SCREENING & PRIORITIZATION OF NANO- AND MICROPLASTIC PARTICLE TOXICITY STUDIES FOR EVALUATING HUMAN HEALTH RISKS

Rob Ellis-Hutchings, Ph.D., DABT
Toxicologist - Dow, Inc.
PROLIFERATION OF MICROPLASTICS SCIENCE

The graph illustrates the proliferation of publications related to microplastics science, distinguishing between overall microplastics (MP Overall) and microplastics in relation to human health (MP + Human). The data shows a significant increase in publications from 2019 onwards.
Microplastics - Human Health Assessments

**EFSA - 2016**

*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)

**BfR - 2018**

Is there a risk to human health from microplastics? More research and scientific data needed  
BfR Communication No 033/2018 of 29.10.2018

**ECCC - 2020**

Science assessment of plastic pollution  
Environment and Climate Change Canada  
Health Canada  
October 2020

**BfR & ANSES - 2021**

Studies quality scored
CONCLUSIONS FROM AUTHORITY ASSESSMENTS

We Need More Data

Relevant exposures

Hazard Identification

External Exposure & Uptake/ Biodistribution

Hazard Characterization

Risk evaluation not possible

Risk Assessment

Target tissues, threshold doses

Well designed, quality controlled, standardized

Useful for all scientists & regulators
LACK OF CONSISTENT AND STANDARD METHODS – TIER BASED SYSTEMATIC REVIEWS NEEDED FOR HUMAN HEALTH RISK EVALUATIONS

Defining/Refining Criteria & Searching in Bibliographic Database

EFSA

Specific Hypothesis

Inclusion or Exclusion based on Relevance

Assessing Reliability of Relevant Studies

Tier 1 Screen

Adapted from Kaltenhäuser et al., 2017: EFSA Systematic Review for Peer-Reviewed Open Literature
LACK OF CONSISTENT AND STANDARD METHODS - TIER BASED

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Tier 2 Expert Evaluation

Individual Studies + Overall Weight of Evidence
Extent to which the hypotheses are/are not supported

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Extent to which the hypotheses are/are not supported

Inclusion in the Risk Assessment

Risk Assessment

Hazard ID

Hazard Characterization

External exposure & Uptake

Adapted from Kaltenhäuser et al., 2017: EFSA Systematic Review for Peer-Reviewed Open Literature
Efforts to date largely focused on regulatory test guideline studies

- Klimisch reliability code (*Klimisch et al.*, 1997)
  - Qualitative
  - Adequacy, Relevance, Reliability
  - Reliable (1 = without, 2 = with restrictions), 3 = Not reliable, 4 = Not assignable

- ToxRTool - Toxicological data Reliability Assessment Tool (*Scheider et al.*, 2009) **21 Criteria**
  - EURL ECVAM (EC JRC); Qualitative & quantitative elements, *in vivo* and *in vitro* ↑ Transparency
  - US EPA evaluated for regulatory hazard assessments (*Segal et al.*, 2015) Useful in initial review steps

- GUIDEnano – ToxRTool adapted for nanomaterials (*Fernandez-Cruz et al.*, 2018) Ecotox + Human health **29-32 Criteria**
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- Microplastics & Human health - No study quality or assessment criteria
**Development of a Nano/Microplastic Screening Approach – Human Health Hazard Studies Tier 1**

- “Poorly documented studies or those with questionable study design and reproducibility should be ID’d as such and not be used” Key elements for Judging the Quality of a Risk Assessment – Fenner-Crisp and Dellarco (2016) EHP 124(8): 1127-1135. Retired US EPA Risk Assessors

- Vast majority of studies can add value, but lack of consistent/standard methods requires a systematic evaluation approach for human health risks
Development of a Nano/Microplastic Screening Approach – Human Health Hazard Studies Tier 1

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➢ Vast majority of studies can add value, but lack of consistent/standard methods requires a systematic evaluation approach for human health risks

