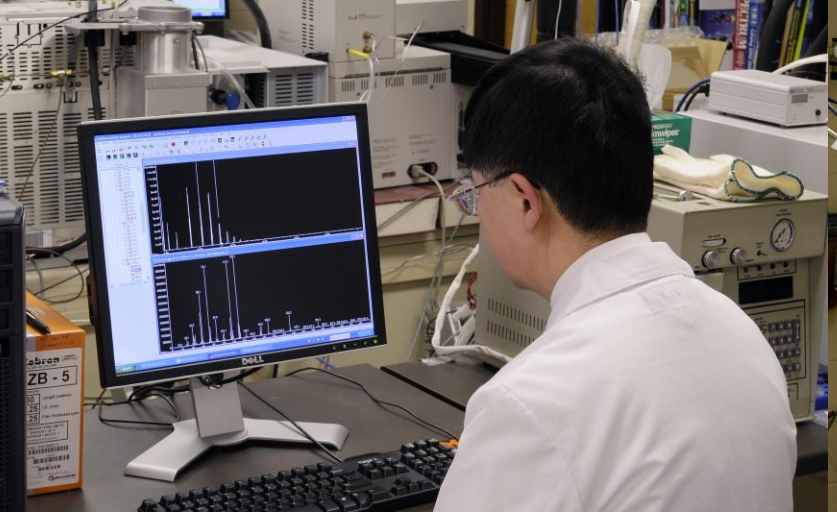
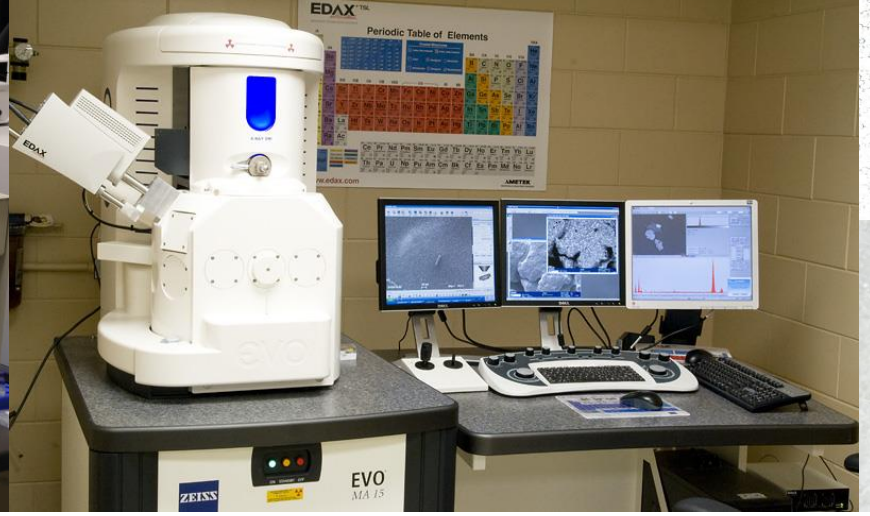
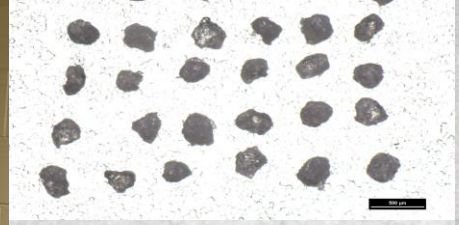
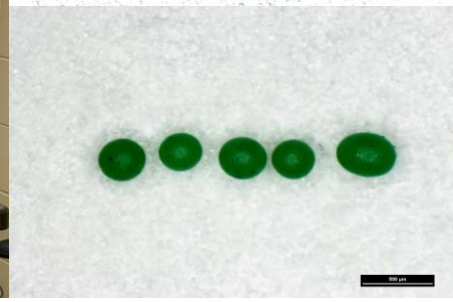
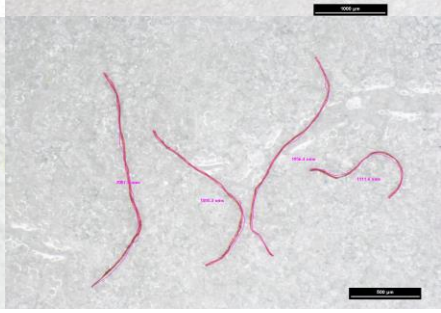





Microplastics and Health Webinar Series 2025:

Webinar 1 – Methodologies for appropriate and improved sampling, analysis, characterization, monitoring, and reporting of microplastics

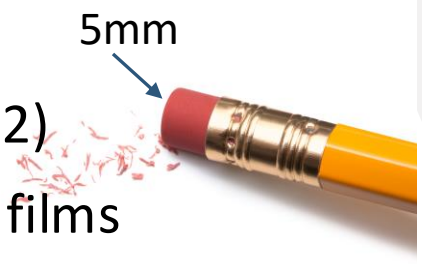
Dr. Imari Walker-Franklin, RTI International
Dr. Jeanne Hankett, BASF Corporation

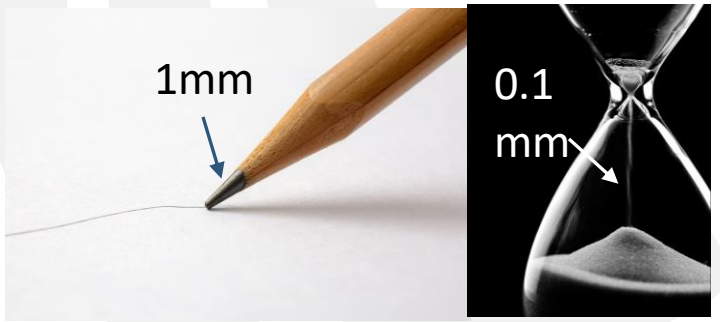
Today's Presentation


- **Brief Re-Introduction to Microplastics**
- **Current Status & Methodologies**
  - The III Parts of Microplastics Characterization
  - I. Collection
  - An aside: QA/QC, Contamination Prevention, and other Open Challenges
  - An aside: Reference & Standard Materials
  - II. Preparation
  - III. Characterization
- **Future Opportunities**
  - Improved quality
  - Reference & standard materials
  - Tissue & other complex matrix measurements
  - Consortia & Global Methods Development

RECALL FROM 2024: DEFINING THE TERMS

WHAT ARE MICROPLASTICS (MNPS)

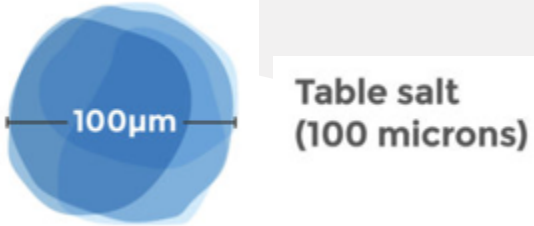




Plastic particles < 5 mm in length (WHO, 2022)

- Shape: Irregular fragments, sphere, fibers, films
- Composition and surface characteristics

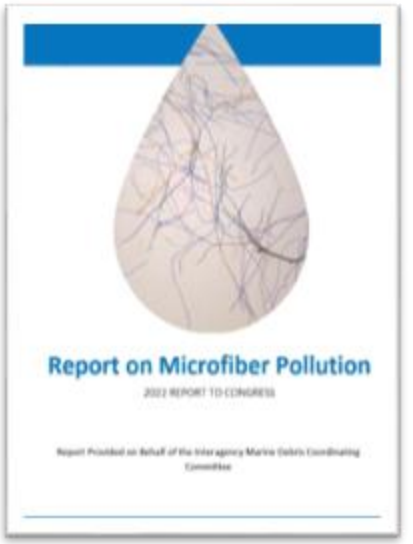
WHAT ARE PARTICLES?



A minute piece of matter with defined physical boundaries and a defined physical boundary as an interface (EU Commission, 2011)

WHAT ARE MICROFIBERS? ARE THEY MICROPLASTICS?

- Solid polymer-containing fiber particle with dimensions: length of 3 nm ≤ x ≥ 15 mm and a length to diameter ratio of > 3 (WHO, 2022)
- Considered microplastic if the size, shape and polymer compositions requirements are met.



EPA and NOAA Interagency Marine Debris Coordinating Committee, 2022

[Environmental Health Matters Initiative Introduction to Microplastics and Health Implications Where are we and where are we going - Webinar 1 | National Academies]

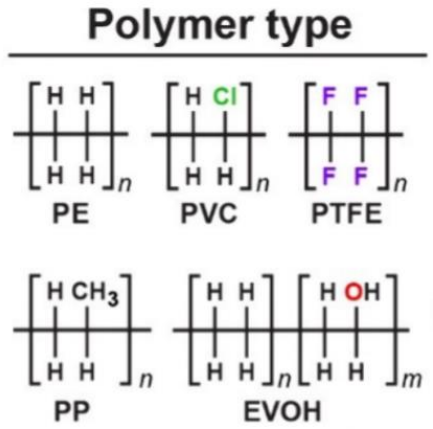
DEFINING THE TERMS

WHAT ARE PLASTICS?

