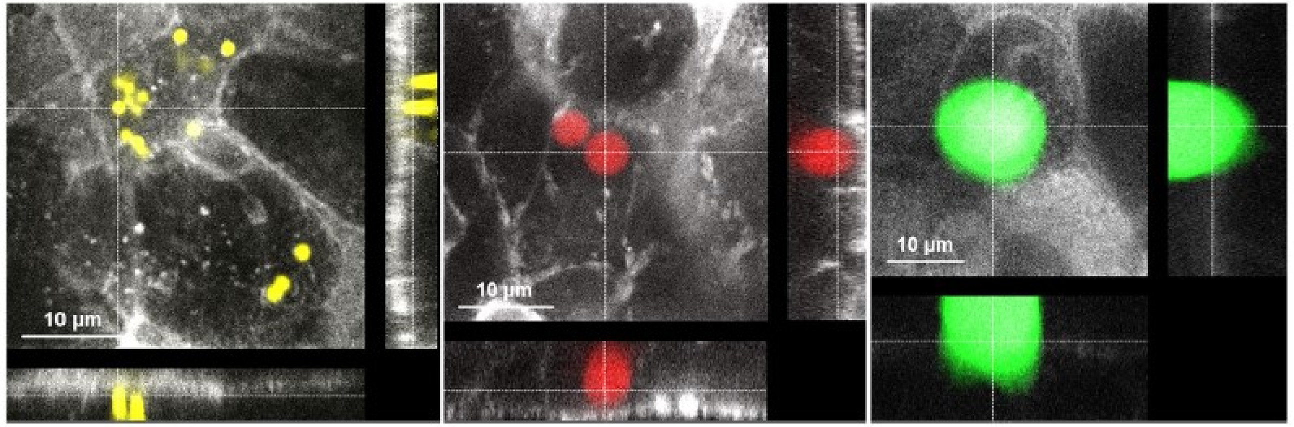
Food Safety Research and Risk Assessment of Micro-, Submicro- and Nanoplastics



4th Joint Symposium on Nanotechnology

Dr. Holger Sieg
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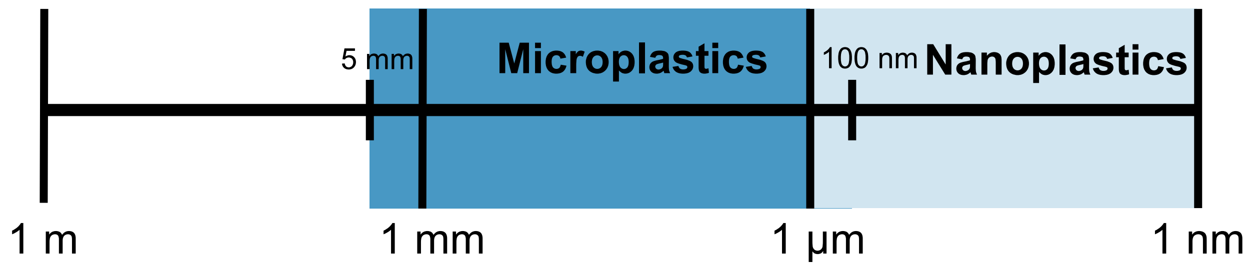
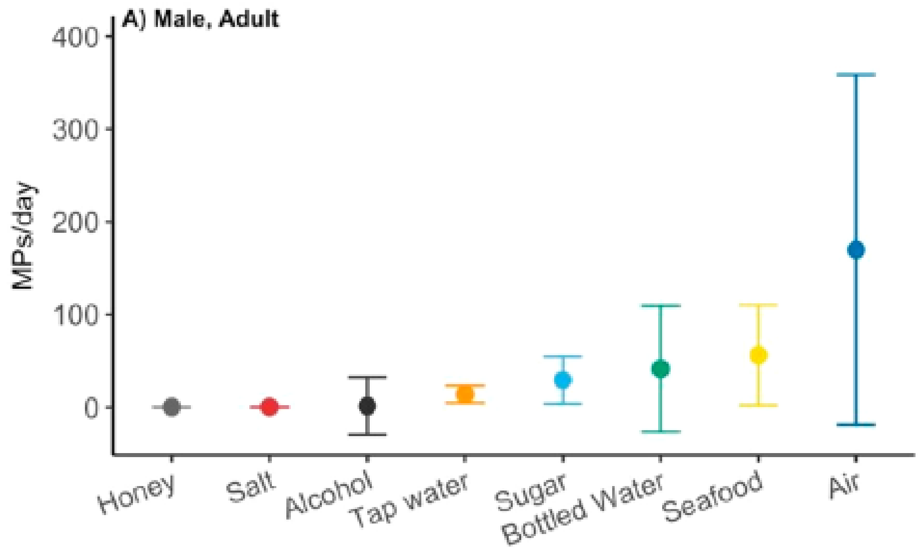
Microplastics: Definition





Cox et al., 2019: „Human Consumption of Microplastics“, Env.Sc.&Tox.

Tap water

Honey

Table salt

Fruits

Bottled water

Sugar

Tea

Seafood
Mussels
Algae

- food
- food production, packaging, food preparation
- food contact materials
- first study in human blood samples

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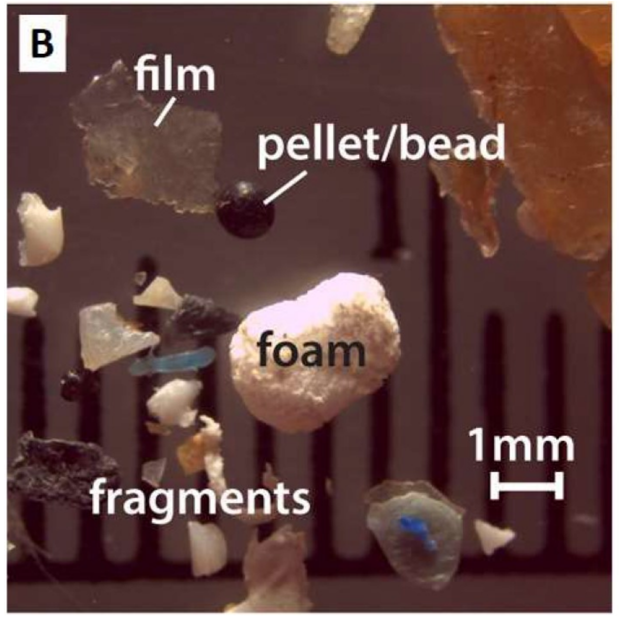
Risk Assessment of Microplastics: Material challenges

Single chemical

*

Microplastics



*

- Hazard Identification: Chemical properties
- Hazard Characterization: Effects, Dose-response-relationships
- Exposure Assessment
- Risk Assessment: Health-Based Guidance Values (HBGV)