➢ Aim: Develop a Tier 1 evaluation tool to screen *in vivo* mammalian and *in vitro* NMP hazard studies for use in human health risk assessments

✓ Transparent, easily understood (qualitative & quantitative)
✓ Relevance, reliability of study results
✓ Criteria: Particle characteristics, Experimental design, Applicability to risk assessment

➢ Combines elements of the ToxRTool + de Ruitjer et al. (2020) screening criteria
**Nano/Microplastic Particle Toxicity Study Assessment Tool (NMP-TSAT)**

### Particle Characterization
1. Size
2. Shape
3. Type
4. Source
5. Surface Chemistry
6. Purity
7. Microbial Contamination

### Experimental Design
1. Particle Concentration Units
2. Particle Stability
3. Test Medium/Vehicle
4. Administered Dose/Concentration
5. Homogeneity of Exposure
6. Administration Route
7. Test Species
8. Feeding/Housing Conditions
9. Sample Size
10. Frequency & Duration of Exposure
11. Controls (Vehicle and/or Particle)
12. Replicates
13. Confirmation of internal dose

### Applicability for Risk Assessment
1. Statistical Analysis
2. Endpoints
3. Dose-response Relationship
4. Concentration Range
5. Effect Thresholds
6. Test Particle Relevance

**Red criteria = Critical.** If scored as 0, then study = unreliable & concluded not fit-for-purpose for use in a risk assessment.

**Proceed to Tier 2 (Expert Review) prior to inclusion in the Risk Assessment.**
NMP-TSAT: In Vivo Studies Assessed

Inhalation
16 studies
- 1 OECD guideline

Oral
24 studies
- 2 OECD guideline

40 NMP + cellulose comparison studies

For each:
- Element: score of 0, 1, or 2
- Category: low, medium, high

Study Fit-for-Purpose

Proceed to Tier 2 (Expert Review) prior to inclusion in the Risk Assessment
### NMP Toxicity Study Assessment Tool (Tier 1) – Learnings about NMP State of the Science

<table>
<thead>
<tr>
<th>Strengths/Sufficient Reporting</th>
</tr>
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<tbody>
<tr>
<td>Studies mostly used biologically-relevant sizes (0.01 – 200 micron)</td>
</tr>
<tr>
<td>Particle characteristics sufficiently reported</td>
</tr>
<tr>
<td>Size, shape, type, source</td>
</tr>
<tr>
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</tr>
<tr>
<td>Particle concentration, test vehicle, route, species/bioassay details, sample size, replicates, controls, frequency/duration</td>
</tr>
<tr>
<td>Applicability to risk assessment sufficiently reported</td>
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<td>Statistical analysis, description of endpoints</td>
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# NMP Toxicity Study Assessment Tool (Tier 1) – Learnings about NMP State of the Science

<table>
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<th>Strengths/Sufficient Reporting</th>
<th>Needed Improvements</th>
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<tr>
<td>Studies mostly used biologically-relevant sizes (0.01 – 200 micron)</td>
<td><strong>Limited particle diversity</strong>: majority using spheres (~60%), primarily of polystyrene (~46%); fibers (~20%)</td>
</tr>
<tr>
<td><strong>Particle characteristics</strong> sufficiently reported</td>
<td><strong>Limited particle sources</strong> (45% from five suppliers)</td>
</tr>
<tr>
<td>Size, shape, type, source</td>
<td><strong>Particles not fit-for-purpose</strong></td>
</tr>
<tr>
<td><strong>Experimental design</strong> sufficiently reported</td>
<td><strong>Equipment calibration</strong>, protein ligand substrate</td>
</tr>
<tr>
<td>Particle concentration, test vehicle, route, species/bioassay details, sample size, replicates, controls, frequency/duration</td>
<td><strong>Test material purity unknowns</strong>: particle, unreacted monomers, surfactants/dispersants, vehicle (e.g. Ethanol)</td>
</tr>
<tr>
<td><strong>Applicability to risk assessment</strong> sufficiently reported</td>
<td><strong>Particle characteristics</strong> insufficiently reported</td>
</tr>
<tr>
<td>Statistical analysis, description of endpoints</td>
<td><strong>Particle surface chemistry</strong>, microbial contamination</td>
</tr>
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<td><strong>Experimental design</strong> insufficiently reported</td>
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</tr>
<tr>
<td><strong>Applicability to risk assessment</strong> insufficiently reported</td>
<td><strong>Particle stability</strong>, homogeneity, internal dose confirmation</td>
</tr>
<tr>
<td></td>
<td><strong>Environmental relevance of particle tested or exposure scenario</strong>, &lt;2 test concentrations (~50%), not possible to evaluate D-R relationship or determine effect thresholds.</td>
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NMP-TSAT: *In Vivo Studies Assessed Vs. Reliable to Proceed to Tier 2*

