CUSP

The European research cluster to understand the health impacts of micro- and nanoplastics

www.cusp-research.eu
hello@cusp-research.eu
https://doi.org/10.5281/zenodo.11035612

Policy brief 2

MICRO- AND NANOPLASTICS AND PUBLIC HEALTH: A REASONABLE CONCERN

Unveiling the risks

The European Commission's Research Cluster to Understand the Health Impacts of Micro- and Nanoplastics (CUSP https://cusp-research.eu) is now three years into its endeavour to develop new analytical tools, share data, conduct inter-laboratory comparisons, and communicate and disseminate research results on the health impacts of micro- and nanoplastics (MNPs).

To date, the five research projects have published more than 60 scientific peer-reviewed research papers and reports, and much more research is underway.

The findings from CUSP have already contributed to filling key knowledge gaps (see Figure 1). It is clear that MNPs are a public health concern, although the health risks remain unclear and the hazards, exposures and risks of individual types of plastics and their specific chemical additives still need to be determined. This is particularly the case in the long term.

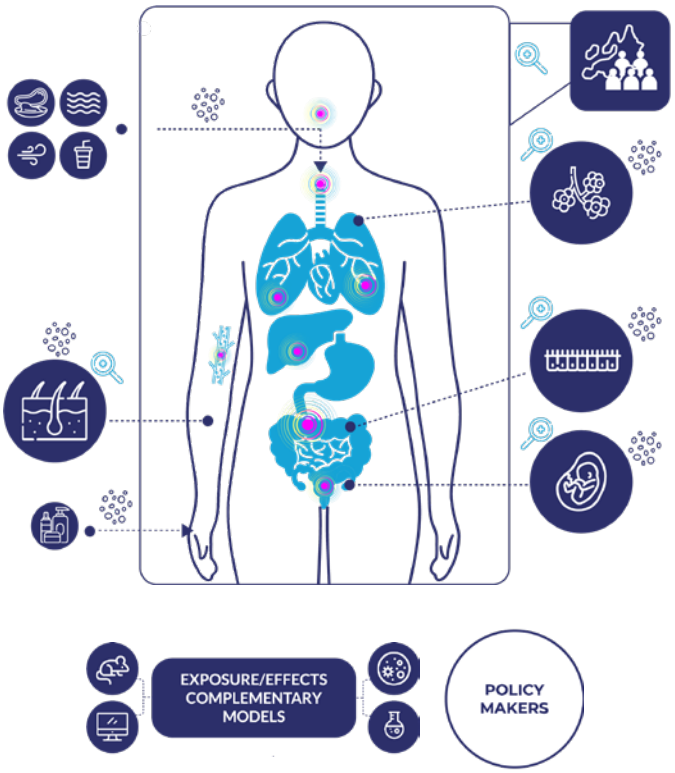


Figure 1: Overview of CUSP main areas of contribution.

Key messages

1. CUSP researchers have documented the potential carcinogenicity, mutagenicity and reproductive toxicity (CMR) of micro- and nanoplastics (MNPs), primarily *in vitro* and with polystyrene.
2. The effects of nanoplastics have been observed to be more pronounced than those of microplastics.
3. The main routes of exposure of MNPs to the human body appear to be inhalation and ingestion. Small MNPs have been found to translocate into the blood.
4. Most of the studies conducted so far are short-term studies and there is a significant gap in understanding the long-term effects of MNPs on human health. More effort is also needed to better establish dose-responses and modes of action.
5. It is important that future studies are conducted on representative MNPs of different chemical compositions, and with physico-chemical characteristics better resembling those MNPs found in the environment.

