics in sediments and sea biota samples in Lebanon. There is no legislation for microplastics as contaminants in food. Knowing that sea salt is produced by the sole evaporation of sea water, growing evidence indicated that it is contaminated with microplastics. To date and to the best of our knowledge only evidence from samples collected in Turkey and Spain were reported in the literature with some contained between 16-84 item/kg, or 50 and 280 item/kg, respectively. Calculated exposures showed that Turkish consumers would consume 249–302 items/year from sea salt.

Accordingly, our aim is to conduct an assessment of microplastic in sea salt collected across the Mediterranean countries and determine the exposure of the consumers to that contaminant.

Sea salt samples will be collected from producing basins across the Mediterranean. The samples will be solubilized in deionized water and filtered. The retained particles will be subjected to Fourier Transform Infrared spectroscopy and their fingerprints will be compared to the ones in the data base. Type, color, shape dimension will be also assessed in order to study further their history. The consumer exposure to microplastic via sea salt consumption will be determined using per capita method.

The overall results obtained to date showed that all (100%) the assessed samples contained plastic fragments including propylene, polyethylene and plasticizers.

This study will allow having a general assessment of the impact of the plastic marine litter on the food safety due to mismanagement. It will also permit the suggestion of actions to be taken based on evidence.

Bibliography

Duncan EM, Arrowsmith J, Bain C, Broderick AC, Lee J, Metcalfe K, Pikesley SK, Snape RTE, van Sebille E, Godley BJ. 2018. The true depth of the Mediterranean plastic problem: Extreme microplastic pollution on marine turtle nesting beaches in Cyprus. Mar Pollut Bull. 136:334-340.

Gündoğdu S. 2018. Contamination of table salts from Turkey with microplastics. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 35(5):1006-1014.

Iñiguez ME, Conesa JA, Fullana A. 2017. Microplastics in Spanish Table Salt. Sci Rep. 7(1):8620.

Nano- and microplastics in the Dutch dairy farms. Sources, concentrations and exposure of dairy cows via water and feed

Antunes Fernandes E [1], Kuijper A [1], van Ede K [1], de Vries Ruben [2]

[1] KeyToxicology (NL), [2] Dutch Dairy Association (NL)

Abstract: It is known that the diet of a dairy cow can be a source of exposure to different environmental pollutants (e.g. dioxins) and it can be expected that the same route of exposure can occur for nano- and microplastics (NMP). On average, per day, the diet of a Dutch dairy cow is composed of approximately 90% of roughage (including fresh grass, maize and silage) and 10% of concentrates (such as maize, soya, citrus, palm and rapeseed meal, beetroots, wheat and by-products of other industries). During grazing, cows can ingest soil and, although soil cannot be considered a traditional feed component, dairy cows may be exposed to NMP via the ingestion of soil. It must be taken into account that all the above-mentioned feed components can be a route of exposure of dairy cows to NMP. In the Netherlands, drinking water production (tap water) mainly uses ground and surface water (KWR, 2019). But a cow may also drink water directly from surface water (e.g. rivers, lakes or canals). Therefore, both tap- and surface water should be considered as possible sources of exposure of dairy cows to NMP. With some feed contaminants there is a carryover from the feed to the milk produced by dairy cows. It is important to assess whether the same may occur with NMP. Therefore, the objective of this literature review is to assess if dairy cows are exposed to NMP via the feed and water, to which levels and if ingested NMP can be excreted via milk.

For an extensive literature review, mainly Google scholar was used to retrieve, preferably, up-to-date articles focusing on the presence of NMP in feed and water of dairy cows. Search was done until September 2019. The key-strings were chosen to include the range in the materials to screen, such as nano- and microplastics and the most common polymers present in plastic particles. Key-strings also included the variability in the feed composition, including the different types of roughage most commonly used in the Netherlands and the most common feed products present in concentrates. Lastly, key strings included the different sources of drinking water available to a dairy cow, from tap- to surface-water. The key-strings were used in a stepwise approach and the retrieved hits were first screened for importance based on their title and abstract. Subsequently, relevant articles were used for data extraction. A regular search on google was done to identify reports prepared by national and international organizations.

There is limited information of microplastics in soil, but values seem to vary between 0,34 ± 0,36 particles / kg dry weight soil and 5 particles / kg soil for farmland soil and flooded plains, respectively. To our knowledge, there are no studies reporting the presence of NMP in roughage or nanoplastics in soil. Also, there is no available data regarding the presence of NMP in concentrates but they may contain up to 0,7 % (w/w) of packaging material (also containing plastic). Concentration of microplastics in drinking water seems to be higher in tap water from surface water (5,45 particles / L) than in groundwater (0,0007 particles / L; WHO, 2019). In the Netherlands, in general, outside urban areas, the highest concentrations of microplastics are found near (effluent) of wastewater treatment plants (81 particles / L). To our knowledge there are no studies reporting the presence of nanoplastics in freshwater systems. During grazing, a cow can eat up to 1 kg of soil per day. Depending on the location of the farm, a cow may ingest between 0,34 and 5 particles per day. A cow drinks, on average, 100 L of water per day. Depending on the source of the water, a cow might ingest between 0,07 and 8100 particles per day. Based on the limited information, water seems to be the most important source of exposure of dairy cows to microplastics and the location of the farm may influence the exposure levels of dairy cows to microplastics.

Based on the limited available information, there are no indications that microplastics in drinking water pose a health risk for humans (WHO, 2019). To our knowledge, at the moment, there are no studies that look at the effect of microplastics on the health of dairy cows, or whether ingested nano- and microplastics reach the milk. It is generally assumed that microplastics > 150 uM are excreted through faeces, but the fate of smaller particles is unsure. It is known that nanoparticles are capable of crossing the gut barrier ending in tissues and milk, but we cannot extrapolate these results to nanoplastics since the properties of nanoparticles are determined by many of the particles characteristics', such as charge, shape, material, etc. and not only by size. Therefore more research is

needed to assess the toxicological properties of NMP, their distribution, kinetics and health effects of NMP, including their capability to cross the gut barrier and reach the milk of dairy cows. At this moment, there are no indications that raw milk is a significant source of exposure of NMP in the human diet, via milk products.

Bibliography

KWR. 2019. De Kwaliteit van bronnen van drinkwater in Nederland. Nieuwegein: KWR 2019.072.

WHO. 2019. Microplastics in drinking-water. Geneva: World health Organization. Licence: CC BY-NC-SA 3.0 IGO.

Session 3: Developments in hazard identification and characterisation for micro- and nanoplastics

A critical review of microbiological colonisation of nano- and microplastics (NMPs) and their significance to the food chain

Walker D [1], Baker-Austin C [1], Smith A [1], Thorpe K [2], Bakir A [1], Galloway T [3], Gaze W [3], Lewis C [3], Quill E [2], Russel J [1], Verner-Jeffreys D [1]

[1] Centre for Environment, Fisheries and Aquaculture Science (CEFAS, UK), [2] Fera Science Ltd. (UK), [3] University of Exeter (UK)

Abstract: Nano- and microplastics (NMPs) are a global concern, with NMPs found in aquatic and terrestrial environments. They can be ingested or otherwise absorbed by both food and non-food plants and livestock and so are capable of entering the human food chain. However, robust comparable and long-term data about the risk that NMPs pose to human health is sadly lacking. One particular area in which the risk posed by NMPs is unclear is their role in the transport of pathogens and their interactions with other micro-organisms of importance to human health. In this Food Standards Agency funded project, we will provide an authoritative and critical review regarding microbial interactions with microplastics and the potential risk that they pose to human health through the food-chain. This review will summarise the available scientific evidence (including peer review and grey literature sources) concerning the diversity of microorganism that colonise microplastics, outline the key pathways that these microbiologically contaminated microplastics are able to enter the food chain from environmental sources (e.g. from water, soil and air) and the associated risks these may pose to human health. We will also focus on areas of particular concern such as the role of NMPs in the spread of AMR in the food chain. The critical review will also identify key evidence gaps by considering both the available peer review scientific and grey literature in this area.

Literature searches will be carried out in Web of Science and other literature databases including OpenGrey, BIOSIS Citation Index, GreenFILE, EThOS, OATD, BASE and Library Hub Discover. To achieve the most inclusive search results, a set of search terms have been developed by the project team which includes experts in food safety, microbiology, ecotoxicology, AMR and microplastics. Initial searches have resulted in several thousand results and so a sift hierarchy method will be used to select only that literature which is relevant. An initial sift based on the article title will removed those articles that are obviously not relevant. These will include articles that deal with the uses of microplastics (for example the use of micro-fibres as a filtration matrix for bacteria) but do not deal with their impact. Articles will be further sifted based on their abstracts and then the main body of the article based on pre-defined exclusion/inclusion criteria. Although search terms have been developed to be as inclusive as possible, it is possible that some relevant literature will not be found using this strategy. Therefore, using the expert knowledge of our project team other articles known to be of importance to the team and their extended networks will also be considered for inclusion in this review. Using the selected literature, we will review the existing knowledge in this important area of public health and identify knowledge gaps that may need future research.