**Inhalation**
- 16 studies
  - 1 OECD guideline

**Oral**
- 24 studies
  - 2 OECD guideline

- **40 NMP + cellulose comparison studies**
  - **13%** proceed to Tier 2 (Expert Review) prior to inclusion in the Risk Assessment
  - **42%** for each:
    - Element: score of 0, 1, or 2
    - Category: low, medium, high

**Study Fit-for-Purpose**

**Risk Assessment Applicability**

**Experimental Design**

**Particle Characterization**

**2 studies**
Nano/Microplastic Particle Toxicity Study Assessment Tool (NMP-TSAT) – 10 Oral in vivo Studies Reliable to Proceed to Tier 2

- Amereh, F.; Eslami, A.; Fazelipour, S.; Rafiee, M.; Zibaii, M. I.; Babaei, M. *Toxicology Research* 2019
- Amereh F; Babaei M, Eslami A, Fazelipour S, Rafiee M *Environ. Pollut.* 2020
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Adequate Subject Matter Expertise during Journal Submission Peer Review?
Deng et al. concluded their data suggest widespread health risks of exposure to microplastics. Study drawbacks have been raised. Potential adversity cannot be evaluated due to lack of details. Concerns regarding causality: Biological plausibility, Coherence of evidence. Conclusion: “a number of issues prevent the usage of the data...for the estimation of gastrointestinal uptake and potential health risks associated with the oral uptake of microplastics”
Tier 2 Expert Evaluation Necessary Prior to Inclusion in the Risk Assessment

Tier 2 Expert Evaluation

Individual Studies + Overall Weight of Evidence

Female & Male Repro.

Oxidative Stress

An et al., 2021

Thyroid

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- Amereh, F.; Eslami, A.; Fazelipour, S.; Rafiee, M.; Zibaii, M. I.; Babaei, M. Toxicology Research 2019
- Park, EJ, Han, JS, Park, EJ, Seong, E, Lee, GH, Kim, DW, et al. Toxicology Letters 2020

**Needed Improvements**

- **Polystyrene**
  - Particles not fit-for-purpose
  - Equipment calibration, protein ligand substrate

- **Polystyrene**
  - Test material purity unknowns: particle, unreacted monomers, surfactants/dispersants, vehicle (e.g. Ethanol)

- **Polystyrene**
  - Particle characteristics insufficiently reported
  - Particle surface chemistry, microbial contamination

- **Polyethylene**
  - Experimental design insufficiently reported
  - Particle stability, homogeneity, internal dose confirmation

- **Polystyrene**
  - Applicability to risk assessment insufficiently reported
  - Environmental relevance of particle tested or exposure scenario
CONCLUSIONS

Nano/Microplastic Particle Toxicity Study Assessment Tool (NMP-TSAT)

Tier 1 Screen

Systematic Assessment Approaches Needed

Proceed to Tier 2 (Expert Review) prior to inclusion in the Risk Assessment

Particle Characterization

Oral NMP

Inhalation

Study Fit-for-Purpose

External Exposure & Uptake/ Biodistribution

Risk Assessment

Hazard Identification

Hazard Characterization

Quality

Polymer Chemistry

SETAC

SOT Society of Toxicology