These projects have received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreements AURORA n° 964827, IMPTOX n° 965173, PLASTICHEAL n° 965196, PLASTICFATE n° 965367, POLYRISK n° 964766.

www.cusp-research.eu
hello@cusp-research.eu
https://doi.org/10.5281/zenodo.7101443

CUSP
The European research cluster to understand the health impacts of micro- and nanoplastics

REGULATORY INSIGHTS: CUSP RESEARCH ON MNPS

The CUSP findings within areas such as carcinogenicity, mutagenicity and reproductive toxicity (CMR), respiratory toxicity and fate and exposure assessment of MNPs can inform European policies and legislation on chemicals, plastics, food, and water (see table 1).

Key areas covered by CUSP research

		CMR	Respiratory toxicity	Immunotox-icology	Cytotoxicity	Human gut microbiota	Sampling, measuring, monitoring	Fate and exposure assessment
Chemicals	Zero pollution action plan	X	X	X	X	X	X	X
	Chemicals Strategy for Sustainability	X	X	X	X	X	X	X
	REACH Regulation (EC) No 1907/2006	X	X	X	X	X	X	X
	Classification labelling and packaging (CLP) Regulation (EC) No 1272/2008	X	X	X				
	Horizon Europe Mission on Cancer	X						
	Chemical Agents Directive (98/24/EC) EU Strategic Framework on Health & Safety at work 21-27	X	X				X	X
	Carcinogens and Mutagens Directive(2004/37/EC)	X						
Plastics	EU Plastic Strategy COM 2018/028	X	X	X	X	X	X	X
	Single Use Plastics Directive (EU) No 2019/904	X	X	X	X	X	X	X
	Bioeconomy Strategy							X
Food	Food Contact Material (FCM) Regulation (EC) No 1935/2004						X	X
	Plastic FCM and articles Commission Regulation (EU) No 10/2011						X	X
	Active and intelligent materials and articles FCM Commission Regulation (EC) No 450/2009	X	X	X	X	X		
	EU-Sustainable food Farm to Fork Strategy						X	X
Water	Drinking Water Directive/Urban Waste Water Treatment Directive and Sewage Sludge Directive/Marine Strategy Framework Directive						X	X
	Horizon Europe Mission on Ocean and Waters	X		X				

Table 1: Overview of CUSP findings that can help inform different EU policy and legislative areas.

Carcinogenicity, Mutagenicity and Reproductive toxicity of MNPs

Three years into the CUSP, researchers have documented the potential carcinogenicity, mutagenicity and reproductive toxicity (CMR) of MNPs.

For example, a review by Domenech *et al.*[1] concluded that most of the studies reviewed indicated the potential of MNPs to induce inflammation and genotoxicity, the latter being recognised as a strong predictor of carcinogenicity. 40% of the studies reviewed were conducted in rodents, although none of them were a 2-year carcinogenicity study, which is traditionally considered the gold standard in carcinogenicity assessment. The ability of MNPs to accumulate in cells and tissues, or their ability to induce fibrosis, may also suggest a relationship between exposure to MNPs and carcinogenic potential. In addition, life-like polyethylene terephthalate (PET) nanoparticles derived from plastic bottles showed carcinogenic capacity through a tumour promoting mechanism when tested in a validated *in vitro* cell transformation assay, whereas no effects were observed for pristine polystyrene and secondary polylactic acid nanoplastics. This suggests that there may be a notable difference between different types of MNPs and between pristine and secondary MNPs[2].

In terms of mutagenicity, adverse effects have also been reported in CUSP research. For example, Alaraby *et al.*[3] investigated the effect of different sizes of polystyrene in an *in vivo* study on fruit fly larvae and observed a broad molecular response altering the expression of genes involved in the general stress response, antioxidant response, genotoxicity response and intestinal damage response. A size-dependent general induction of reactive oxygen species (ROS) production and DNA damage was also observed, with the small polystyrene particles inducing a higher response. Similar gene disruption was observed in fruit flies following exposure to PET nanoplastics from plastic bottles, providing more realistic data on the potential adverse effects of environmental MNPs[4]. A number of CUSP studies have also investigated the combined effects of exposure to MNPs and different environmental pollutants. Barguilla *et al.*[5] found that co-exposure to nanoplastics and arsenic increased DNA damage and the aggressive features of the initially transformed phenotype in the connective tissue of developing mouse embryos. In contrast, Alaraby *et al.*[6] found that oxidative stress and DNA damage caused by silver, especially nanosilver, were dramatically reduced by co-exposure with polystyrene nanoparticles.