- High relative molecular mass polymer (20,000-200,000 Da)
- Shaped by flow at some stage in its processing into finished products (ISO 472:2013)

Microparticles

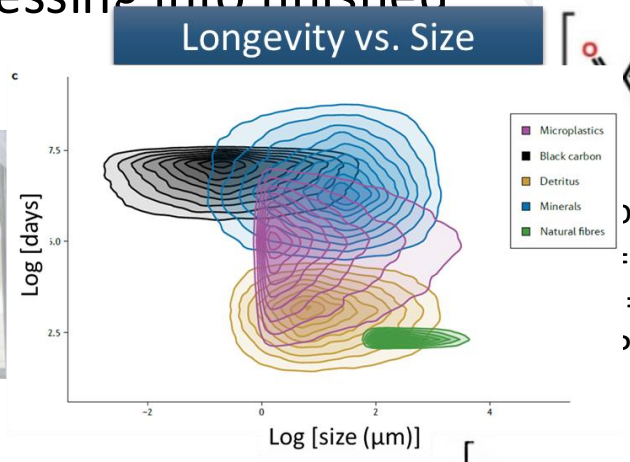
Plastics

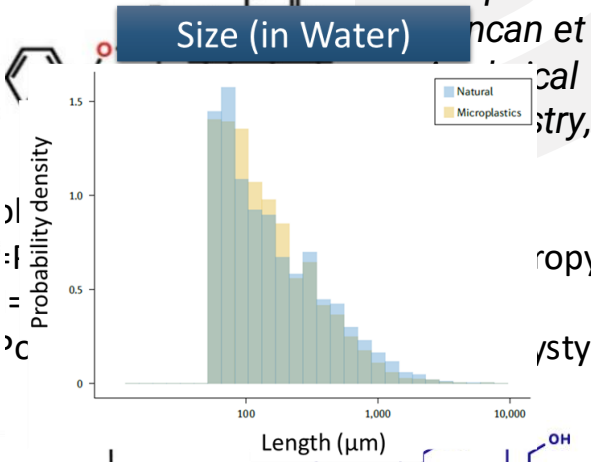


Adapted from ncan et al., ical stry, 2024

ropylene,

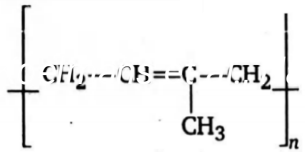
ystyrene



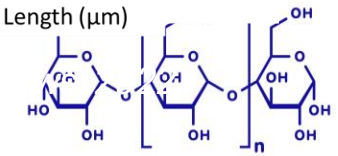


WHAT ARE POLYMERS?

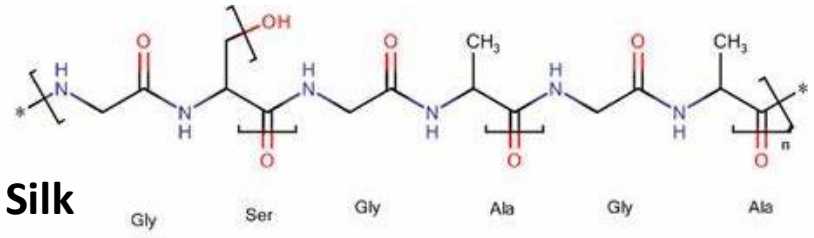
- Molecule of high relative molecular mass
- Multiple repeating units of low relative molecular mass molecules (IUPAC, 2008)



Natural rubber



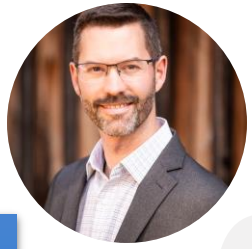
Polysaccharide

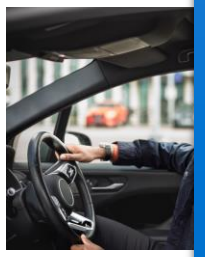


Silk

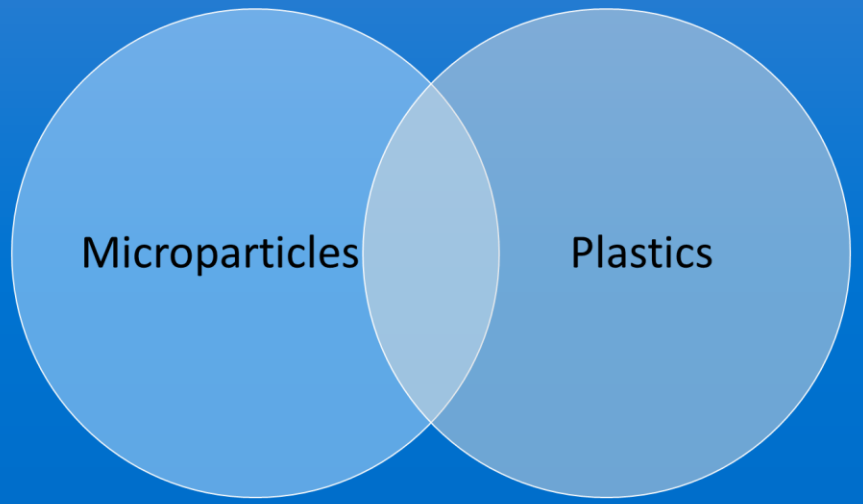
[Environmental Health Matters Initiative Introduction to Microplastics and Health Implications Where are we and where are we going - Webinar 1 | National Academies]

DEFINING THE TERMS

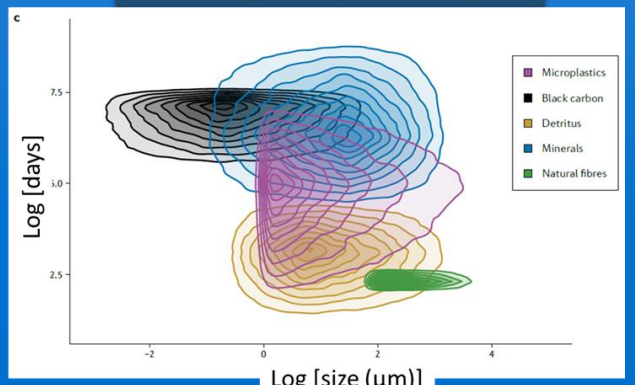




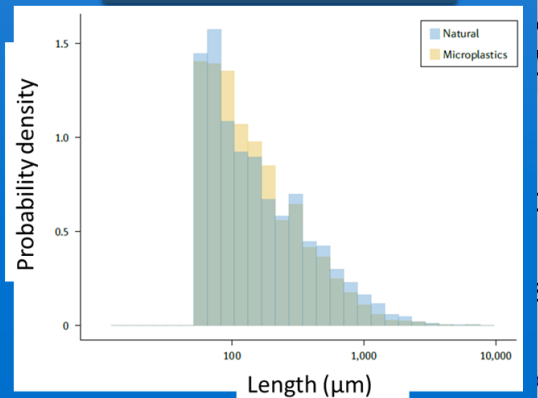
Microplastics have features both of microparticles and plastics.



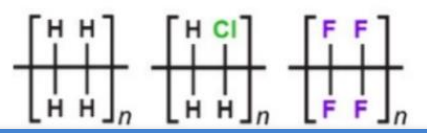
Longevity vs. Size



Size (in Water)



Adapted from Koelmans et al., Nature Reviews, 2022

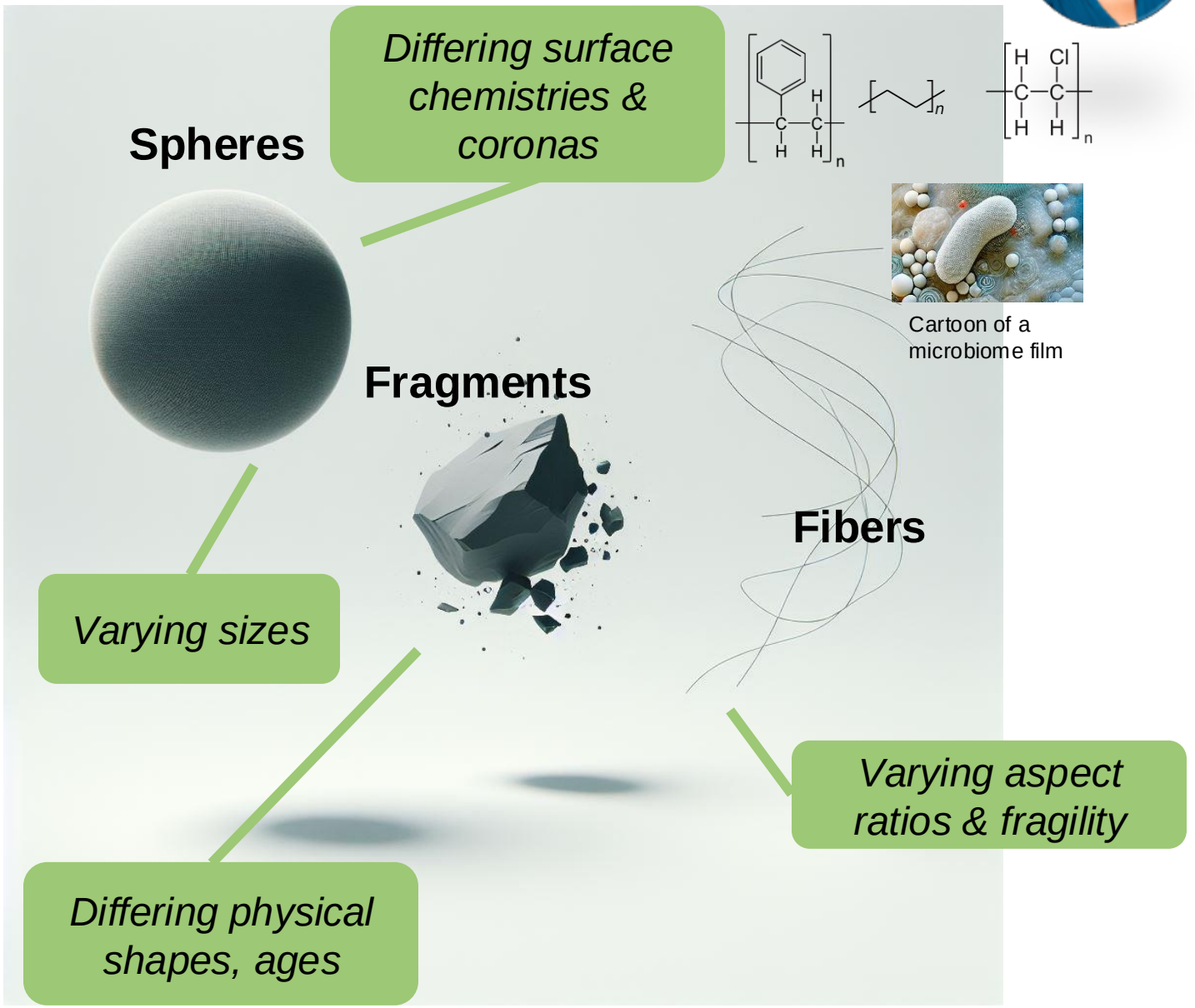


[Environmental Health Matters Initiative Introduction to Microplastics and Health Implications Where are we and where are we going - Webinar 1 | National Academies]

Why Are Microplastics So Unique from an Analytical Perspective?

