Gut Passage of Microplastics and Bioavailability of Co-contaminants Associated with Microplastics in Organisms Exposed via Diet or Aqueous Phase

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Microplastics

**Manufactured microplastics**
- Industrial abrasives, exfoliants,
- Precursors (resin pellets etc.)

**Breakdown of plastics**
- Fibers (e.g., clothing)
- Weathering of larger pieces in environment

Project Kaisei (www.projectkaisei.org)
Size Comparison: Particle of Diameter 1 nm versus 1 mm

1 nanometer

1 millimeter

Earth $10^7$ m

Fullerene $10^{-9}$ m

Football $0.1$ m

Neutrophil $\sim 10$ μm

Whale shark $10$ m

Zebrafish embryo $1$ mm
Comparison

**Nanoparticles**
- Definition debatable
- One dimension < 100 nm
- Source:
  - Deliberate manufacture
- Presence in surface waters
  - TiO$_2$-NPs, Ag-NPs: sub ppb
  - Carbon NPs??
  - **Anticipated** to become an environmental issue

**Microplastics**
- Definition debatable
- Plastic particles < 1-5 mm
- Source:
  - Deliberate manufacture
  - Breakdown of larger plastics
- Presence in surface waters
  - Marine environments in suspension and associated with sediments
  - Recognized as significant environmental debris
## Comparison: Aquatic Toxicity

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Microplastics</th>
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<tbody>
<tr>
<td><strong>Toxicity concerns:</strong></td>
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# Study Objectives

## Nanoparticles
- **Toxicity concerns:**
  1. Ingestion
  2. Occlusion of gut/gills
  3. Absorption across epithelia?
  4. Trophic transfer?
  5. Transfer of co-contaminants
     - Associated during manufacture
     - From environment

## Microplastics
- **Toxicity concerns:**
  1. Ingestion?
  2. Occlusion of gut
  3. Absorption across epithelia?
  4. Trophic transfer?
  5. Transfer of co-contaminants?
     - Substances from plastics
     - From environment?
SEM Images of Microplastic Particles

- Amorphous
- Complex/porous surface
- Metals not detected by X-ray spect.
- Same particles used in sorption studies (Bakir et al.)
Do Co-Contaminants Associate (sorption) with MPs?

Analytical chemistry: Yes (Bakir et al., 2014; others)

- Amount depends on plastic type (PVC < PE)
- Specific co-contaminant
- Salinity [not significant effect on sorption (phenanthrene)]

Effect on co-contaminant bioavailability?
Do Co-Contaminants Associate (sorption) with MPs?

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Effect on co-contaminant bioavailability?

Bioavailability assessed using gene expression analysis. Larval zebrafish, *Danio rerio*, used as an analytical tool to investigate bioavailability (not a toxicity test).
Do Co-Contaminants Associate (sorption) with MPs?

Analytical chemistry: Yes (Bakir et al., 2014; others)
- Amount depends on plastic type (PVC < PE)
- Specific co-contaminant
- Salinity [not significant effect on sorption (phenanthrene)]

Effect on co-contaminant bioavailability?

Note: fish used as detector of bioavailability, not a toxicity test
ZF Gene Expression: Bioavailability

Microparticle + Co-contaminant ⇌ [Phenanthrene] [17α-Ethinylestradiol]
ZF Gene Expression: Bioavailability

Is expression related to concentration?
First need to characterize response

\[ y = 1.7627x + 1.0772 \]
\[ R^2 = 0.9364 \]

Vicky Sleight
ZF Exposure: Bioavailability

Microparticle (400 mg/L) + Co-contaminant

Stirred 5 days in the dark (20°C)

[Phenanthrene] = 500 ppb
or
[17α-Ethinylestradiol] = 1 ppb
ZF Exposure: Bioavailability

Microparticle (400 mg/L) + Co-contaminant

Sedimentation

Aqueous

Vicky Sleight
Phenanthrene Bioavailability

Sedimentation + Aqueous

Microparticle (400 mg/L) + Co-contaminant

ZF cyp1A (fold change)

