Analysis, prevalence and impact of microplastics in freshwater and estuarine environments: an evidence review.

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Systematic review procedure to address three questions

• Are the current sampling and analytical methods scientifically robust and appropriate?
• What are the sources of the microplastics found in freshwater environments?
• What is/are the impact(s) of microplastics on freshwater and estuarine biota?

[Microplastics = all plastic particles sizes ≤ 5 mm including nano-sized (≤ 0.1 μm) plastic particles?]
Systematic review procedure

Objective way of searching for, reviewing and summarising evidence to help answer specific questions

Pre-defined protocol
Set of clearly defined questions
Set of pre-defined search terms
Consistent approach for evaluating the relevance of evidence to the questions
Consistent approach for evaluating the robustness of evidence
Capturing the evidence base

<table>
<thead>
<tr>
<th>PICO elements</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td></td>
</tr>
<tr>
<td>Comparator</td>
<td></td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
</tr>
</tbody>
</table>
An initial wide search to establish the population of evidence in published and grey literature

<table>
<thead>
<tr>
<th>Population</th>
<th>plastic*</th>
<th>freshwater*</th>
<th>wetland</th>
<th>potable</th>
<th>micro*</th>
<th>river*</th>
<th>marsh</th>
<th>reservoir</th>
<th>microplastic</th>
<th>stream*</th>
<th>swamp</th>
<th>aquifer</th>
<th>nanoplastic</th>
<th>brook</th>
<th>wastewater*</th>
<th>groundwater</th>
<th>*plastic</th>
<th>lake*</th>
<th>drinking water</th>
<th>sewage</th>
<th>pool</th>
<th>aquatic</th>
<th>outfall</th>
<th>pond</th>
<th>ecosystem*</th>
<th>estuar*</th>
<th>transitional</th>
</tr>
</thead>
</table>
holdings of relevant environmental regulators
Stages of evidence gathering

Identification
Screening
Eligibility
Identification
Scoring
Are the current sampling and analytical methods scientifically robust and appropriate?
A second set of pre-defined search terms relevant to the question

<table>
<thead>
<tr>
<th>Population</th>
<th>Intervention</th>
<th>Comparator</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>aggregate*</td>
<td>spectroscop*</td>
<td></td>
<td>count</td>
</tr>
<tr>
<td>colloid*</td>
<td>raman</td>
<td></td>
<td>quantif*</td>
</tr>
<tr>
<td>floc*</td>
<td>particle analysis</td>
<td></td>
<td>abundance</td>
</tr>
<tr>
<td>plankton*</td>
<td>pyrolysis</td>
<td></td>
<td>concentrat*</td>
</tr>
<tr>
<td>sediment*</td>
<td>sampI*</td>
<td></td>
<td>density</td>
</tr>
<tr>
<td>diet*</td>
<td>separat*</td>
<td></td>
<td>substance</td>
</tr>
<tr>
<td>content</td>
<td>identif*</td>
<td></td>
<td>state</td>
</tr>
<tr>
<td>*fibre</td>
<td>flotat*</td>
<td></td>
<td>morphology</td>
</tr>
<tr>
<td>*fiber</td>
<td>floatat*</td>
<td></td>
<td>dimension</td>
</tr>
<tr>
<td>*bead</td>
<td>microscop*</td>
<td></td>
<td>composition</td>
</tr>
<tr>
<td>fragment*</td>
<td>digest*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pellet*</td>
<td>centrifug*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>flake*</td>
<td>buoyan*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nurdle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dust</td>
<td></td>
<td></td>
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</tbody>
</table>
Map of evidence identified as relevant

Records identified through database searches using general search terms (n = 6,280)

Records after duplicates removed (n = 3,456)

Records after keyword exclusion of irrelevant topics (n = 1,844)

Records after manual removal of irrelevant topics (n = 1,177)

Records after screening on title/abstract for relevance to ER1 (n = 414)

Records relevant to freshwaters (n= 175) or estuaries (n = 107)

Full text articles after removal of text read at title, abstract (n= 283)

Additional sources from ER2 & ER3 screening (n = 35)

Records after removal of duplicate articles reporting same study

Hierarchy of evidence (n = 237)

Records after removal of articles with confounding factors (n= 209)

Duplicates removed (n = 2,842)

Sources excluded (n = 1,612)

Sources removed (n = 667)

Sources removed (n = 764)

Sources removed (n = 131)

Sources removed (n = 46)

Sources removed (n = 63)

Evidence sources per year

[Cut-off date April 2019]
Relationships among particle size, volume of water sampled and reported concentration