Dusza *et al.*[7] noted that *in vivo* rodent studies have demonstrated MNPs' ability to cross the placental barrier and accumulate in the internal organs of offspring, leading to metabolic disturbances, behavioural changes, neurotoxicity, and increased mortality. These effects are believed to be related to changes in gene expression, oxidative stress, inflammation and cell death. MNPs have been found in human blood, placental tissue, and meconium. However, the potential impact of MNPs on pregnancy and foetal development remains largely unexplored. Given that pregnancy and early life are the most vulnerable periods of human development, the effects observed in *in vivo* studies are of concern.

1 Domenech, J. et al. Mutat Res Rev Mutat Res 2023 791:108453
2 Domenech, J. et al. J. Hazard Mater 2024 469:134030
3 Alaraby, M. et al. Environ Sci: Nano 2022 9:1845-1857
4 Alaraby, M. et al. Sci Total Environ 2023 863:160954
5 Barguilla, I. et al. Int J Mol Sci 2022 23: 2958
6 Alaraby, M. et al. Sci Total Environ 2022 842:156923
7 Dusza, H.M. et al. Sci Total Environ 2023 860:160403

Respiratory toxicology

In addition to the CMR properties of MNPs, CUSP has also investigated the respiratory and immunotoxicity of MNPs. Human primary nasal epithelial cells were used by Annangi *et al.*[8,9] as a model of the first barrier of the respiratory system to study polystyrene and life-like PET MNPs. Significant cellular uptake and increased levels of intracellular radical oxygen species were observed, as well as a loss of mitochondrial membrane potential in exposed cells. Identifying the potential drivers of airborne MNP toxicity is a critical step in better assessing the potential health risks posed by respirable microplastic pollution. Wieland *et al.*[10] investigated the potential role of size, shape, ζ-potential, adsorbed molecules and microorganisms, and biopersistence in the toxicity of airborne microplastics and found that all are likely to be relevant. Therefore, special efforts must be made to understand the particular physicochemical form in which airborne MNP particles occur in our environment (homes, urban air, workplaces).

8 Annangi, B. et al. Environ Toxicol Pharmacol 2023 100:104140
9 Annangi, B. et al. Biomolecules 2023 13:220
10 Wieland, S. et al. J Hazard Mater 2022 428:128151

Immunotoxicology

Using polystyrene ranging in size from a few hundred nanometres to several microns, Collin-Faure *et al.*[11] showed that polystyrene altered the normal function of macrophages in a size- and dose-dependent manner, even at non-toxic concentrations of polystyrene. Macrophages are important because they capture particular material to facilitate the removal of foreign substances from the body. Specifically, changes in oxidative stress, lysosomal and mitochondrial function, and the expression of various surface markers involved in the immune response were observed. Van den Berg *et al.*[12] investigated the effect of environmentally weathered polystyrene on dendritic cells, which are antigen-presenting cells involved in the regulation of immune responses in humans. 0.2, 1 and 10 µm polystyrene particles were found to induce phenotypic and functional maturation of the cells at various concentrations ranging from 1 to 100 µg/ml. No acute cytotoxicity or ROS production was observed for PET NPs (200 nm) in human peripheral blood mononuclear cells tested at various concentrations up to 100 µg/ml[13].