- Microplastics create additional challenges compared to traditional analyses
  - **Chemistry**
    +
  - **Particle**

- The materials category of microplastics is **extremely diverse!**
- Recovery and analysis is dependent on both: physical & chemical parameters
- Contamination potential is prevalent
- Ideally, methods should perturb the microplastics as little as possible, with robust recoveries



How Do We Quantify & Chemically Identify Microplastics?

- Before MPs from the environment or biological samples can be analyzed, they must first be appropriately **sampled**, undergo rigorous **extraction** procedures in the laboratory, and the appropriate analytical technologies be chosen to characterize the **number & weight** MPs present (both important to hazard & risk)*

- **Chemical-based identification**, avoiding false positives and negatives is critical

*Microplastics Health Effects Panel, SCCWRP, California, 8 Sep 2021

I. Sampling: Starting Your Microplastics Journey

I. Sampling: How Microplastics Are Collected

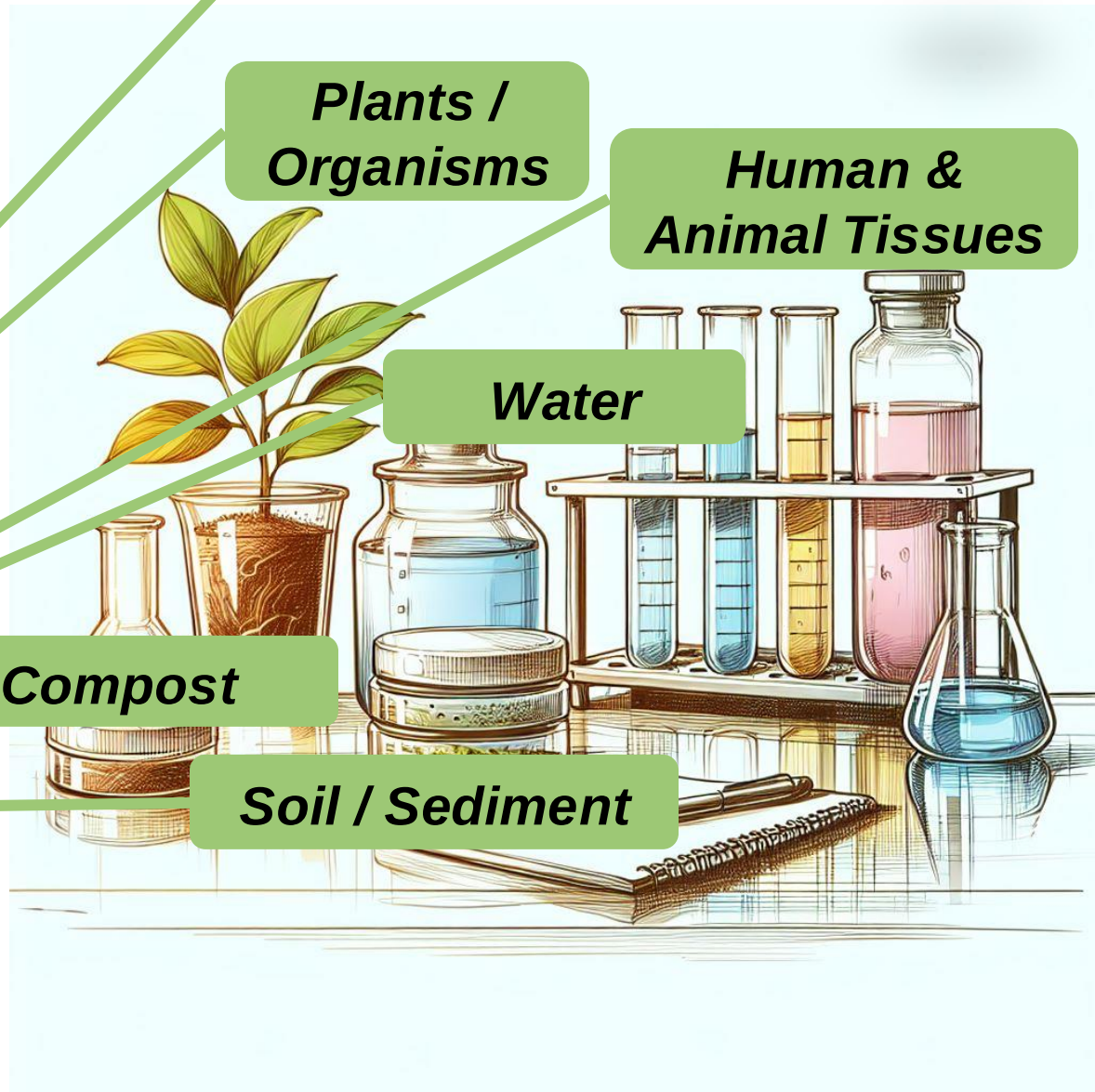
- Very few standard methods exist from sample collection beyond water matrices
  - (ASTM **D8332-20**, ISO/CD **16094-1**, ISO **5667-27**)
- Collection categories are divided into **Environmental** & **Biological** Sampling
- ISO **24187:2023** provides general guidance for sampling
- Method of sampling directly impacts relevance of results

Each compartment can be sampled by a variety of unique methodologies, some of which are well-accepted, but ***rarely standardized for microplastics-specific applications***

Air

Plants / Organisms

Human & Animal Tissues

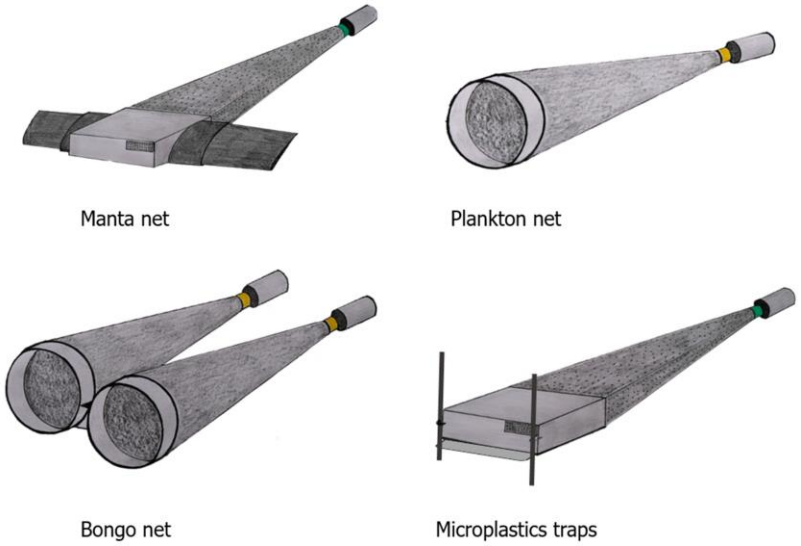


Water

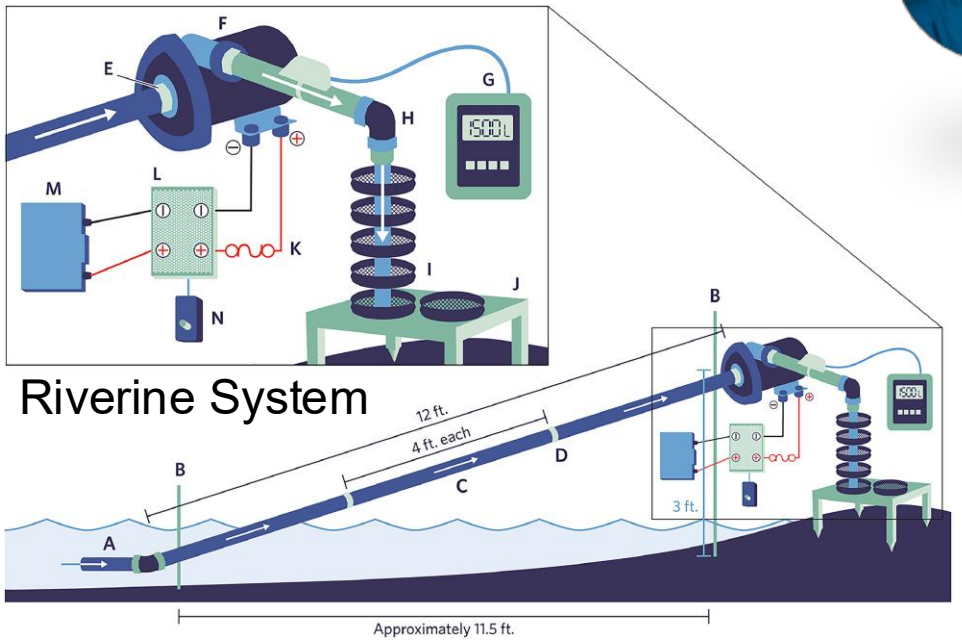
Compost

Soil / Sediment

I. Collection of Samples in Aqueous Environments



(Campanale, C., et al. *Sustainability* , 2020)



(Bryksa, J., et al. *MethodsX*, 2024)

- Majority of MNP water sampling has focused on environmental freshwater and marine environments. Limited analysis for drinking water or wastewater.