Broad mixture of

- Material
- Particle size and shape
- Abundance

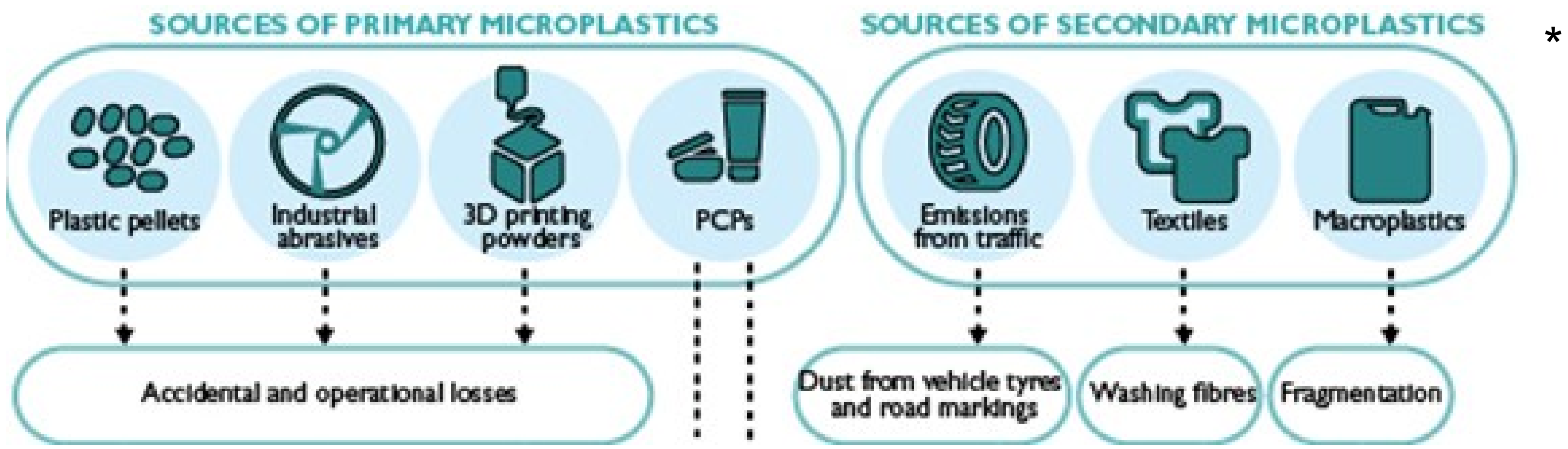
Plastics does not equal plastics !

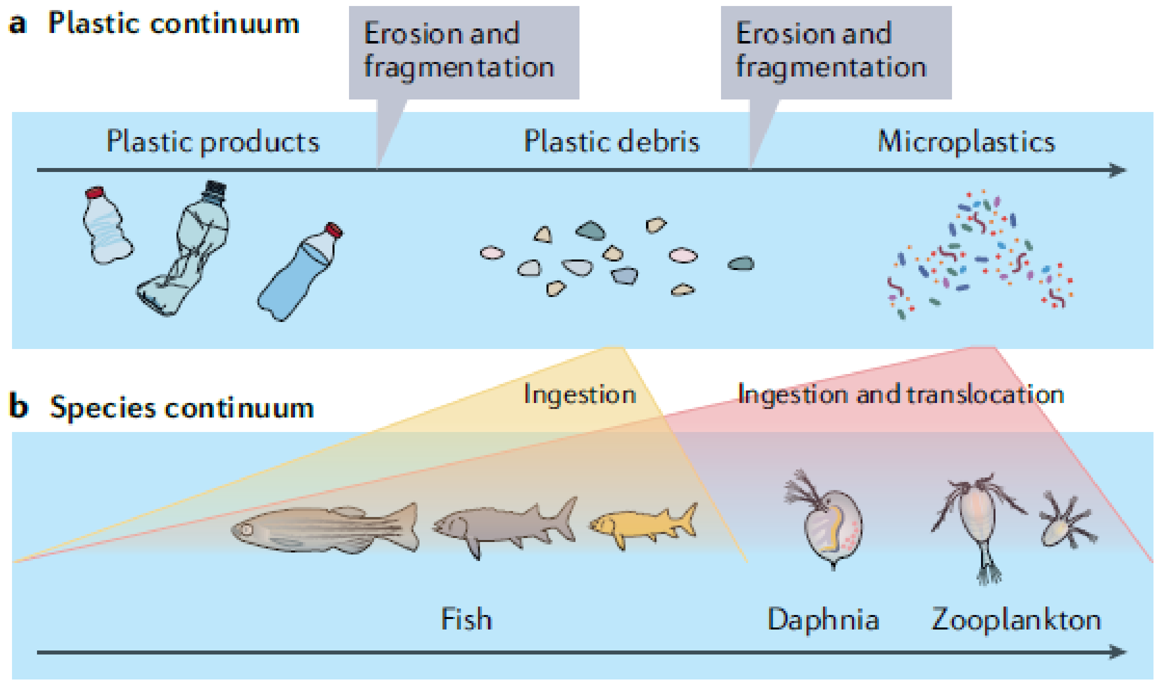
Risk assessment is challenging !

**calroth.com*

**Baldwin et al. 2016. Plastic debris in 29 Great Lakes tributaries: Relations to watershed attributes and hydrology. Environ Sci Technol. 50(19):10377-10385.*

Microplastics: Abundance





Risk assessment of microplastic particles

Albert A. Koelmans, Paula E. Redondo-Hasselerharm, Nur Hazimah Mohamed Nor, Vera N. de Ruijter, Svenja M. Mintenig and Merel Kooi

*Image source: Finnish Environment Institute (SYKE) 2017.

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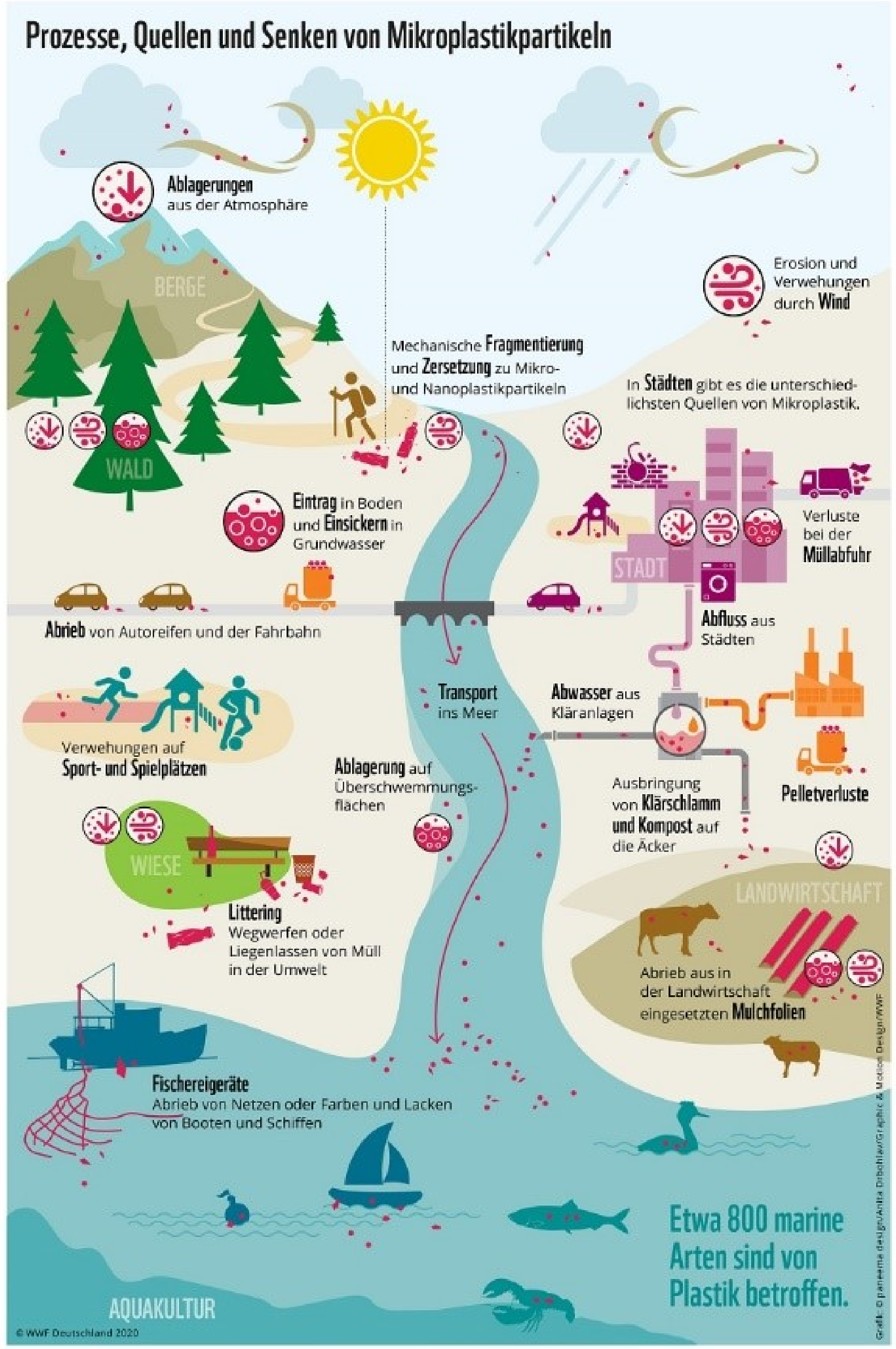
Microplastics: Abundance

