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*"Micro- and Nanoplastics in Focus:*

*Bridging Allergic Disease, Toxicology, and Environmental Concerns"*

# Toxicology of micro/nanoplastics: what we know, what we need to know


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# Defining the topic of this talk

- **Micro-nanoplastics (MP/NP) a pervasive and increasing problem of environmental pollution**

- **A concern also for risk assessment in regard of human health.**

**Understanding and assessing adverse health effects *increasingly complex*, as more evidence is collected.**

**Let's avoid *drowning into complexity*!**

**many important uncertainties do exist, *but***

**evidence leads to the definition of critical concepts**

# Absorption of MP/NP in mammalian organisms? To what extent do they reach internal tissues?

**Bioavailability of MP/NP depends on size**
**It is considered to reach 1% and above only for MP below 1.5 microns**

**Low absorption, but**
***slow continuous*, low-level, long-term due to pervasive exposure-accumulation in the body, possibly including comparts considered as "sheltered" (e.g., sperm).**

**So, in parallel with bioaccumulating pollutants (PCB) and for-ever chemicals (PFAS)**
**MP/NP might be assessed in terms of body burden**

**NP below 0.1 microns are obviously more bioavailable,**
**but difficult to measure reliably.**
***Sensitive harmonized methods* for measuring NP are needed because of their potential toxicological relevance (see below)**

# Ingestion

**Mostly consumption of *foodstuffs (*clams and similar molluscs - sedentary filtering organisms- fish, table salt, sugar, packaging, etc.etc.)**

***After ingestion***

**specialized epithelial Microfold (M) cells actively transport luminal antigens to the underlying lymphoid follicles (Peyer's patches) to initiate an immune response;**

**Insoluble particles may penetrate the intestinal mucus through the increase in solubility due to the adsorption of a "corona" of biocompatible intestinal contents (Ca,P salts) or *small size*;**

**Internalization of particles by paracellular transfer of particles through the single layer of the intestinal epithelium (persorption)**

**Also a*fter inhalation* (generally *lower exposure than ingestion*, yet not to be overlooked):**
**clearance by macrophages or**
**migration to blood and/or lymph circulation may contribute to body burden**

# MP in a *sheltered* compart: human semen

**(*Montano et al., Sci Total Env, 2023*)**

**semen from men living in a polluted area of Southern Italy**

***Raman Microspectroscopy:*** **16 pigmented MP (2-6 µm) with spheric or irregular shapes in six out of ten samples (60%).**

**Chemical composition *very diverse*: polypropylene, PET, polystyrene, PVC, polycarbonate, polyoxymethylene, acrylic, suggesting dietary/environmental exposure (rather than occupational)**

***Hypothesis*: rather than passing the blood-testis barrier,**

**MPs pass into the semen through the epididymis and seminal vesicles, which are also tissues highly susceptible to inflammation.**

# Besides size, *other factors* involved in bioavailibility and toxicity?

- **Chemistry: for instance, in mussels, (*Wei et al., Mar Environ Res, 2021*)**
- **Both polyethylene and polystyrene increased oxidative stress biomarkers (eg, superoxide dismutase) and altered energy and lipid metabolism**
- ***Polyethylene was more potent***

**very low toxicity of *biodegradable* polylactic acid MP in human cells (*BFR, 2020*) and mussels (*Khalid et al., Mar Pollut Bull, 2021*)**

**(overall, might be quantitative, rather than qualitative difference)**

- **MP can be "*classified" upon their potency* on a *standardized in vitro system* and their mixture effect be assessed (like dioxins): binding affinity/energy to CYP450 receptor protein of zebrafish (*Enyoh, Chemosphere, 2022: potentially interesting, can it really work?)***

**Shape and mass may influence absorption and surely the *extent of the body burden***

**How to use these informations to interpret *biomarkers of (internal) exposure* (e,g., MP in urine, *Wu et al., J Hazard Mater 2022*)**

# Adverse effects of MP can be consistent with three possible pathways

- **- Direct effect on the digestive system: highly relevant, because *not related to systemic bioavailability***

- **- effects on internal organs and tissues, related to the *systemic bioavailability and body burden***

- **Trojan Horse effect**

# Digestive system effects

- **altered gut microbiota (biodiversity, abundance) (*Li et al. Chemosphere. 2020; Qiao et al. Nanoscale, 2021*)**

**intestinal mucus (e.g., reduced mucus secretion and expression of secretion-related genes, altered faecal metabolite profiles, *Chen et al., Ecotoxicol Environ Saf 2022*)**

**integrity of epithelial barrier, e.g., decreased expression of *tight junction proteins* (enhanced entry of pathogenic bacteria?) (*Qiao et al. 2021*)**

**intestinal inflammation (enhanced *interleukin-1α*, produced by activated macrophages and neutrophyls) (*Li et al., 2020*)**

**allergy-like response (with activation of IgA and mast cells, *Park et al., Toxicol Lett. 2020*)**

**could be main targets of MP (*Fournier et al., J Hazard Mater 2021*)**

# Digestive system effects

- **Effects observed in laboratory rodents (that have different microbiota than humans)**
- **Often treated with high doses**

- **Provided that conditions of exposure are realistic (e.g., not leading to MP overload)**
- **They should be considered as highly relevant, because the digestive system is the first and most exposed site**