11 Collin-Faure, V. et al. Front. Immunol. 14:1092743

12 Van der Berg, A.E.T. et al. J Immunotoxicol 2022 19(1):125–133
13 Djapovic et al. Polymers 2023 15(24): 4703

Human gut microbiota

The detection of MNPs in most categories of consumed food and drinking water is indicative of widespread human intake. It is therefore important to understand how the gastrointestinal tract, particularly the intestine, interacts with these small particles. Recent *in vivo* studies and *in vitro* models of the gastrointestinal tract have shown that MNPs of different types and sizes affect intestinal bacteria, thereby affecting intestinal homeostasis[14]. Biotransformation of e.g. PET and polylactic acid (PLA) microplastics has also been reported, and feeding of MP has been shown to alter the composition of human colonic microbial communities, suggesting that microplastics are indeed capable of exerting health effects at the digestive level[15,16].

14 Jiménez-Arroyo, C. et al. Microb Biotechnol 2023 16:34–53
15 Tamargo, A. et al. Sci Rep 2022 12:528
16 Jiménez-Arroyo, C. et al. Sci Total Environ 2023 902:166003

Fate and exposure assessment (1/2)

In chemical risk assessment[17], the concentrations used in *in vitro* tests should be compared with *in vivo* exposure conditions[18]. The main routes of exposure of MNPs to the human body are via the respiratory and gastrointestinal tracts (GIT) through inhalation or ingestion, but also via the skin through the use of personal care products (PCPs) containing MNPs. Once MNPs have entered the human body, they may be translocated from the exposed organ to other body compartments. Based on current knowledge, CUSP researchers have concluded that dermal translocation of MNPs is unlikely. In contrast, small MPs and NPs can generally translocate from the gastrointestinal and respiratory systems to the blood and thus to other tissues and organs[19]. CUSP researchers have quantitatively tracked the uptake and transport of 200 nm polystyrene particles doped with the europium chelate Eu-β-diketonate (polystyrene-Eu) in wheat and lettuce[20]. It was found that polystyrene Eu particles accumulated mainly in the roots, while transport to the shoots was limited (e.g. <3% for 5,000 µg polystyrene particles per litre exposure). The study also showed that 250 nm gadolinium-labelled polystyrene was taken up by lettuce and that the type of polymer affected the biodistribution of particles in lettuce (roots and leaves) and the number of particles transferred from plants to insects feeding on treated lettuce and from insects to insect-feeding fish, accumulating mainly in the fish liver[21].

Once in the environment, MNPs have been shown to be covered by a layer of molecules, the so-called corona, consisting mainly of natural organic matter (NOM), polysaccharides and proteins. This bio-corona has been observed to be very efficient in stabilising microplastics in solution. The stability depends on several parameters such as the intrinsic properties of the particle (size, density, hydrophobicity) and the corona formation, which changes the particle wettability, electrostatic charge and steric hindrance[22].

Vela *et al.*[23] studied the potential effects of digestion on the physicochemical/biological properties of polystyrene nanoplastics and found a high tendency of digested polystyrene nanoplastics to agglomerate and a differential presence of proteins on their surface. The digested polystyrene nanoparticles

17 ECHA 2017. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance. DOI: 10.2823/337352
18 (See note number 15).
19 Romero-Andrada, A.L. et al. Arch Bronconeumol 2023 59(11):709-711; Ramsperger, A.F.R.M. et al. NanoImpact 2023 29: 100441; Jiménez-Arroyo, C. et al. Sci Total Environ 2023 902:166003
20 Luo, Y. et al. Nat Nanotechnol 2022 17:424–431

21 Monikh et al. 2022 Nano Today 2022 46:101611
22 Schwartz, M. et al. Langmuir 2023 39(12):4291–4303
23 Vela, L. et al. Environ Pollut 329 (2023):121656

CUSP
The European research cluster to understand the health impacts of micro- and nanoplastics