- Need for a differentiated view:
    - Plastic waste ⟷ Microplastics
  - Environmental protection ⟷ Human consumer protection
  - Exposure route: Oral ⟷ Dermal ⟷ Inhalative
  - Material: Inert Polymer ⟷ Additives, Contaminants, Biofilms
  - Size range: Larger particles ⟷ Small particles



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Microplastics: Detection methods

Early studies: Liebezeit 2013/14: Microplastics in honey and beer

Microscopic detection without material analysis

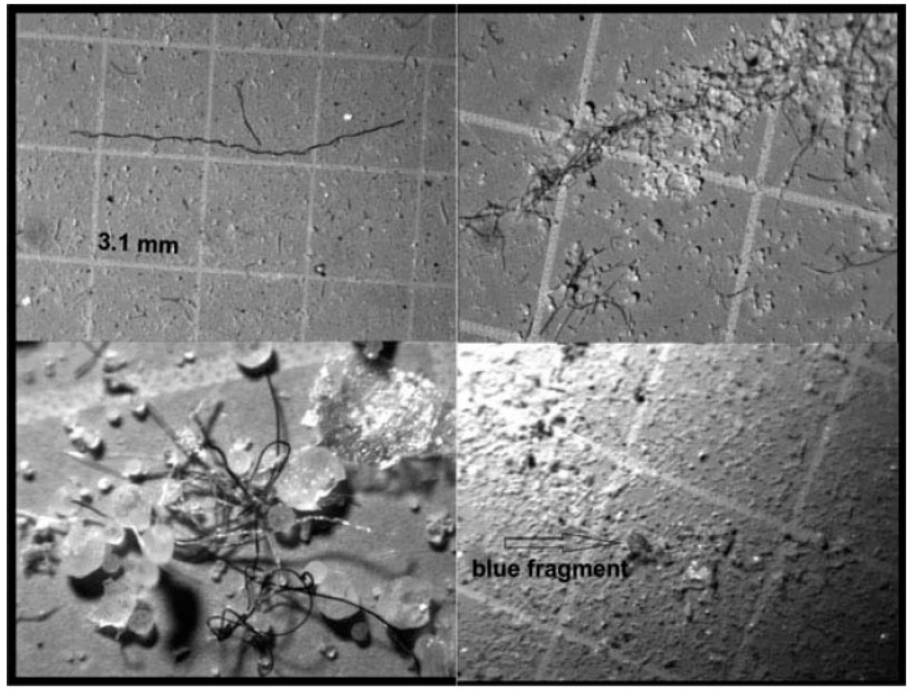

Figure 1. Examples of fibres and fragments in four honey samples after peroxide treatment before wax melting.

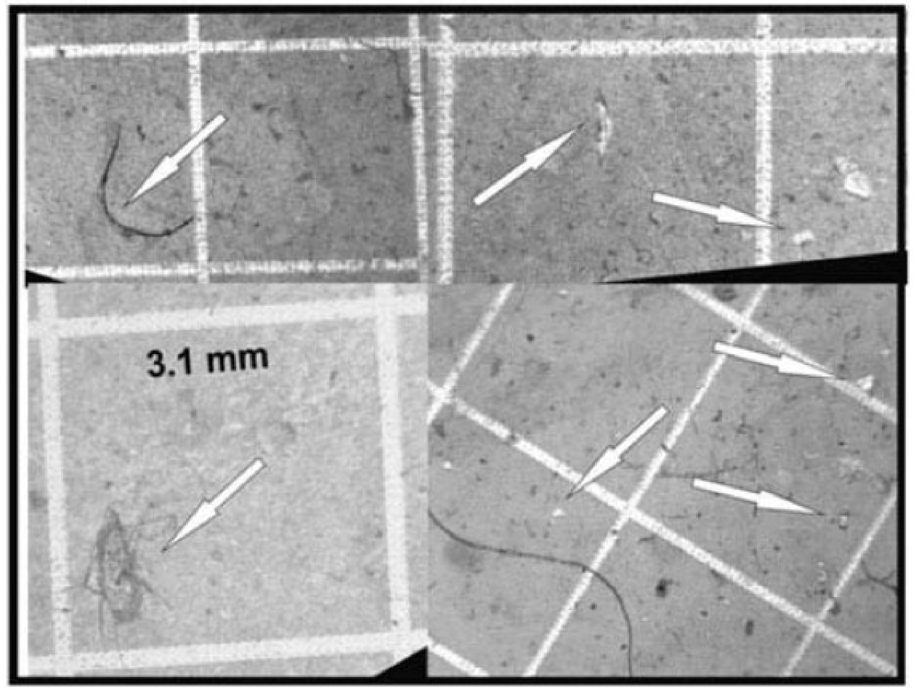

Figure 1. Examples of microplastic contaminations in German beers. White arrows indicate non-stained synthetic material.

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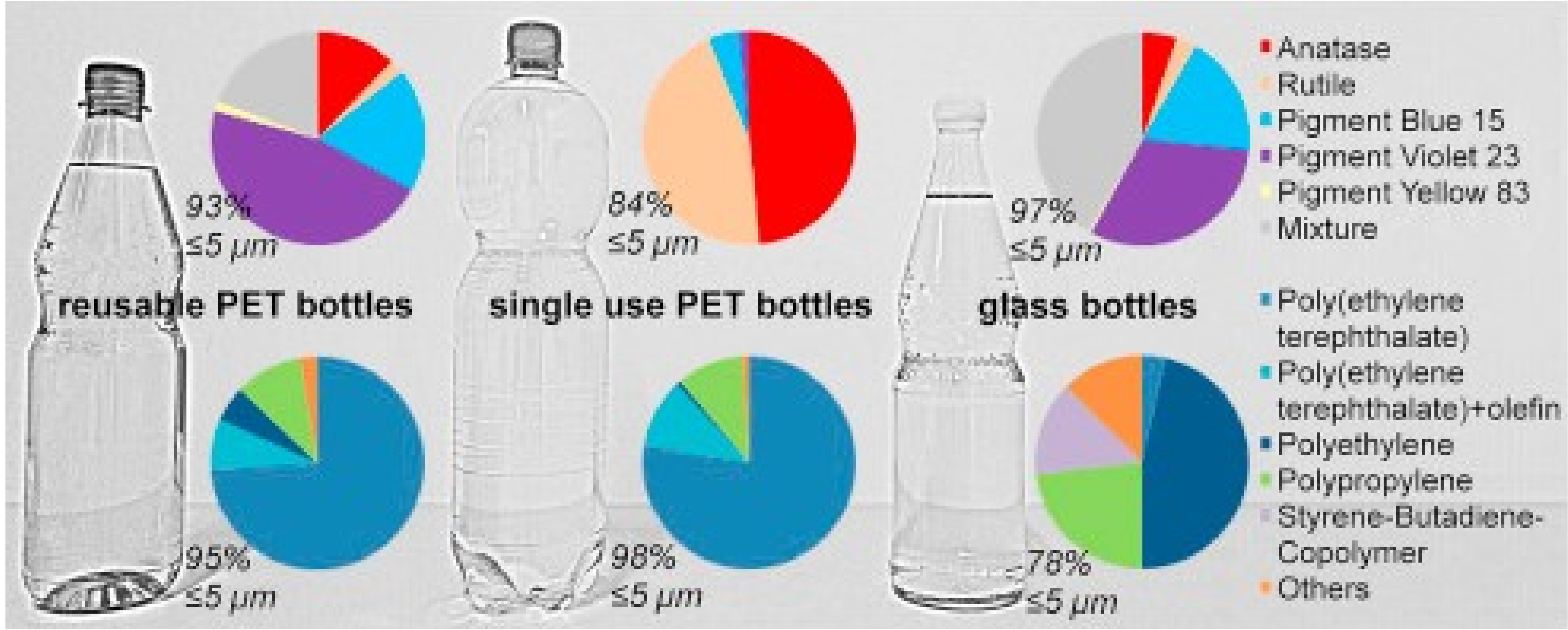
Microplastics: Detection methods

More recent studies use a variety of methods including material characterization.



