25th EFSA colloquium

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

6-7 May 2021

Online event

BRIEFING NOTES FOR DISCUSSION GROUPS

These briefing notes aim to provide participants with the relevant background information so as to be prepared for an interactive exchange of views and expertise during the Colloquium.

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Disclaimer

The briefing notes have been prepared by the colloquium scientific programme committee and break-out session committees to provide background information to the participants, and to facilitate an exchange of views and expertise during the colloquium. They were not designed to compile all relevant scientific literature, or to review in detail each single relevant scientific publication. The briefing notes do not disclose any confidential information or data. Mention of proprietary products is solely for the purpose of providing specific information and does not constitute an endorsement or a recommendation by EFSA for their use. The briefing notes do not necessarily represent the official position of EFSA, and EFSA assumes no responsibility or liability for any errors or inaccuracies that may appear.

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1) Background

Humans are exposed to plastic particles via food, water and air, but possible health effects are yet to be determined. The issue of possible food safety risks, linked to pollutants possibility to migrate to the edible parts of marine animals and the effect of the plastic particles on animal health and ecosystems, was firstly brought to the attention of the European Food Safety Authority (EFSA) in 2013 at its Emerging Risks Exchange network. In 2015, EFSA was requested by the German Federal Institute for Risk Assessment (BfR) to provide a statement on the presence of micro- and nanoplastics in food, with particular focus on seafood. The terms of reference included an extensive review of the available information on micro- and nanoplastics in food and their potential hazards to human health, together with the identification of data gaps and proposals for research recommendations.

To be able to perform a comprehensive assessment of the risks posed by micro- and nanoplastics to human health via the food chain, the EFSA CONTAM Panel statement[1] provided the following recommendations related to:

- Analytical methods – Whereas there is not yet a harmonised definition[2] of micro- and nanoplastics, standardised analytical methods for the quantification and characterisation of micro- and nanoplastics in food and feed matrices are necessary to generate reliable data.
- Exposure assessment – The assessment of dietary exposure to micro- and nanoplastics is essential for a comprehensive risk assessment. Occurrence data in food and feed are limited (for microplastics) or lacking (for nanoplastics). A better understanding of possible contamination of food as the result of processing/distribution/packaging is also needed. More occurrence data should be generated by reliable analytical methods and on the sources of food and feed contamination, in particular for particles below <150 µm.
- Hazard identification and characterisation - Information on the fate (ADME; absorption, distribution, metabolism and excretion) and biological activity of micro- and nanoplastics following oral exposure is needed to identify and characterise the possible adverse effects on human health of nano- and microplastics in food and feed. In particular, research was recommended on the degradation of microplastics and potential formation of nanoplastics in the human Gastrointestinal (GI) tract. Information on plastic additives and contaminants needs to be evaluated as well.

The environmental and human health risks posed by micro- and nanoplastics have become subject to increasing regulatory and scientific scrutiny. At EU level, tackling plastic pollution and its impact on the health of ecosystems has become a policy priority, as highlighted by the European Strategy for Plastics in a Circular Economy[3] and of its complementary Bioeconomy Strategy[4]. In pursuing an ambitious approach for plastic

[1] https://www.efsa.europa.eu/it/efsajournal/pub/4501

[2] ECHA's proposed restriction of intentionally added microplastics includes a regulatory definition of micro and nanoplastics. This is currently with the European Commission and Member States for decision making. Also the State of California has adopted one: https://www.natlawreview.com/article/california-adopts-definition-microplastics-drinking-water#:~:text='Microplastics%20in%20Drinking%20Water'%20are,5%2C000%20micrometers%20(%C2%B5m)3

[3] A European Strategy for Plastics in a Circular Economy: https://ec.europa.eu/environment/circular-economy/pdf/plastics-strategy-brochure.pdf. It also contained ECHA's mandate to develop a restriction proposal for intentionally added microplastics.

[4] A sustainable Bioeconomy for Europe: strengthening the connection between economy, society and the environment. Updated Bioeconomy Strategy: https://ec.europa.eu/research/bioeconomy/pdf/ec_bioeconomy_strategy_2018.pdf

packaging recyclability, the Strategy for Plastics contains a strong response on microplastics, while the Bioeconomy Strategy is meant to sustain the European priority of supporting healthy ecosystems, by, for instance, achieving plastic-free seas and oceans. In this context, policy relevant research and data on the sources and impacts of microplastics are considered pivotal in sustaining the health-relevant aims of such strategies.

Many recent reports have been published (such as the ones from the SAM[5], SAPEA[6], FAO[7], ECHA[8], JRC[9], WHO[10], EEA[11] and VKM[12]). Although some reports indicate lack of evidence for public health risks from consumption of microplastics, many of the food safety aspects remain yet to be addressed and the public concern around this matter is still very high.

EFSA's 2019 Eurobarometer on Food Safety in the EU found that microplastics in food was a topic of concern among EU consumers, particularly in Northern Europe. These findings corroborate insights from the BfR Consumer Monitor, where high awareness and concern on the issue are observed.

Five Horizon 2020 research and innovation projects have been funded under the topic "Micro- and nano-plastics in our environment: Understanding exposures and impacts on human health" as a part of the call "Better Health and care, economic growth and sustainable health systems (H2020-SC1-BHC-2018-2020)"[13] (Table 1). The objective is to provide policy-relevant scientific data in support of improved human health hazard and risk assessment of micro and/or nanoplastics, in agreement with research recommendations provided in the EFSA CONTAM Panel statement in 2016. The projects were launched on 1/04/2021 and form a collaborative cluster focusing on delivering better insights into food as a source for micro- and/or nano-plastics[14], the assessment of possible contaminants and pathogens, its fate and possible health effects and preliminary investigations into long-term effects of micro- and/or nano-plastics.