Fate and exposure assessment (2/2)

showed greater cell uptake than undigested particles in all three cell lines tested (TK6, Raji-B and THP-1), but no differences in toxicity were observed, except for high - and assumed unrealistic - exposures. The effect of polystyrene MPs (10 µm) on food digestion was assessed by monitoring the kinetics of protein hydrolysis through static *in vitro* gastric digestion of cow's milk. Digestion of cow's milk mixed with polystyrene resulted in transient accumulation of larger peptides and reduced bioavailability of short peptides in the gastric phase. The casein epitopes relevant to persistent cow's milk allergy (αS2-casein), which are otherwise rapidly digested, were identified in the hard corona of polystyrene[24]. The effect was polystyrene concentration and size dependent, with smaller particles and only at higher concentrations inducing measurable effects.

24 De Guzman, M.K. et al. Environ Poll 2023 335:122282

ADDRESSING KNOWLEDGE GAPS (1/2)

The available evidence indicates that MNPs may have adverse effects on human health, as demonstrated by both *in vitro* and *in vivo* animal studies. It is important to acknowledge this evidence, especially for submicron-sized particles.

However, it is worth noting that the strength of the evidence is difficult to assess due to the high concentrations of tested particles, which some may consider unrealistic. Several scientific knowledge gaps remain:

Representative materials. Most **studies** on CMR, respiratory toxicity, etc. were **performed on one size and shape** (often spherical) polystyrene MNPs. It is important that these studies are performed with other MNPs of **varying sizes and chemical compositions.**[25]

25 Busch, M. *et al.* Front Toxicol 2023 5:1112212

Information transparency. There is a general **lack of use, production, health and safety information** on different types of commercially available MNPs and the additives that are used in plastics. This lack of information may be hindering the testing of **relevant combinations of MNPs and additives**.

***In vitro* studies.** In line with the EU's commitment to replace, reduce, and refine the use of animals in research and testing (Directive 2010/63/EU), **the majority of CUSP studies are carried out in vitro**.

CUSP have identified[26] several challenges and considerations for *In vitro* testing of plastic particles. These include the **low density of polymers**, which affects particle behaviour in cell culture media; **differences between applied and effective doses** due to particle sedimentation rates and aggregation, the influence of the biomolecular corona on particle interaction with cells, **potential assay interference by particles**, the use of fluorescently labelled nanoplastics for **tracking internalisation**, **size limitations** of particles for organ-specific studies, **chemicals in nanoplastic samples**, and the **relevance of model particles** compared to environmental occurrences.

These factors particularly complicate the hazard assessment of nanoplastics and **require careful experimental design and interpretation**.

26 Busch, M. *et al.* Front Toxicol 2023 5:1112212.

Body barriers. There is a need for systematic studies to understand the fate of MNPs and what happens during their journey through the **intestinal tract**. The study of their interactions with **midgut bacteria, intestinal enzymes** and the **acidic intestinal environment**, as well as their **potential effects on the gut microbiota** and **digestive diseases**, remains an area requiring more comprehensive research. Likewise, the fate of inhaled MNPLs in the **respiratory tract**, its behavior in the context of **pulmonary surfactant**, and its potential role in **respiratory pathologies** and **lung microbiome alterations** needs to be better determined.

Long-term studies. Most studies conducted so far are **short-term studies** and **there is a significant gap in understanding the long-term effects of MNPs on human health and the environment**.

www.cusp-research.eu
hello@cusp-research.eu
https://doi.org/10.5281/zenodo.7101443

CUSP
The European research cluster to understand the health impacts of micro- and nanoplastics

ADDRESSING KNOWLEDGE GAPS (2/2)

Causal linkages. The exact **dose-response relationship and specific mechanisms of action** by which MNPs exert their final adverse outcomes are still not fully understood. Strategies to improve this area will be highly beneficial to identify and predict more efficiently the adverse effects of MNPs, and to facilitate the use of the information for regulatory purposes.

Analytics. Despite of the progress, the analytical workflow to determine the MNPs exposure in the environment and inside the human body with sufficient spatial resolution, specificity and sensitivity, require further optimization and standardization efforts.