Small-sized microplastics and pigmented particles in bottled mineral water, Barbara E.Oßmann et al., DOI:10.1016/j.watres.2018.05.027

Microplastics: Detection methods


Eine Initiative des Bundesministeriums für Bildung und Forschung

Plastik in der Umwelt

Quellen • Senken • Lösungsansätze

PlastikNet

Discussion Paper
Within the scope of the research focus
Plastics in the Environment
Sources • Sinks • Solutions

Microplastics Analytics

Sampling, Preparation and Detection Methods

Table 3: Generation of results for different detection methods (for abbreviations of methods, see appendix)

Characteristics	**Spectroscopic**						**Thermoanalytical**				**Chemical**
	µ Raman	µ FTIR (trans)	FPA FTIR (trans)	µ ATR-FTIR	ATR-FTIR	NIR / Hyper-spectral Imaging	Py-GC-MS	Mod. Py-GC-MS	TED-GC-MS	DSC	ICP-MS
Type of polymer	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Only PE, PP	Only tyre abrasion
Detectable additives	Pigments	No	No	No	No	No	Yes	No	No	No	No
Particle surface (chemical)	Yes	No	No	No	Yes	Yes	No	No	No	No	No
State of degradation*	Surface Oxidation	No	No	Surface Oxi-dation	Surface Oxi-dation	No	Oxi-dation	No	No	Mol. weight	No
Particle number, particle size, particle shape, particle surface morphology	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No
Mass balances	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes

- No universal detection method -> Combined methods needed
- Up to now no validation or standardisation
- Size limitations, complex food matrix
- Still no routine food control possible

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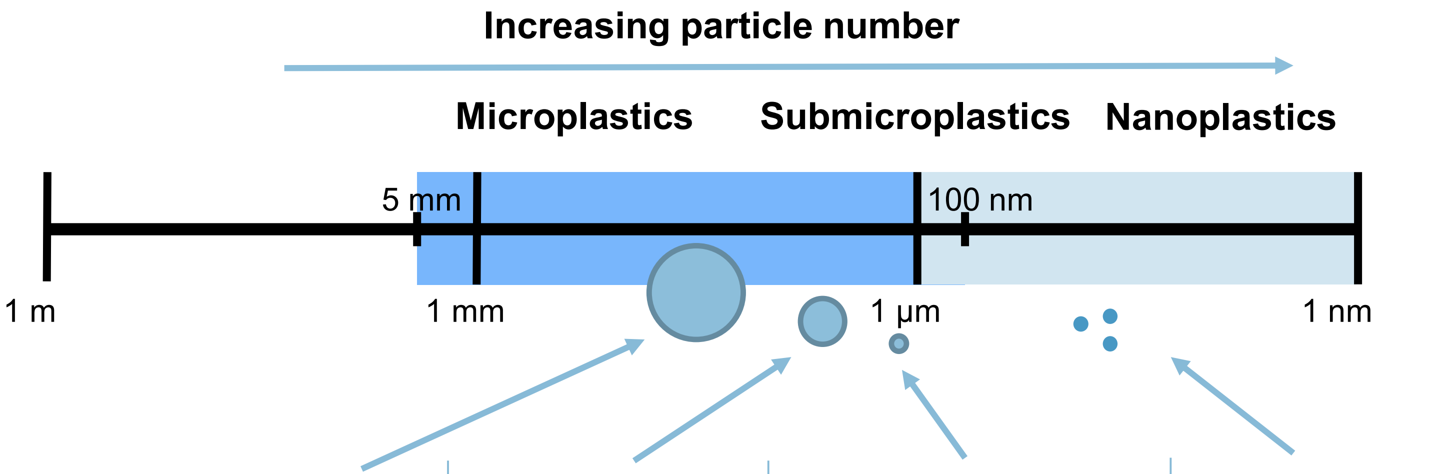
Microplastics: Size limitations of detection methods

Microplastics: Size-dependent bioavailability



150 µm	10 µm	1,5 µm	< 1,5 µm
Upper size limit for persorption*	Upper size limit for cellular uptake	Prospected upper size limit for systemic bioavailability*	Very limited data*


EFSA Journal

STATEMENT

ADOPTED: 11 May 2016

doi: 10.2903/j.efsa.2016.4501

Presence of microplastics and nanoplastics in food, with particular focus on seafood

EFSA Panel on Contaminants in the Food Chain (CONTAM)

*EFSA

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Microplastics: BfR studies on cellular uptake