Results will be presented as per the status of the work at the time of the meeting

Discussions will be presented as per the status of the work at the time of the meeting

Oral Uptake of Micro- and Nanoplastics - Recent Developments in Research and Risk Assessment

Holger S [1]; Stock V [1], Paul M [1], Böhmert L [1], Braeuning A [1], Lampen A [1]

[1] *German Federal Institute for Risk Assessment (BfR; DE)*

Abstract: The topic of micro- and nanoplastics gained much public attention, since it has been discovered recently that microplastic particles reach the human gastrointestinal tract via food products. Quantification of oral uptake of such particles, however, is still very challenging. Very little is known about the bioavailability and the fate of microplastic particles in the human body or potential adverse effects. There are major knowledge gaps regarding the correlations between bioavailability, particle size, material type and potential mechanisms of action. Therefore, a profound risk assessment is currently not possible. Even less is known about nanoplastics, due to the lack of characterized materials and analytical methods.

The best known material is polystyrene, which is available in many sizes and chemical variations, such as surface modifications and fluorescent labels. An in vivo study with transgenic HOTT-reporter mice exposed to different fluorescent polystyrene particles (1, 4 and 10 µm) was performed to determine particle uptake and oxidative stress1. Analyses were extended by experiments using Caco-2-based cellular Transwell models of the gastrointestinal barrier to determine uptake and transport in vitro. Other, higher abundant, plastic materials like PE, PP, PVC and PET are only available as polydisperse powders without labeling or surface modifications (Figure 25). For these particles, method development was performed including fluorescent staining, milling and grinding, as well as the adaption of physico-chemical and cellular methods on particles with low density 2. An *in vitro* digestion system based on artificial fluids simulating mouth, stomach and intestine was used to investigate particle fate during the passage through the digestive tract 3. A variety of cellular in vitro testing systems are available to analyze mechanistic effects for hazard identification, but they need to be adapted to plastic particles as part of a series of profound method validations.

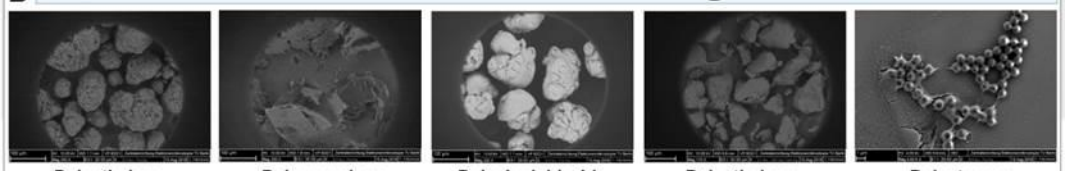
In vitro and *in vivo* studies focusing on the uptake of polystyrene microparticles indicated a very limited cellular uptake into cells of the gastrointestinal barrier and therefore a very low systemic bioavailability in a size range between 1 and 10 µm. The intestinal conditions, simulated by artificial saliva, stomach fluid and intestinal fluid, led to noteworthy structural changes of the particles, which, however, were caused by attachment of organic matter and not by particle degradation. After a digestion of organic compounds by hydrogen peroxide, the microplastic particles remained unaffected. Cellular investigations showed effects on intestinal and hepatic cell lines only in overload situations beyond realistic exposure scenarios. No oxidative stress could be detected *in vivo*.

Although an oral exposure to plastic particles has been proven, there are still big knowledge gaps regarding the quantity of exposure, bioavailability and fate of the particles, and potential toxicological modes of action, which could lead to a hazardous potential for consumers. This potential might derive from the particles themselves, but also from a release of toxic contaminants or additives (Figure 26). The gastrointestinal barrier function plays a meaningful role to answer these questions. A decisive improvement of cellular and analytical methods and technical progress in the applicability of toxicological testing systems, including the availability of reference materials, is needed. Due to a lack of data, a sound risk assessment of microplastics cannot be performed yet. Nevertheless, the general principles of risk assessment are applicable. It is expected that the state of knowledge will develop significantly in the next years, needed for a complete risk assessment of orally ingested micro- and nanoplastics in the near future.

A

Material	Size (µm)	Surface-Modification	Fluorescence	Diameter DLS (nm)	Image-Analysis
Polystyrene (PS)	10	SO_4		✗	✓
	4	neutral / COOH / SO_4		3800 (± 430) ✓	✓
	1	COOH		871 (± 253) ✓	✓
	0.1	neutral / COOH / SO_4 / NH_2	NANO!	61.2 - 79.1 (± 0.6 - 0.8) ✓	✓
	0.02	COOH	NANO!	35 (±1.9) ✓	✓
Polyethylene (PE)	Powder			✗	✓
Polypropylene (PP)	Powder			✗	✓
Polyvinylchloride (PVC)	Powder			✗	✓
Polyethylene-terephthalate (PET)	Powder			✗	✓

B



Polyethylene (PE) | Polypropylene (PP) | Polyvinylchloride (PVC) | Polyethylene-terephthalate (PET) | Polystyrene (PS)

Figure 25: Model particles of different plastic materials. A: Assortment of model particles of polystyrene with defined size, surface modification and fluorescence labeling. Other plastic materials are only available as polydisperse powders. B: Electron microscopy pictures of different plastic materials made by SEM (PhD project Valerie Stock)

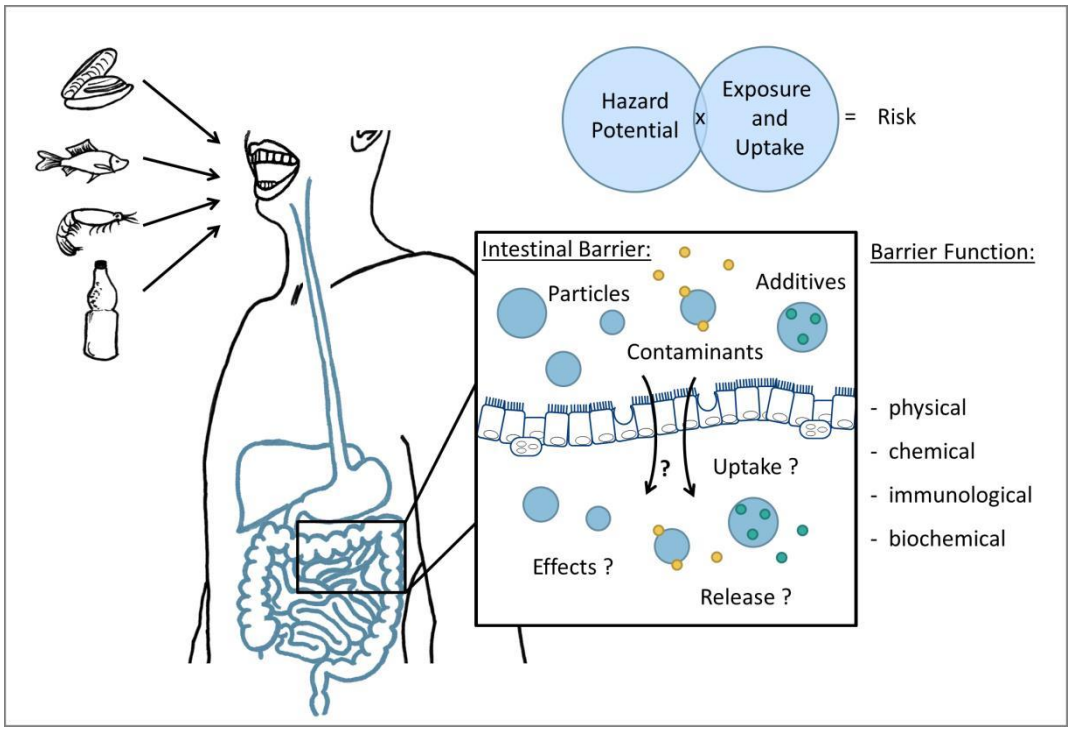


Figure 26: Scheme of oral uptake of microplastics and the passage of the gastrointestinal barrier.

Bibliography

Stock V, Böhmert L, Lisicki E, Block R, Cara-Carmona J, Pack LK, Selb R, Lichtenstein D, Voss L, Henderson CJ, Zabinsky E, Sieg H, Braeuning A, Lampen A. 2019. Uptake and effects of orally ingested polystyrene microplastic particles *in vitro* and *in vivo*. Arch Toxicol. 93(7):1817-1833.

Stock V, Böhmert L, Dönmez MH, Lampen A, Sieg H. 2020. An inverse cell culture model for floating plastic particles. Anal Biochem. 591:113545.

Stock V, Fahrenson C, Thuenemann A, Dönmez MH, Voss L, Böhmert L, Braeuning A, Lampen A, Sieg H. 2020. Impact of artificial digestion on the sizes and shapes of microplastic particles. Food Chem Toxicol. 135:111010.

