PATROLS

Advanced Tools for NanoSafety Testing

Ecotoxicity testing – exposure control matters

Lars M. Skjolding and Anders Baun

The need

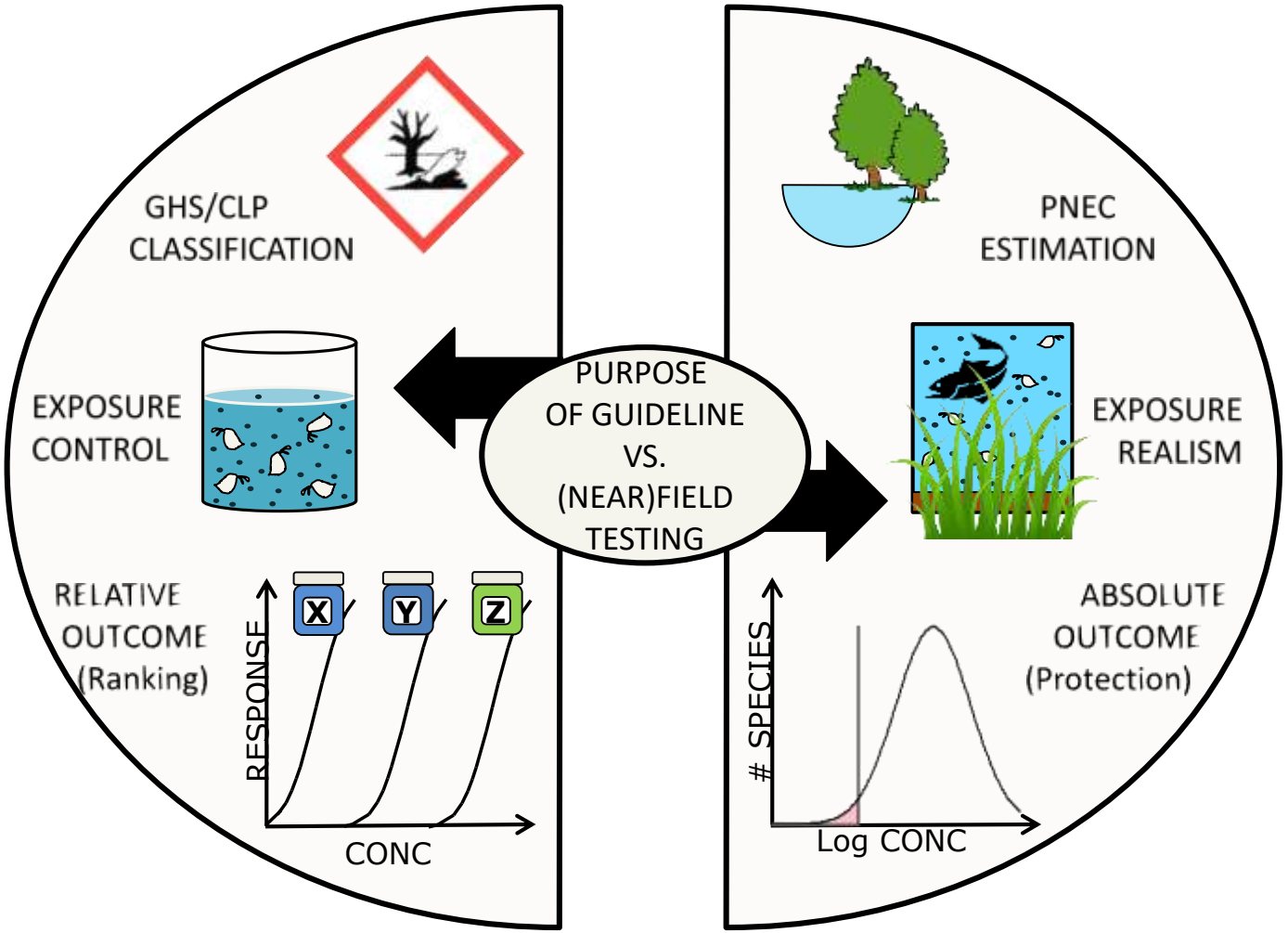
- An urgent need for reliable and reproducible results of ecotoxicological experiments
    - To identify
    - To rank
    - To classify

  - the environmental hazards of nanomaterials

  AND:
    - To quantify environmental safe-levels (PNEC)

- (Modified) standardized methods and guideline tests traditionally used for chemicals the tools in the toolbox...