in vitro: Intestinal cell lines

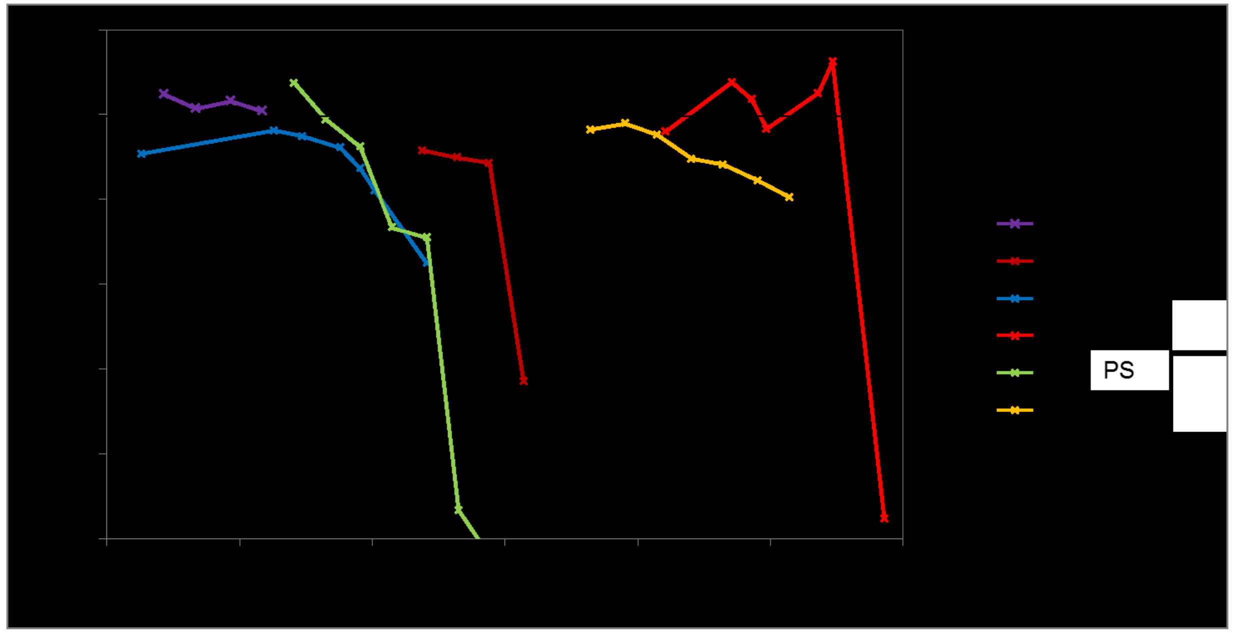
in vivo: Mouse study

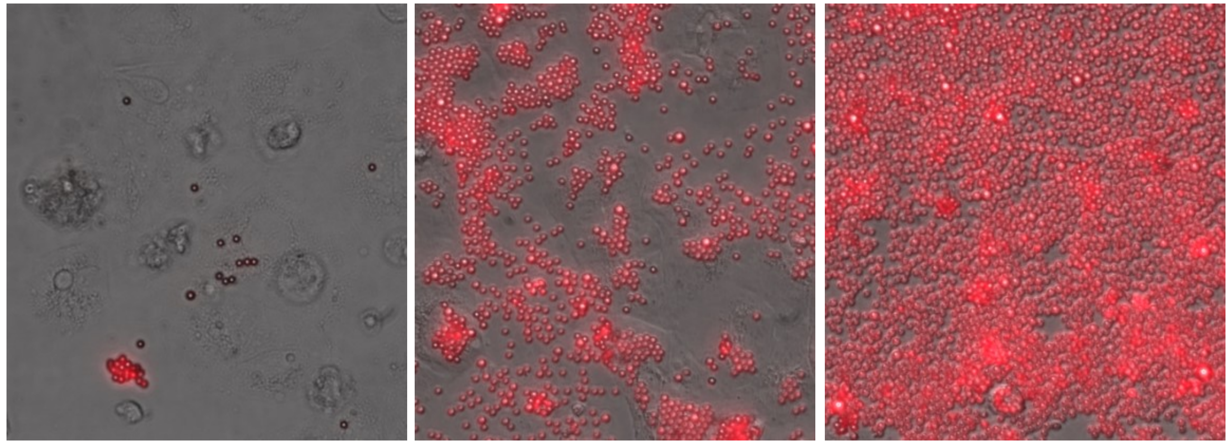
- 28-day oral feeding study (HOTT-mice)
- Only a few particles(1 µm) in organs of the GI-tract

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

Characterization of toxicological impact

in vitro: Cell viability measurements





Effects only in „overload“ situations

Characterization of toxicological impact

EU-FORA SERIES 3 — BfR Bundesinstitut für Risikobewertung — EU-FORA — EFSA Journal

APPROVED: 7 September 2020
doi: 10.2903/j.efsa.2020.e181102

Risk assessment and toxicological research on micro- and nanoplastics after oral exposure via food products

German Federal Institute for Risk Assessment (BfR), Department of Food Safety, Unit Effect-based Analytics and Toxicogenomics Unit and Nanotoxicology Junior Research Group, Berlin, Germany,
Sofiya Shopova, Holger Sieg and Albert Braeuning

Table 3: Selected toxicological effects of micro- and nanoplastics

Toxic effects	Microplastics	Model	Main findings	References
Gastrointestinal toxicity	PE	Blue mussel *Mytilus edulis* L.	Notable histological change and a strong inflammatory response	von Moos et al. (2012)
	PS	Adult male zebrafish	PS microplastics increased the expression of IL-1α, IL-1β and interferon in the gut; indicated microbiota dysbiosis and inflammation	Jin et al. (2018)
	PA, PE, PP, PVC and PS	Zebrafish and nematode	Villi cracking and splitting of enterocytes	Lei et al. (2018)
	PS	Male mice	Accumulation of PS microplastics in mice guts, consequently caused the reduction of intestinal mucus secretion damage of gut barrier function; metabolic disorders in mice	Jin et al. (2019)
	PS	AGS cells	Inflammatory gene expressions such as IL-6 and IL-8	Forte et al. (2016)
Liver toxicity	PS	Zebrafish	Inflammation and lipid accumulation both in 5 μm and 70 nm; oxidative stress and alterations in their metabolic profiles; disturbance of lipid and energy metabolism	Lu et al. (2016)
	PS	*Eriocheir sinensis*	Decreased activities of AChE, CAT, and ALT in *Eriocheir sinensis* liver; antioxidants CAT, SOD, GPx and GST level decreased in the liver; expressions of the genes encoding p38 in the MAPK signalling pathway was upregulated while significantly declined in ERK, AKT and MEK	Yu et al. (2018)
Liver toxicity	PS	Mouse	TG and TCH levels decreased; decreases on key gene expressions related to lipogenesis and TG synthesis in liver indicating mouse hepatic lipid disorder	Lu et al. (2018)

- *in vivo:* different effects detected in a variety of species

- Not according to OECD-criteria
- Often invertebrates, no human studies
- Not under controlled experimental conditions
- Size distributions often not investigated
- Often very high doses

↓

- Not aplicable for risk assessment
- No dose-response-relationships available yet
- No Health-Based Guidance Values derivable

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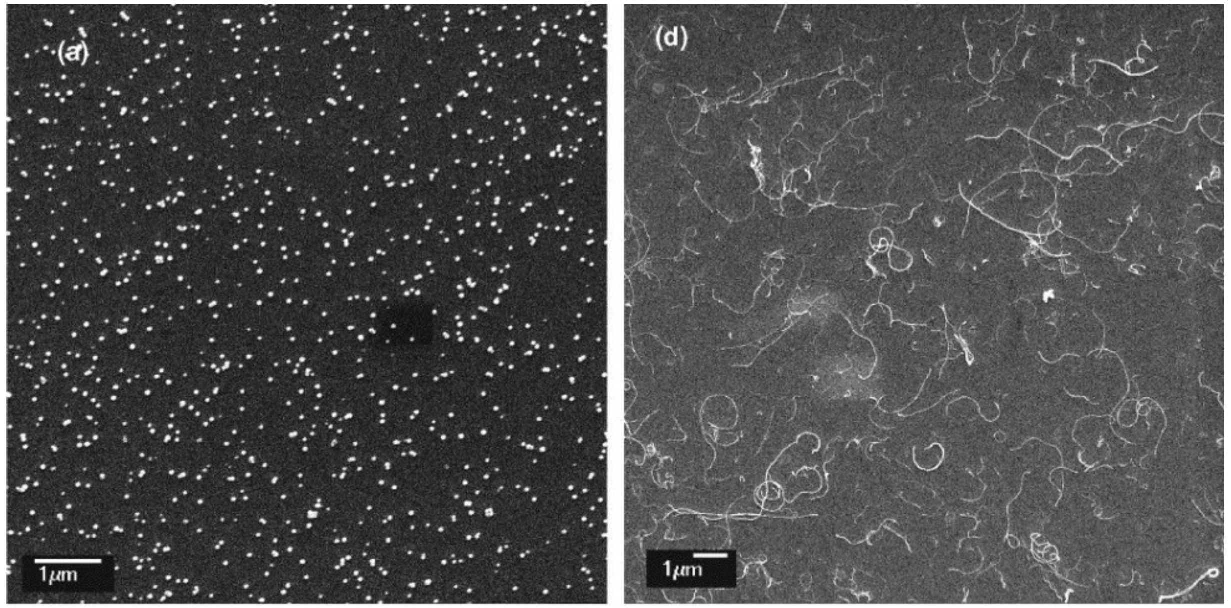
From Micro- to Nanoplastics

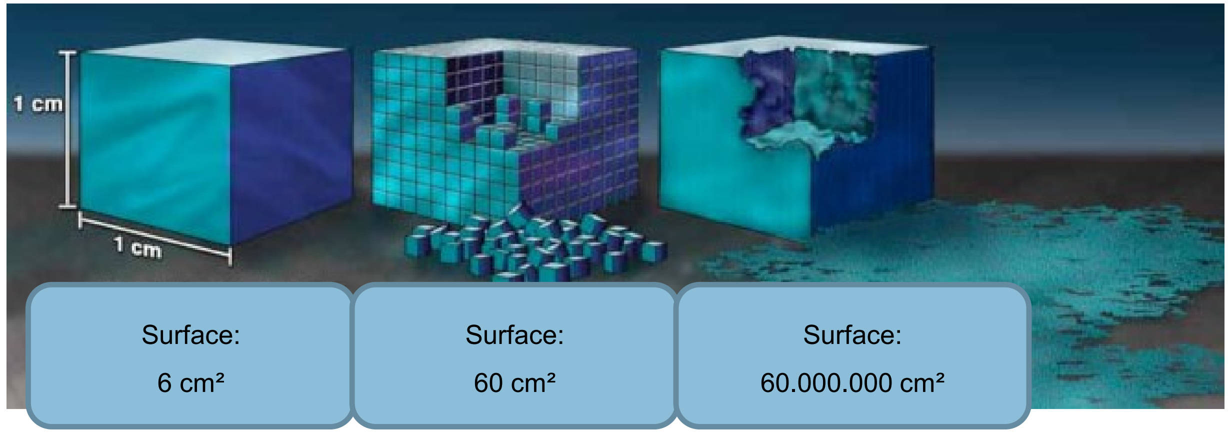
- **Microplastics**: Preliminary assumptions
  - Available exposure studies indicate low uptake:
    - Only few particles detectable
    - Often in non-edible organs
    - Cellular uptake of particles > 1.5 µm very low
    - Systemic bioavailability unlikely
  - Acute effects are low:
    - Effects only after very high exposure measurable.