Simgi® as an advanced model for the study of the interaction between food-derived microplastics, the human gastrointestinal tract and gut microbiota

Tamargo A [1], Cueva C [1], Alcolea V [2], Portela R [2], Bañares MA [2], Reinosa JJ [3], del Campo A [3], Fernandez JF [3], Moreno-Arribas MV [1]

[1] Institute of Food Science Research (CIAL) – CSIC (ES), [2] ICP-CSIC, Instituto de Catalisis y Petroleoquimica (ES), [3] Instituto de Ceramica y Vidrio (ICV-CSIC, ES)

Abstract: The prevalence of plastic particles in the food chain is already evident. Therefore, the scientific community is concerned about the health risks of food-use microplastics; although the risk assessment of microplastics is not possible, it is a current global challenge. The dynamic gastrointestinal simulator (simgi®) pursues the need for a dynamic in vitro simulation of the human gastrointestinal tract adapted to food safety and health fields (www.cial.uam-csic.es/simgi/). The simgi® system can simulate separately or in continuous gastric, intestinal digestion, and colonic fermentation, under computer control of the physicochemical parameters. Furthermore, the simgi® platform has confirmed its models suitability since its first studies showed the consistency between the simulated digestion of food products and the metabolic evolution observed with the intake of the same products in human intervention studies. Its modular configuration has allowed the study of miscellaneous products as dietary fibres, probiotics, proteins and polyphenol-rich food matrices to evaluate their gastrointestinal digestion and impact on gut microbiota. Moreover, simgi®; dynamic operation allows the simulation of acute and chronic intakes, which are used to study short- and medium-term effects of digested products. Therefore, the simgi® system is an exclusive tool to monitor avant-garde products of interest, such as nanoparticles (Cueva et al., 2019) and microplastics, which food safety is yet to determine. This study aims to evaluate the impact of the main stages of the human gastrointestinal tract on different relevant food-use plastic materials, also to ascertain the effect of digested microplastics on gut microbiota composition and functionality.

Two microplastic size ranges have been used as prototypes: pellets between 2.8 and 3.6 mm and milled plastics between 50 and 300 µm. Polyethylene terephthalate (PET) was considered as a model for non-biodegradable microplastic, whereas polylactic acid (PLA) was used as biodegradable. Initial steps focused on performing the studies needed to configure the simgi®; system as a useful tool for monitoring microplastics in the human gastrointestinal tract. Thus PET pellets, milled PET, PLA pellets, and milled PLA were digested in vitro according to the standardised static gastrointestinal digestion food protocol (Brodkorb et al., 2019) to establish digestion influence on the microplastics and simgi®; digestion parameters. Microplastics size, shape, and stability (using FESEM, FT-IR Spectroscopy, and Raman spectra) were evaluated after gastric and small intestinal digestion for the microplastic models. The physiological conditions of the ascending, transverse, and descending colon were reproduced sequentially on the simgi®; colonic bioreactors, which included a simulated human colonic microbial community, to study the interaction between the gut microbiota and microplastics. Each colonic bioreactor was fed with a dose of 0.166 mg digested milled PET particles. The milled PET dose, was selected considering previous estimations of human microplastic intake from food and beverages (Luo et al., 2019). Changes in microbiota composition (microbial plate counting and sequencing 16S rRNA), microbial metabolic activity (ammonium and SCFA production), and microplastics shape and stability (using FESEM, FT-IR Spectroscopy, and Raman spectra) were evaluated in the ascending, transverse, and descending colon after feeding the system with microplastics.

In this study, a novel lab practical guidance, designed for operation with microplastics, on simgi®; was proposed. At the gastric and intestinal levels, there were no remarkable changes for the size or structure of the studied particles. However, all microplastics showed organic material deposits on their surface after in vitro gastrointestinal digestions (Figure 1 as an example of milled PET after gastric and intestinal digestion). At the colonic level, milled PET particles induced several changes in colonic microbiota composition/diversity. Using plate counting, total aerobic and total anaerobic microorganisms considerably decreased (Δlog (CFU/mL) ≥-3) during 72 hours of colonic fermentation in all colonic compartments. Furthermore, a notable reduction of Bifidobacterium spp. and *Clostridium spp*. groups were observed after the first 48 hours of fermentation. Likewise, 16S rRNA gene

sequence analysis showed reductions in alpha-diversity indexes, especially for the transverse and descending colon in the first 24 hours of fermentation. Phylogenetic analysis at the phylum level exhibited differences in the relative abundances of important taxa after being fed microplastic, although they were compartment dependent. Bacteroidetes was reduced for all colonic compartments, whereas Firmicutes abundance increased in the ascending colon, Proteobacteria and Synergistetes in the transverse colon, and Desulfobacteria in the descending colon. Regarding the most important members at the genus level, results revealed differences in the relative levels of Bacteroides and Parabacteroides, with an important decrease in all colonic compartments being detected, and the most evident effect in transverse and descending colon, reaching values below 5%.

In vitro static gastrointestinal digestion simulations (Brodkorb et al., 2019) allowed the study of the interaction between microplastics and the human gastrointestinal tract. Furthermore, results presented at the gastrointestinal level, agree with those reported for microplastic gastrointestinal digestions (Senathirajah et al., 2021), suggesting the formation of an organic corona in particles by the simulated digestive fluids. However, neither the colonic stage nor colonic microbiota are included in previous digestion models, which also cannot simulate the effects of a chronic intake, which is the actual situation of microplastics intake, considering the latest data estimated for the inadvertent ingestion of microplastics (Luo et al., 2019). Therefore, it is noteworthy to be able to simulate the colonic stages, as it was simulated on the dynamic simgi® system. In fact, this study has evaluated for the first time, to the best of our knowledge, the microplastic effect on the human colonic microbiota. Colonic bacterial populations showed a decrease in alpha-diversity and Firmicutes/Bacteroidetes ratio, which are considered as health-related indexes. Furthermore, the analysis of the key members of the microbiota at family and genus levels corroborated the results which point to a possible intestinal dysbiosis after being fed microplastics, as was reported for mice models (Stock et al., 2020). Although this project is still in its infancy, the simgi® platform appears a useful tool to study the effects of microplastics along the gastrointestinal tract, and an effective support on research and food industry development by acting complementary and/or as a previous level to human studies, given their ethical and economic restrictions.

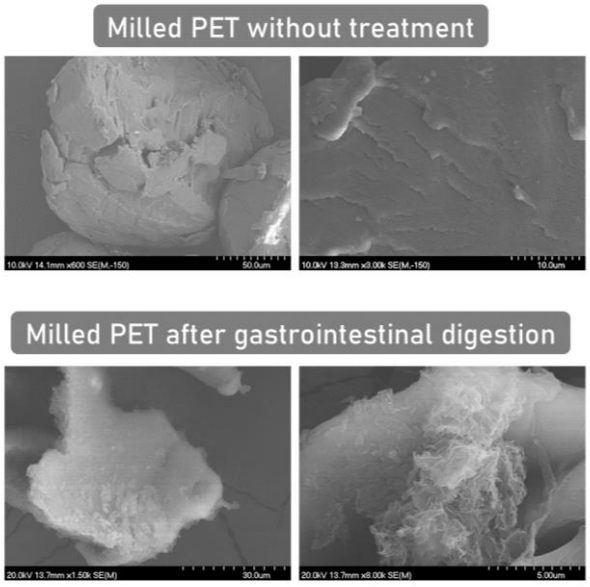


Figure 27

Bibliography

Cueva C, Gil-Sánchez I, Tamargo A, Miralles B, Crespo J, Bartolomé B, Moreno-Arribas MV. 2019. Gastrointestinal digestion of food-use silver nanoparticles in the dynamic SIMulator of the GastroIntestinal tract (simgi®). Impact on human gut microbiota. Food Chem Toxicol. 132:110657.

Brodkorb A, Egger L, Alminger M, Alvito P, Assunção R, Ballance S, Bohn T, Bourlieu-Lacanal C, Boutrou R, Carrière F, Clemente A, Corredig M, Dupont D, Dufour C, Edwards C, Golding M, Karakaya S, Kirkhus B, Le Feunteun S, Lesmes U, Macierzanka A, Mackie AR, Martins C, Marze S, McClements DJ, Ménard O, Minekus M, Portmann R, Santos CN, Souchon I, Singh RP, Vegarud GE, Wickham MSJ, Weitschies W, Recio I. 2019. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc. 14(4):991-1014.

Luo T, Wang C, Pan Z, Jin C, Fu Z, Jin Y. 2019. Maternal Polystyrene Microplastic Exposure during Gestation and Lactation Altered Metabolic Homeostasis in the Dams and Their F1 and F2 Offspring. Environ Sci Technol. 53(18):10978-10992.

Senathirajah K, Attwood S, Bhagwat G, Carbery M, Wilson S, Palanisami T. 2021. Estimation of the mass of microplastics ingested - A pivotal first step towards human health risk assessment. J Hazard Mater. 2021 404(Pt B):124004.

Stock V, Fahrenson C, Thuenemann A, Dönmez MH, Voss L, Böhmert L, Braeuning A, Lampen A, Sieg H. 2020. Impact of artificial digestion on the sizes and shapes of microplastic particles. Food Chem Toxicol. 135:111010.

Towards improved understanding of the ingestion and trophic transfer of microplastic particles – Critical review and implications for future research

Cassart M [1], Gouin T [2]

[1] PlasticsEurope (BE), [2] TG Environmental Research (UK)

Abstract: Observations of microplastic particles (MPs) in the environment and their detection in the stomachs and intestines of aquatic organisms have been observed routinely over the last 50 years. The bioaccumulation potential of MPs thus a concern to regulators assessing the environmental risks of MPs, as their bioaccumulation may result in internal levels that can impact both individuals and populations. In this review, the ingestion of plastic debris of varying sizes is collated.