[5]European Commission's Scientific Advice Mechanism (SAM) Group of Chief Scientific Advisors: https://ec.europa.eu/info/sites/info/files/research_and_innovation/groups/sam/ec_rtd_sam-mnp-opinion_042019.pdf

[6] SAPEA report: https://www.sapea.info/wp-content/uploads/report.pdf

[7] FAO – Report on microplastics in fisheries & aquaculture (2017): http://www.fao.org/3/a-i7677e.pdf

[8] ECHA: microplastics - Registry of restriction intentions until outcome - ECHA (europa.eu)

[9]DG JRC - Dir. F: Toussaint et al.: Review of micro- and nanoplastic contamination in the food chain. Food Additives and Contaminants – Part A. 36 (2019) 639-673. DOI: 10.1080/19440049.2019.1583381

[10]WHO: Microplastics in drinking-water https://www.who.int/water_sanitation_health/publications/microplastics-in-drinking-water/en/

[11]EEA - Plastics, the circular economy and Europe's environment — A priority for action https://www.eea.europa.eu/publications/plastics-the-circular-economy-and/

[12] https://vkm.no/download/18.345f76de16df2bc85a513b4e/1571823698421/20191023.pdf

[13] https://ec.europa.eu/info/funding-tenders/opportunities/portal/screen/opportunities/topic-details/sc1-bhc-36-2020

[14] NanoSafety Cluster Newsletter 22 March 2021, p. 24-27, https://zenodo.org/record/4607937#.YH7mnGgzY2x

Table 1. Horizon 2020 projects launched on 1/04/2021, under the call Better Health and care, economic growth and sustainable health systems (H2020-SC1-BHC-2018-2020), topic: Micro- and nano-plastics in our environment: Understanding exposures and impacts on human health (SC1-BHC-36-2020)

PROJECT	PARTICIPATING INSTITUTIONS	Analytics Activities
964827 AURORA *Actionable European roadmap for early-life health risk assessment of micro- and nanoplastics* Coordinator: Prof Roel Vermeulen Utrecht University, NL	1. Medical Center Utrecht NL 2. Barcelona Institute for Global Health - ISGlobal ES 3. Hasselt University BE 4. Mount Sinai School of Medicine, NY, USA 5. Utrecht University NL 6. Masaryk University CZ 7. VU University NL 8. University of Eastern Finland FI 9. Food Packaging Forum CH 10. Institute of Occupational Medicine UK 11. University of Oldenburg DE	The scale of micro- and nanoplastic (MNP) pollution is becoming increasingly clear yet little is known about how this pollution impacts health. The AURORA project will deliver an actionable European roadmap for early-life health risk assessment of MNPs to support regulation of MNPs and the products and processes that generate secondary MNPs, and development of safer alternatives. We will focus on MNP exposures and toxicological and health effects during pregnancy, in utero, and in early life. These periods are critical for development and health later in life and are of heightened vulnerability to environmental insults. We have recently shown that MNPs are likely to cross the placental barrier in vitro and in vivo, underlying the urgent need to understand the impact of MNPs on reproductive and early-life health. AURORA will do so 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, 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. AURORA is part of the European MNP cluster on human health
965173 iMPtox *An innovative analytical platform to investigate the effect and toxicity of micro and nano plastics combined with environmental contaminants on the risk of allergic disease in preclinical and clinical studies*	1. University of Belgrad, RS 2. Moverim consulting sprl BE 3. Promoscience srl IT 4. Medical University of Vienna AT 5. Catholic University of Leuven BE 6. Ghent University BE 7. Karolinska Institute, SE 8. University of Vienna AT 9. Sciensano BE 10. French National Centre for	There is an urgent need to understand the risks on human health of micro- and nano-plastic particles (MNPs) that contaminate food and environment. We aim to create a cross-disciplinary platform to design suitable analytical approaches for determining the extent of the problem in the environment and to our health by evaluating the influence of ingested and inhaled exposure of MNPs contaminated with metals, allergens, pathogenic bacteria and toxins on allergic responses. Our strategy includes developing a novel combination of tools to identify, extract, characterize, and quantify MNPs from selected foods, environmental

Coordinator: Prof. Tanja Cirkovic Velickovic University of Belgrad, RS	Scientific Research (CNRS) FR 11. Srebrnjak Children's Hospital HR 12. University of Applied Sciences and Arts of Western Switzerland CH	media, and tissues of exposed animals to assess MNP prevalence based on size, shape and type. We will produce and label MNPs for use in a variety of pre-clinical studies to investigate toxicity and responses to MNPs, contaminants and allergens. We plan to use novel stable isotope 13C labelling and detection to determine MNP fate and accumulation in the gastrointestinal and respiratory tracts and secondary organs after immediate and chronic exposure and the effect on allergic immune responses at the cellular and molecular levels. Additionally, we designed a clinical study to evaluate the influence of MNP exposure in humans. The outcome of this state-of-the-art project includes - novel tools for MNP detection - improved understanding of the effects of NMPs combined with critical contaminants in the air, water and food on human health and discovery of predictive biomarkers - increased awareness of disease risk in response to MNPs and contaminants - improved communication strategies between science and relevant stakeholders and contribution to blue growth and the health-relevant aims of the European Strategy for Plastics in a Circular Economy - policy-relevant scientific data in support of improved human health hazard and risk assessment and for response and mitigation policies at the national and EU level for policymakers. ImpTox is part of the European MNP cluster on human health.
965196 PLASTICHEAL *Innovative tools to study the impact and mode of action of micro and nanoplastics on human health: towards a knowledge base for risk assessment* Coordinator: Prof Ricard Marcos, Autonomous University of Barcelona, ES	1. Autonomous University of Barcelona, ES 2. Finnish Institute of Occupational Health TTL FI 3. Wageningen University NL 4. Technical University of Denmark DK 5. French Alternative Energies and Atomic Energy Commission (CEA) FR 6. BioMedical Research Institute of Murcia ES 7. University of Manchester UK 8. AIMPLAS ES 9. Cancer Research Centre of Lyon FR 10. Helmholtz-Centre for Environmental Research DE 11. University of Leipzig DE	The exponential increase in the production/use of plastic translates into a parallel increase of environmental plastic-waste that is continuously degraded into micro and nanoplastics (MNPLs). Information on the MNPLs effects on human health is still preliminary and, furthermore, the limitations in current methodologies prevent accurate human exposure/risk assessment. In this context, PLASTICHEAL aims at providing new methodologies and solid scientific evidence to regulators by combining the use of breakthrough research and validated test methods to set the knowledge basis for adequate risk assessment of MNPLs. PLASTICHEAL will be supported by an innovative experimental approach that will first generate human exposure estimates after identification, measurement, and characterization of MNPLs present in the environmental air, water and food sources, as well as in human biological samples of population groups with potential high MNPLs exposure levels (biomonitoring study) by means of adapting the existing analytical methodology proven useful for fibres and nanomaterials. Those estimates will then be complemented/ correlated with the output of kinetic models using data on MNPLs translocation, accumulation and destabilization of the Gastrointestinal and Respiratory Tracts, and with the MNPLs toxicokinetic in blood and secondary organs using in vivo models. Thereafter, immune effects,