  - but: little known about **Nanoplastics**
    - Methodological challenges in detection and quantification
    - Cellular effects acute and long-term
    - Cellular uptake mechanisms and quantity

From Micro- to Nanoplastics



Quelle: KIM, S. C., (...), OBERDORSTER, G. & PUI, D. Y. 2010.
A nano-particle dispersion method for in vitro and in vivo nanotoxicity study.
Nanotoxicology, 4, 42-51.



Quelle: www.nano.gov

Knowledge from Nanotoxicology

- Nanoparticles can have nano-specific properties

- Enlarged surface-to-mass-ratio
- High reactivity
- Different uptake mechanisms
- Ability to cross biological barriers

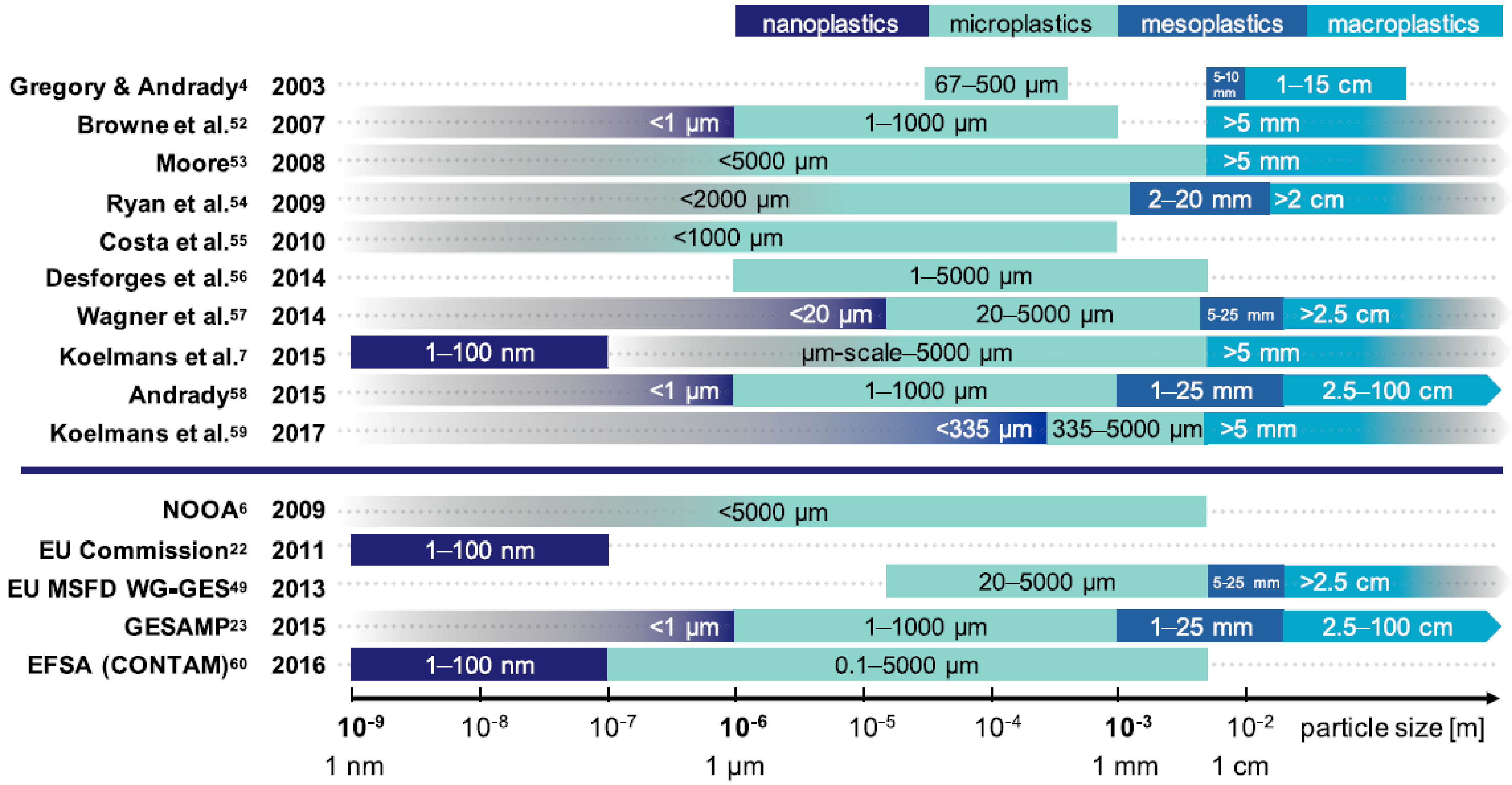
Nano-specific effects?

From Micro- to Nanoplastics

Environmental Science & Technology
Feature
Cite This: Environ. Sci. Technol. 2019, 53, 1039–1047
pubs.acs.org/est

Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris

Nanna B. Hartmann,*,† Thorsten Hüffer,*,‡ Richard C. Thompson,§ Martin Hassellöv,∥



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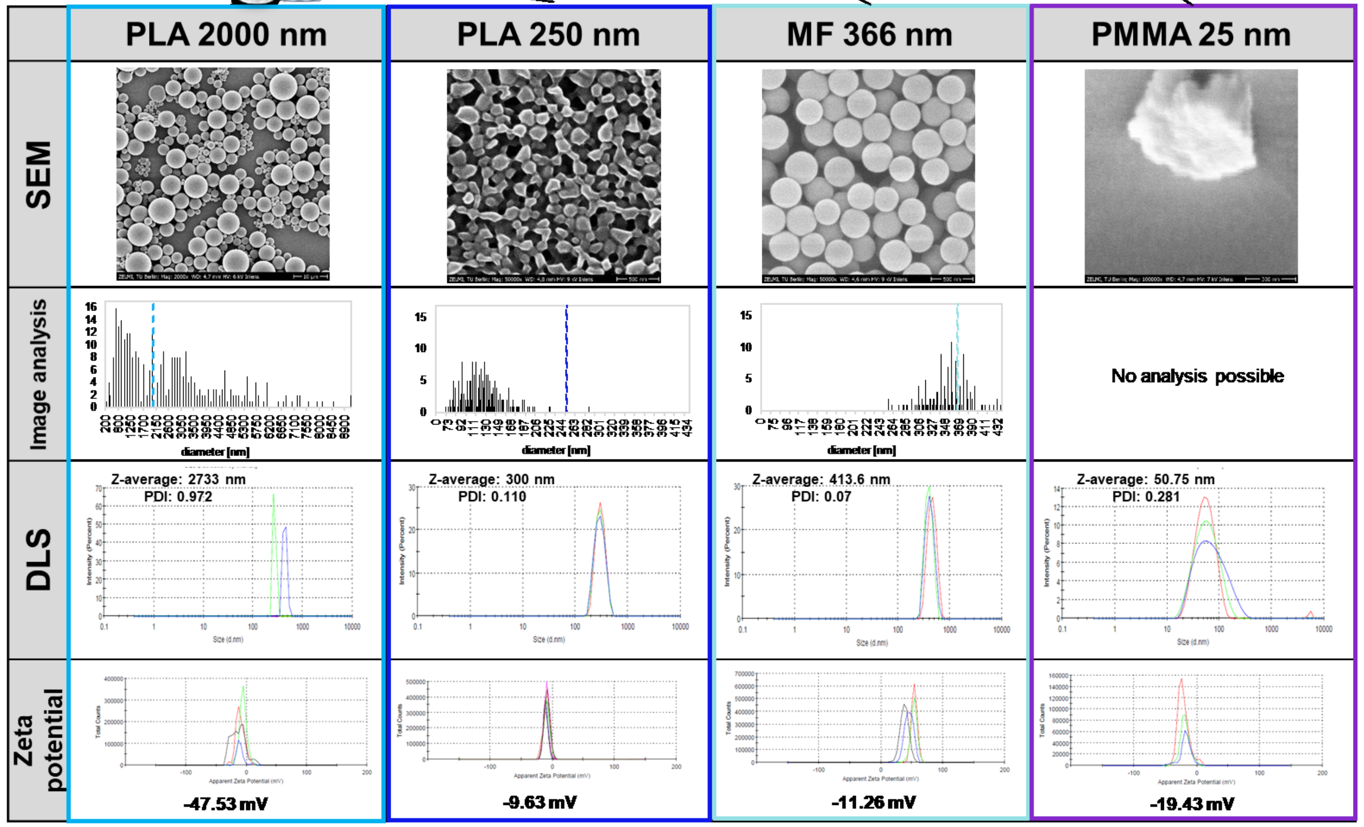
From Micro- to Nanoplastics: Own Research