The sometimes forgotten ”why”



Hjorth, Skjolding, Sørensen, Baun (2017) Regulatory adequacy of aquatic ecotoxicity testing of nanomaterials. NanoImpact 8, 28-37

Example:

ISO 6341 – *D. magna* immobilization

"This method is applicable to: chemical substances which are soluble under the conditions of the test, or can be maintained as a stable suspension or dispersion under the conditions of the test"

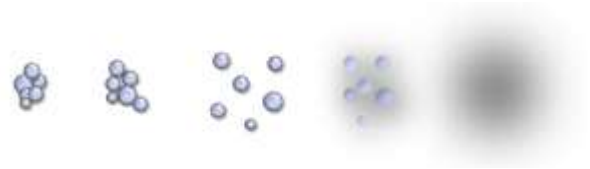
CONTROL!

(and describe to assure control)

Control

What's preventing it?

- **Particles**
  - Not dissolved
  - Particles do not "behave well"

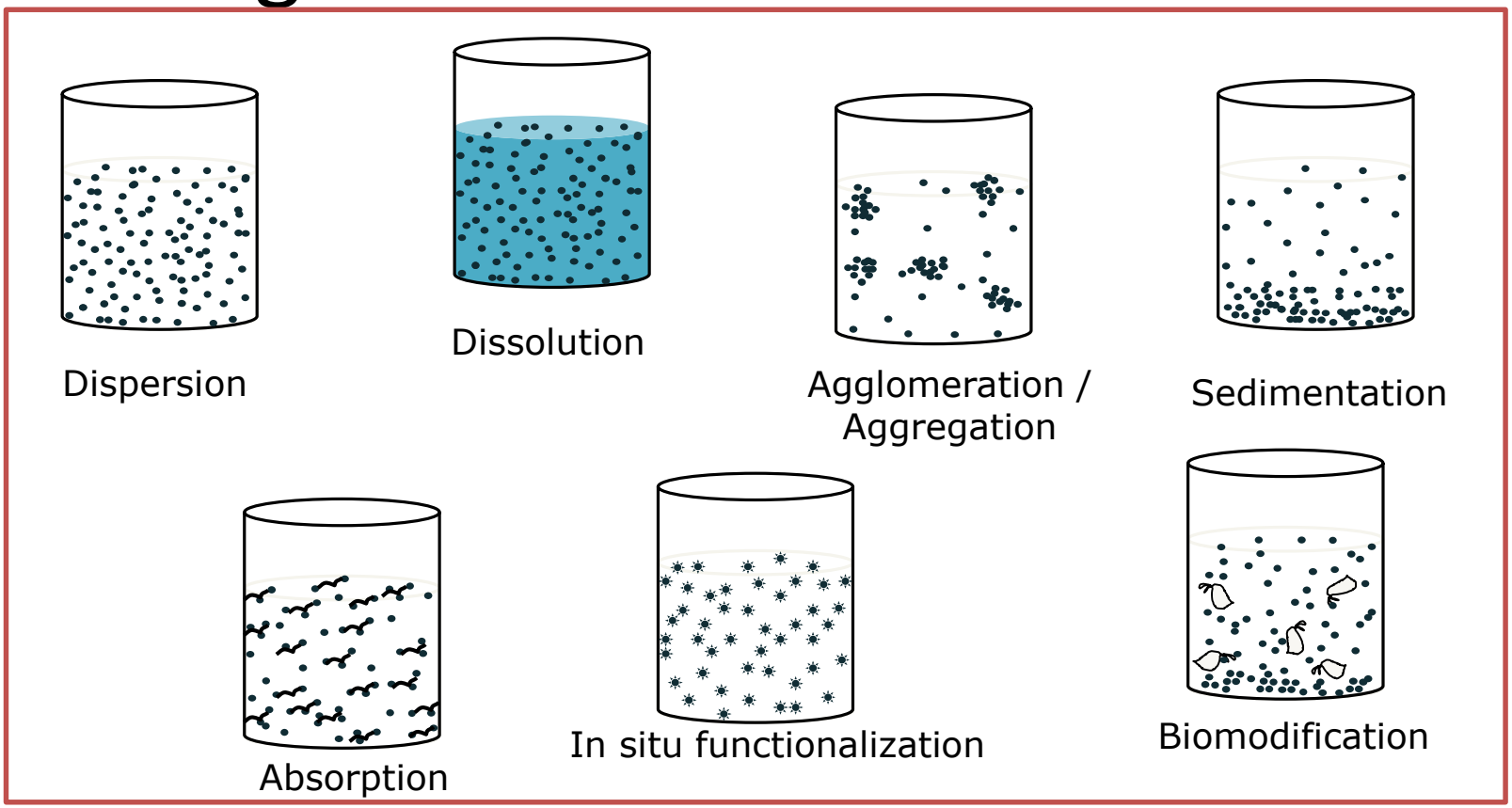


- **Testing media**
- **Dilution…**
- **Organisms**
- **Time!**
  - Non-equlibrium
  - Uncontrollable dynamic changes of key parameters during testing

Effect = f(conc|t,org,media) ?