The objective of this work is to summarize observations obtained from a critical review of the literature related to the biological uptake and bioaccumulation of MPs reported over the last 50 years in both aquatic and terrestrial species at all levels of biological organization. Publications referring to interactions between plastic debris and aquatic organisms date as early as the 1920s with increased awareness beginning in the late 1960's and early 1970's. Exponential increase in publications from about 2007. More than 400 publications were reviewed representing data for approximately 87,000 individual organisms at all biological levels of organisation and regions of the world for more than 900 species

The average of reported MP/individual across all studies is estimated at about 4, with studies typically reporting averages ranging between 0 and 10 MP/individual. (See **Error! Reference source not found.**) A general observation is that while strong evidence exists for the biological ingestion of MPs they do not bioaccumulate and do not appear to be subject to biomagnification as a result of trophic transfer through food webs, with >99% of observations from field-based studies reporting MPs to be located within the gastrointestinal tract.

Overall, there is substantial heterogeneity in how samples are collected, processed, analyzed, and reported, causing significant challenges in attempting to assess temporal and spatial trends or in helping to inform mechanistic understanding.(see the second figure) Nevertheless, several studies suggest that the characteristics of MPs ingested by organisms is generally representative of plastic debris in the vicinity of where individuals are collected. Monitoring spatial and temporal trends of ingested MPs could thus potentially be useful in assessing mitigation efforts aimed at reducing the emission of plastic and MPs to the environment. The development and application of standardized analytical methods are urgently needed to better understand spatial and temporal trends. The ingestion of particles of varying sizes for aquatic organisms can vary from species to species and is strongly influenced by physiological and behavioural traits that are related to the size of the organism and its feeding strategy. Consequently, some species may be susceptible to ingesting and accumulating MPs, however, there currently exists limited mechanistic understanding related to the potential of MPs to bioaccumulate. Suggestion that future research target a specific fish species as biomonitor used to assess relative level of plastic contamination in the environment, analogous to the

use of northern fulmars in assessing good environmental status within the Marine Strategy Framework Directive.

Dynamic Imaging Analysis of In Vivo Behaviour of Micro/Nanoparticles Labelled with Over-1000 nm Near-Infrared Fluorophore

Umezawa M [1], Soga K [1]

[1] *Tokyo University of Science (JP)*

Abstract: Understanding the in vivo behaviour of micro- and nanoparticles after oral exposure is important to assess the human health risk of micro- and nanoplastics in food. We propose that the use of particle models labelled with over-thousand-nanometre (OTN-) near-infrared (NIR) fluorophore to visualize in vivo dynamic behaviour of the particles. The most advantage of using OTN-NIR light (wavelength: 1000-1350 and 1500-1650 nm) is high penetration through biological tissues owing to less scattering compared to the light of visible and shorter-wavelength NIR (380-1000 nm). Therefore OTN-NIR enables live and video-rate imaging of the particle behaviours in biological deep organs. Rare-earth-based inorganic materials (Soga et al., 2010; Sekiyama et al., 2018; Chihara et al., 2019) and organic dye-based phosphors (Kamimura et al., 2017, 2019) have been reported as representatives of OTN-NIR fluorescent contrast agents. In this paper, our demonstrative imaging data of OTN-NIR fluorescence particles in live small animals. The use of OTN-NIR emissive phosphors for labelling micro- and nanoplastic partide models will enable to analyse comparatively their in vivo dynamic behaviour related to their hazards on health of humans and animals.

Rare-earth-based inorganic phosphors were synthesized by the coprecipitation method [1, 3] or the thermal decomposition method (Sekiyama et al., 2018) as previously described. Briefly, solutions of rare-earth ions (Y^{3+}, Yb^{3+}, Nd^{3+}, Er^{3+}, and Ho^{3+}) were mixed at designed ratios to obtain optimal OTN-NIR emission as desired. Then fluorescence inorganic materials were collected after heating. To prepare organic dye-based phosphors, an IR-1061 dye was dissolved with amphiphilic polymers in acetonitrile, dropped into water, stirred overnight to remove the acetonitrile and to collect the phosphors in aqueous/physiological environment (Kamimura et al., 2017). The hydrodynamic diameters of particle models with OTN-NIR phosphors were analysed by dynamic light scattering. The optical properties (light absorption and emission) were analysed by spectrometers. The OTN-NIR fluorescence particles were orally or intravenously administered into mice. To determine the dynamic change in distribution of the particles in mice, the OTN-NIR fluorescence images were collected by a camera with InGaAs CCD sensor, that is sensitive to OTN-NIR, under irradiation of light for excitation (808 or 980 nm) of phosphors. As a commercial device of portable in vivo OTN-NIR fluorescence imaging system, SAI-1000 (Shimadzu Co., Tokyo, Japan) was also used the imaging analyses.

Inorganic particles Y_2O_3 and $NaYF_4$ co-doped with Yb^{3+} and Er^{3+} emits OTN-NIR at 1550 nm under 980-nm excitation. $NaYF_4$ co-doped with Yb^{3+}, Ho^{3+}, and Ho^{3+} emits OTN-NIR at 1150 and 1550 nm under 980-nm excitation. $NaYF_4$ co-doped with Nd^{3+} and Yb^{3+} emits OTN-NIR at 1000 and 1064 nm under 808-nm excitation. The $NaYF_4$ material synthesized by the thermal decomposition method was nanosized (<40 nm in diameter) and can be mixed with various organic materials for OTN-NIR fluorescence labelling. The organic phosphors loaded with IR-1061 also emit OTN-NIR at 1100 nm under 980-nm excitation and can be used for labelling other organic compounds. Because of high penetration of the NIR lights of excitation and emission through the biological tissues, the time-dependent change in the distribution of the OTN-NIR fluorescence labelled particles in various organs in mice was successfully observed. The results of fluorescence imaging have clearly shown the dynamic in vivo particle behaviour, such as retention in the intestinal tract (Figure 28a) and the blood (Figure 28b) followed by distribution in the liver (Figure 28c). The dynamic behaviour is dependent on size and chemical composition of particles.

This paper showed the demonstrative data of in vivo OTN-NIR fluorescence imaging that visualized the distribution of particle matter in deep tissues of live mice. Because a highly emissive OTN-NIR phosphors, rare-earth-based nanomaterials and organic molecular fluorescence dye (IR-1061), work well in hydrophobic materials, these phosphors will visualize the difference in time-dependent change in in vivo distribution of particulate plastic models composed of polyethylene, polypropylene, polyvinyl chloride, polystyrene, and polyethylene terephthalate under video-rate imaging. In addition, some of the phosphors are also work as luminescence thermometer for contactless temperature imaging of deep tissues (Sekiyama et al., 2018; Chihara et al., 2019). Labelling of the particle models by these

phosphors may be also useful for clarifying temperature change with inflammation, tissue injury, and other biological phenomena related to their hazard.

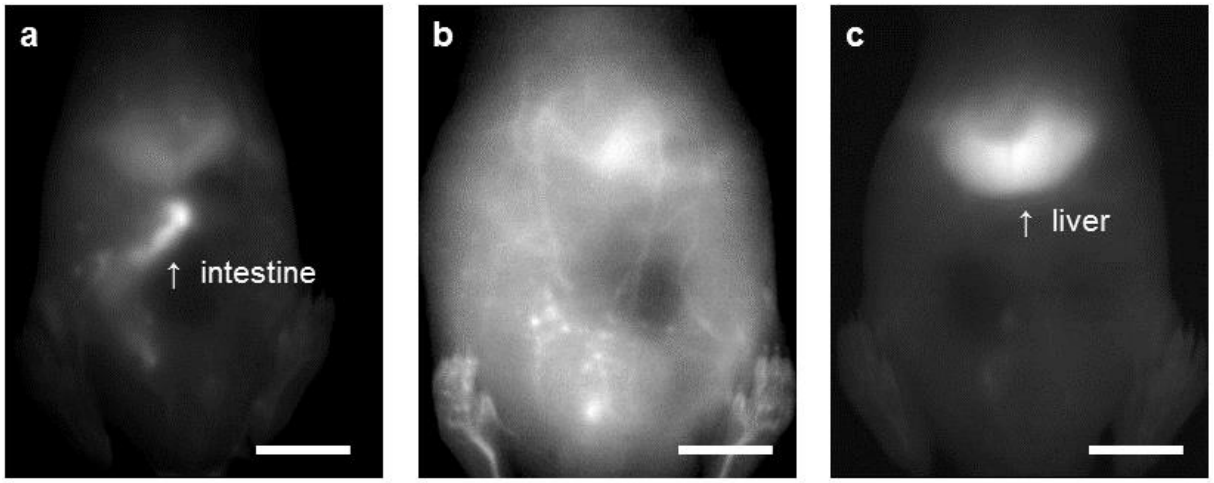


Figure 28: Examples of in vivo fluorescence images that display the distribution of OTN-NIR fluorescence-labelled particles in mie. The fluorescence imaging of OTN-NIR enables to visualize the particle behaviours in deep organs of live mice such as (a) the small intestine, (b) whole-body blood circulation, and (c) the liver, because of low scattering by biological tissues in the wavelength range, also called 'second biological window'. The time-dependent change in the distribution of particles is dependent on the size and chemical composition of the particles. Scale bars indicate 10 mm.