**Freshwater**

- **Particle Size – Sample Vol**
  - $R^2 = 0.324$
- **Sample Vol – Conc**
  - $R^2 = 0.701$
- **Particle Size – Conc**
  - $R^2 = 0.358$

**Estuaries**

- **Particle Size – Sample Vol**
  - $R^2 = 0.543$
- **Sample Vol – Conc**
  - $R^2 = 0.630$
- **Particle Size – Conc**
  - $R^2 = 0.485$

Points represent mean per study/technique/habitat.
Relationships among particle size, volume of sediment sampled and reported concentration

- **Particle Size – Sample Vol**
- **Sample Vol – Conc**
- **Particle Size – Conc**

**Points represent mean per study / technique / habitat**

**Freshwater**: 
- R² = 0.215

**Estuaries**: 

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The size range of particles captured by the sampling and processing method used influences the mean abundance of microplastic particles reported.

Comparison among studies is not possible without consideration of the size of particles considered.

A range of sample volumes may be necessary to quantify the abundance of different sized particles adequately.

More research into appropriate sample volumes for sediment is required.
Techniques used to quantify and characterise microplastic particles

A range of techniques have been used to quantify and characterise particles:

Spectroscopic
  (e.g. FTIR, Raman, near infrared)
Thermoanalytical
  (e.g. Py-GC-MS, TED-GC-MS)
Chemical
  (e.g. ICP-MS)

Each return information on different characteristics of the microplastics present in the sample
Due to the variety of techniques that have been used to quantify and characterise microplastics, as well as variation in the volume sampled and size of particles considered, it is not possible to assess differences in the microplastic profile among studies using the data currently available.
Reliability of Studies

Scored 0 – 2 per criterion following Hermsen et al. (2018) and Koelmans et al. (2019)

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<table>
<thead>
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<tbody>
<tr>
<td>1.</td>
<td>Sampling methods</td>
</tr>
<tr>
<td>2.</td>
<td>Sample size</td>
</tr>
<tr>
<td>3.</td>
<td>Sample processing and storage</td>
</tr>
<tr>
<td>4.</td>
<td>Laboratory preparation</td>
</tr>
<tr>
<td>5.</td>
<td>Clean air conditions</td>
</tr>
<tr>
<td>6.</td>
<td>Negative control</td>
</tr>
<tr>
<td>7.</td>
<td>Positive controls</td>
</tr>
<tr>
<td>8.</td>
<td>Target component (for Biota only)</td>
</tr>
<tr>
<td>9.</td>
<td>Sample treatment</td>
</tr>
<tr>
<td>10.</td>
<td>Polymer identification</td>
</tr>
</tbody>
</table>
Change in Reliability Scores over time

Two measures of reliability:

Total Accumulated Score (0 – 18)
Number of Zeros (9 – 0)
Reliability of studies of microplastics in freshwaters and estuaries has increased over time
What is/are the impact(s) of microplastics on freshwater and estuarine biota?
Map of evidence identified as relevant

- Records identified through database searches using general search terms (n = 6,282)
- Records after duplicates removed (n = 4,211)
- Records after keyword exclusion of irrelevant topics (n = 2,172)
- Records after screening on title/abstract for relevance to ER2 and manual removal of irrelevant topics (n = 423)
- Records relevant to freshwaters and estuaries (n = 274)
- Full text articles after removal of text read at title, abstract (n = 274)
- Records after removal of duplicate articles reporting same study
  - Hierarchy of evidence (n = 154)
- Records after removal of articles with confounding factors (n = 105)
- Duplicates removed (n = 2,071)
- Sources excluded (n = 2,039)
- Sources removed (n = 1,749)
- Sources removed (n = 149)
- Sources removed (n = 120)
- Sources removed (n = 49)

Evidence Sources per Year

[Cut-off date April 2019]
Reliability of Experimental Studies

Scored 0 – 2 per criterion following CRED (Criteria for reporting and evaluating ecotoxicity data) method of Moermond et al. (2016)

| 1. Validity criteria                          |
| 2. Adequate controls                         |
| 3. Identity of test substance                |
| 4. Source of test substance                  |
| 5. Identity of test organisms                |
| 6. Source of test organisms                  |
| 7. Appropriate for test substance            |
| 8. Appropriate for test organism             |
| 9. Gradient of exposure                      |
| 10. Exposure duration                        |
| 11. Verification of exposure                 |
| 12. Biomass loading                          |
| 13. Adequate replication                     |
| 14. Appropriate statistical methods          |
| 15. Raw data available                       |
Reliability of Experimental Studies

Total Cumulative Score (0 – 30) and Number of Zero scored categories (15 – 0)

Box indicates 25th and 75th percentiles, whiskers minimum and maximum, and line median size of particles (n = 103)
Two measures of reliability:

- Total Accumulated Score (0 – 30)
- Number of Zeros (10 – 0)
The majority of studies of the impact of microplastics on freshwater and estuarine biota were unreliable in several aspects.

