AURORA

Researching early-life health impacts of micro- and nanoplastic

Scientific Summary

Quick Facts – What is the AURORA research project?

- Project Name: Actionable EUropean ROadmap for early-life health Risk Assessment of micro- and nanoplastics (AURORA)
- Focus on researching early-life human health impacts of micro- and nanoplastics exposure
- It is a Horizon 2020 research project funded by the European Union (Grant ID 964827; CORDIS)
- AURORA is one of five projects funded in the European Cluster to Understand the health impacts of micro- and nanoplastics (CUSP)
- Timeline: 1 April 2021 – 31 March 2026 (five years)
- 11 project partner organizations, 30+ people, 9 countries
- Coordinated by Prof. Roel Vermeulen, University Medical Center Utrecht, the Netherlands
- Project website: www.auroraresearch.eu

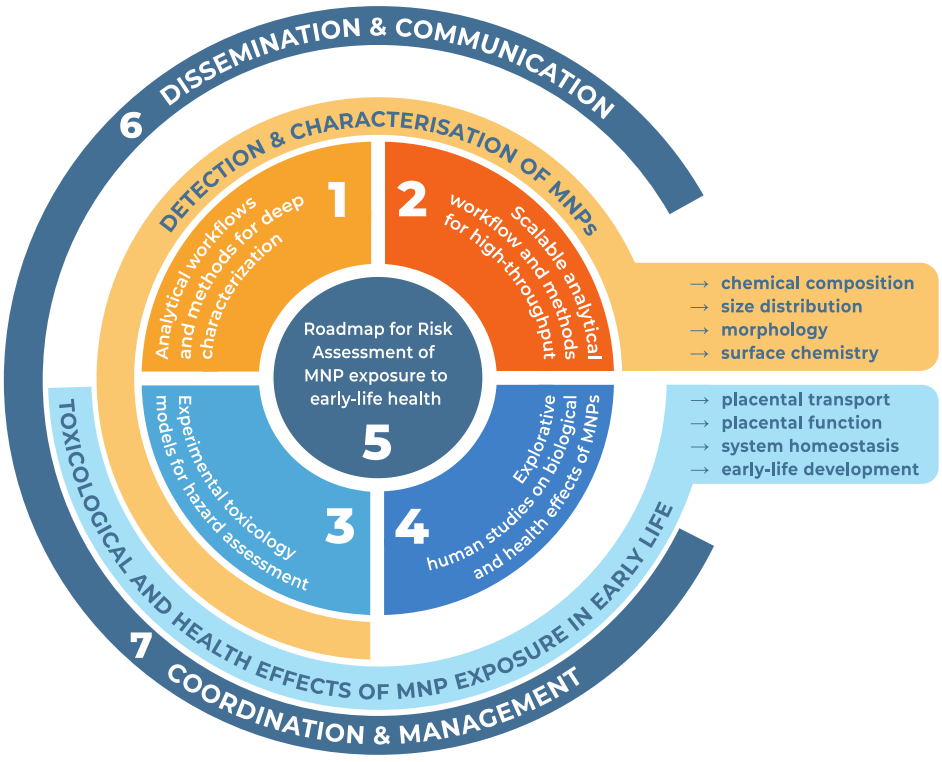

Figure 1. Overview of the AURORA project research objectives and work packages (WP)

Introduction

Micro- and nanoplastics (MNPs) are everywhere, and humans are increasingly exposed to them through air, water, and food. Disconcertingly, very little is known about the uptake of MNPs in the human body, nor about their kinetics (distribution), accumulation, and biological and health consequences. There is currently no roadmap indicating research directions and priorities.

We will address roadblocks in MNP exposure assessment by advancing analytical methods for measuring MNPs in human tissues. We will focus on MNP exposure and toxicological effects during pregnancy, in utero, and in early life. These are periods that are critical for development and health later in life, and of heightened vulnerability to environmental insults.

AURORA will assess MNP exposure at three levels (maternal, maternal/fetal interface, and fetal) and will address the effects of MNPs on early-life and female reproductive health (including perturbations to placental function, immune-inflammatory responses, oxidative stress, accelerated aging, endocrine function, and growth and development) (Fig. 2).