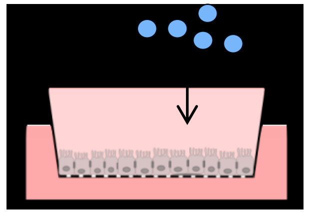
Polydisperse material from micrometer to nanometer range

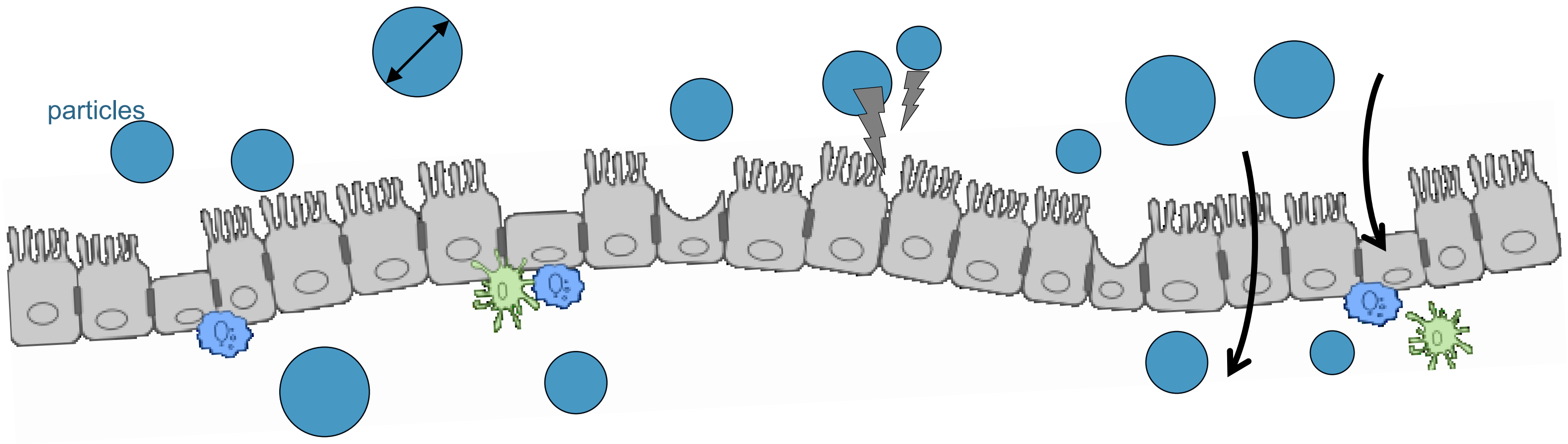
Submicroparticles Same size range, but different materials

Example for nanoplastic particles

Uptake and transport

Intestinal barrier models





Cellular effects
Intestine and Liver

PhD thesis of Maxi Paul

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Cellular uptake of submicrometer particles

Differences in cellular uptake:

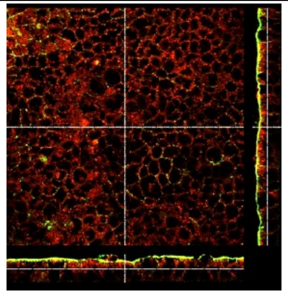
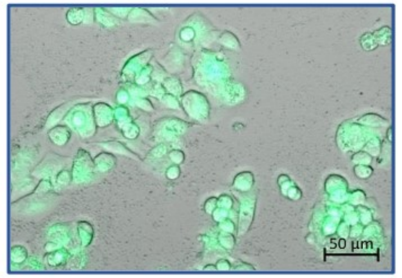
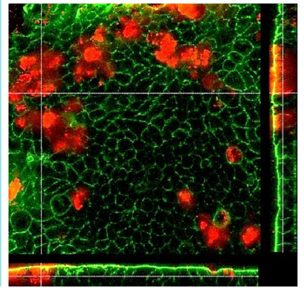
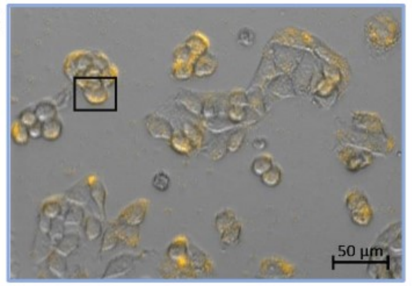
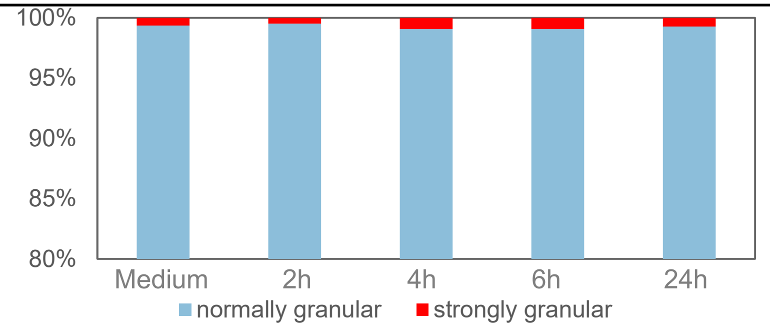
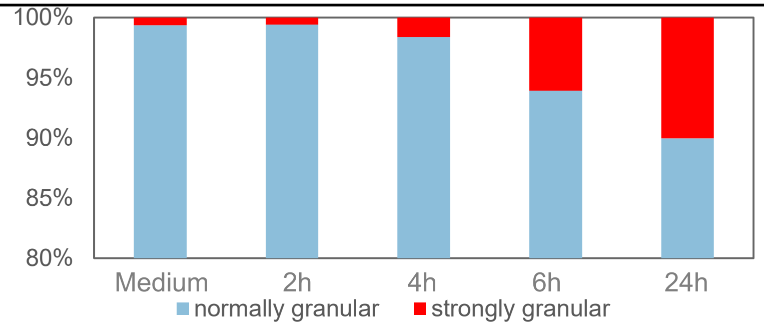
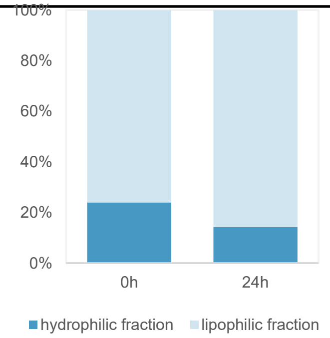
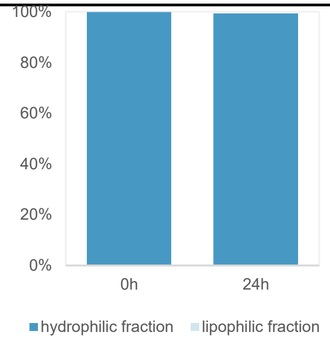
Submicrometer particles show different uptake behavior?