Published studies have become less reliable over time.
Relationships between particle size and reported ecotoxicological threshold concentrations

Ecotoxicological endpoints behaviour, feeding, growth, reproduction, survival

Lines fitted by least squares regression
Less reliable studies (<median score) shown (red symbols) but excluded
Under experimental conditions, high concentrations microplastics can have a negative impact on the feeding, behaviour, growth, reproduction and survival of freshwater and estuarine biota.

The concentration required to cause such impacts is related to the size of the particles of microplastic.
Effect of taxonomic group on size specific threshold concentrations

Sufficient data to test effect of taxonomic group on relationship between particle size and threshold concentration for Crustacea, fish and algae using ANCOVA.

<table>
<thead>
<tr>
<th></th>
<th>F value</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Particle Size</td>
<td>569.85</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td>Particle Size * Endpoint</td>
<td>0.30</td>
<td>0.8778</td>
</tr>
<tr>
<td>Particle Size * TaxaGp</td>
<td>1.71</td>
<td>0.2109</td>
</tr>
</tbody>
</table>

No effect of taxonomic group or ecotoxicological endpoint on relationship with particle size.
Effect of polymer on size specific threshold concentrations

Sufficient data to test effect of polymer on relationship between particle size and threshold concentration for polyethylene (PE), polystyrene (PS), polyamide (PA) using ANCOVA

No effect polymer or ecotoxicological endpoint on relationship with particle size
Differences in reported thresholds could not be attributed to differences in the taxonomic group of the test organism or to the polymer used.
Size of particles used in ecotoxicological studies (n = 125), and smallest particles considered in studies of microplastics in estuaries and freshwaters (n = 185).

Nanoparticles ≤0.1 μm.

Box indicates 25th and 75th percentiles, whiskers minimum and maximum, and line median size of particles.
The majority of laboratory based toxicological studies have been undertaken using plastic particles that do not reflect the size of the microplastic particles that have been described from environmental samples collected in estuaries and freshwaters.

This mismatch adds uncertainty to our understanding of risk from microplastics.
Concentrations used in ecotoxicological studies

Threshold concentrations reported from laboratory studies

Mean concentrations reported from field collections of microplastics in estuaries and freshwaters

Lines fitted by least squares regression
Laboratory based toxicological studies have been undertaken using concentrations of microplastics that are many orders of magnitude greater than the concentrations that have been reported from samples collected from freshwater and estuarine environments.
Size specific threshold concentrations

Species Sensitivity Distribution approach not appropriate

Size Specific Thresholds fitted to 10%ile by quantile regression, i.e. concentration that is lower than 90% of reported lethal (dashed line) and all thresholds (red line) for that specific particle size

Less reliable studies (<median score) excluded

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Establishing Risk
Comparison of reported environmental concentrations and size specific thresholds

Size Specific Thresholds fitted to 10%ile by quantile regression, i.e. concentration that is lower than 90% of reported lethal and all thresholds.

Concentrations reported from field collections of microplastics in estuaries and freshwaters

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Establishing Risk
Comparison of reported environmental concentrations and size specific thresholds

Size Specific Thresholds fitted to 10%ile by quantile regression, i.e. concentration that is lower than 90% of reported lethal and all thresholds.

Quantiles (99%, 95%, 90%, 75%) fitted to concentrations reported from field collections of microplastics in estuaries and freshwaters.
The calculated size specific threshold concentration for lethal effects was higher than 99% of reported environmental concentrations, suggesting that lethal effects of microplastics on freshwater and estuarine biota are highly unlikely.

Over certain size ranges the calculated size specific threshold concentration for sublethal effects was exceeded by the highest 10% of concentrations reported from environmental samples, suggesting that there may be a possible risk of some sublethal effects in a small proportion of sites.
Three Reports to be available from Department for Environment, Food and Rural Affairs here

Evidence Reviews on Analysis, Prevalence & Impact of Microplastics in Freshwater and Estuarine Environments Evidence Review 1 Are the current sampling and analytical methods scientifically robust and appropriate?

Evidence Reviews on Analysis, Prevalence & Impact of Microplastics in Freshwater and Estuarine Environments Evidence Review 2 What are the sources of the microplastics found in freshwater environments?

Evidence Reviews on Analysis, Prevalence & Impact of Microplastics in Freshwater and Estuarine Environments Evidence Review 3 What is/are the impact(s) of microplastics on freshwater and estuarine biota?
Acknowledgments

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We acknowledge the experts who contributed their opinions to the reviews.