Alternative Metrics for the Physicochemical Characterization of UFP
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AIME: provide a tool box of relevant instruments for risk assessment, Characterisation, toxicity testing and exposure measurements of MNMs

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Impact on in vitro and in vivo toxicity tests: **Metrics and Dosage**
DEFINITIONS AND UNITS
4. The water solubility of a substance is the saturation mass concentration of the substance in water at a given temperature.
Solubility of Nanoparticles depends from size (Ostwald–Freundlich)

\[
\ln \left( \frac{C_i(sat)}{C_i} \right) = \frac{V_i^*}{RT} \cdot \frac{2}{3} \cdot \frac{\gamma_SL}{4\pi r^2} \cdot \frac{4\pi r^2}{3\pi r^3} = \frac{V_i^*}{RT} \cdot \frac{2\gamma_{SL}}{r}
\]

Commercial powder as used in sun screen (25 nm)

Measurement of solubility of Nanomaterial following the OECD TG 105 is in principle not possible
solubility of NM110 in synthetic saliva at 37ºC, pH 6.84 (48h)

- $\text{Zn}_3(\text{PO}_4)_2(s)$

Dissolved Zn (mg/L) vs. Total Zn (mg/L)
Method for determination of the solubility kinetic
Particle size

• No individual technique can satisfy a meaningful characterization.

• A combination of methods for primary particle size measurement and methods for hydrodynamic size measurement is necessary.

• **For primary particle size measurement, transmission electron microscopy techniques is the appropriate method.**

• In the case of granulometry of NM dispersions, there are 4 available techniques based on robust detection technologies: Centrifugal Liquid Sedimentation (CLS), **Dynamic Light Scattering (DLS)**, Nanoparticle Tracking Analysis (NTA), Coulter counter

• Each technique requires proper calibration standards for the desired zones of interest.

• Use of a common dispersion protocol is mandatory

  **SOP’s are established and in validation phase**
OECD TG109 Density of solids

- **Poured density**, Schüttedichte, densité apparente,
- **Tapped density**, Stampfdichte, densité après tassement,
- **Density**, (Wahre) Dichte (Massendichte), densité,
- **New**: Density of agglomerates, skeletal density, effective density

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Density

• The only method which gives reproducible results: He-Pycnometer

Protocol for true density measurement with He-Pycnometer

Method

This method measures the volume of gas (Helium) displaced by a known mass of powder, and gives the true density of the material. The sample must be completely dried.
Densities of agglomerates

Estimating the effective density of engineered nanomaterials for in vitro dosimetry

Glen DeLoid\textsuperscript{1,}\textsuperscript{*}, Joel M. Cohen\textsuperscript{1,}\textsuperscript{*}, Tom Darrah\textsuperscript{2}, Raymond Derk\textsuperscript{3}, Liying Rojanasakul\textsuperscript{3}, Georgios Pyrgiotakis\textsuperscript{1}, Wendel Wohlleben\textsuperscript{4} & Philip Demokritou\textsuperscript{1,}\textsuperscript{*}

[Image of a PCV tube with centrifugation and sections labeled as intra-agglomerate media and inter-agglomerate media]
Several reasons exist why for nanoparticles this Guideline is not applicable:

- Nanoparticles form colloidal dispersions, which are thermodynamically unstable.
- The results of a test following the OECD guidelines will depend strongly from the sample preparation and the energy added during the shaking process.
- The colloidal stability influences the behaviour in the liquid.

**Conclusion:**
The OECD Guideline is not applicable to nanoparticle suspension. The guideline has to be replaced by a determination of the colloidal stability of the nanoparticles in different liquids.


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Metrics and Dosage

Mass/area !!!
Administrated mass
Deposited mass

\[ v_{\text{sed}} = \frac{2g(\rho_{NP} - \rho_{\text{media}})d^2}{9\mu} \]

All particles which are in a distance of \( t \times v_{\text{sed}} \) could reach the cell membrane.

\[ c / c_0 = \text{erf}(x / 2\sqrt{Dt}) \]

\[ M(t) = 2c_0\sqrt{\frac{Dt}{\pi}} \]

Sticking coefficient = 1, newer model with SC < 1

\[ \frac{\partial n}{\partial t} = A \frac{\partial^2 n}{\partial x^2} - B \frac{\partial n}{\partial x}, \quad A = \frac{RT}{N6\pi \mu a} ; \quad B = \frac{X}{6\pi \mu a} = \frac{4\pi a^3 g \delta}{6\pi \mu a} = \frac{2g\delta a^2}{9\mu}, \]
Advanced computational modeling for in vitro nanomaterial dosimetry

Glen M. DeLoid*, Joel M. Cohen, Georgios Pyrgiotakis, Sandra V. Pirela, Anoop Pal, Jiying Liu, Jelena Srebric and Philip Demokritou

Particle and Fibre Toxicology (2015) 12:32
Viability A549 cells: MTS assay
Exposure to poly-styreneamine NP, 50 nm

**standard metric:** $\mu g/mL$  
**new metric:** $\mu g/cm^2$ (ISDD)

A common European approach to the regulatory testing of nanomaterials
Viability A549 cells: Annexin V/PI assay
Exposure to poly-styreneamine NP, 50nm

**standard metric:** µg/mL  
**new metric:** µg/cm² (ISDD)
Conclusions

• Most of the OECD technical guidelines for physical and chemical characterization of NP needs modifications or are from a thermodynamic point of view not applicable

• Primary particles size, size distribution (shape) should be determined by TEM

• Agglomerate size and size distribution DLS or equivalent methods are useful (hydrodynamic diameter)

• Density of primary particles and effective density has to be known

• For toxicity tests deposited mass/area is adequate metrics. If the particles are correctly characterized, mass could be transferred into number, surface and volume.
Thank you for your Attention

A common European approach to the regulatory testing of nanomaterials

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