As much of the research on the impact of MNPs on early-life health is in its infancy, AURORA will provide a first and important step in method and knowledge development in relation to MNP risk assessment of early-life health. The newly developed understanding, methods, and tools will inform remaining knowledge and technology gaps that need to be addressed for coming to a comprehensive risk assessment of MNP exposure and early-life health and that will be disseminated through an Actionable eUropean ROadmap for early-life health Risk Assessment of micro- and nanoplastics (AURORA).

Objectives Overview

AURORA is working towards five research objectives and two support objectives:

- Objective 1: develop and advance new, low-throughput METHODS for in-depth characterization of micro- and nanoplastics in complex matricies (e.g. human tissues, foodstuffs)
- Objective 2: innovate high-throughput METHODS for use in large-scale biomonitoring and health studies of diverse human populations
- Objective 3: assess health EFFECTS in the placenta and the developing fetus using toxicological models
- Objective 4: EPIDEMIOLOGY – study effects of MNPs exposure in human populations (including two birth cohort studies)
- Objective 5: deliver an actionable roadmap for RISK ASSESSMENT by integration of results from the other objectives
- Objective 6: COMMUNICATE research findings, make results actionable to stakeholders, and support stakeholder dialogue
- Objective 7: MANAGE the project, ethics, and coordinate with other CUSP research cluster projects

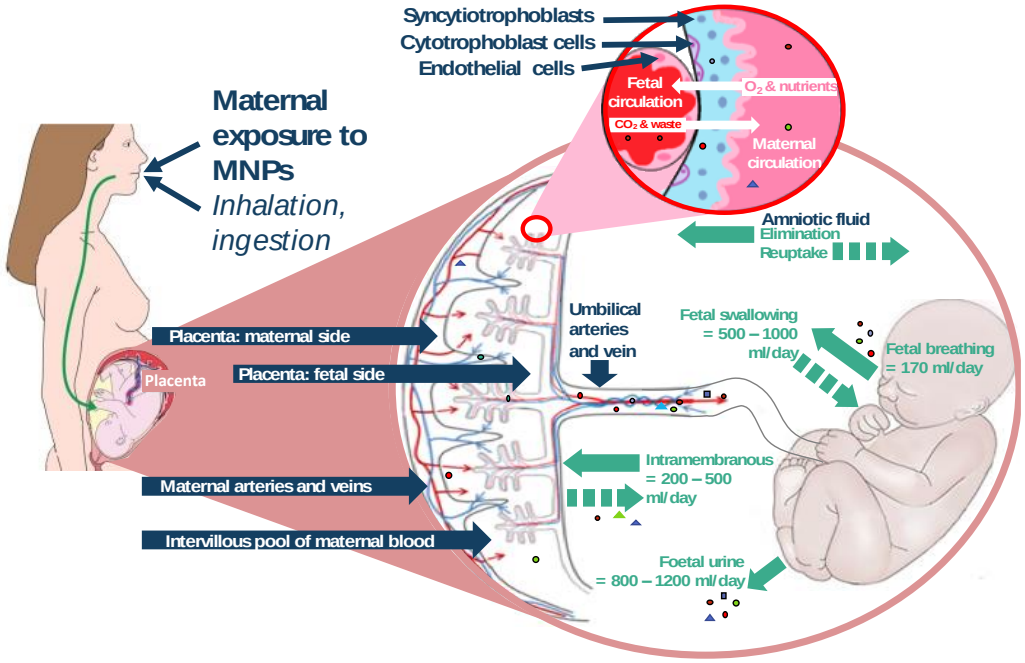

Figure 2. The routes of fetal exposure to MNPs during pregnancy. Maternal blood, urine, whole placentas, cord blood, amniotic fluid, meconium (first stools after birth; not shown in this picture), and fetal tissue will be analyzed for the presence of MNPs in Work Packages (WPs) 1-2. Toxicokinetic (i.e. distribution) and effect studies, focusing on the placental barrier and assessing the toxic effects of MNPs will be addressed in WP3. WP4 will for the first time explore possible associations between fetal MNP exposure and early-life health in human observational studies.