		transforming effects, genotoxic effects, impact on transcriptome/epigenome/secretome (i.e. omics), stemness imbalance and potential molecular mechanisms of action and adverse outcome pathways in blood, primary and secondary organs will be studied under in vitro, in vivo, and ex vivo short and long-term (co)exposure settings. To ensure the impact of PLASTICHEAL's developed methodology and gained knowledge on current and future regulation, a continuous dialogue will be established from the beginning of the project with policy makers and other key stakeholders of the plastic value chain. PLASTICHEAL is part of the European MNP cluster on human health (Name and acronym to be decided)
965367 **PLASTICSFATE** *Plastics fate and effects in the human body* Coordinator: Dr Rudolf Reuther Environmental Assessments (ENAS) DE	1. Environmental Assessments DE 2. European Research Services DE 3. National Institute of Occupational Health NO 4. National Research Center for the Working Environment DK 5. Institute of Functional and Clinical Anatomy DE 6. University Medical Center Utrecht NL 7. Wageningen Food Safety Research NL 8. Federal Institute for Materials Research and Testing (BAM) DE 9. Spanish National Research Council (CSIC) ES 10. Institute of Science and Technology for Ceramics IT 11. Fraunhofer Institute for Ceramic Technologies and Systems DE 12. Helmholtz-Centre for Environmental Research DE 13. Leibniz Institute of Freshwater Ecology and Inland Fisheries DE 14. University of Leiden NL 15. University of Ljubljana SI 16. University of Natural Resources and Life Sciences Vienna AT 17. University of Bayreuth DE 18. University of Turin IT 19. University of Rome IT 20. University of Paris FR 21. National Technical University of Athens EL 22. Itene ES 23. Ecamricert IT 24. Gaiker ES	"The main goal of PlasticsFatE (Plastics Fate and Effects in the Human Body) is to improve our present understanding of the impact of micro- and nano-plastics (MP/NP) and associated additives/adsorbed contaminants (A/C) in the human body. Human exposure to MP/NP may result from the widespread use of plastic products and their release to the environment, where they degrade to MP/NP particles. But plastics particles reach natural systems also as secondary by-products, e.g., from tyre wear or abrasion of textiles. As a consequence, these particles are found in food, drinking water, air and environmental media (food chain, soils). Despite recent efforts to assess the real dimension of human risks associated with MP/NP, our current knowledge is still insufficient. One of the reasons is the lack of reliable and validated methods that are able to generate the science-based data we need. PlasticsFatE will address this challenge and associated uncertainties by implementing a comprehensive measurement and testing program (""test the test""), including inter-laboratory studies, to improve and validate the performance and applicability of available methods and tools to MP/NP. The tested and validated approaches will be used to (1) identify and detect MP/NP and A/C in a variety of complex matrices, such as food (vegetables, fruits, beverages, fish etc.), human tissues and consumer products (tooth paste, beauty products), as well as relevant environmental media (air, drinking water, soils), and to (2) assess their (also long-term) fate and toxicity in the human body by using advanced cell culture and organ models that simulate real exposure to MP/NP in the respiratory and gastro-intestinal tract. The newly developed innovative approaches will be integrated into a novel risk assessment strategy specifically designed for MP/NP to provide the policy relevant and scientifically sound data needed to support the health-relevant aims of European strategies for plastics. PlasticsFatE is part of the European MNP cluster on human health."

	25. Innosieve Diagnostics NL 26. Society for Chemical Engineering and Biotechnology DE 27. OPTIMAT Limited UK 28. Indian Institute of Toxicology Research IN	
964766 **POLYRISK** *POLYRISK - Understanding human exposure and health hazard of micro- and nanoplastic contaminants in our environment* Coordinator: Dr Raymond Pieters Utrecht University NL	1. Utrecht University NL 2. VU University Amsterdam NL 3. VU Medical Centre NL 4. German Federal Institute for Risk Assessment (BfR) DE 5. German Federal Institute for Materials Research and Testing (BAM) DE 6. Federal Institute for Occupational Safety and Health (BAuA) DE 7. Norwegian Institute of Public Health NO 8. National Research & Development Institute for Textiles and Leather RO 9. Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) IT 10. Ideaconsult Ltd. BG 11. Health and Environment Alliance (HEAL) BE 12. Fraunhofer Institute for Microstructure of Materials and Systems DE 13. European Research Services DE	The POLYRISK project aims unraveling the risks of microplastic and nanoplastic particles (MNP) that are ubiquitous in our environment and are likely to be entering the human body via inhalation and ingestion. The most bioavailable low-micron and nano-sized MNP, pose the biggest analytical challenges or today's analytical chemists. Existing knowledge about the adverse pro-inflammatory effects of airborne particulate matter and nanoparticles, combined with pro-inflammatory evidence of MNP exposure observed in animal models and in vitro pilot tests with human immune cells, suggests that MNP may cause immunotoxicity in humans. Occupational exposure of workers to fibrous MNP can indeed lead to granulomatous lesions, causing respiratory irritation, functional abnormalities and flock worker's lung. Currently, human health risk assessment protocols specific to MNP are not available and key data is missing. This hampers science-based decision making. On this backdrop, POLYRISK's human risk assessment strategy will combine highly advanced sampling, sample pretreatment and analytical methods to detect MNP in complex matrices, up-to-date fit-for-purpose hazard assessment technologies and multiple real-life human exposure scenarios. We will focus on key toxic events linked to several chronic inflammatory diseases. The consortium uniquely brings together interdisciplinary experience and know-how on quality-controlled chemical analyses of MNP and additives, intestinal and respiratory toxicity models, human exposure epidemiology, immunotoxicology and real-life high-exposure studies. POLYRISK's novel human risk assessment strategy is based on mechanistic reasoning and pragmatically accommodates the complexity of the MNP toxicant class. Building with ground-breaking science, stakeholder engagement and strong communication, POLYRISK aims to rapidly reduce current MNP risk uncertainties and support EU efforts to ensure public health is adequately protected from the potential risks of MNP pollution. POLYRISK is a part of the European cluster on Health Impacts of Micro- and Nanoplastics.

The issue of micro- and nanoplastics in food and feed is still affected by many uncertainties on their effects on human health and by consumers concerns about their ubiquitous presence in the environment. Communication on the topic of plastics is sustained, shaping the public discourse and opinion.