- **Alterations of the digestive tract may, at least, contribute to systemic adverse effects of MP**
  **role of the *microbiome* in the organism' health**
  ***immune* dysregulation**
  **altered *cross-talk* between the intestine and other (e.g., nervous) systems**
  **impaired absorption of *nutrients***

**(an intestinal disease with a lot of systemic impact? *Celiac Disease!)***

# Effects on internal organs and tisues

- **A growing number of rodent studies show that MP can adversely affect *almost all internal organs and tissues***

- **Common features: inflammation, oxidative stress**
- **Which might be consistent with a "*foreign body*" action**

**These studies have been criticized for employing very high, unrealistic dose levels**
**(which may a problem for their use in risk assessment)**
**no or inadequate measurement of absorption**
**or of MP burden in the investigated organ(s)/tissue(s)**
**(which can be more relevant)**

**However, the results are *consistent***
**And the studies should not be dismissed**
**at least for *hazard identification***

# Effects on internal organs and tisues: two examples

- **Male Reproductive System (*Hu et al., J Hazard Mater. 2024*)**
- **a meta-analysis of 39 studies, identified**
- **increased reactive oxygen species as the initiating event (MIE)**
- **this triggered oxidative stress, *mitochondrial* dysfunction**
- ***sperm DNA* damage, *apoptosis and autophagy* of testicular cells**

- **The tissue damage led to downregulation of the testicular *endocrine signalling* (steroidogenic enzymes and steroidogenic acute regulatory protein), that disrupted hypothalamic-pituitary-testicular axis.**

- **Consequences at tissue/organ levels**
- **(endocrine) reduction of *testosterone***
- **(testis) impaired *blood-testis barrier*, inflammation,**
- **and impaired *sperm quantity/quality* (sperm concentration motility, viability, abnormality rate)**

# Effects on internal organs and tisues: two examples

- **Kidney:**
- **pathway of excretion and site of direct effect**
- **(*Wang et al., Environ Health Perspect. 2021*) Polystyrene MP bioaccumulate in renal tubules in mice, increasing oxidative stress and causing inflammation**

- **Affected by *gut disruption* (gut-kidney axis)**
  **(*Liang et al., Environ Pollut. 2024* )**
  **polystyrene MP in mice impair the gut barrier,**
  **increased urinary complement-activated product C5a levels and renal C5aR expression, leading to chronic kidney dysfunction**
  **The complement system, in particular the *C5a/C5aR pathway***
  **(powerful inflammatory mediator: chemotaxis, TNF-alpha upregulation),**
  **may be a key component in MP-kidney injury through gut-kidney axis**

# TROJAN HORSE

# Trojan Horse effect

- **absorbed MP may carry contaminants into the organism**
- **the actual concerns depending from the *internal exposure of critical target organs for specific pollutants***

- **MP, especially when originating from fragmentation of old plastic waste, can contain on average 4% of additives (including *endocrine disruptors, such as phthalates and bisphenols*)**

- **MP can adsorb inorganic/organic/biological contaminants from the environment**
- **Heavy metals, dioxins, PCBs,**
- **films on the MP rough surface can contain pathogens, bacteria carrying transferrable antibiotic resistance..**

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# Trojan Horse effect

- **In general, *and unfortunately,***
- **In most cases the Trojan horse effect seems to represents *only a minor fraction* of the overall exposure from foods/environment**

- ***But careful***
- **On 2016 EFSA assessed the exposure to bisphenol A through highly contaminated mussels, making a worst-case analysis**
  **The exposure was largely below the TDI (4 µg/kg body weight)**

**but on 2023 EFSA has defined a *new, much lower TDI***
**(0.2 ng/kg bw)**

**It would better to make a new assessment**

# Nanoplastics: specific aspects

- **evidence still limited point to intracellular accessibility**
- **and in particular interactions with *subcellular organelles***
- **Mitochondria**
- **Lysosomes,**

**leading to altered homeostatic mechanisms, e.g., *redox-related* or *autophagy-related*.**

**Mitochondria: disrupting energy metabolism (zebrafish: *Trevisan et al. Front Env Sci 2020*; human lung and liver cells, *Halimu et al al., J Hazard Mater 2022; Lin et al., Environ Sci Technol 2022*)**

**Lysosomes: inhibition of *mTORC1 signaling*, a central regulator of cellular metabolism located in the lysosome: impact on glycolysis, oxidative phosphorylation, amino acid metabolism (*Wang et al Environ Int 2022)***

# Main current questions for toxicological research

**Extended and updated from**

**TAIEX Workshop on Microplastics and One-Health: what's next!**

***Priorities for research and actions in the SEEHN Member States (Western Balkans and beyond)***

**organised in co-operation with**
**Institute of Public Health ofAlbania and**
**South Eastern Europe Health Network (SEEHN)**

**Tirana (October 2022)**

# Toxicology of MP/NP what's next?

- **first of all, the full picture of adverse effects.**

**the relationships between external dose, absorbed dose, time-related body burden, and adverse responses:**
**can *"tolerable" intake levels* be envisaged?**

**the definition of adverse outcome pathways (*Hu et al., J Hazard Mater. 2024*) can help predicting the apical outcomes of sub/cellular changes and identifying *early biomarkers.***

**modelling the "Trojan Horse" effect for main plastic-related contaminants for an up-to-date risk assessment.**

**While research is badly needed, the available evidence calls for a precautionary *yet energic* reduction of MP/NP emissions**

# HVALA for attention and interest!