Objectives In Depth & Methodology

Objective 1. Exposure Characterization: to develop analytical methods and techniques for in-depth characterization of MNPs in maternal and fetal human sample matrices

Reliable detection and suitable characterization techniques for MNPs are essential to understand their possible uptake and kinetics and the biological effects of exposure to MNPs. However, many methods available for larger sized particles reach their detection limits at sizes <0.1 µm and/or cannot unambiguously distinguish sub-micron sized MNPs (i.e. NPs) from contaminating particles of similar size that are expected to be present in real-world samples. We will develop and apply an analytical workflow for detection and in-depth characterization of MNPs, covering tissue sample collection, pre-processing, and low-throughput optical and analytical chemistry techniques for human sample matrices at the maternal, maternal/fetal interface, and fetal level (see Fig. 3).

Physico-chemical properties: We will use microscopic, spectroscopic, and high-resolution mass spectrometry methods to characterize MNPs. These 10 methods will generate data on:

- quantity (particle count, mass)
- chemical composition
- size distribution
- morphology
- surface chemistry

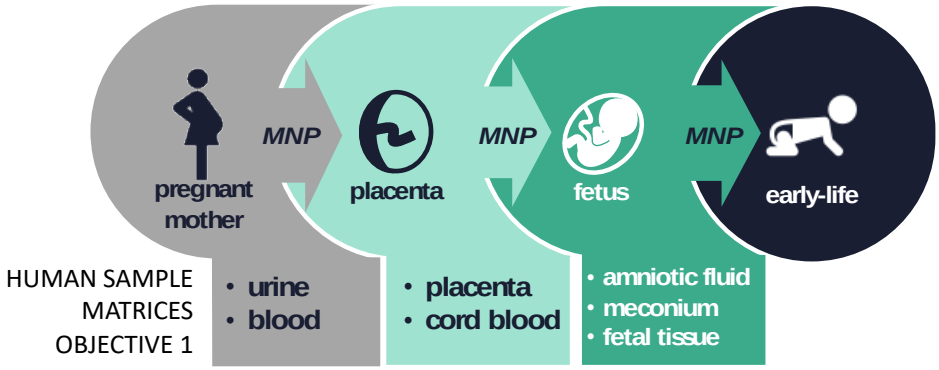

Figure 3. Human sample matrices covered in in-depth exposure characterization workflows of Objective 1

Testing materials: The following MNPs will be comprehensively characterized (Objective 1) and undergo toxicological testing (Objective 3), using a tiered approach:

- Pristine: beads and irregularly shaped; multiple polymers and sizes
- Pristine: fibers (synthesized)
- Degraded: mimicking environmental weathering and biological (gastric) degradation
- Extracted: household dust
- Extracted: human tissue
- Bioplastics: PHA & PLA
- Non-synthetic homologues: including wool fibers

Objective 2. Scalable Exposure Assessment: to develop a high-throughput analytical workflow with scalable methods and techniques for quantitively assessing maternal and fetal exposure to MNPs in large population cohorts

We will develop and validate analytical protocols and techniques for high-throughput exposure assessment of MNPs, their additives, and plastic related compounds in maternal urine, maternal blood, placenta, and cord blood. High- throughput methods are essential for comprehensive exposure surveys and to reach the scale necessary to conduct meaningful human studies.

Using high-resolution mass spectrometry platforms (Pyr-, GC-, and LC-HRMS), we will (semi-)quantitatively measure MNPs while allowing for the characterization of particle bound monomers and additives, non-intentionally added substances (NIAS), and free plastic-related compounds.

Over 5300 polymer formulations are commercially available (Groh et al. 2019 Sci Tot Environment, 651, 3253-3268). Furthermore, MNPs not only include the component polymers themselves (e.g., polyethylene, polypropylene) but also residual monomers and oligomers, chemical additives (e.g., phthalates), and non-intentionally added substances (NIAS, i.e., impurities, reaction by-products, and degradation products). Therefore, AURORA will leverage recent advances in untargeted chemical screening to comprehensively assess MNP-associated chemicals.

Adapting the untargeted HRMS approaches to MNP research will not only allow detection of a diverse series of plastic particles, but also characterization of bound additives, free plastic related compounds, and biological response measures from alterations in metabolic pathways.