<table>
<thead>
<tr>
<th></th>
<th>Phe+PVC (n=3)</th>
<th>Filtered (n=3)</th>
<th>Phe+PVC (n=3)</th>
<th>Filtered (n=3)</th>
<th>control (n=6)</th>
<th>control (n=18)</th>
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<tbody>
<tr>
<td>Sediment</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Aqueous</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a</td>
<td>a</td>
<td>b</td>
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<tr>
<td>Positive</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Negative</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
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<td>b</td>
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Vicky Sleight
Indication is lower bioavailability of phenanthrene in presence of MPs

Vicky Sleight
**17α-Ethinylestradiol Bioavailability**

<table>
<thead>
<tr>
<th>Microparticle (400 mg/L)</th>
<th>Co-contaminant</th>
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### Sedimentation

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<th>Sample Type</th>
<th>Number of Replicates</th>
<th>ZF vtg (fold change)</th>
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<tr>
<td>EE2+PVC (n=3)</td>
<td>Sediment</td>
<td>a,b</td>
</tr>
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### Aqueous

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<tr>
<td>control (n=3)</td>
<td>Positive</td>
<td>a</td>
</tr>
<tr>
<td>control (n=18)</td>
<td>Negative</td>
<td></td>
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</table>

*Vicky Sleight*
17α-Ethinylestradiol Bioavailability

Indication is lower bioavailability of 17α-Ethinylestradiol in presence of MPs
So, if substances sorb to MPs (indicated by analytical chemistry and reduced bioavailability tests) can they be transferred to organisms?

Aqueous phase

\( nC_{60} \) + 17α-ethinylestradiol (EE2) → \( nC_{60} \)-EE2 complex

Questions for MPs:

- Do organisms ingest MPs?
- Are ingested MPs absorbed across epithelial membranes?
- How long do MPs reside within digestive tract before egestion?
- Can sorbed co-contaminants become bioavailable?

Park et al., 2010, 2011

Evaluate vtg expression (liver)

Dietary exposure

Adult zebrafish

Brine shrimp
Do organisms ingest MPs?
Are ingested MPs absorbed across epithelia?
How long do MPs reside within GI tract before egestion?

Test organism:
Shore crab *Carcinus maenas*

**Approach:**
- **Feed pellets:** fish paste and gelatine (pellet weight 0.25 g)
  - Particles counted and specific amount added to each pellet
  - Sub-sample of pellets examined to verify numbers of particles/pellet
- **Treatments:**
  - Control without particles
  - Large sand (LS) 1000-1230 µm
  - Large plastic (LP) polyethylene microspheres 850-1000 µm
  - Small plastic (SP) polyethylene microspheres 47-53 µm
- **Procedure**
  - Crabs fed particle-free food for > 5 days
  - Crabs starved for 3 days before being fed a single treatment pellet
  - After feed pellet administered, control pellet given every 24 hours
  - Crab washed, faeces and water collected, filtered, particles counted/time

Joseph Hatfield
Crabs did ingest these MPs.
These MPs not appreciably absorbed.
Gut retention varied by particle type.

- Large sand (LS)
- Large plastic (LP)
- Small plastic (SP)

Time when 50% particles egested:

What about sorbed co-contaminants?

Joseph Hatfield
### Summary/Conclusions

#### Nanoparticles
- **Toxicity concerns:**

1. Ingestion
2. Occlusion of gut/gills
3. Absorption across epithelia?
4. Trophic transfer?
5. Transfer of co-contaminants
   - Associated during manufacture
6. From environment

#### Microplastics
- **Toxicity concerns:**

1. Ingestion: **YES**
2. Occlusion of gut
3. Absorption across epithelia? **NO**
4. Trophic transfer?
5. Transfer of co-contaminants
   - Substances from plastics
   - From water: associated with MPs, transfer to organisms?
Acknowledgments:

- Stan McMahon; Helena Reinardy; Gabriela Aguirre; Robyn Wright; Andy Atfield (University of Plymouth)

Future Research:
- Dr. Ana Catarino: Marie Curie Research Fellow (June 2014-2016)
- “Marine microplastics toxicity: investigating microplastics and their co-contaminants in marine organisms (MARMICROTOX)”

SETAC North America (November 2014):
- Microplastics Session: “Environmental Impacts of Microplastics: an Issue of Local, Regional, and Global Concern”
- Session Chairs: Drs. Chelsea Rochman and Ted Henry
- Submit abstracts by 28 May 2014 (Platform and Poster session)