Nanomaterial Categories:
Context, State of Play, and Future Directions

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Nano Categories: Overview

- Category Use in Traditional Chemical Assessment
  - Category Concepts
  - Utility, and Bases

- Potential Uses for Nanomaterial Categories
  - Range of Options
  - What May work
  - An Example

- Summary and Next Steps
Categories for Traditional Chemicals: OECD

- **Drivers**

- A group of chemicals whose physicochemical and (1) health, (2) ecotoxicological, or (3) fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity
  - Metal Ions, Breakdown product, increasing Chain length, **MOA**

- **2014 OECD Workshop On MN Categories**
Assessment of Values may be quantitative, or qualitative (Hazard)

Justification Must be Provided for Analog Groups/Endpoint Trends

Extrapolation more difficult to justify than Interpolation

“A category with increasing chain length, with a common functional group, will affect solubility / log Kow, which in turn may affect bioavailability and hence toxicity, both mammalian and aquatic.”  → ECOSAR
<table>
<thead>
<tr>
<th>Property</th>
<th>Related to</th>
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<tbody>
<tr>
<td>logKow</td>
<td>Adsorption, estimation of bioconcentration in gill respiring animals, aquatic toxicity, mammalian absorption (oral and dermal)</td>
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<tr>
<td>Log Koa</td>
<td>Estimation of potential for bioaccumulation of non metabolisable substances in air breathing animals</td>
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<tr>
<td>Water solubility</td>
<td>Adsorption, Henry’s law constant, aquatic toxicity, hydrolysis</td>
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<tr>
<td>Molecular weight</td>
<td>Bioavailability, absorption or bioaccumulation, steric hindrance</td>
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<tr>
<td>Molecular dimensions</td>
<td>i.e. 3 D structural characteristics such as Dmax and molecular length (distribution or probability)</td>
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<tr>
<td>Vapour pressure</td>
<td>Volatility with respect to choice of test conditions, inhalation</td>
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<tr>
<td></td>
<td>Distribution coefficient between air and water, potential for exposure from water based formulation and hence relevant for considering inhalation route of exposure.</td>
</tr>
<tr>
<td>Henry’s Law constant</td>
<td>Degree of ionization, relationship to irritation and corrosion, hydrolysis of ionisable substances (see sections 3.3.6), potential for uptake (including bioconcentration and accumulation), and sorption to soil (e.g. clay)</td>
</tr>
<tr>
<td>Acid dissociation constant (pKa)</td>
<td>Lipophilicity, solubility, absorption, membrane penetration, plasma protein binding, distribution</td>
</tr>
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How do we Proceed with MN Categories?

- **Possibilities, but many Physicochemical parameters:**
  - Size $\rightarrow$ Toxicity???
  - Biologically Reactive Surface Area $\rightarrow$ Toxicity
  - Shape $\rightarrow$ Toxicity
  - Zeta Potential $\rightarrow$ Stability/Fate

- **Narrow Categories, for Narrow Purposes (for now):**
  - Which Phys Chem properties, MOAs hold., for which MNs?
  - Data Quality Issues with Tests Conducted
  - Changes through the Life Cycle
  - To build Toxicity Categories, consider AOP Framework?
Fig. 1. Conceptual diagram of key features of an adverse outcome pathway (AOP). Each AOP begins with a molecular initiating event in which a chemical interacts with a biological target (anchor 1) leading to a sequential series of higher order effects to produce an adverse outcome with direct relevance to a given risk assessment context (e.g., survival, development, reproduction, etc.; anchor 2). The first three boxes are the parameters that define a toxicity pathway, as described by the National Research Council [3].
Ooman et al. 2014. *Nanotox*, 8

**Source to Adverse Outcome Pathway**

- **Powder**
  - Embedded in matrix or on surfaces
  - Changes of phys.-chem. properties

- **Aerosol**
- **Suspension**

- **Deposition in the lung**, alveolar, intestinal, dermal absorption

- **Surface coating changes**
- **Agglomeration, desagglomeration**

- **Crossing of biological barriers**
- **Tissue distribution**, intracellular distribution

- **Inflammation**
- Catalysing formation of reactive compounds
- Ion release
- Direct interaction with cellular structures