Objective 3. Toxicology: to assess the toxicity as well as the toxicokinetics and -dynamics of MNPs in experimental models

This objective focuses on the potential uptake, accumulation, and transport of MNPs in the placenta (i.e. the maternal/fetal interface), as well as toxic effects on the placenta and developmental toxicity of MNPs. Here, the size, morphology, and surface chemistry of MNPs are deemed relevant as well as chemical composition as effects may also arise from the chemicals that are adsorbed to and/or are leaching from MNPs.

We will use a suite of complementary experimental models with increasing complexity, from single-cell to whole embryo, to assess toxicokinetics, toxicodynamics, and toxicity. These include in vitro placental mono- and multicellular models, placental perfusion models using fresh human placentas, and embryonic toxicity models using mouse embryonic stem cells (functional units) and (in-vivo) zebrafish embryos (organism level).

Toxicological responses: Using a tiered approach, AURORA will evaluate if MNPs exert toxic responses related to:

- Placental transport
- Placental function
- System homeostasis
- Early-life development

Figure 4. Tiered approach to investigate transport, accumulation, and toxicity of MNPs in placenta and embryo using toxicological models with varying complexity and origin

Objective 4. Epidemiology: to determine the relationship between MNP exposure and female reproductive and early-life health

AURORA will be the first to generate preliminary evidence of the potential (long-term) effects of MNPs on early-life health and during pregnancy. We will use European birth cohorts that have collected and are currently collecting placenta and other tissue samples and will measure MNPs in these samples to assess early-life and maternal exposures: BISC (Spain, 2019–ongoing) and ENVIRONAGE (2010–ongoing). We will exploit the uniquely rich data on placental function available in these cohorts, along with other biomarkers and outcomes hypothesized to be affected by MNPs:

- Reproductive health effects: including perturbations to placental function, premature placental aging, and immune-inflammatory responses
- Early-life health effects: including perturbations of immune-inflammatory responses, oxidative stress, accelerated aging, endocrine function, and growth and development

We will also generate new outcome data, including metabolomics data, to also identify changes in the system homeostasis associated with MNP and MNP-associated chemical mixture exposures.

Objective 5. Advance Risk Assessment: to develop an actionable roadmap for risk assessment of MNPs

Together with the results from the comprehensive exposure assessment (Objectives 1 and 2) and hazard identification and dose-response efforts (Objectives 3 and 4), in AURORA we will create a general framework and actionable roadmap for early-life health risk assessment of MNP exposure by i) identifying the key remaining knowledge and technology gaps and ii) formulating recommendations to further advance the field of MNP and early-life health risk assessment (Fig. 5). This roadmap will cover approaches/techniques relevant to a) chemicals (including non-intentionally added substances), b) micro- /nano-particles and fibers, c) mixtures, d) the combination of chemicals and micro-/nano-particles and fibers, and e) developmental and reproductive toxicity.

Intervention study: In addition to assessing the impact of MNPs on child and maternal health, we will conduct a proof-of-principle intervention study to evaluate if the body burden MNPs can be reduced by switching to non-plastic food packaging – an exploratory risk management effort.