Insights relevant to food safety risk assessment in the area of micro- and nanoplastics, along with social and behavioural factors behind concerns regarding human health, will be presented in the colloquium, attempting to describe each of these factors and stimulate further research in both life and social sciences.

EFSA is responsible for providing scientific advice on health risks in food and feed to EU policymakers. Public concern regarding potential risks cannot be ignored. The organization of the EFSA Scientific colloquium is an opportunity to foster collaboration and knowledge sharing to facilitate food and feed safety risk assessment for the development of policy solutions that address the societal concerns.

2) Objectives and expected outcome

The goal of the Scientific Colloquium is to bring researchers, risk assessors and risk managers together, understand the current state of play and ongoing research in micro and nanoplastics, foster collaboration and build synergies. This will contribute to filling gaps in scientific knowledge and facilitate the assessment of the risks of micro and nanoplastics to human health and facilitate the translation of new data into policy decisions.

The EFSA Scientific Colloquium on micro- and nanoplastics in food and feed envisages to:

- Review available research and existing research gaps to assess the evolution of scientific evidence since the EFSA CONTAM Panel 2016 statement
- Anticipate possible collaboration opportunities and synergies with ongoing and planned research activities in order to ensure closing the existing data and knowledge gaps
- Identify the challenges and regulatory constraints for lifecycle/food system risk assessment of micro- and nanoplastics
- Stimulate discussion and identify priorities for the generation of the data necessary to perform a comprehensive assessment of the risks to human health of micro- and nanoplastics in food and feed
- Explore possible ways to make data generated by research available for risk assessors and risk managers.
- Consider the risk perceptions about micro- and nanoplastics in food and feed into an overall view on priority setting

The colloquium outcomes will be published in a special issue of the EFSA Journal. The outcomes will be used to define a strategy for research collaboration and knowledge sharing to facilitate food and feed safety risk assessment acknowledging the related societal concerns.

3) Organising Committee

EFSA Scientific Programme Committee (SPC)

Angelo Maggiore, Aleksandra Lewandowska and Hans Verhagen *(till 31/10/2021), SCER unit*
***Marco Binaglia and Karen Mackay**, BIOCONTAM unit*
***Ana Afonso**, GMO unit*
***Sandra Rainieri**, FIP unit*

***Domagoj Vrbos and* Giorgia Zamariola**, *COM unit*
***Marta Hugas and* Stef Bronzwaer**, *ED Office*

External Advisory Committee (AC)

***Peter Simpson**, European Chemicals Agency (ECHA)*
***Birgit Sokull-Klüttgen**, Joint Research Centre (JRC), European Commission*
***Uta Faure**, Directorate-General for Research and Innovation, European Commission*
***Veerle Vanheusden**, Directorate-General for Health and Food Safety, European Commission (DG-SANTE)*
***Lisa Scheuermann**, World Health Organization*

Technical organising Committee (TC)

***Vanessa Descy, Cinzia Percivaldi** and **Lucia Parrino,** CORSER unit*
***Anthony Smith**, COM unit*

4) Colloquium 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**	Pietro Giuseppe Gucciardi

	Nanoplastics Identification in liquids Followed by Q&A	(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**	

5) Breakout groups

Structure

The programme includes the following breakout groups, exploring questions on microplastics and nanoplastics in food and feed:

1. Developments in analytical methods for micro- and nanoplastics
2. Developments in dietary exposure of humans to micro- and nanoplastics
3. Developments in hazard identification and characterisation for micro- and nanoplastics

The participatory format of the breakout session will foster collaborative thinking on several complementary dimensions of the subject matters. In each group, discussion and interaction among participants will be facilitated by a pre-appointed chairperson, supported by a rapporteur, who will be responsible for taking notes and preparing report-back slides.

Insights and conclusions from each parallel group will be shared during plenary report-back moments and discussions following the break-out sessions.

The work of the breakout groups must:

- *Provide an overview of the scientific evidence since the EFSA CONTAM PANEL 2016 statement.*
- *Identify collaboration opportunities with ongoing and planned research activities*
- *Identify the challenges and regulatory constraints for lifecycle/food system risk assessment of micro and nanoplastics*
- *Identify priorities for the generation of data necessary to perform a comprehensive assessment of the risks to human health of micro- and nanoplastics in food and feed*
- *Identify the most relevant risk assessment questions considering risk perceptions and stakeholders priorities*
- *Discuss the need of a dedicated strategy for nanoplastics, what knowledge can be transferred from microplastics, and what needs re-learning or starting from fresh, in how far micro and nanoplastic particles are different from or similar to naturally occurring micro- and nano particles (e.g. from dust).*

The number of registered attendees is 147. They will be subdivided in 6 break out (BO) groups of around 20 persons each.

BREAKOUT GROUPS 1 AND 2 - DEVELOPMENTS IN ANALYTICAL METHODS FOR MICRO- AND NANOPLASTICS

Introduction

Harmonised definitions of micro- and nanoplastics and reliable, standardised and validated analytical methods for their extraction, detection, identification, quantification and characterisation in food and feed matrices are urgently needed. Together with standardized metrics, data generated using such methods will ensure consistency and study comparability and help conduct a robust risk assessment for micro- and nanoplastics in food and feed.

Discussion points

The discussion should focus on ongoing and planned developments since the EFSA CONTAM Panel 2016 statement regarding analytical methods used for extraction, detection, quantification and characterisation of the micro and nano plastic particles in food/feed, the analytical challenges and the possible standardisation and harmonisation of these methods, together with current regulatory data gaps. At the same time, the challenges for adoption of a single definition, including micro vs nano, primary micro and nano versus secondary, plastic components versus contaminant should also be considered.

The two breakout groups will address the following questions:

1. **What are the gaps in knowledge about analytical methods for (micro/nano) plastics?**

   Examples:

   - Better physical sample extraction methods and/or chemical or enzymatic digestion methods to destroy biogenic matter without altering the plastic
   - Limited methods for the identification and quantification of microplastics in food/feed and lacking methods for nanoplastics.

2. **What can be done in order to overcome the gaps in knowledge?**

   Examples:

   - Analytical methods should be further developed and standardised.
   - Reference materials should be developed.
   - Avoidance of plastic contamination from equipment used during sampling and analysis.
   - Quality assurance should be in place and demonstrated e.g. use of method blanks.

3. **What needs to be prioritised in relation to overcoming the gaps in knowledge? The following criteria could be considered for the prioritisation:**
   The following criteria could be considered for the prioritisation:
   The results from the research recommendations for priority activities will

Criteria 1 - have benefits on the establishment of harmonised definitions in Europe

Criteria 2 - increase our understanding of the hazards and exposure linked with the presence of plastic in food and feed

Criteria 3 - facilitate the implementation of monitoring programmes at EU level.