Bibliography

Soga K, Tokuzen K, Tsuji K, Yamano T, Venkatachalam N, Hyodo H, Kishimoto H. 2010. Application of ceramic phosphors for near infrared biomedical imaging technologies. Proc. SPIE 7598, Optical Components and Materials VII, 759807.

Sekiyama S, Umezawa M, Kuraoka S, Ube T, Kamimura M, Soga K. 2018. Temperature Sensing of Deep Abdominal Region in Mice by Using Over-1000 nm Near-Infrared Luminescence of Rare-Earth-Doped $NaYF_4$ Nanothermometer. Sci Rep. 8(1):16979.

Chihara T, Umezawa M, Miyata K, Sekiyama S, Hosokawa N, Okubo K, Kamimura M, Soga K. 2019. Biological Deep Temperature Imaging with Fluorescence Lifetime of Rare-Earth-Doped Ceramics Particles in the Second NIR Biological Window. Sci Rep. 9(1):12806.

Kamimura M, Takahiro S, Yoshida M, Hashimoto Y, Fukushima R, Soga K 2017.. Over-1000 nm near-infrared fluorescent biodegradable polymer nanoparticles for deep tissue *in vivo* imaging in the second biological window. Polym J 49, 799–803.

Kamimura M, Ueya Y, Takamoto E, Iso K, Yoshida M, Umezawa M, Soga K. 2019. Fluorescent Polystyrene Latex Nanopartides for NIR-II *in vivo* Imaging. Journal of Photopolymer Science and Technology 32(1): 93-96.

Microplastics; occurence, levels, and implications for the environment and human health related to food. An opinion from VKM.

Rakkestad KE [1], Skåre JU [1], Alexander J [1], Haave M [1], Jakubowicz I [1], Knutsen HK [1], Lusher A [1], Ogonowski M [1], Skaar I [1], Sverdrup LET [1], Wagner M [1], Agdestein A [1], Bodin J [1], Elvevoll E [1], Hemre GI [1], Hessen DO [1], Hofshagen M [1], Husøy T [1], Krogdahl Å [1], Nilsen AM [1], Rafoss T [1], Skjerdal T [1], Steffensen IL [1], Strand TA [1], Vandvik V [1], Yngvild W [1]

[1] *The Norwegian Scientific Committee for Food and Environment (NO)*

Abstract: In 2017, The Norwegian Scientific Committee on Food and Environment (VKM) self-initiated a mandate for an opinion on microplastics (MPs) and nanoplastics (NP) in food and environment. The opinion were to be based on recently published international and/or national reports, complemented with peer-reviewed literature from December 2016 to February 2019. The mandate included a summary of the state of knowledge on the presence of MPs in the environment and the implications for the ecosystem, terrestrial and aquatic organisms, food production and human health.

VKM published their opinion in October 2019. The opinion summarises and discusses analytical, experimental and sampling methods, describes levels of MPs and NPs in various environments, as well as sources, transport, distribution and fate of MPs. It also includes a chapter on biofilms and rafting, in addition to the hazard identification and characterisation, exposure assessment and risk characterisation. Uncertainties in each step of the assessment were described, and knowledge gaps identified.

Knowledge based on the assessments done by EFSA (2016), FAO (2017) and SAPEA (2019) was summarized. A systemic literature search was performed for the period December 2016 to February 2019, resulting in 2116 unique papers used as basis for both human and environmental risk assessments.

Two sets of quality criteria were developed for papers on occurrence/levels and on toxicology, respectively. Papers with poor total score according to these criteria were excluded. Papers were also excluded based on justified expert judgement. This resulted in a final acceptance of 270 papers.

For the environmental hazard assessment toxicity data from 122 peer-reviewed publications were extracted. Parameters like experimental concentrations, lowest observed effect concentration (LOEC), polymer type, shape, size and endpoint were collected. Generally, the data extraction for a particular study was performed factorially, meaning that LOEC data for a particular endpoint was retrieved for each combination of particle shape, particle size, polymer type and exposure duration. However, this was not always possible due to the vast amount of data generated by gene expression or enzyme activity profiling. In these cases, the lowest LOEC for any one of these endpoints was extracted. Hence, in these cases, the toxicity, operationally defined as the LOEC, was defined by the most sensitive endpoint in the enzyme activity and gene expression category.

The LOECs were used to construct species sensitivity distributions (SSDs). VKM decided to make use of most available data by pooling all species and endpoints, and the SSDs were constructed from 63 studies covering 39-40 species.

Almost 60% of the scientific papers identified were excluded, as they were not of sufficient quality.

Regarding human hazard assessment, VKM addressed only oral exposure and uptake via the gastrointestinal tract. However, very few relevant papers were identified, and VKM concluded that the available information did not provide sufficient basis to perform a reliable human hazard assessment. Also, reliable occurrence data were too limited to form basis for a meaningful exposure assessment. Hence no human risk assessment could be done.

For the environmental hazard assessment VKM derived a predicted no effect concentrations (PNEC) for nano- and microplastics of 0.14 µg/L (95% confidence interval: 0.04-0.64 µg/L) for mass-based concentrations and 71.6 particles/L (95% confidence interval: 3.45-1991 particles/L) for numerical concentrations. The PNECs were based on the SSDs, and were set at the hazard concentration 5% level.

Measured environmental concentrations (MECs) of microplastics were derived from cumulative distributions of the measured concentrations in surface and water columns globally, or from locations relevant to Norway (Atlantic, Arctic, North Atlantic, North Sea).

Comparing the PNEC with PECs (=MECs) in different scenarios resulted in risk characterisation ratios (RCRs) of 5.41×10^{-6}, 2.80×10^{-3} and 1.455 for 95, 50 and 5% of locations on a global scale. Thus, the environmental risks on nano- and microplastics are low for most locations as the RCRs are well below one in most scenarios. For the 6% most heavily polluted locations, the RCR was estimated to exceed one, implying a risk from nano- and microplastics exists at those places.

VKM acknowledges that many different approaches are used to study microplastics depending on the matrix of interest. While this is inherent to an evolving field of research, this also poses a challenge to risk assessment, as data comparability is limited.

The few studies relevant for human hazard assessment that have become available since EFSA's assessment in 2016 used pristine nano- and microparticles. Micro- and nano-sized particles present in food are generally not pristine, and the relevance of studies on pristine particles for toxicity of weathered particles present under natural exposure conditions is unknown. There is a general lack of information on the toxicokinetics of MP and NP, and a lack of relevant experimental toxicity data. Furthermore, there is a lack of reliable occurrence data in food and drinking water.

Although the environmental hazard assessment has a number of limitations, the PNEC estimates should be relatively robust.

VKM acknowledges that it has not been established to which extent the observed effects of MP and NP exposure are specific to plastic materials. The effects may be driven by the presence of non-nutritious particles, which also are naturally ubiquitous in the environment. Moreover, it is important to highlight that assessing "microplastics" as one entity is clearly ignoring their physico-chemical heterogeneity. To perform a more differentiated environmental hazard assessment was, at this point, not feasible due to the limited available data of acceptable quality.

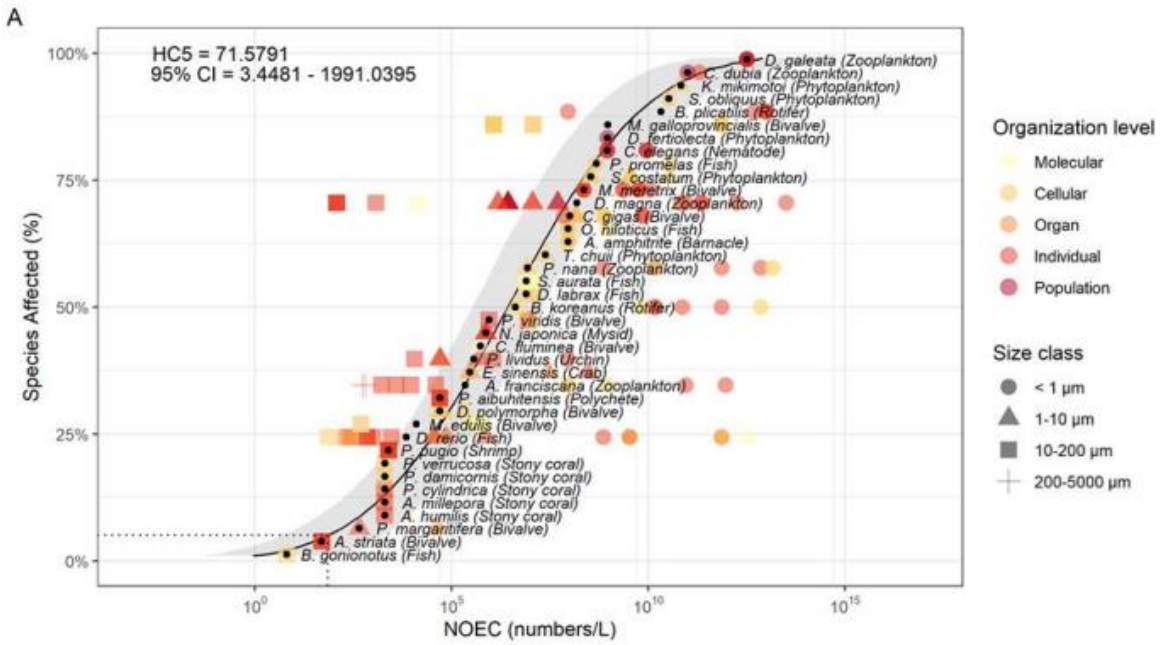


Figure 29: Species sensitivity distribution for species exposed via the water phase. Data is presented as numerical (items/L) concentrations. Black points represent the geometric mean of the NOECs reported for a particular species if this species occurred in more than one study. If a species was unique to one study but several NOECs were reported, the black dot represents the minimum NOEC. The symbols show all NOECs recorded for a particular species. The colour code indicatesindicates the level of biological organisation at which the NOECNOEC was observed. The shapes indicate the size class of nano - and microplastics used in a particular study. The black line represents the weighted fitted regression curve (weighted average of several different distributions) and the grey band represents the bootstrapped.