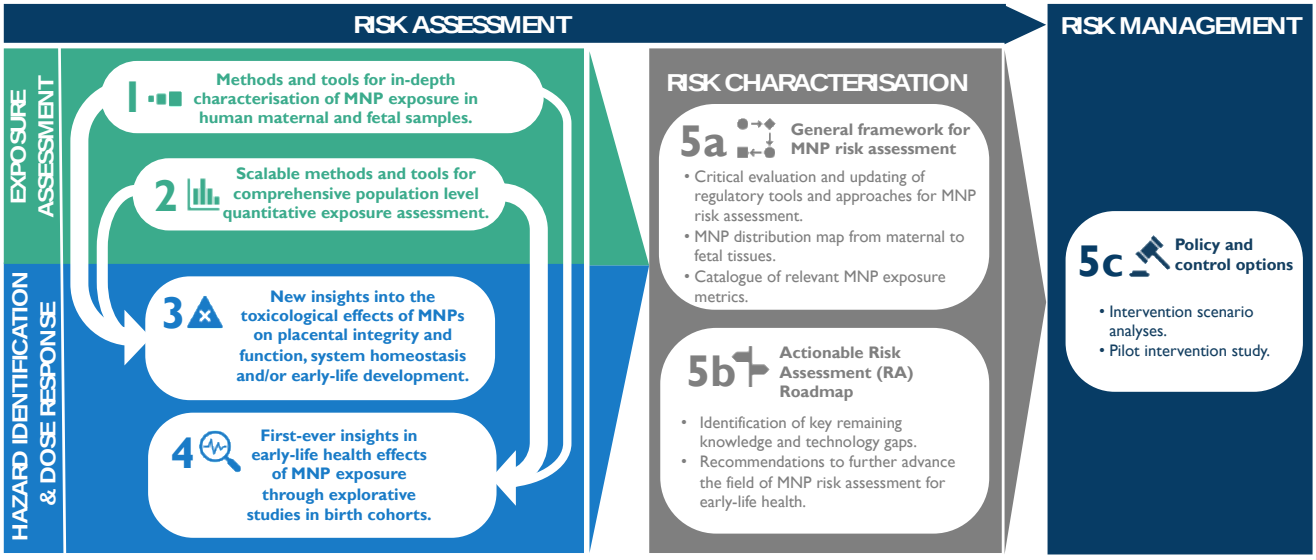

Figure 5. Overview of the main components of the AURORA risk assessment roadmap for MNP exposure for early life health

Project Partners & Executive Board

The AURORA consortium comprises eleven partners from eight European countries and one from the United States (Fig. 6).

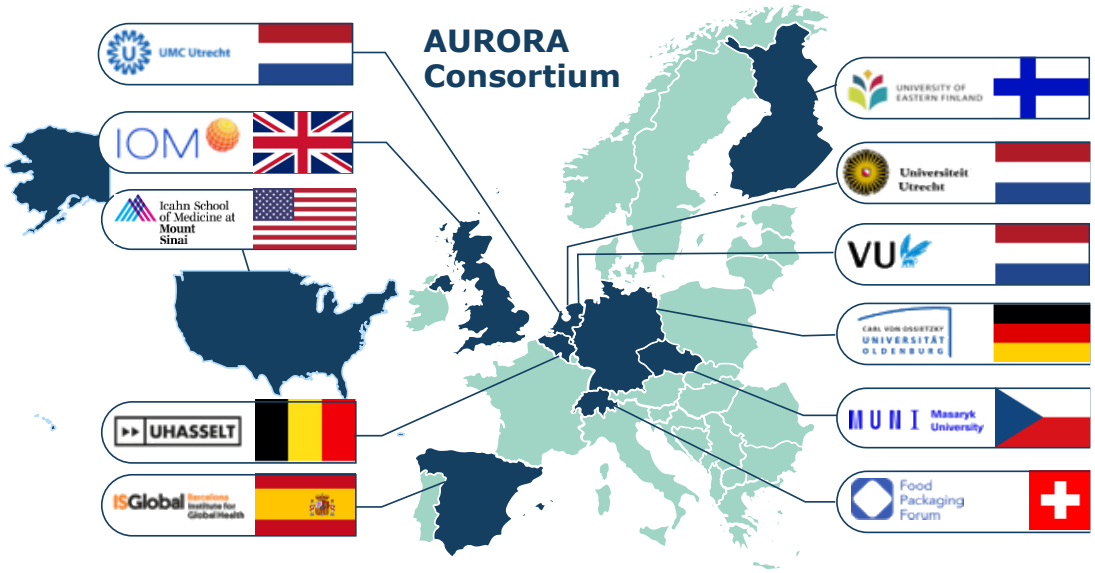

Figure 6. Partners within the AURORA consortium

The consortium brings together leading institutions in exposure assessment and biomonitoring (i.e. MSSM, MU, HU), exposome science (UMCU, MSSM, ISGlobal), catalysis (UU), spectroscopy (UU, HU), analytical chemistry (MSSM, UO, MU), reproductive and developmental toxicology (UU, VU, UEF), early-life health (UMCU, ISGlobal, HU), risk assessment (UMCU, IOM, FPF), and communication (FPF) (see Table 1).