An EU Survey questionnaire has been distributed to the registered participants of breakout groups 1 and 2 before the event to streamline the discussion during the event and collect a preliminary list of items for the prioritisation exercise requested in question 3. This will be facilitated through an on-line poll during the breakout session.

The breakout groups should focus the discussion on ongoing and planned research activities, collaboration opportunities to make research available to regulatory science and measures required for the implementation of methods.

Gaps in knowledge identified by participants though a survey on 16-23/04/2021

- Unified definitions for nanoplastics, microplastics, 'molecular nanoplastics' (oligomers, 1-10 nm)
- Definition of optimum analytical methods for identification of microplastics in each food category (best to use multiple analytical methods but there should be affordable practical standards developed
- Adequate methodologies for analysis of biodistribution of micro and nanoplastics inside the organisms, tissues and cells (animals/humans) and in biological fluids - milk, egg, meat, seafood. For the small sizes (small micro and nanoplastics) there are few or no reference methods specialized: SEM, TEM, AFM, microRaman, microFTIR are proposed, but are not definite for the smaller sizes
- Feasible approach for the digestion of biological tissue, with relatively fast and high efficiency
- Standardisation of analytical methods (for sampling, isolation and identification) for each product type; standard units, protocols and guidelines should be prepared in order to harmonize results from human health and ecotoxicology. Including: which spectra library is used, and what minimum accepted similarity index to the spectra library is allowed
- Geolocalisation of sampling activities and detailed description of investigated species/matrices
- Large-scale studies covering large geographical area and/or multiple species, large sample size
- Method validation and performance criteria: various methods are being used, but not all enable a safe and reliable identification of microplastics. QA/QC procedures are not applied consequently, raising the question of validity of results.
- Analytical methods need to be able to sample, sort and distinguish between micro- or nanoplastic on or in food
- Integrated approach for detecting micro and nanoplastics as well as contaminants at the same time
- Standardized methods for extraction, purification and concentration of nanoplastics from environmental media from trace level up to sufficient concentration for instrumental detection. Includes sampling of representative sample and extrapolation of the results, for example how much of sample needed extrapolate the results for whole fish or batch of fish meal (500kg)
- Tools for detection and chemical identification of small microplastics (< 10 µm) and nanoplastics in liquid digested samples (e.g. seafood, GI tracts)

- Understanding of micro-to-nano fragmentation processes due to photooxidation and photothermal processes
- Methods to identify the proportion of different plastics present in a mixture of microplastics and in complex mixtures
- Light-based methods for counting micro and nanoplastics, including selective counting of different types of MNPs. Current methods are often not sensitive enough for nanoplastic detection
- Identification of source of contamination
- Precise food categories of interest should be define in order to solve the problem of optimal digestion protocols required for complex food matrices
- More rapid and cost-effective sample processing and identification (size, chemical species) methods
- Methods that can measure the leaching of additives, impurities etc from the material into human tissues
- Quantification of micro/nanoplastic mass, not only no. of particles (ideally both should be measured for a full view on the exposure)
- Biomarkers of exposure
- Development of methods without the extraction/degradation step which could influence chemical composition of plastics and, and which would allow to investigate the distribution of of micro/nano plastics in the organic matrix (heteroaggregation, association with soil, cellular components....)
- Limited studies on development of alternative (more sustainable) materials to plastics as the production keeps increasing
- Relevant reference material (available material can be different than weathered particles (e.g. different hydrophobicity; aAlso fibers as reference material is missing)

Recommended reading:

1. Danopolous et all 2020 (Environ Health Perspect 2020 Dec;128(12):126002. doi: 10.1289/EHP7171. Epub 2020 Dec 23.)
2. Dawson et al., 2021 (Environmental Pollution 276 (2021) 116684 https://doi.org/10.1016/j.envpol.2021.116684)
3. Dehaut et al., 2019, Dehaut et al. / Trends in Analytical Chemistry 116 (2019) 346e359 https://doi.org/10.1016/j.trac.2018.11.011
4. G.R. Bitencourt, P.A. Mello, E.M. M. Flores et al. Science of the Total Environment 749 (2020) 142301

Ways to overcome the gaps in knowledge, as identified through the survey

- Determination of microplastic/nanoplastic size range that poses risk to health to health and develop methods specifically for this range
- Analytical method comparison to select techniques for the developed standards
- Analytical methods for imaging (combination of spectroscopies and microscopies) specialized in plastics on biological matrix
- Rapid methods for detecting micro/nanoparticles based on size and/or composition and/or aggregations, and/or contaminants occurrence/leaching and/or any other parameters relevant for hazard characterisation or exposure studies.
- Methods for distinguishing between micro- and nanoplastics in food
- Developing nomenclature, protocols, guidelines, and standards (including units) for micro and nanoplastic isolation and characterization (in environmental samples, food matrices, solid/liquid/air samples)
- Validation criteria for microplastic analysis
- Method performance assessment
- Using model micro/nanoplastics to optimize existing methods, developing reference materials (nanoplastics standards with or without specific additives for instrumental development)

- Seek sustainable alternatives to plastics in order to lower the amount of micro and nanoplastics released to the environment
- Applying techniques for both mass and particle number evaluation, to give more comparable results among different investigations (standardized protocols should combine these two approaches)
- Micro/nano plastics geolocalisation system shared among research and governmental institute actively working in micro/nanoparticle detection
- Developing dye/probe/labelling technology that selectively binds to specific micro/nano plastics
- Ecouraging the research community to investigate on MP occurrence in EU waters
- Raise citizen awareness about microplastics as a public health issue
- Lessons learned from nanotechnology/nanospectroscopy:
    - give nanoplastics definition that tailors the definition of microplastics, and is compatible with physical /chemical properties (e.g. capability to view and discern particles with an optical microscope, related the concept of spatial resolution and diffraction limit in optical microscopy)
    - combination of Raman spectroscopy with optical trapping for analysis and sorting of nanoplastics based on their composition (e.g. Raman cytometry) in a stream of fluid
    - use of surface-sensitive nanospectroscopies, Surface Enhanced IR / Raman, for particles detection and for determination of surface-pollutants
    - use and standardization of spectroscopic scanning probe microscopies such as AFM-IR, Nano-FTIR, Tip-Enhanced Raman Spectroscopy
    - methodologies for materials-selective staining of nanoplastics with dyes and application of optical nanoscopies (STED/PALM/STORM) for quantification and size determination
    - combination of multiple analytical and metrological techniques in cascade (e.g. Raman + tweezers + DLS) for chemical/dimensional analysis
    - develop new tools to study the fragmentation process at the single particle level

Relevant reading:

1. BAM/JRC exercise (https://ec.europa.eu/jrc/en/science-update/call-laboratories-participate-proficiency-testsmicroplastics- drinking-water-and-sediments)
2. Quasimeme scheme (https://science.vu.nl/en/research/environment-and-health/projects/microplastics-ws-and-ils/index.aspx)
3. Dehaut et al. / Trends in Analytical Chemistry 116 (2019) 346e359 https://doi.org/10.1016/j.trac.2018.11.011

Background documents

EFSA (European and Food Safety and Authority), 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal 2016;14, e04501.