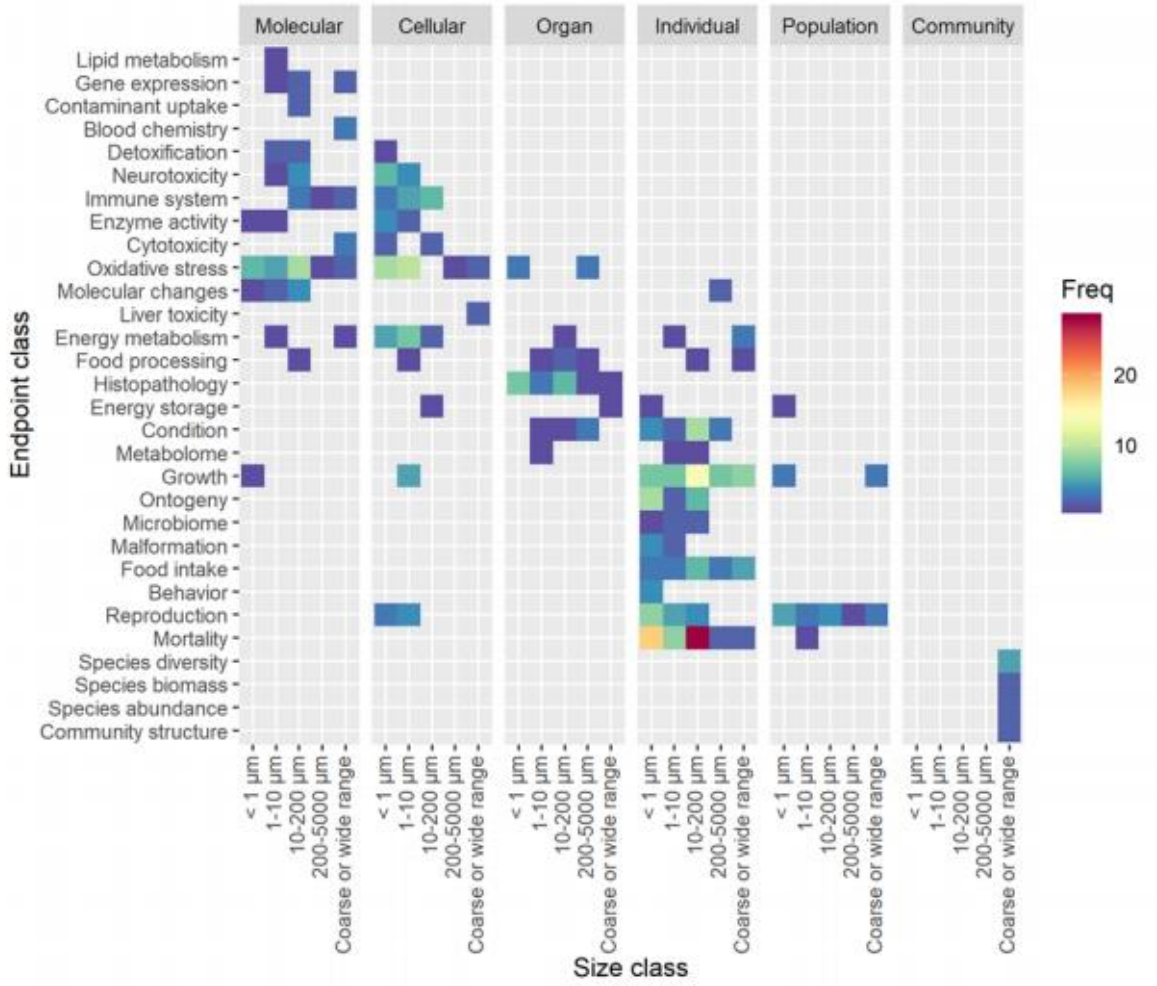


Figure 30: Heatmap showing the frequency of observed effects per endpoint class divided by particle size class and level of biological organisation. One observation is equal to a measured biological endpoint for a unique set of experimental conditions. There can be more than one endpoint and several experimental conditions within a particular study. For example, if mortality and growth effects have been observed for two different microplastics in two different organisms, then there would be 2 x 2 x 2 = 8 observations at the individual level in that study.

Bibliography

Hoogenboom LAP. 2016. Statement: Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal, 14(6), e04501.

Lusher AL, Hollman PCH, Mendoza-Hill JJ. 2017. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fisheries and Aquaculture Technical Paper. No. 615. Rome, Italy.

SAPEA. 2019. Science Advice for Policy by European Academies. A Scientific Perspective on Microplastics in Nature and Society. Berlin: SAPEA. https://doi.org/10.26356/microplastics.

Micro- and nanoplastics and early-life human health: the AURORA Horizon 2020 research project

Muncke J [1], Boucher J [1], Groh K [1], Vermeulen R [2]

[1] Food Packaging Forum (CH), [2] Julius Center for Health Sciences and Primary Care, University of Utrecht (NL), on behalf of the AURORA consortium

Abstract: The scale of micro- and nanoplastic (MNP) pollution is becoming increasingly clear yet little is known about how this pollution impacts human health during early-life stages. The AURORA project will deliver an actionable European roadmap for early-life health risk assessment of MNPs to support regulation of MNPs, the products and processes that generate secondary MNPs, and development of safer alternatives. Within the project, we will focus on MNP exposures and toxicological and health

effects during pregnancy, in utero, and in early life. Preliminary studies have shown that MNPs are likely to cross the placental barrier, underlying the urgent need to understand the impact of MNPs on reproductive and early-life health.

AURORA will advance this understanding by significantly enhancing exposure assessment capabilities for measuring MNPs and MNP-associated chemicals (e.g., additives) in tissues relevant for early-life development (placenta, cord blood, amniotic fluid, meconium, fetal tissue). It will take a unique approach by combining in-depth characterization methods (microscopy and spectroscopy) and scalable methods (mass spectrometry) to develop methods for both detailed and large-scale toxicological characterization, exposure assessment, and epidemiological studies. This will be combined with a novel tiered-testing approach and epidemiological investigations to provide the first extensive evaluation of maternal and fetal MNP exposures and health perturbations, including placental function, immune-inflammatory responses, oxidative stress, accelerated aging, endocrine disruption, and child development.

In the course of developing and applying the tools and methodological workflows of the AURORA research program, we will create a risk assessment framework specific to MNPs and identify the remaining knowledge gaps and priorities needed for comprehensively evaluating the impact of MNPs on early-life health.

The AURORA project starts on April 1, 2020 and is part of the EU Horizon 2020 funded research cluster. The project is a collaboration between 11 partners based in Europe, the UK and the US, coordinated by the University Medical Center Utrecht, Netherlands, and will run for 5 years. One of the core activities of AURORA is effective dissemination of the research findings, and for this reason we will closely collaborate with different stakeholders from academia, policy arenas, civil society and industry.

Bibliography

Vermeulen R, Schymanski EL, Barabási AL, Miller GW. 2020. The exposome and health: Where chemistry meets biology. Science. 367(6476):392-396.

Van den Brink PJ, Boxall ABA, Maltby L, Brooks BW, Rudd MA, Backhaus T, Spurgeon D, Verougstraete V, Ajao C, Ankley GT, Apitz SE, Arnold K, Brodin T, Cañedo-Argüelles M, Chapman J, Corrales J, Coutellec MA, Fernandes TF, Fick J, Ford AT, Giménez Papiol G, Groh KJ, Hutchinson TH, Kruger H, Kukkonen JVK, Loutseti S, Marshall S, Muir D, Ortiz-Santaliestra ME, Paul KB, Rico A, Rodea-Palomares I, Römbke J, Rydberg T, Segner H, Smit M, van Gestel CAM, Vighi M, Werner I, Zimmer EI, van Wensem J. 2018. Toward sustainable environmental quality: Priority research questions for Europe. Environ Toxicol Chem. 37(9):2281-2295.

Lenters V, Portengen L, Rignell-Hydbom A, Jönsson BA, Lindh CH, Piersma AH, Toft G, Bonde JP, Heederik D, Rylander L, Vermeulen R. 2016. Prenatal Phthalate, Perfluoroalkyl Acid, and Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models Based on Elastic Net Regression. Environ Health Perspect. 124(3):365-72.