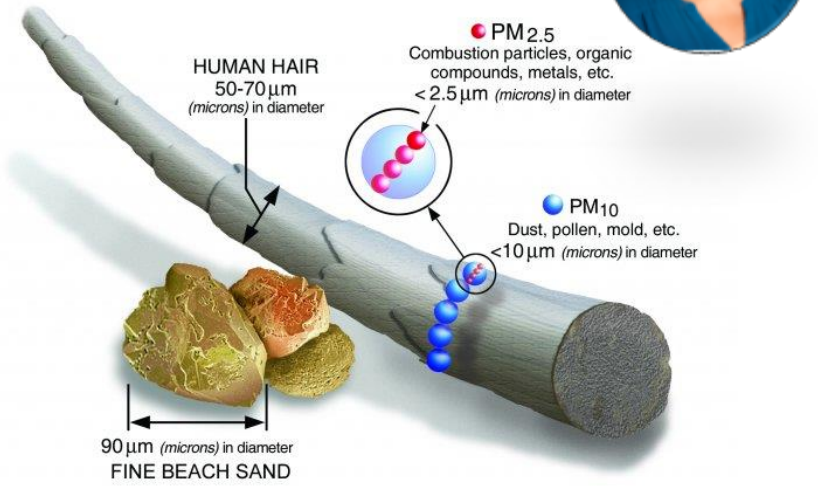
- Environmental sampling techniques include
  - Neuston or Manta Nets (< 330 µm)
  - Plankton Nets (< 100 µm)
  - Sieving (< 20 µm)
    - Clogging
    - Sample volume challenges
  - Grab and Filter *ex situ*
    - Lower volume of water
    - Labor intensive/Costly

ASTM **D8332 – 20** Standard Practice for Collection of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers

- Sample Pump System
  - Controlled volume and depth
  - Sieve system
  - Lose small MNPs (< 20 µm)
  - Thousands of L collection possible

I. Collection of Samples in Air: Industrial Hygiene Inspired

- Many **Active** and **Passive** sampling techniques for microplastics are based on OSHA or NIOSH methodologies, e.g. NIOSH 500 and 600 for dust collection
  - Common measurement devices for dust / particles in air include:
    - Filter cassettes
    - Cyclones
    - Particle Impactors
    - Wet/dry collector buckets
- Measurements have been obtained in outdoor & indoor air, as well as occupational-specific settings
- Filter cascades can be applied in parallel to capture particles of differing size ranges

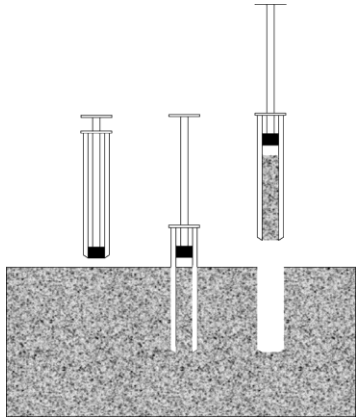


Particulate Matter (PM) Basics | US EPA

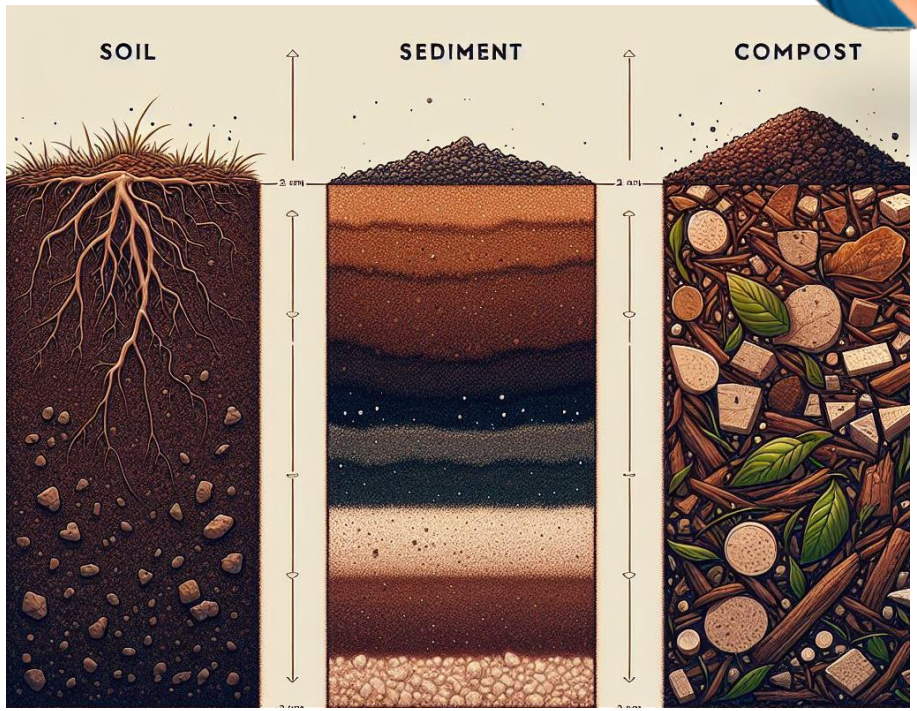
- For human health, focus on ≤ 2.5 µm
- In many cases, the volume of air filtered can be calculated to extrapolate concentration of particles (e.g. PM 2.5 values, ug/m^3)
- *Note: virtually all air sampling methods for particles are not chemistry-specific.* ***Thus, analytical confirmation of chemical identity as a microplastic is critical***

I. Collection of Samples in Soil, Sediment, & Compost

- Sediment
  - NOAA recommends 400 g ww per replicate
  - MFSD recommends at least 5 replicates within top 5 cm
  - Sampler options
    - Box Corer
    - Grab sampler
- Soil
  - No standard methods exist
- Compost
  - Ensure homogenous sampling
    - Varying degrees of organic & inorganic components
  - ISO/WD **24899** discusses methods for compost extraction for small amounts of compost



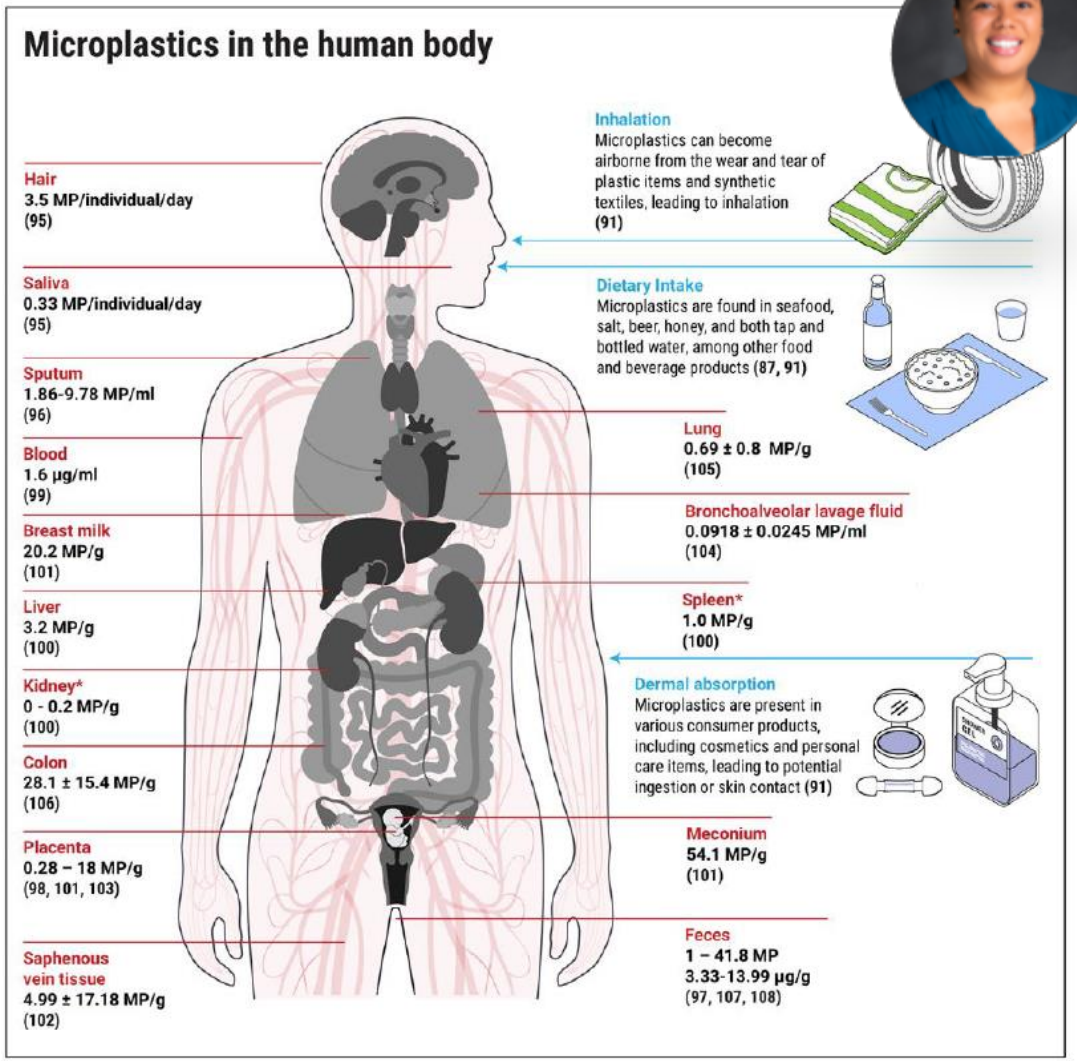
Piston Corer



I. Collection of Biological Samples

Focus on Humans

- Controlled toxicology studies
  - There are unique sampling/exposure differences in animal vs non-animal (ex. *in vitro*) based MNP studies
    - Quartz well plates ($$) for cell studies
    - Limited plastic environment for animal studies
  - Many currently use PS beads or fluorescently tagged MNPs
- Exploratory biological sampling
  - No standard methods for biological sampling i.r.t. microplastics exist
  - Often occurs under typical medical operating conditions (non-ideal, high contamination risk).
- Newer studies reduce potential contamination during sampling (plastic-free samplers, glass blood tubes, metal bins, e.g.)
- More information in: Twenty years of microplastic pollution research—what have we learned? | Science
- Quality ranking for Human Health MNP associated papers review



"Figure 4. Quantities are as reported in each study and **have not been further QA/QC screened for this review**."