BREAKOUT GROUPS 3 AND 4 - EXPOSURE TO MICRO- AND NANOPLASTICS

Introduction

Occurrence data in food (including drinking water) and feed are limited (for microplastics) or lacking (for nanoplastics). Most of them focus on microplastic content in seafood, specifically fish and shellfish. There is a need to quantify the amount (and characterise the type) of particles in those animal tissues and organs that are typically consumed. The occurrence of microplastics has also been reported in other food items, including honey, sugar, beer, and salt. Few studies have evaluated the occurrence of microplastics also in bottled water samples.

A few new studies reported microplastics occurrence also in fruits and vegetables, and packaged meat[15].

The point sources of microplastics in food and feed are currently unknown, although it is likely that microplastics enter the food chain through plastic waste breaking down in environmental matrices, such as water and air. Food-chain transfer in aquatic environment is partially understood, whereas dynamics in terrestrial food chains are largely unknown. Microplastics in the air might also settle on food items. A better understanding of possible contamination of food as the result of food manufacturing, processing, and handling, as well as food packaging materials, is needed. Data are lacking also on the impact of the food matrix on microplastic bioavailability (e.g., water-based versus solid/dry foods).

Particles <150 µm are particularly affected by such a lack of occurrence data. This is even more true for the smallest microparticles within this size range, which might be the most relevant in terms of health effects. Studies investigating the occurrence of microplastics focus on different size ranges and often use different units to report plastic amounts, thus limiting comparisons between results obtained in different studies. Data on nanoplastics are lacking.

Microplastics particles are highly diverse and different methods measure different fractions of the full size, shape and density continuum. However, it is not always possible to align the effect data obtained for monodisperse nano or microplastics for certain polymers, with exposure data for irregularly shaped, polydisperse nano or microplastics that we find in drinking water or food.

Discussion points

Taking into consideration ongoing and planned developments since the EFSA CONTAM Panel 2016 statement, the breakout groups will address the following questions:

1. What are the gaps in knowledge on exposure to (micro/nano) plastics?

   Examples:

- How much are humans exposed to?
- What is relevant regarding exposure to (micro/nano) plastics: e.g. particle size ranges, chemical characterization of particle polymers
- Which are the relevant contaminants carried by plastics (or plastic additives)
- How to approach complex exposure patterns (combined exposure)?

[15] https://dx.doi.org/10.1021/acs.est.0c07384

- What is the relevance of dietary exposure versus other sources/routes of exposure (e.g. inhalation)?
- What should the right metric be (e.g. mg/kg bodyweight, number of particles, surface area)?

2. What can be done in order to overcome the gaps in knowledge on dietary exposure?

   Examples:

- Identification of critical ranges of particle sizes to focus on
- Identification of critical particle compositions to focus on
- Main food items contributing to (lifetime) exposure and eventual accumulation?
- Research bioavailability of the chemicals onto/in the particles
- What's the chemical contribution of microplastics in relation to total chemical intake?

3. What needs to be prioritised in relation to overcoming the gaps in knowledge? The following criteria could be considered for the prioritisation:

   Criteria 1 - The results from the research recommendations for priority activities will have benefits on the establishment of harmonised definitions in Europe

   Criteria 2 - The results from the research recommendation for priority activities will increase our understanding of the exposure linked with the presence of plastic in food and feed

   Criteria 3 - The results from the research recommendation for priority activities will facilitate the implementation of monitoring programs at EU level.

An EU Survey questionnaire has been distributed to the registered participants of breakout groups 3 and 4 prior the event to streamline the discussion during the event and collect a preliminary list of items for the prioritisation exercise requested in question 3. This will be facilitated through an on-line poll during the breakout session.

The breakout groups should focus the discussion on ongoing and planned research activities, collaboration opportunities to make research available to regulatory science and measures required for the implementation of monitoring programs.

Gaps in knowledge identified by participants though a survey on 16-23/04/2021

- Information on exposure to atmospheric small micro and nanoplastics
- Main human exposure pathways and their contribution to total exposure (literature mainly focuses on ecotoxicology)
- What type of particles are humans and animals exposed to? Which are the most harmful and where to they accumulate? What are the adsorbed substances of concern?
- Exposure to toxic contaminants caused by micro and nanoplastics
- Comparison of the relative exposure of nanoplastics vs microplastics as well as hazards related to them
- Content of micro and nanoplastics in complex matrices (meat, fish, beverages, other edible animal tissues)

- To what extent is food plastic packaging a source of exposure to chemicals carried on plastics and do processes like UV treatments contribute to leaching into food
- Influence of food processing (pasteurization, washing, cooking etc.) on micro and nanoplastic content in food, and the release of toxic contaminants into food
- Main food/water sources that are contaminated with micro and nanoplastics, total dietary exposure (taking into account the reference intake, and not just the content in the product) – also to contaminants
- Contamination of feedstock with micro and nanoplastics
- Human exposure calculated on up-to-date consumption and taking into accounts trends and habits on a local level
- Transfer of micro- or nanoplastics from contaminated soil to plants
- Absorption of micro and nanoplastic through the gastrointestinal tract (most particles expected to be internalized in tissues are very small and often left out from current works)
- Excretion rates: How big is the proportion of orally ingested microplastics that leaves the body via excretion? Does excretion depend on size and shape of the particle?
- Lack of awareness of the micro and nanoplastics in food/feed
- A coordinated approach for monitoring/reduction/mitigation measures for primary and secondary micro/nano plastics exposure
- Health based guidance values