- **Organ toxicity**

**Material properties**

**Use**

**Release**

**Uptake**

**Distribution**

**Early event**

**Apical effect**
CNTs and Aquatic Toxicity: Modes of Action?

- Direct chemical toxicity
- Production of reactive oxygen species
- Interaction with tissues, cells, and subcellular components
- Whole organism effects associated with mechanical obstruction of gill structures and gut
- Effects on other species in food chain due to CNT bioaccumulation potential?
Protocol Variations for CNT Aquatic Toxicity Tests

- Varying methods exist for dispersing CNTs in test media
- Measuring concentrations of CNTs in suspension can be challenging
- Settling of CNTs / adherence to vessel walls, etc.
- Static renewal vs. flow through toxicity test systems, vs. no media exchanges
- Dose relevance
- Acute vs. chronic values: use in specific risk assessments?
- Which species are most relevant?
CNT Regulatory Protocols & Toxicity Results?

- EPA protocol \([EPA \text{ 821-R-02-013}]\)
- Static renewal
- Purity = 95%
- MWCNT Size: 20-30 nm x 2 µm singlets
- Method: Sonication methods well-described; measured concentrations in test solutions; accounted for settling

Protocol adjustments, and material characterization methods, appropriate for CNTs:
- Good material characterization
- Sonication well described, acid treatments
- Natural organic matter, GA, SDC, SDS, Tween 20, HCO-40
- Heavy metals, carbon impurities
- Controls (for material, contaminants, and dispersant)
- Measurement of concentrations in exposure media
- Agitation, and/or exposure medium renewal
MOA: Postulated that *D. magna* > Sensitive > *C. dubia*: *C. dubia* has a greater mass-based filtration rate; *D. magna* more famine-sensitive

Is there Potential for a Category for this type of MWCNT?

- **D. magna** 96-hr EC$_{50}$ = 2 mg/L; 21-day EC$_{50}$ ~ 0.5 mg/L
- **C. dubia** 7-day LOEC = 0.25 mg/L
Thank You

- Potential
- Many Individual Physicochemical Properties
- Consider Unifying Physicochemical Properties like Log $K_{ow}$ for Traditional Chemicals $\rightarrow$ MNs?
- Build with results from Selected Studies
- Consider Use of Extended AOP concept
- Start Narrow

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• Increasing Agglomeration with increasing Zeta Potential
• Differences in Particle size between Different Particles
• Different Zeta Potentials reflect different Transport in Sand Columns
Mode and mechanism of action concepts can also facilitate the read-across for aquatic toxicity. The term “mode of action” is understood in a broader sense than “mechanism of action”, the first being seen as an integrator of the general type of interaction of a chemical with the organism, while the second is perceived as the precise (bio)chemical molecular interaction related to the Molecular Initiating (MIE) or Key Event (KE) of an Adverse Outcome Pathway. According to one of the earliest classification schemes (Verhaar et al., 1992), four modes of actions are distinguished for acute aquatic toxicity: inert substances, relatively inert substances, reactive substances, and specifically acting substances. The toxicity of the substances in the first two groups (later known also as non-polar and polar narcotics, respectively) is mainly hydrophobicity driven, while the second two groups (i.e. the reactive chemical substances and the specifically acting substances) form specific domains, and read-across between such domains is not trivial. The more precise definition of the mechanisms of aquatic toxicity can further facilitate the filling of data gaps. Some authors distinguish, instead of between reactive and specifically acting substances in relation to fish, between uncoupling of the oxidative phosphorylation, respiratory inhibition and electrophilic/nucleophilic mechanisms, electrophiles/proelectrophiles, acetylcholinesterase inhibitors, or central nervous system seizure agents (Russom et al., 1977). Other authors split further the electrophilic reactivity in specific reactivity mechanisms such as Michael type-addition, Schiff-base formation, etc. (Schultz et al., 2005). Different types of models could be used within a specific mechanistic domain (Netzeva et al., 2008). For substances within the same reactive mechanism of action, the potency of protein binding as predictor for e.g. acute aquatic toxicity, can be estimated in (semi-)quantitative manner (OECD QSAR Toolbox ver. 3.2).