R. C. Thompson *et al.*, *Science* 10.1126/science.adl2746 (**2024**).

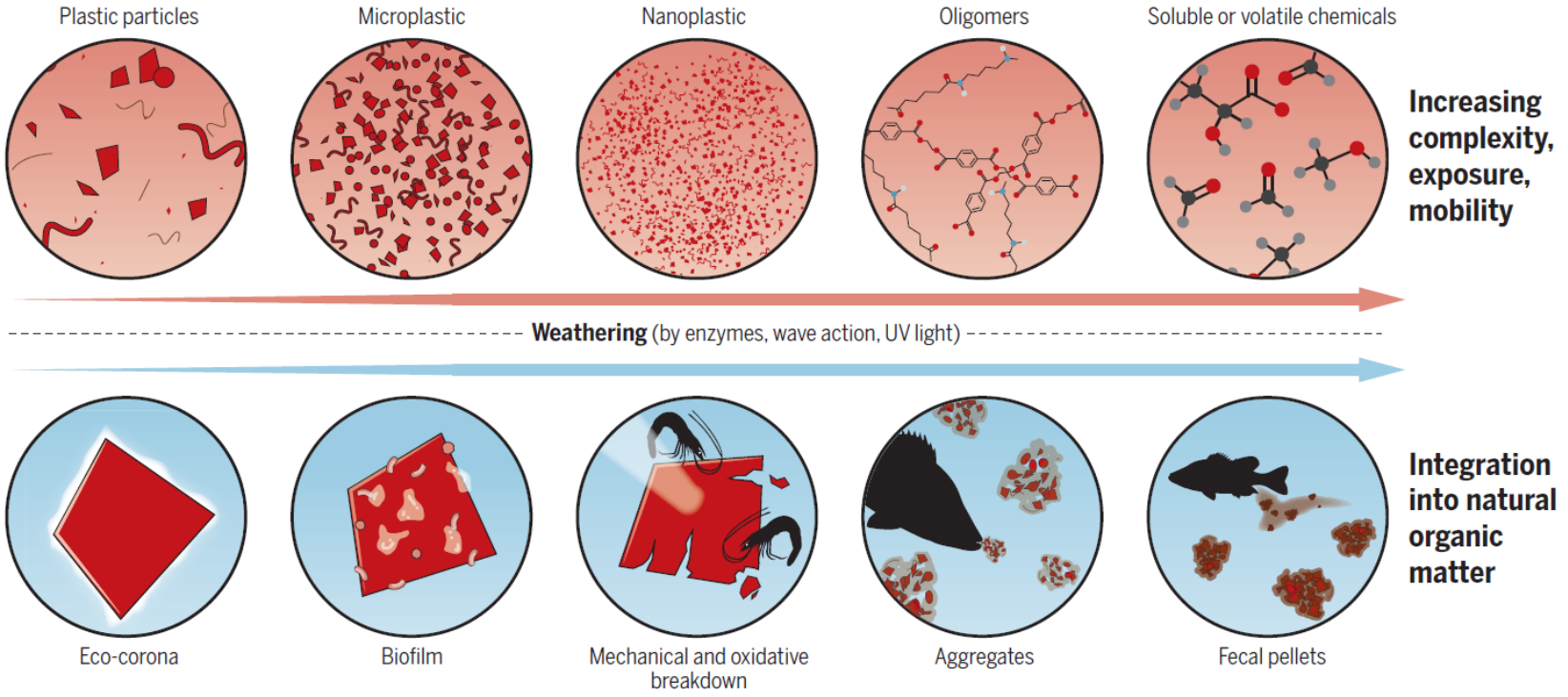
II. Microplastics Extraction

II. Microplastic Sample Preparation

Whether MPs are located in biological tissues or the environment, they have an associated matrix around them which should be removed prior to analysis

- MPs should be prepared considering:
  - Type of **matrix**
  - Method of **analysis**
  - Resulting potential for **false positives or negatives**
  - **Chemistry** of MPs

- **Ideally**, matrix removal should be reproduceable, allow MPs to remain intact with **high % recoveries**, and **low signal interference**



MacLeod, et. al, Science 373, 61-65 (2021)

An Aside…Microplastic Sample Preparation & Proper Protocols

Whether MPs are located in biological tissues or the environment, they have an associated matrix around them which should be removed prior to analysis

In-depth literature reviews indicate critical needs for quality development of the following areas associated with Parts: I (Sampling), II (Extraction), III (Analysis)

Contamination Prevention	***Blanks Protocols***	***QA/QC***	***Reference & Standard Materials***
Part I, II, III	**Part I, II, III**	**Part I, II, III**	**Part II, III**

The reality is: as more complex samples are analyzed by advanced analytics, **data interpretation is increasingly complex**. Some representative impacts from the lack of quality checks will be discussed in Part III

- The 2022 WHO Report *Dietary and inhalation exposure to nano- and microplastic particles, and potential implications for human health* indicates states: "…the available data are **of only very limited use for assessing the risk of NMP to human health.** Several shortcomings were identified, the most important of which was the heterogeneity of the methods used, including **use of "bespoke" methods** for analysing data…"

An Aside… Blanks & Lab Perturbation Tests: An Ideal Situation

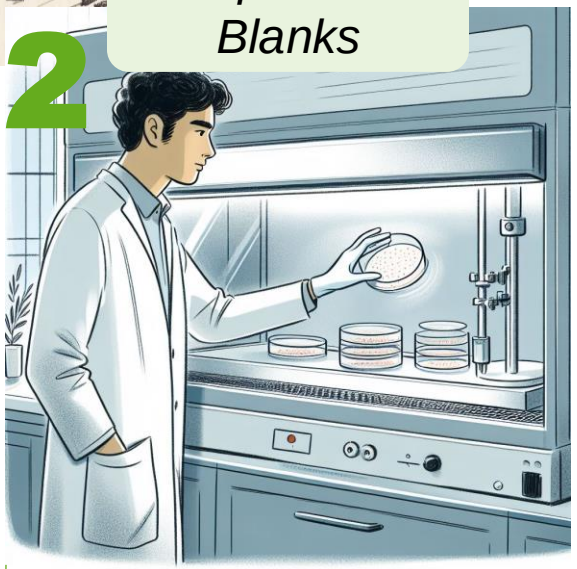
Blanks Protocols

QA/QC

1 *Field Blanks*

3 *Instrument Blanks*

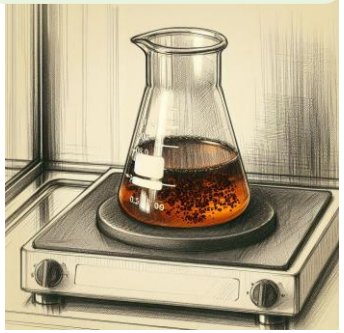
2 *Preparation Blanks*



Lab or "Process" Blanks

Tests for perturbations to reference MPs should be completed in parallel to procedure blanks

Chemical Effects



*Environmental Effects**

** Note: there is no way to fully mimic environmental / biological impact on MPs, but we try our best in the lab!*

An Aside…Contamination Prevention and Basic QA/QC Principles

Higher Quality Research = More Relevant Results

Example Spike Recovery

Density Gradient Step
PP % Recovery (50 mg): 89% ± 10%
PE % Recovery (50 mg): 56% ± 23%
LOD spike PP (5 mg) 30% ± 12%
LOD spike PE (5 mg) 22% ± 14%

Contamination Prevention

Best Practices

- Method Transparency
- Note taking: clothing, observations
- Excellent lab & field hygiene
- Non-plastic clothing
- Laminar flow hoods / benches
- Good air filtration
- Use no plastic supplies or reduced quantities of plastic supplies, isolated room
- Masking (tox)

- Guidance in: ISO **24187:2023**, **A study to inform best practices for microplastic analysis, 2023**

QA/QC

Best Practices: Consider ISO Principles

- % Recoveries should be conducted for **each** extraction / transfer procedure (experimental spikes)
  - Relevant particle size(s)
  - Relevant particle chemistries
  - Relevant (multiple) concentrations
- **Control standards** in addition to **experimental spikes** should be analyzed against calibration curves
- Process blanks, instrumental, and analysis blanks should be run
- **The chemical & environmental impacts on reference materials should be elucidated** (qual and/or quant evaluation)
- **Evaluation of matrix background for potential false positive / negative effects is CRITICAL**

An Aside…Reference & Standard Materials

- Reference materials are manufactured according to strict specifications and, for e.g. certified by NIST for one or more quantities of interest
- Standard materials are often analyzed / certified with traceability, and can be used for instrument calibration & analysis, and compared to reference standards
- References & standards needs for tox differ significantly from enviro. applications
- Current microplastics “Kits” include:
  - Polymer Kit 1.0 (22 polymers, Hawai'i Pacific University)
  - Polymer Kit 2.0 in development, Polymer Kit 2.0: Reference materials
  - Frontier Microplastic Calibration Standard kit
  - NIST Polymer pellet SRM
  - Companies like Sigma, Microplastic Solutions
  - JRC (Study) Reference materials for microplastics in water
- Reference / comparison vibrational spectroscopy data can be found in:
  - Raman SLOPP and SLOPP-E and FTIR FLOPP and FLOPP-E
- Reference & standards needs discussion in: Microplastic Reference Materials Invited Expert Workshop

II. Sample Preparation for Tissue / Organ Samples

- Matrix removal for biological samples often focuses on organic contaminants which can impact multiple types of analytics from properly identifying micro and nanoplastics:
  - E.g. fats, oils, cellulose, oligomers, lipids, other natural polymers
- No **standard methods exist** for microplastics extraction from biological tissues, and new methods are constantly being developed. Partial digestion of matrix remains a major challenge
- Common digestion methods include (with some combinations of below):
  - Basic digestion: potassium hydroxide, sodium hydroxide
  - Acidic digestion: nitric acid
  - Oxidative digestion: hydrogen peroxide, sodium hypochlorite
  - Enzymatic digestion
  - Microwave-assisted digestion
  - Increased temperatures
- Time scales for digestion range days to weeks but digestion efficiency has limited information
- Some methods include a density separation step or further processing depending on analysis
- More information can be found in: C. Fiore, et. al. 2024. A review on methods for extracting and quantifying microplastic in biological tissues

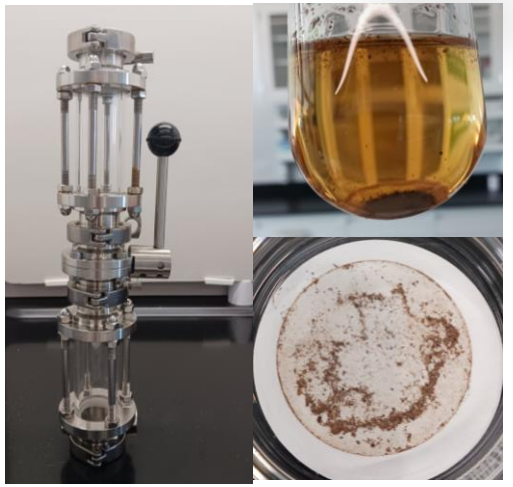
II. Sample Preparation for Additional Matrices

- Existing standards for extraction from **water** include: ASTM **D8333-20**, ISO **16094-1 to 3**
- Typical extractions include multiple steps:
  - Density gradients
  - Oxidation or matrix removal, e.g.
    - Fenton reaction
    - Schweizer reaction
    - Acid or base digestion
- The more contaminated a sample is, the greater the need for rigorous extraction procedures (i.e. MPs in clean water require much less prep than with high suspended solids)
- Reproducible extraction of MPs from sediment / soil is notoriously difficult
- One method ISO/WD **24899** is in development for extraction of microplastics from **compost**
- No standard methods for extraction from sediments or soils exist; however, a Density Separation Device was developed by K. Shaw, et. al. at NIST / Hawaii Pacific University for sediments

Table2. Separation of polymer types by solutions used in density separation.