Ways to overcome the gaps in knowledge, as identified through the survey

- Standards for sample collection, reporting the results, extraction, identification
- Data on exposure thorough the whole diet/in a representative food basket (incl. food packaging)
- Comparison between dietary vs non-dietary exposure (e.g. through water, air; weather data and models to investigate causalities and co-ocurrances needed)
- Studies on micro and nanoplastic occurrence in human placenta and whether they may trigger immune responses or may lead to the release of toxic contaminants, resulting harmful for pregnancy
- Data on egestion rates of microplastics in a large group of consumers
- The effects on the contribution of microplastics and nanoplastics of the different food processing technologies – at which stage of the food chain are the micro and nanoplastics introduced?
- Integrating *in vivo* and *in vitro* data
- Using relevant standards and controls
- Understanding of the physicochemical properties of real world nanoplastic samples occurring in food and feed and identifying the properties that are relevant for uptake/toxicity
- Data on behaviour of MPs in soil and transfer to plants
- Veryfying interaction with human microbiome and other contaminants
- Appropriate risk values for the population including the different types (Ej. children, pregnant women...)
- Funding studies on particle behavior and contaminants release related to human health and method development
- Preventive measures for micro and nanoplastic exposure
- Geolocalisation system combined with artificial intelligence (AI) should provide a good set of tools to locate and follow the fate of micro/nano plastics released to the environment and provide insight into where exposure to harmful micro and nano plastics (primary or secondary) are more likely to occur
- Regulatory action based on the health effect studies
- Increased public awareness campaign at all levels by all stakeholders; industry, NGO and government

- Identification of the most relevant particle characteristics explaining bioavailability and/or toxicity (e.g. size, surface charge, polymer type, shape, the presence of additives)

Background documents

EFSA (European and Food Safety and Authority), 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal 2016;14, e04501.

Nur Hazimah Mohamed Nor, Merel Kooi, Noël J. Diepens, and Albert A. Koelmans, 2021. Lifetime Accumulation of Microplastic in Children and Adults. Environmental Science & Technology Article ASAP DOI: 10.1021/acs.est.0c07384

BREAKOUT GROUPS 5 AND 6 – HAZARD IDENTIFICATION AND CHARACTERISATION FOR MICRO- AND NANOPLASTICS

Introduction

There is a lack of information on the possible adverse effects on human health of micro/nanoplastics in food and feed. In order to identify and characterise them, information on the fate (Absorption, Distribution, Metabolism and Excretion - ADME), impact of the physicochemical properties of the ingested particles (e.g. size, length, shape, polymer type and surface chemistry), biological activity of micro- and nanoplastics following oral exposure is needed. Toxicological research is needed to better inform human health risk assessment. This includes the identification of target tissues, threshold doses, and mode of action. The fate and transport of ingested particles in the human body remains largely unknown. Accumulation of microplastics in the body tissues could cause a variety of adverse outcomes such as inflammation, oxidative stress, and immune responses[16]. Another source of concern is the chemical toxicity of intentionally added or adsorbed chemicals. Furthermore, biofilms growing on aquatic microplastics may be a source of fungi, viruses and opportunistic bacterial pathogens and antibiotic resistance genes that may interact with gut microbiota.

Available literature suggests that following oral ingestion, microplastics may remain confined to the GI tract, translocate from the GI tract into organs or tissues, and/or be excreted. There is currently a lack of understanding of the size ranges associated to particle ADME, as well as of the role played by the chemical composition of the particles on toxicity. Studies in experimental animals are often based on test concentrations of microplastics that are several orders of magnitude higher than would be anticipated for humans. Moreover, conflicting observations of ecotoxicological effects of microplastics were reported, likely due to difficulties that exist in testing the effects of microplastics in organisms and the lack of standardized test methods. There is also a need to understand to which extent the effect of microplastics on living organisms is due to their physical effects, or due to them being a vector for other chemicals (e.g. chemicals additives or adsorbed environmental contaminants).

Discussion points

Taking in consideration ongoing and planned developments since the EFSA CONTAM Panel 2016 statement, the breakout groups will address the following questions:

1. What are the gaps in knowledge on hazard identification and characterisation of (micro/nano) plastics?

   Examples:

- Standardisation of protocols for toxicity testing
- How hazardous are the chemical contaminants adsorbed to microplastics?
- How hazardous are the microbiological contaminants adsorbed to microplastics?
- How relevant is the immune response triggered by microplastic exposure?
- What is the stability of microbial contaminants within the human body?
- Which are the effects on gut function and microbiota?
- Is there a dose-response relationship in the effects produced by microplastic particles?

[16] https://dx.doi.org/10.1021/acs.est.0c07384

2. What can be done in order to overcome the gaps in knowledge?

   Examples:

- Produce experimental data enabling the understanding of:
    a. their ability to cross the epithelial barrier in the gastrointestinal tract.
    b. the role of the physicochemical properties (size, size distribution, chemical composition, etc.)
    c. their mode of action in the human body
    d. their long-term effects…
- Make use of the expertise of other fields of knowledge, such as;
    e. Nanoparticles toxicity
    f. Medical applications (drug delivery systems)
    g. Studies on polymers and their applications and behaviour….

3. What needs to be prioritised in relation to overcoming the gaps in knowledge? The following criteria could be considered for the prioritisation:

   Criteria 1 - The results from the research recommendations for priority actions will have benefits on the establishment of harmonised definitions in Europe

   Criteria 2 - The results from the research recommendation for priority actions will increase our understanding of the hazards linked with the presence of plastic in food and feed (including insights in dose-response)

   Criteria 3 - The results from the research recommendation for priority actions will facilitate the implementation of monitoring programs at EU level.

An EU Survey questionnaire has been distributed to the registered participants of breakout groups 5 and 6 prior the event to streamline the discussion during the event and collect a preliminary list of items for the prioritisation exercise requested in question 3. This will be facilitated through an on-line poll during the breakout session.

The breakout groups should focus the discussion on ongoing and planned research activities and collaboration opportunities to make research available to regulatory science. They should consider the identification of regulatory challenges that may impact data availability for risk assessment.