Vector effects. The **direct implications of MNPs** and their interactions with other contaminants on human health, beyond the cellular level to more systemic effects, are not yet fully understood. Their **potential to act as carriers of other hazardous contaminants**, including human pathogens, and to modulate their uptake and harmful effects is underexplored.

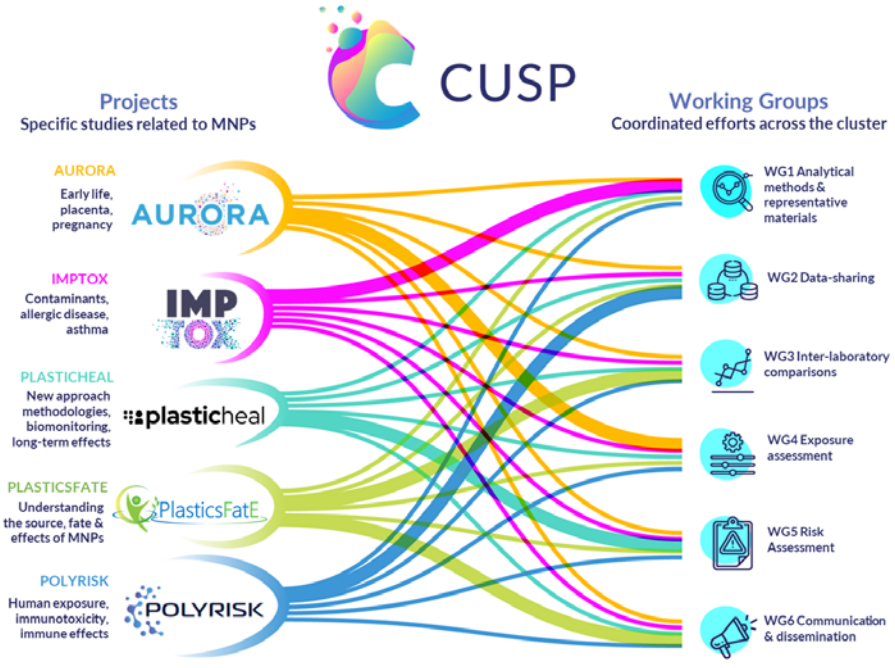
Regulatory acceptance. Most of the studies conducted to date have been *In vitro*, and data from currently available *In vitro* tools are not yet considered adequate to be used alone for regulatory decision making regarding risk assessment and classification and labelling for repeated dose toxicity[27]. All **barriers that currently impede the use of in vitro studies for risk assessment and classification and labelling of substances** need to be understood and addressed.

27 See note number 17.

Environmental fate and exposure. Uncertainties exist regarding the **environmental distribution of MNPs**, their **persistence** and the main **environmental pathways** by which humans and other organisms important for human nutrition are exposed to these particles[28]. There is a need for **long-term exposure models** to better mimic real-world scenarios and for chemical assessment methods for complex matrices[29].

28 Barguilla, I. et al. Int J Mol Sci 2022, 23:2958
29 Barguilla, I. et al. Int J Mol Sci 2023 24: 7851

High-risk population groups. Determining the factors able to modulate the individual risk to MNPLs exposure is very relevant to make proper decision-making. CUSP investigations indicate the necessity to protect early life for the health of future generations, but other population groups with potential higher susceptibility need to be explored in the future.



Contact us at
www.cusp-research.eu
hello@cusp-reseach.eu
CuspResearch
CuspResearch

AURORA www.auroraresearch.eu
IMPTOX www.imptox.eu
plasticheal www.plasticheal.eu
PlasticsFatE www.plasticsfate.eu
POLYRISK www.polyrisk.science

These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 964827 (AURORA), No. 965173 (IMPTOX), No. 965196 (PlasticHeal), No. 965367 (PlasticsFatE), and No. 964766 (POLYRISK).