Polymer	Density (g cm^{-3})	Water	NaCl	NaI	$ZnBr_2$
		1 gcm^{-3}	1.2 gcm^{-3}	1.6 gcm^{-3}	1.7 gcm^{-3}
PP	0.9–0.91	+	+	+	+
PE	0.92–0.97	+	+	+	+
PA	1.02–1.05	–	+	+	+
PS	1.04–1.1	–	+	+	+
Acrylic	1.09–1.20	–	+	+	+
PMA	1.17–1.20	–	+	+	+
PU	1.2	–	+	+	+
PVC	1.16–1.58	–	±	+	+
PVA	1.19–1.31	–	±	+	+
Alkyd	1.24–2.10	–	–	+	+
Polyester	1.24–2.3	–	–	+	+
PET	1.37–1.45	–	–	+	+
POM	1.41–1.61	–	–	±	+

Label: +: separation, ±: possible separation, –: not separated. Polymers: PE: polyethylene, PP: polypropylene, PS: polystyrene; PA: polyamide (nylon), POM: polyoxymethylene, PVA: polyvinyl alcohol, PVC: polyvinylchloride, PMA: poly methyl acrylate, PET: polyethylene terephthalate, PU: polyurethane (polymer density adapted from Ref.[44]).



NIST / HPU Density Separation Device for Sediments with Sodium Polytungstate (Density: 2.3 g cm^{-3}) at BASF Corp Labs

III. Analysis – Completing Your Microplastics Journey

Microplastic Quantitative Analytics Rundown

Count-Based

How many?

Microscopy / Imaging Methods

µ-infrared imaging
µ-Raman imaging
laser diode infrared imaging
multispectral imaging
fluorescence imaging
scanning electron microscopy transmission electron microscopy
optical light microscopies

Flow Measurements

light scattering (dynamic light scattering / electrophoretic light scattering)
flow cytometry
flow cams
nanoparticle tracking analysis

Materials Characterization

What are the physical characteristics?

Thermal Methods

thermogravimetric analysis, differential scanning calorimetry

Surface & Others atomic force microscopy (with infrared), melt tests, refractive index, nuclear magnetic resonance spectroscopy

Mass Based

Weight?

Mass Spectrometry Methods

thermal extraction/desorption gas chromatography mass spectrometry (TED GC/MS)
Pyrolysis GC/MS

Gravimetric Analysis

Weigh it!

- *No size limits*
- *Sample weight limits vary per instrument*

At minimum, an MP study should have confirmed chemical identity of microplastics and confirmed presence of solid particles

A Quick Dive into Count-Based Spectroscopic Methods

Current Status:

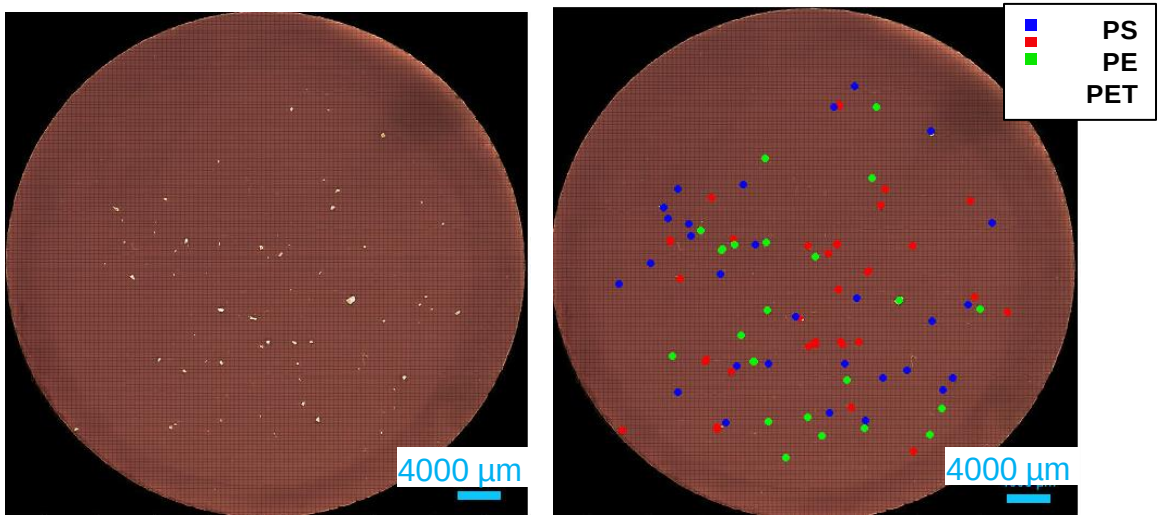
- Raman and µIR spectroscopic imaging remain dominant methods
- **Expertise & experience** is still required for proper analysis
- Machine learning / AI & automated imaging & particle counting / statistical analysis improving
- Subsampling is still a critical issue
- No reference materials readily available for complex plastics and/or aged plastics
- Standard methods for MPs originating from **water only**: ISO/DIS **16094-2**, ASTM **WK87463**

IR Benefits & Limitations:

- Theoretical lateral LOD tens of µms
- Interference from water / moisture & dyes / additives possible
- False positives possible: oligomers, stearates, oils, fats, surfactants, etc.
- Should have high % match to relevant reference materials **(> 80%)**

Raman Benefits & Limitations:

- Theoretical lateral LOD 1 µm
- Advances beyond diffraction limit with model systems
- Water not a major interference
- Dyes (fluorescence) can cause interferences or sample melting
- False positives possible: oligomers, stearates, oils, fats, surfactants, etc.
- Should have high % match to relevant reference materials **(> 80%)**



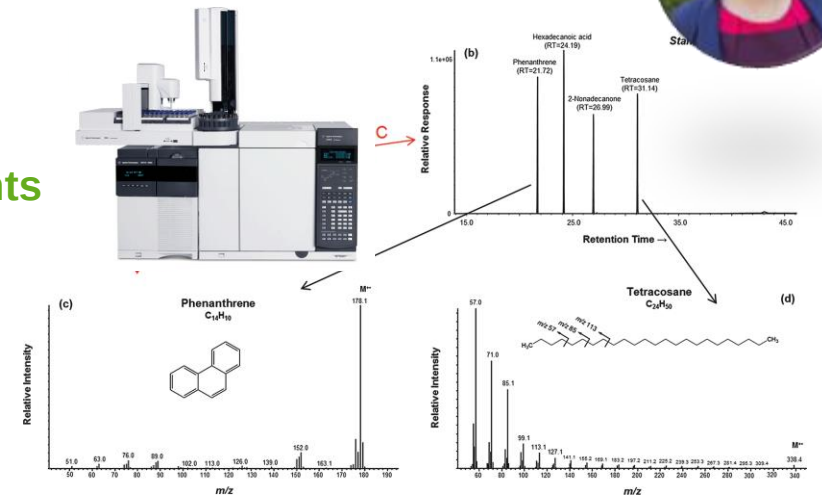
Raman mosaic image demonstrating different MP chemistries potentially indistinguishable by light microscopy
Kyle Akred, MS – BASF Corp

A Quick Dive into Mass-Based Methods (Pyrolysis Highlight)

Current Status:

- Standard Methods for **water** matrices only: ASTM **D8401-24**, ISO/DIS **16094-3**
- Simultaneous detection, ID, & quant of individual polymer types possible
- Many questions remain on impact of matrices for output

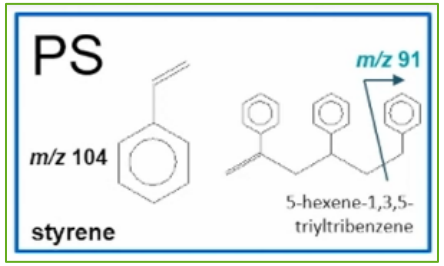
1. **Heat sample**
2. **Separate fragments on column**
3. **Detect by MS**

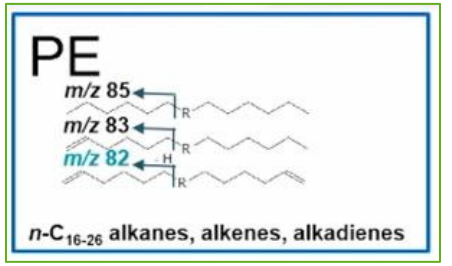


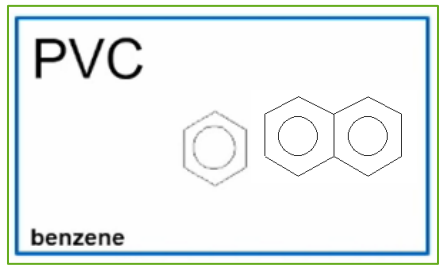
Benefits & Limitations:

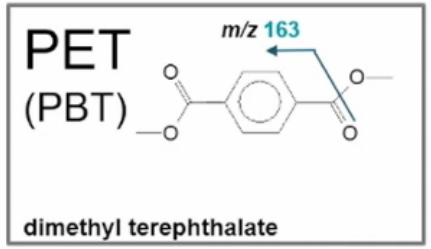
- Powerful! Chemical specificity, can ID smaller molecules, additives
- **Extremely low LOD**, theoretically (<0.005 mg), but narrow calibration range
- Trace biofilms, fats, organic & inorganic molecules, pH can all impact quant & qual ID **(false positives AND negatives)**
- Some polymers require reactive pyrolysis
- Biological samples often require significant adaptation of sample preparation for matrix removal & QA/QC before pyrolysis can be used **(not nearly as simple as vibrational spectroscopies)!**

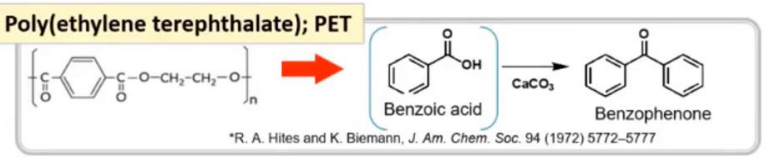
Resulting pyrolyzate patterns are unique, but must be carefully compared to similar patterns from organic molecules, making expertise a critical component of analysis!