Gaps in knowledge identified by participants though a survey on 16-23/04/2021

- Defining the 'harm' done by micro/nanoplastics (including effects on site of contact) and biomarkers of exposure (including changes in transcriptome, metabolome, proteome)
- Toxicity of nanoplastics/microplastic themselves (not the chemical contaminants; both human toxicity and ecotoxicity should be studied, experimental animals/models): minimal concentrations to elicit toxic effects, acute toxicity, repeat dose and chronic toxicity (Neurotoxicity; Cardiovascular effects; Nephrotoxicity; Inmune Toxicity Genotoxicity; Carcinogenicity, among others) – current data is inconclusive
- Standardization of biodegradability tests for bioplastics
- Bioaccumulation: in which organs and tissues microplastics and nanoplastics accumulate most likely and which are the processes through which they are removed from the human body, translocation of micro- and nanoplastics into cells, experimental sorption and desoprtion kinetics studies are required to develop bioaccumulation models

(also including endocrine disruptors such as BPA found in many plastics). Analyse the influence and differences of plastic types (PE, PP, PET etc.) of microplastics to cross epithelial barriers, to accumulate in organs and tissues, and to cause toxic effects. Beside particle size and material type, do also analyse shape, surface charge, biocorona. What is the size limit for nano/microplastics to enter the blood system? It is often stated that smaller particles are toxicologically more relevant. But a size-dependent evaluation is needed, taking into account multiple size classes (<200 nm, 200-1000 nm; 1-5 µm; 5-20 µm, <20 µm)

- Standardization of biodegradability tests for bioplastics
- Effect of cooking on the amount and fate of microplastics (and if possible nanoplastics) and contaminants after:industrial processing and home cooking (steam cooking, pan cooking, microwave...)
- Environmental contaminants and biofilms adsorbed to nanoplastics: adsorption studies should be done for different types of nanoplastics Polystyrene is still the one with more data, but little is known with other plastics; what is their fate after ingestion of contaminated food? The digestive processes may contribute for the increase of desorption and/or leaching of chemical toxicants. Acute and long-term exposure should both be considered.
- Toxicity of mixtures and aggregates, relevance of the composition of micro and nanoplastics, as well as the different additives used in their formulation. Cross-reactivity with other pollutants
- The human placental epigenetic response to MPs additives of concern: BPA and phtalates
- In vivo protocols for toxicity testing including toxicokinetics, acute and subacute protocols (taking into account ethical considerations that prohibit human studies)
- Setting health-based guidance values for dietary exposure
- Lack of control groups in studies on microplastics (e.g. other naturally occurring particles), using primary MP standards, which do not reflect those ingested by humans
- Testing toxicity of microplastics at environmentally relevant concentrations and of different polymer type, size, shape, composition, adsorbed contaminants, etc
- Classification and fate of micro/nanoparticles through their life cycle, based on toxicological profile (e.g., cancerogenic, teratogen, mutagenic, allergic, etc.)
- Long term-effects of micro- and nanoplastics
- Fate of nanoplastics through the end of life
- Available technologies to reduce the hazard risk of micro and nano pollutants
- Relation between micro- and microplastics and treatment technologies

Relevant reading:

1. SAPEA, Science Advice for Policy by European Academies, 2019. A Scientific Perspective on Microplastics in Nature and Society. Berlin: SAPEA. 10.26356/microplastics. Available at: https://www.sapea.info/microplastics-launch/.
2. World Health Organization, 2019. Microplastics in drinking-water. Geneva. License: CC BY-NC-SA 3.0 IGO. Available at: https://www.who.int/water_sanitation_health/publications/microplastics-in-drinking-water/en/.
3. Leslie, H. A., & Depledge, M. H. (2020). Where is the evidence that human exposure to microplastics is safe?. Environment International, 142, 105807.

Ways to overcome the gaps in knowledge, as identified through the survey

- Analyse the influence of microplastics after oral intake in the development of gastrointestinal inflammation diseases
- Dose Response modelling studies

- Analyse the influence of gastrointestinal inflammation, stomach ulcer and intestinal ulcer on the absorption and systemic bioavailability of microplastic particles in human body
- Labelling nanoplastics with fluorescent dyes to track and visualize uptake
- Produce experimental data on:
    - their ability to cross epithelial barrier, gastrointestinal tract (including the particle size able to cross these barriers; appropiate size range to be considered in the standardization)
    - their mode of action
    - their long-term effects identifying target organs/tissues and specific biomarkers of effects
    - their capacity to carry and release other chemicals (bisphenols and phthalates, additives of concern, plasticizers and endocrine-disrupting chemicals (EDCs))
    - toxicity of mixtures based on complex matrix composition
    - bioaccumulation in organs
    - effects on microbiome
    - genotoxicity
- Creating a group of reference materials with diverse characteristics (relevant particles of known toxicity) and a group of representative characteristics of microplastics to be tested
- Using control groups with naturally occuring particles, use whethered MPs and environmentally relevant doses.
- Testing for biofilm growth, and for opportunistic human pathogens in the biofirms on micro and nanoplastics (Several studies have suggested that the plastisphere may contribute to the spread of antibiotic resistance)
- Assessment of disinfection treatment on NPs/MPs (on location, at wasterwater treatment plants) – effect on particle content and adsorbed contaminants
- Addressing the methodological gaps for nanoplastic testing *in vitro*: currently used cell lines CaCo2 and HT 29 have limitations and cannot reflect the interaction with human immune system or with gut microbiota. Novel in vitro systems are needed to evaluate inflammation, necrosis, coagulation, etc
- Use adequate models for ecotoxicity, uptake and bioaccumulation
- Standards for aging and biodegradation prior to toxicity tests
- Life cycle assessment mandatory for every manufacturer (including research groups) using or producing nano/microplastics
- Classification of micro and nanoplastics based on their harmful effects
- Clear information for consumers to facilitate the choice of safe products, develop legislation on the labelling

Background documents

Vethaak AD and Legler J. (2021) Microplastics and human health. Science, 371, 672-674. DOI: 10.1126/science.abe5041

Yong CQY, Valiyaveetill S and Tang BL. (2020) Toxicity of microplastics and nanoplastics in mammalian systems. International Journal of Environmental Research and Public Health, 17, 1509 https://doi.org/10.3390/ijerph17051509

Yee MS-L, Hii L-W, Looi CK, Lim W-M, Wong S-F, Kok Y-Y, Tan B-K, Wong C-Y, Leong C-O. Impact of Microplastics and Nanoplastics on Human Health. Nanomaterials. 2021; 11(2):496. https://doi.org/10.3390/nano11020496

Nur Hazimah Mohamed Nor, Merel Kooi, Noël J. Diepens, and Albert A. Koelmans, 2021. Lifetime Accumulation of Microplastic in Children and Adults. Environmental Science & Technology Article ASAP DOI: 10.1021/acs.est.0c07384