Different intracellular localization?

Different mechanisms?

PhD thesis of Maxi Paul

Cellular uptake of submicrometer particles

	PLA 250 nm	MF 366 nm
	 Caco-2  HepG2	 Caco-2  HepG2
	Strong differences in uptake and cellular interaction of the particles	
side scatter anaylsis		
Octanol water distribution	 lipophilic fraction hydrophilic fraction	

Membrane interaction?

Endosomal deposition?

Hypothesis: Differences in Hydrophobicity

Maxi Paul, paper accepted
Microplastics and Nanoplastics Journal

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Cellular uptake of submicrometer particles

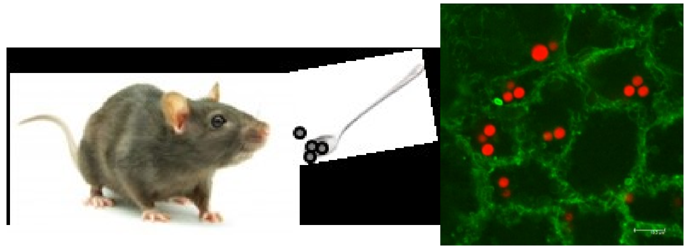
Application in combined Transwell model:

Transport to hepatic cells after crossing the intestinal barrier?

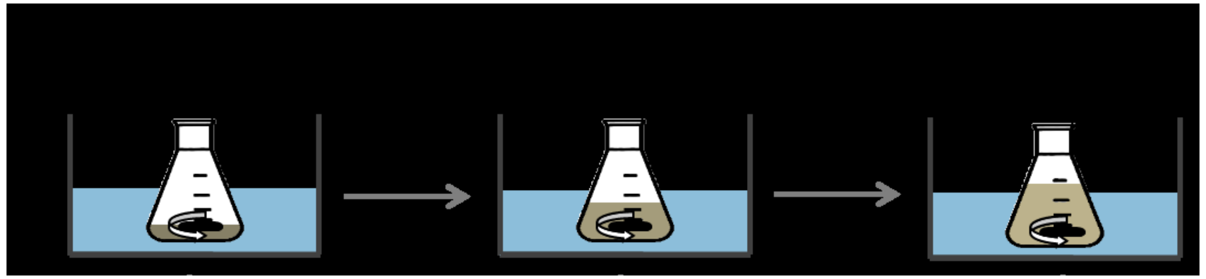
Maxi Paul, unpublished data

Next Steps

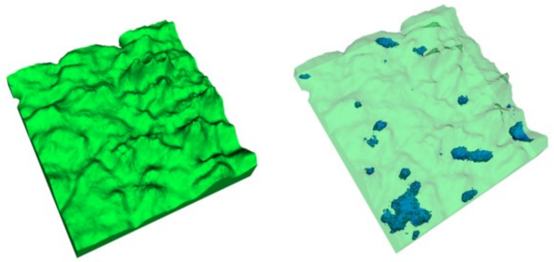
- Rat Study with Fraunhofer ITEM



- *In vitro* Digestion with BAM



- TOF-SIMS with Taipei Medical University

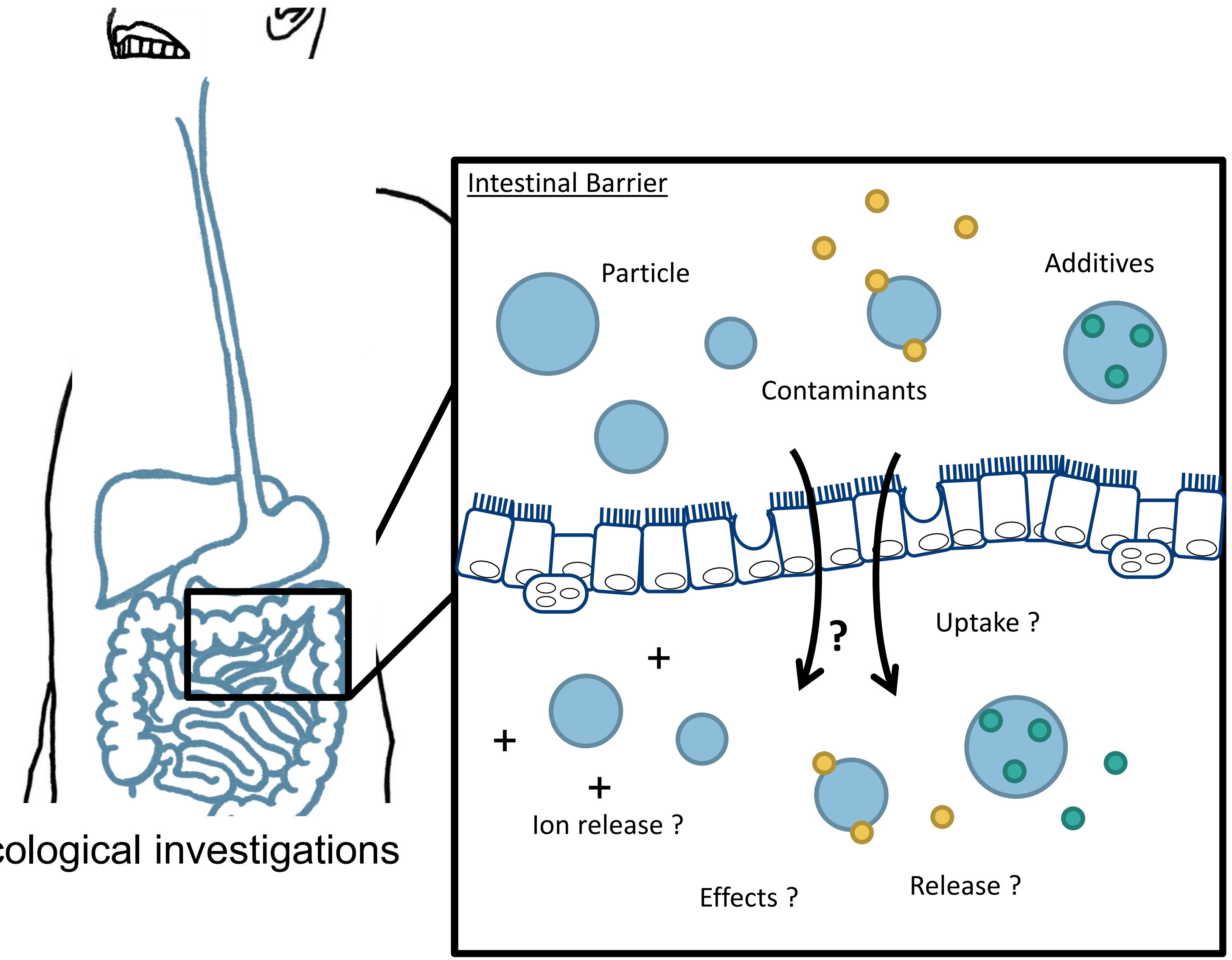


Summary from the view of Risk Assessment

- State of knowledge:
  - Microplastics rather ubiquiteously present in food chain
  - Complex mixture of chemicals
  - Exposure level unclear
  - Effects only measurable in overload situations

- Regulatory view:
  - Avaliable studies are not applicable for risk assessment yet
  - No validated quantification methods
  - Routine food control and monitoring not possible yet
  - Method development ongoing for: Analytics, quantification and toxicological investigations

- Major research needs:
  - Detection and quantification methods, exposure in food matrix
  - Mechanisms of action and dose response values
  - Submicro- and Nanoplastics, small size fractions



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Micro- and nanoplastics – current state of knowledge with the focus on oral uptake and toxicity

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