Table 1. AURORA partners and an overview of their expertise and roles

Partner	Country	Specific expertise and technology provided and associated roles	Relevant WPs
University Medical Center Utrecht (UMC Utrecht) (Coordinator)	The Netherlands	› Epidemiology, exposomics, statistical modelling › Risk assessment › Overall project management › Access to human samples from A-RISK	4 5 7
ISGlobal	Spain	› Epidemiological analyses › Access to human samples from BISC Birth cohort	4
Hasselt University (HU)	Belgium	› Epidemiological and statistical chemistry analyses › Analytical chemistry, in-depth characterization of MNPs in human samples › Access to human samples from the ENVIRONAGE Birth Cohort	4 1
Mount Sinai (MSSM)	United States	› Analytical chemistry with focus on scalable methods for characterization of MNPs in human samples › Untargeted methods for exposomics and emerging exposures	1, 2 1, 2, 4

Table 1. Continued

Partner	Country	Specific expertise and technology provided and associated roles	Relevant WPs
Utrecht University (UU)	The Netherlands	› Toxicological analyses › Analytical chemistry, in-depth characterization of MNPs in human samples	3 1
Masaryk University (MU)	Czech Republic	› Measuring MNPs using targeted HRMS › Risk Assessment strategies and methodologies	2 6
Vrije Universiteit (VU)	The Netherlands	› Toxicological analyses, in vitro models › Access to fetal samples from FREIA	3 1
University of Eastern Finland (UEF)	Finland	› Provision of placental perfusion model for toxicological endpoints	3
Food Packaging Forum (FPF)	Switzerland	› Systematic scientific literature review and data extraction › Risk Assessment strategies and methodologies › Wider stakeholder engagement, translation of scientific results into policies, communication, and dissemination	5 5 6
Institute of Occupational Medicine (IOM)	United Kingdom	› Risk Assessment strategies and methodologies	5
University of Oldenburg (UO)	Germany	› Analytical chemistry, in-depth characterization and scalable methods of MNPs	1, 2

The AURORA project will be run by an Executive Board composed of work package (co-) leaders (see Table 2). The project is coordinated by UMC Utrecht under the leadership of Prof. Roel Vermeulen.

Table 2. Composition of the AURORA Executive Board

Work Package (WP)	WP Lead	WP co-lead(s)
1. In-depth characterization workflows and methods	Florian Meirer (UU)	Tim Nawrot (HU)
2. Scalable Exposure Assessment	Petra Přibylová (MU)	Doug Walker (MSSM)
3. Experimental Toxicology methods	Markus Forsberg (UEF)	Majorie van Duursen (VU)
4. Explorative Human Studies	Tim Nawrot (HU)	Martine Vrijheid (ISGlobal)
5. Advanced Risk Assessment	Virissa Lenters (UMCU)	Matthew Boyles (IOM)
6. Dissemination & Impact	Jane Muncke (FPF)	Roel Vermeulen (UMCU)
7. Consortium Management	Roel Vermeulen (UMCU)	Virissa Lenters (UMCU)

Project Planning

The Gantt chart shown in Table 3 depicts the timing of the scientific tasks (with abbreviated titles) involved in work packages one through five. Tasks related to Dissemination, Project Management and Ethics run throughout the project.

Table 3. Gantt chart of scientific tasks within the first five AURORA work packages

Work Package / Tasks		Project Month																			
		3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
1	1.1 Establish fingerprints																				
	1.2 Optimization methods																				
	1.3 Develop workflow and SOPs																				
	1.4 In-depth characterization of MNPs																				
2	2.1 Optimization HT protocols																				
	2.2 MNP fetal exposure assessment																				
	2.3 Develop and validate statistical classifiers																				
	2.5 Toolset for MNP biomonitoring																				
3	3.1 Tier 1 analyses																				
	3.2 Tier 2 analyses																				
	3.3 Tier 3 analyses																				
	3.4 Tier 4 analyses																				
4	4.1 Determinants and sources of MNP exposure																				
	4.2 Placental function																				
	4.3 Placental inflam. and biomol. markers of ageing																				
	4.4 Metabolomic perturbations																				
	4.5 Early life growth and development																				
5	5.1 RA framework																				
	5.2 Proof-of-Principle intervention study																				
	5.3 Update methodologies to assess MNP exposure																				
	5.4 Update methodologies for MNP hazard assessment																				
	5.5 Actionable roadmap																				

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