FPF dossier. 2020. Microplastics. DOI: 10.5281/zenodo.3725591

Muncke J, Andersson AM, Backhaus T, Boucher JM, Carney Almroth B, Castillo Castillo A, Chevrier J, Demeneix BA, Emmanuel JA, Fini JB, Gee D, Geueke B, Groh K, Heindel JJ, Houlihan J, Kassotis CD, Kwiatkowski CF, Lefferts LY, Maffini MV, Martin OV, Myers JP, Nadal A, Nerin C, Pelch KE, Fernández SR, Sargis RM, Soto AM, Trasande L, Vandenberg LN, Wagner M, Wu C, Zoeller RT, Scheringer M. 2020. Impacts of food contact chemicals on human health: a consensus statement. Environ Health. 19(1):25.

Contribution to micro- and nanoplastics hazard assessment: lessons learnt from nanomaterials toxicity investigation

Silva MJ [1], Alvito P [2], Louro H [1]

[1] National Institute of Health Doutor Ricardo Jorge (INSA), and Centre for Toxicogenomics and Human Health (ToxOmics), NOVA Medical School, New University of Lisbon (PT), [2] INSA and Centre for Environmental and Marine Studies (CESAM), University of Aveiro (PT)

Abstract: The last decade has witness growing scientific concerns and public debate over the adverse effects that may result from marine and freshwater organisms' exposure to plastics and

microplastics. Moreover, due to microplastics degradation or direct release from domestic and industrial sources, aquatic organisms are equally exposed to nano-sized plastics (size between 1 and 100 nm, EFSA 2016). These micro- and nanoplastics have the potential to accumulate in edible tissues of the exposed organisms, including those that are part of the human diet, thus entering the human food chain (Toussaint et al., 2019). Apart from this indirect exposure route, humans are additionally exposed to tiny plastic particles through water consumption and consumer products that incorporate the so-called microbeads. In contrast to the wide range of investigation on the impact of plastics/microplastics on the ecosystem, a major question is whether micro- and nanoplastics will have a negative impact on human health. This negative impact may derive from the physicochemical nature of the micro- and nanoplastics, that resemble those of engineered nanomaterials, including their capacity to cross barriers and reach all organs and tissues, inducing deleterious effects e.g., inflammation or genotoxic effects that may lead long-term disease, such as cancer. Moreover, these plastic particles might be associated with a wide range of substances, including chemicals present in the plastic composition, e.g., metals, polychlorinated biphenyls and plasticizers or chemicals adsorbed to their surface, which may also be hazardous to human health.

An effective risk assessment of micro- and nanoplastics needs to be supported by a robust hazard assessment. The experimental approach to be applied should rely on previously acquired knowledge from the toxicity assessment of different engineered nanomaterials. Indeed, the potential cytotoxic, immunotoxic and genotoxic effects of these plastic particles have to be assessed using complementary in vitro and in vivo assays and considering their specific physicochemical properties.

In this work, we will present the testing strategy that has been recently applied to the genotoxicity characterization of nanomaterials in human cell models, e.g., a co-culture of human intestinal epithelial cells and mucus-producing cells, and in an integrative in vivo model (Louro et al., 2014) . Particularly, the approach used to the toxicity assessment of titanium dioxide following a harmonized in vitro digestion process will be presented and its advantages and pitfalls discussed. Furthermore, the results of our previous approaches to the toxicity assessment of nanomaterials have supported the view that a thorough understanding of the relationship between the physicochemical properties, the behaviour of nanoparticles in biological systems and their mechanism of action is of utmost importance to predict their biological activity (Louro et al., 2019). Particularly, the investigation of genotoxic and epigenetic effects will be focused, given their strong association with carcinogenic effects.

In summary, we propose the use of a predictive toxicology approach based on defining the key events at cellular and molecular levels, to identify and characterize the hazard of micro- and nanoplastics, reducing the in vivo experimentation to the lowest possible level.

Bibliography

Hoogenboom LAP. 2016. Statement: Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal, 14(6), e04501.

Louro H, Saruga A, Santos J, Pinhão M, Silva MJ. 2019. Biological impact of metal nanomaterials in relation to their physicochemical characteristics. Toxicol In Vitro. 56:172-183.

Louro H, Tavares A, Vital N, Costa PM, Alverca E, Zwart E, de Jong WH, Fessard V, Lavinha J, Silva MJ. 2014. Integrated approach to the *in vivo* genotoxic effects of a titanium dioxide nanomaterial using LacZ plasmid-based transgenic mice. Environ Mol Mutagen. 55(6):500-9.

Toussaint B, Raffael B, Angers-Loustau A, Gilliland D, Kestens V, Petrillo M, Rio-Echevarria IM, Van den Eede G. 2019. Review of micro- and nanoplastic contamination in the food chain. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 36(5):639-673.

Developments in understanding consumer perceptions of micro- and nanoplastics in food

Micro- and nanoplastics: and environment and food safety threat. A consumer perception study

Ojinnaka D [1], Aouzelleg A [1]

[1] *London South Bank University (UK)*

Abstract: Microplastics and nanoplastics are very tiny fragments of plastics about 5mm in length and very much less (~nm). They are produced by mechanical actions, biodegradation, and photo-oxidative degradation of plastics over time. Additionally, some microplastics such as exfoliating agents are purposely manufactured. Microplastics are ubiquitous and their accumulation in the environment threatens the ecosystems, food safety and security, significantly. It is estimated that 7000 to 35,000 tons of plastics float in the open ocean and most of these invariably will degenerate to microplastics and nanoplastics. The environmental threats are attributable to the properties, microplastics are insoluble and chemically stable. Their large surface areas make them excellent carriers of toxic organic chemicals; numerous heavy metals, can be adsorbed onto the surfaces. The old adage "we are what we eat" is very true. The health hazards presented by some chemical compounds in foods and the environment are well documented with the focus shifting from one type of hazard to another. Microplastics are the emerging food and environment contaminants, the threats from them are unfolding, pollution of the aquatic environment and ultimately food safety and supply, are increasingly alarming. However, there is a thin evidence on the food safety and security threats. There is still not enough information on the consumers' perception and level of awareness of the toxicological risk of micro- nanoplastics. Thus an informed awareness is essential. So this paper seeks to provide some data on consumer perception and knowledge through a very rigorous, in-depth and varied surveys and literature review.

The research will be both qualitative and quantitative. It will be achieved through the use of surveys, polls, interviews, case studies, and longitudinal studies. The survey, polls, and interviews will be administered in person and through online platforms like Bristol online and Qualtrics. It also aims to critically review relevant literature on the environment, food safety and supply threats with a consideration of the awareness, control and management systems in place.

Information will be gathered from surveys, interviews, and polls will be collected through the use of questionnaires, which will be shared through online platforms as well as through personal interactions with the proposed sample population. The information will be analyzed through SPSS version 18, which is a software package employed when carrying out statistical information. The analysis will be aided through the use of food frequency data; a factor that will be included in the questionnaires dispensed to the sample population. During the analysis, the relationship between variables will be determined through the use of a chi-squared test.

There are controls in place to tackle plastic marine litter, but they are not enough as the use of plastics remains popular and unabated. Many consumers use and dispose plastic without much thought about the consequences, this must changed. Plastic accumulation in oceans and seas causing animal ingestion and entanglement has been repeatedly reported by scientists and environmental associations. The consumers' perception and level of awareness of the toxicological risk of micro-nanoplastics remains poor. An informed awareness is will be provided. The outcomes would also support informed national and international campaigns that depart from the unfiltered rhetoric.

Bibliography

Alimi OS, Farner Budarz J, Hernandez LM, Tufenkji N. 2018. Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. Environ Sci Technol. 52(4):1704-1724.

Barboza LGA, Dick Vethaak A, Lavorante BRBO, Lundebye AK, Guilhermino L. 2018. Marine microplastic debris: An emerging issue for food security, food safety and human health. Mar Pollut Bull. 133:336-348.

da Costa JP. 2018. Micro- and nanoplastics in the environment: Research and policymaking. Curr Opin Environ Sci Health 1;12-16.

Hoogenboom LAP. 2016. Statement: Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal, 14(6), e04501.

Our Blue Planet. 2017. Blue Planet II, episode 7. [Documentary] BBC1, 10 December 2017.

Perceptions of Bulgarian consumers on microplastics in food: a preliminary study

Georgieva S [1], Peteva Z [1], Trifonova T [1], Stancheva M [1], Markova N [1]

[1] *Medical University Varna*

Abstract: The intensive use of plastics in recent decades has led to the widespread distribution of plastic particles that remain in the environment indefinitely. Due to their small size (<5 mm), microplastics are considered bioavailable to organisms in aquatic ecosystems. Ingestion of microplastics by marine organisms can lead to the introduction of toxic substances at the base of the food chain and the potential for bioaccumulation (Cole, et al., 2011).

Plastic pollution is usually more visible and therefore different in this respect from other classical risks, such as those caused by chemicals (Syberg, et al., 2018).

The societal interest on the need to solve the problem of plastic pollution is driven by a combination of factors – e.g. reduction of plastic pollution visible in waters and coastal zones; concern about negative physiological and behavioural impacts on animals and other biota; and conflict with the moral convictions of individuals (European Union, 2019).