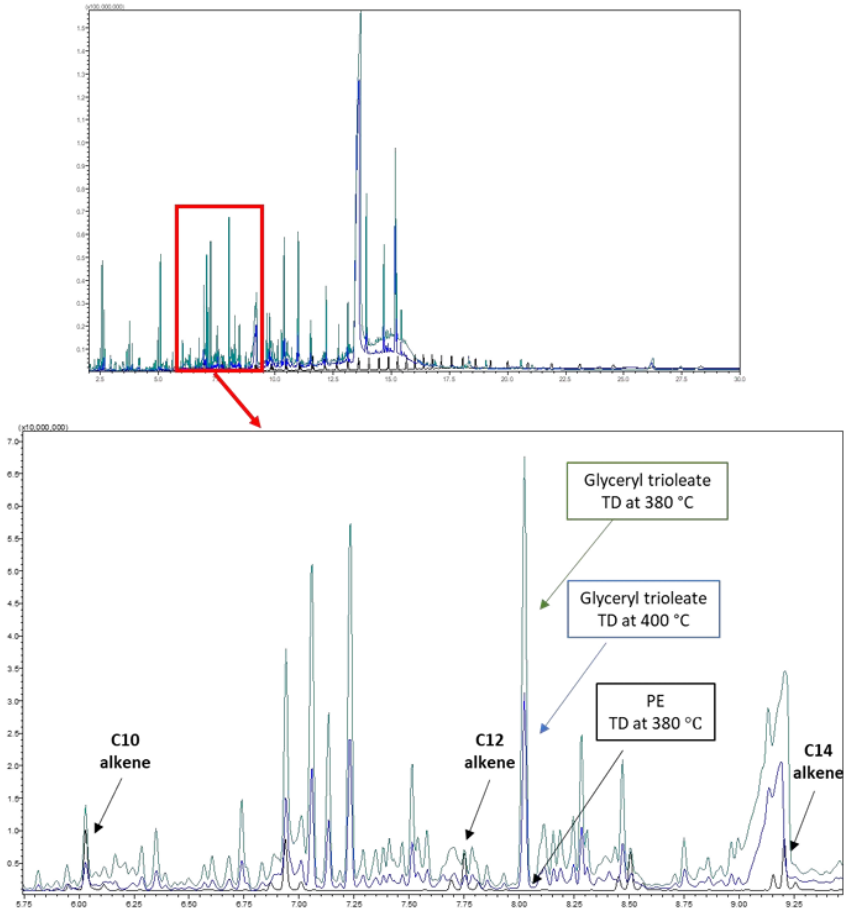






Polymers transform under pyrolytic conditions

A Quick Dive into Mass-Based Methods (Pyrolysis Highlight)



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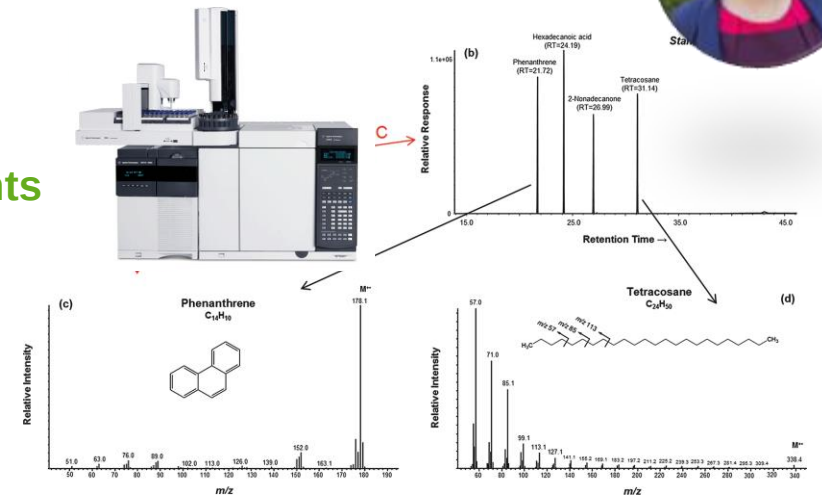
2. Separate fragments on column
3. Detect by MS



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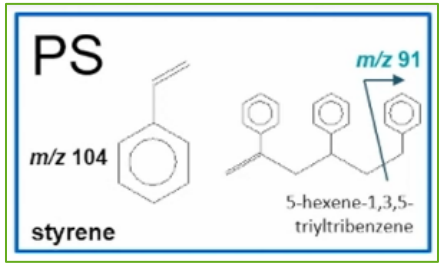
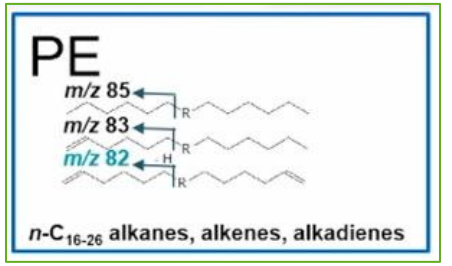
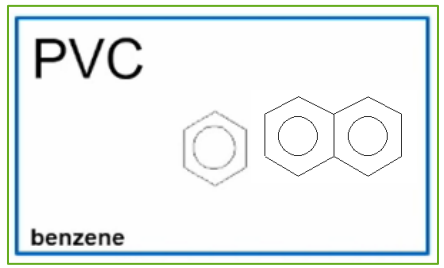
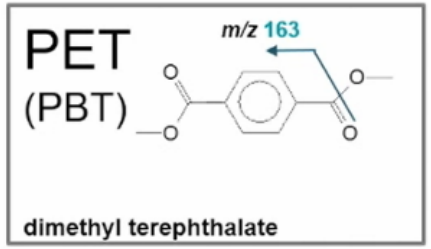
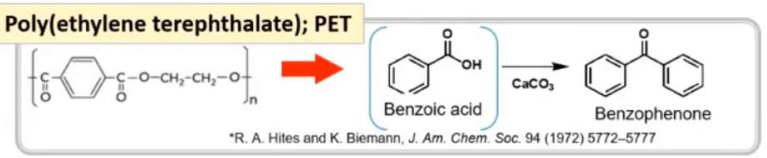










Polymers transform under pyrolytic conditions

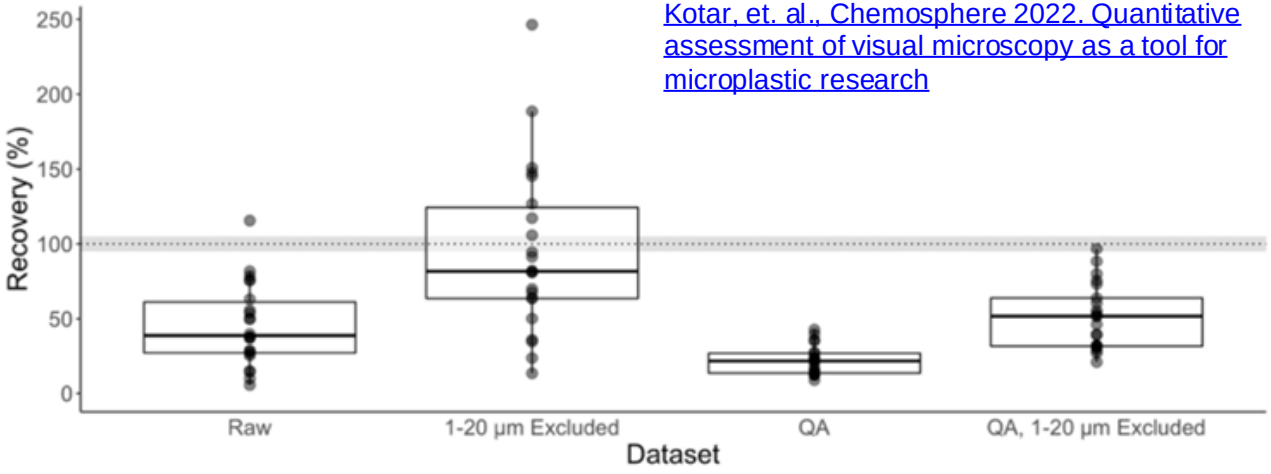
C. Rauert, et. Al. J. Environ. Exp. Assmt. 2022, Extraction and Pyrolysis-GC-MS analysis of polyethylene in samples with medium to high lipid content

C. Rauert, ES&T 1015. Assessing the Efficacy of Pyrolysis–Gas Chromatography–Mass Spectrometry for Nanoplastic and Microplastic Analysis in Human Blood | Environmental Science & Technology

Previous Interlaboratory Studies Tell Us Quantitation is Complicated

- We've improved since these ILSs; historically, they tell us reproducibility in results even in controlled conditions is difficult!
- Further guidance is required to improve outcomes for reproducibility & robustness
- Methods are often complex, and further streamlining is needed to make analyses "routine"

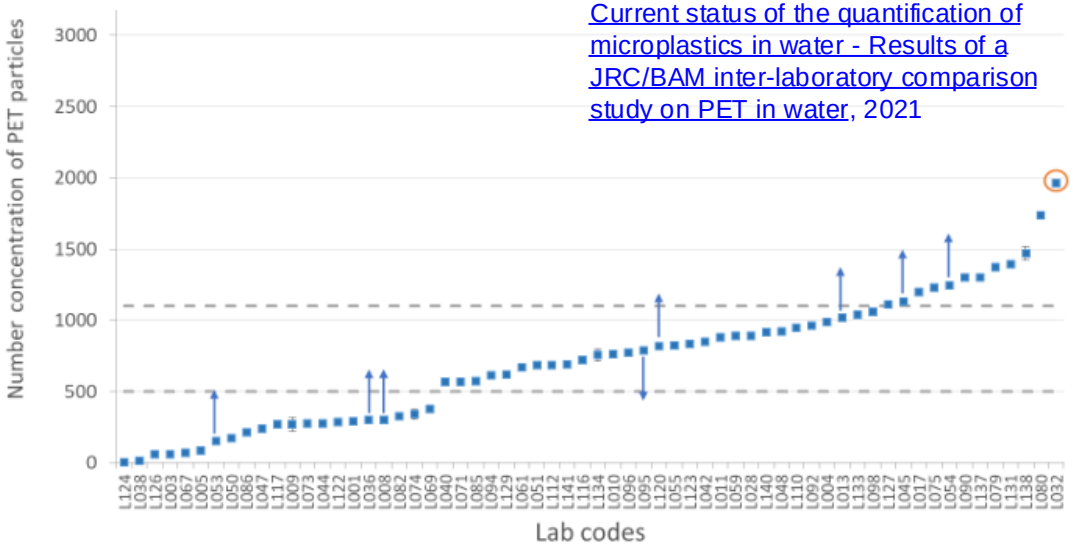
SCCWRP ILS



Kotar, et. al., Chemosphere 2022. Quantitative assessment of visual microscopy as a tool for microplastic research

"Average recovery (±StDev) for all reported particles >50 µm was **94.5 ± 56.3%.** After quality checks, recovery for >50 µm spiked particles was **51.3 ± 21.7%.** Recovery varied based on morphology and color, with poorest recovery for fibers and the largest deviations for clear and white particles. **Experience mattered**…"

JRC/BAM ILS



Current status of the quantification of microplastics in water - Results of a JRC/BAM inter-laboratory comparison study on PET in water, 2021

"The scatter of measurement results is very high, revealing a **substantial lack of inter-laboratory reproducibility** that seems to be largely independent of the analysis technique applied. This is a severe problem for the comparability of measurement results…"

Future Growth Opportunities

- Growth in the 4 Opportunity for Improvement Areas will continue to increase the quality level and relevance of future studies, especially in the lens of biological samples

Contamination Prevention | ***Blanks Protocols*** | ***QA/QC*** | ***Reference & Standard Materials***

- Analytical & technological advances continue to lower LODs and improve ease of analysis
- Advances in the complexity of modeling have the potential to improve our understanding of hazard & risk
- Standards Organizations & Government Orgs will continue to develop methods & guidance
- With such a complex topic, no one group can improve the level of scientific knowledge alone. Increasing collaborations between government, academia, and industry may be key to advancing this scientific field, e.g.
  - Southern California Coastal Water Research Project
  - **MARII**
  - **Operation Clean Sweep**

Future Opportunities – Industry Efforts

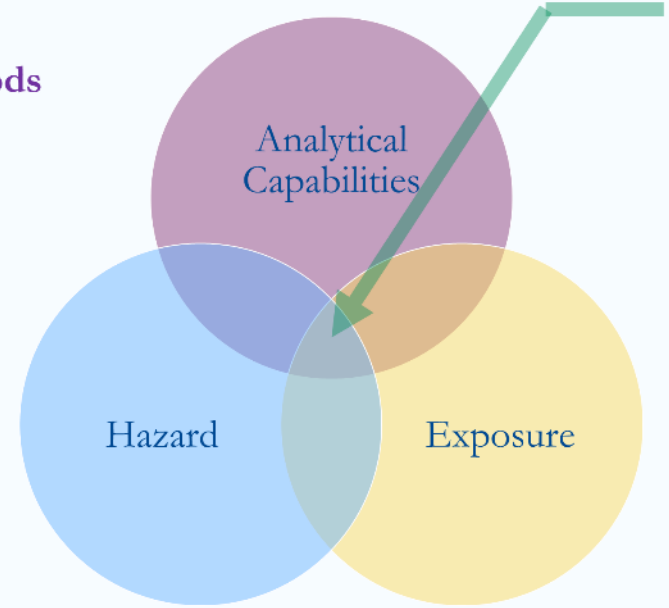
Microplastics Advanced Research & Innovation Initiative (MARII)

International Council of Chemical Associations

Developing a Holistic Risk Assessment Framework

American Chemistry Council
- Standard Sampling Methods
- Analytical Methods
- Reference Materials

PLASTICS EUROPE
cefic
- Approaches to identify human health hazards
  - Ingestion & Inhalation
- Approaches to identify environmental hazards
  - Aquatic ecotoxicity
  - Model organisms



Risk Assessment
- Probabilistic Risk Modelling
- Environmental Risk Framework

cefic
JCIA
PLASTICS EUROPE

- **Human Health**
  - Uptake and internal distribution
  - Composition of indoor and outdoor air
- **Environmental Exposure**
  - Fate, transport, and sediment deposition
  - Adsorption and bioaccumulation

JCIA
American Chemistry Council
cefic
PLASTICS EUROPE

[Microplastics Advanced Research and Innovation Initiative (MARII) - International Council of Chemical Associations (ICCA)]

Future Opportunities – Industry Efforts

Operation Clean Sweep®

- Operation Clean Sweep® (OCS) – a global program for the prevention and containment of plastic/resin pellet/flake/powder loss during production, loading, distribution, processing and recycling
- Initiated by the US plastics industry association in 1991, globally adopted by other associations as program licensees and more than **5,000 companies** participating
- Signed associations and pellet-handling value chain commit to pro-actively implement good housekeeping practices
- **Available: Manuals, best practices, reporting, third-party auditing & certification**

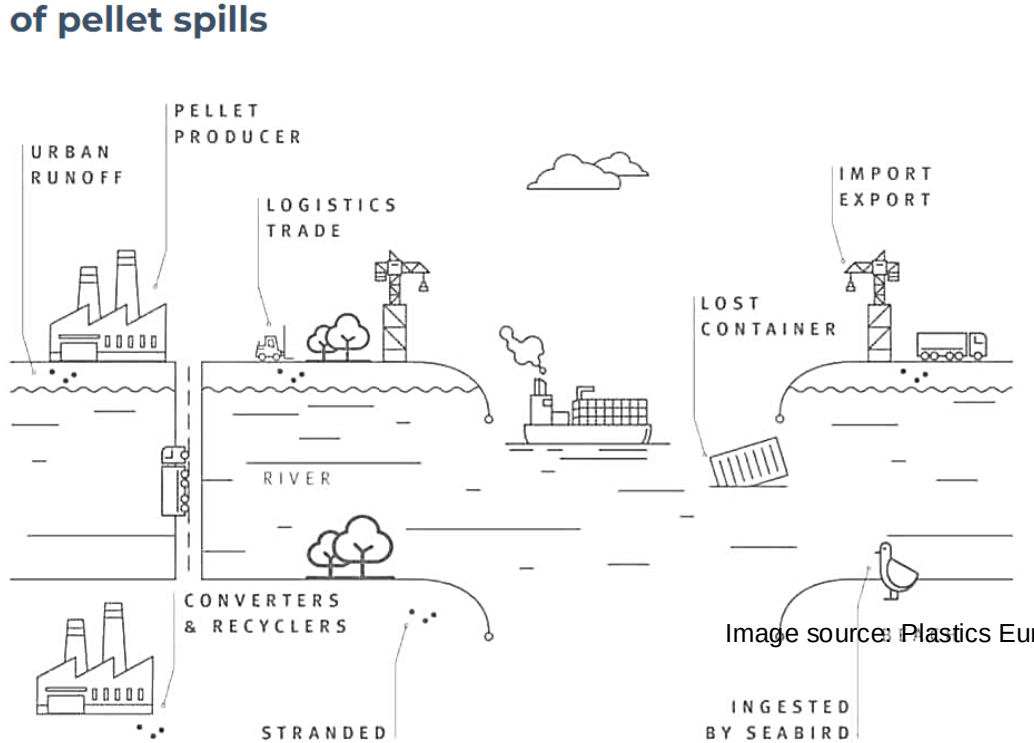


Image source: Plastics Europe

Advances in Analytical Technologies

Next generation analytical technologies are constantly being developed & evolved to reduce detection limits, improve instrumentation runs, and interpret data sets. E.g.

- Microcavity size selection with Raman microscopy (European Commission, Joint Research Centre)
- Nanoflow assays (NIST)
- Hyperspectral imaging flow cytometry (DoE and EPA)
- Photothermal IR (multiple academic labs)
- Nonlinear spectroscopies (academic labs)
- Single particle chemical analysis
- Machine Learning & AI-supported automated data analyses

Take-Home Messages & Acknowledgements

- Microplastics investigations began in aqueous environments; unsurprisingly, the next frontier to tackle with increasing harmonization is quantitation and chemical ID of microplastics in more complex matrices (e.g. additional environmental compartments, biological samples)
- Advances in analytical technologies have allowed for improved analysis (targeted ID, lower detection limits) but multiple challenges for false positives and negatives, contamination prevention, QA/QC, and reference and standard materials remain.
- Increasing levels of quality and transparency in research will be critical to aid in the improved understanding of exposure, hazard, and risk, and improved relevance for results
  - The generation of new standard methods, guidance, and reference and standard materials moves us in the right direction
- Lessons / exchange between multiple sectors will further enhance breakthroughs in MP science

Thank You

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