Changing individuals' perceptions and concerns about the risk of microplastics would lead to changes in consumer behavior. This implies the possibility of more effective control of microplastic pollution. Study of the general public's knowledge and risk perception of microplastics are very limited (European Commission, 2019).

The aim of this study is to conduct a preliminary evaluation of the perceptions and attitudes of consumers in Bulgaria towards microplastics as a problem in general and microplastics in food.

A survey was conducted in six town including the capital city of Bulgaria in the period September 2019 - January 2020 by means of questionnaires. A preliminary validation of the questionnaire was done among 15 respondents and some questions were corrected.

In the present survey, respondents were contacted personally and asked to complete a self-administered anonymous questionnaire. The total sample consists of 191 persons. Part I of questionnaire (4 questions) asks for socio-demographic characteristics of each respondent. Part II (10 questions) collects information about the perception of the respondents on microplastics and their distribution in food. In this part, two types of questions were provided. Using a five point interval scale ranging from "no knowledge" to "expert" or "not concerned /interested" to "very concerned/interested", the respondent was asked to evaluate his/her knowledge about microplastics, his/her concern on microplastics as a problem, if food products contain microplastics and if microplastics present in food are believed to be a health issue. The other six questions are multi select multiple-choice type. Hereby, was collected data about the main sources of information regarding microplastics, the distribution and presence of microplastics in food products, etc.

Approximate time for answering questions- 10-15 minutes.

Data was analyzed using MS Excel 2013.

In total 194 respondents were contacted but because of missing answers in 3 questionnaires, they were removed. The total sample analyzed consists of 191 persons whereas 30 % are men and 70% are women. Data analysis of other personal information shows that 42% of respondents are on age 18-30, 35 % - 30-45, 19%- 45-60 and 4% are over 60 years of age. Most of the respondents have a university degree (54%) and 44% - secondary education.

Average value of self-evaluation of respondents of their knowledge of microplastics is 2.4. Highest mean level of this indicator is established by respondents in the age group 30-45 – 2.6.

Answers on respondents concern on microplastics averaged 3.6.

Mean results about the interest of respondents if food contains microplastics and if microplastics present in food could cause a health problem are resp. 3.5 and 4.1. Most interested in the topic are respondents with high school degree. Respondents in the age group 30-45 mostly agree that microplastics in food are a health issue. However, no significant differences are established between the different respondent groups.

Internet is pointed as the main source of information about microplastics for all respondent groups. Most respondents would like to receive more information about microplastics via social media (27 %), television/radio (21%) and scientific publications (19 %).

Among 191 respondents answers analyzed, the number of participants in age groups 18-30 and 30-45 as well with high school and university diploma is close. Therefore, their answers are discussed further.

In general, respondents evaluate their level of knowledge of microplastics as lower than average (2.4). Despite their level of concern and interest if food consumed contains microplastics are above but closer to average (resp. 3.6 and 3.5). Furthermore, most respondents agree that ingested microplastics could pose a health risk.

Comparison of respondents' opinion on distribution of microplastics (Figure 31) shows that lakes, ground, everyday products, food and drinks are thought to contain microplastics. Young people (18-30 years) point lakes, seas and oceans, whereas respondents with age 30-45 think that mostly everyday products contain microplastics. Respondents with university degree think also that ground, food and drinks are main sources of microplastics.

Comparison of respondents' opinion on which food products could contain microplastics (Figure 32), interviewers mostly often indicate drinking water, beverages, fish, and other seafood. Hereby, respondents with higher education more often point more than four possible food products that might contain microplastics.

Despite of the small sample size of the present study and its exclusively exploratory nature results in this paper indicate that Bulgarian consumers strongly believe that drinking water contains microplastics and microplastics in food could be a serious health threat.

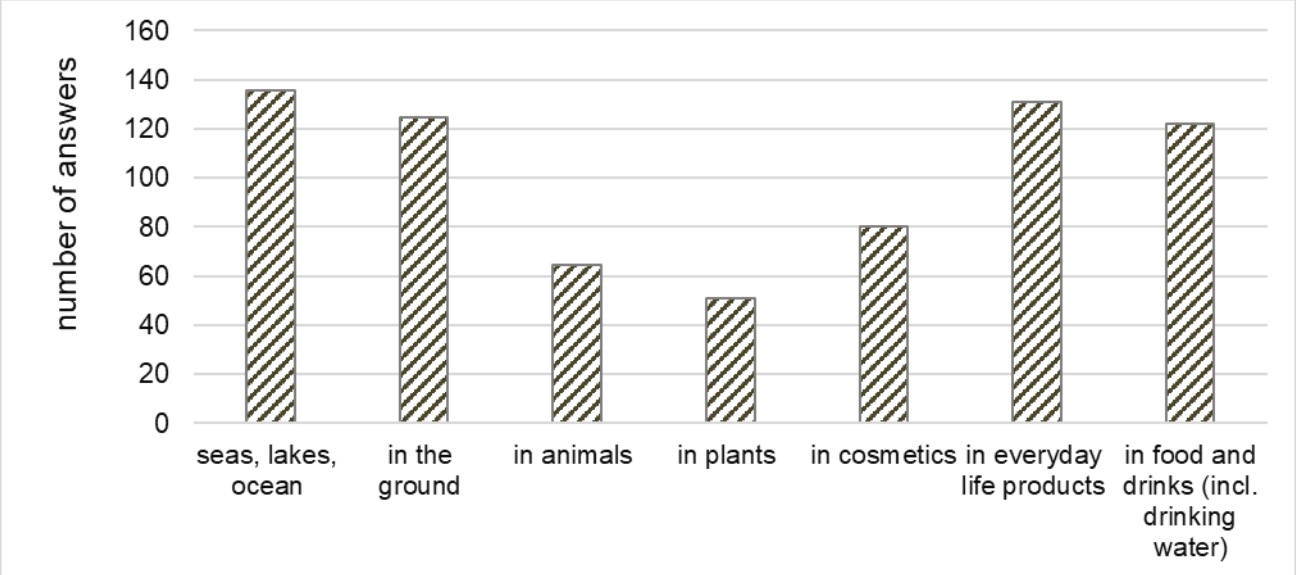


Figure 31: Comparison of respondents' opinion on distribution of microplastics

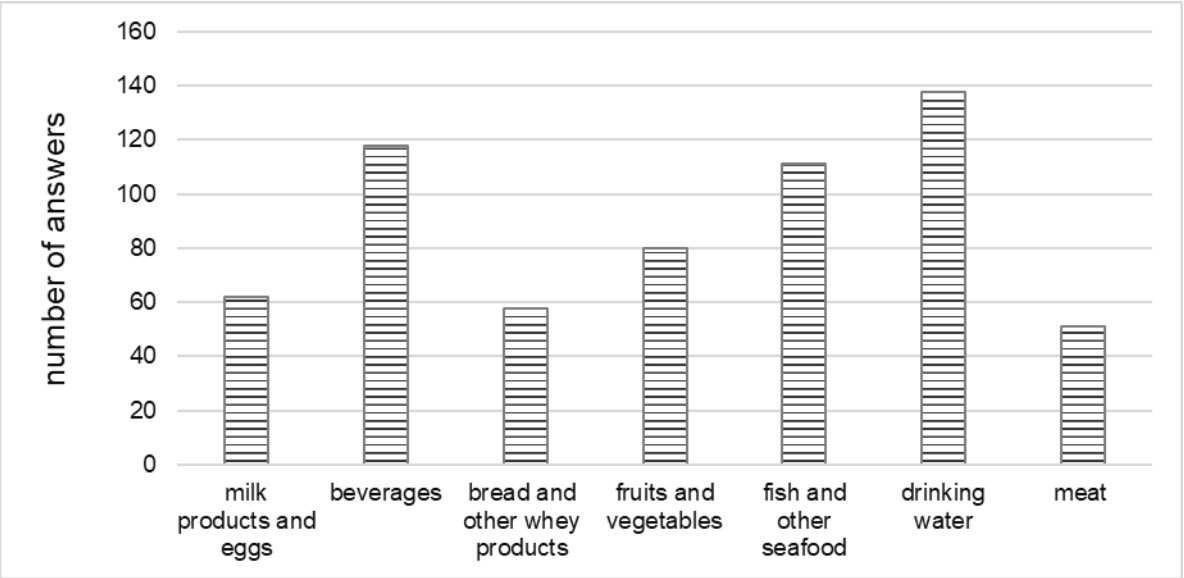


Figure 32: Comparison of respondents' opinion on food products that contain microplastics

Bibliography

Cole M, Lindeque P, Halsband C, Galloway TS. 2011. Microplastics as contaminants in the marine environment: a review. Mar Pollut Bull. 62(12):2588-97.

Syberg K, Hansen SF, Christensen TB, Khan FR. 2018. Risk Perception of Plastic Pollution: Importance of Stakeholder Involvement and Citizen Science. In: Wagner M., Lambert S. (eds) Freshwater Microplastics. The Handbook of Environmental Chemistry, vol 58. Springer, Cham

European Commission. 2019. Environmental and Health Impacts of Microplastic Pollution – from scientific evidence to policy advice. Workshop Report

European Union. 2019. Environmental and Health Risks of Microplastic Pollution. Group of Chief Scientific Advisors, Scientific Opinion 6/2019