Baalousha & Lead (2013). Nature Nanotechnology, 8, 308-309
Baun, et al. (2008).. Ecotoxicology, 17 (5), 387-395
Hartmann et al.. (2013)..Nanotoxicology DOI: 10.3109/17435390.2012.710657

Transformation processes occur during the test…

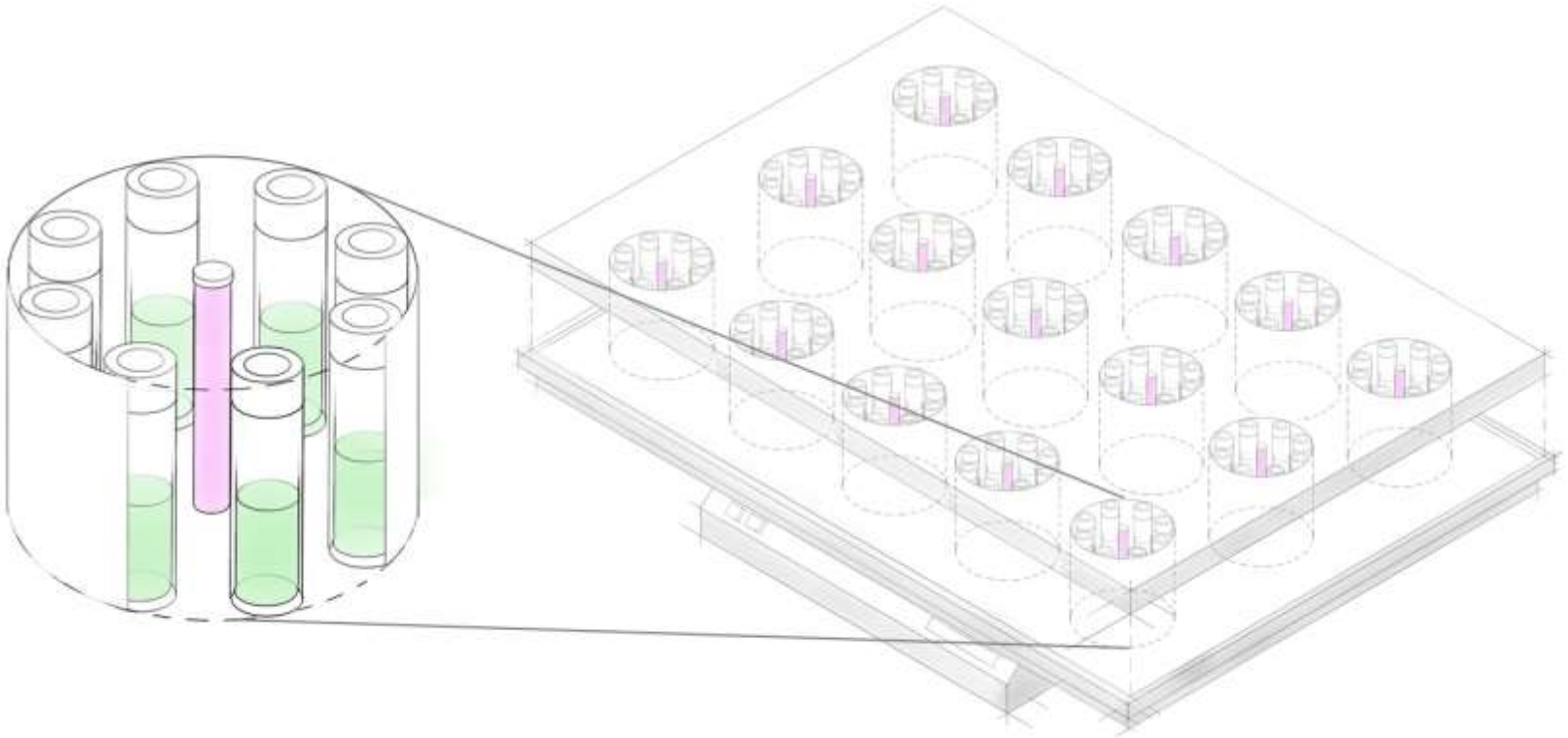


Modified from Sørensen, Hjorth, Delgado, Hartmann, Baun (2015) Nanoparticle ecotoxicity – physical and/or chemical effects? Integrated Environmental Assessment and Management, 11, 722-724

The algal test issues

- The algal toxicity test is one of only three mandatory tests used to generate the ecotoxicity data required for pre- and post-market registration of chemicals by European and international regulations, e.g. REACH, CLP, TSCA (USA), and NICNAS (Australia). For this purpose, standardised algal test guidelines have been developed by international organisations, e.g. ISO and OECD. These testing standards and guidelines prescribe ideal test conditions in terms of pH, temperature, carbon dioxide levels and light intensity. However maintaining stable test conditions during algal testing is in practice difficult and results suffer from problems with reproducibility and reliability for a range of chemical substances and nanomaterials (often referred to as "difficult substances").

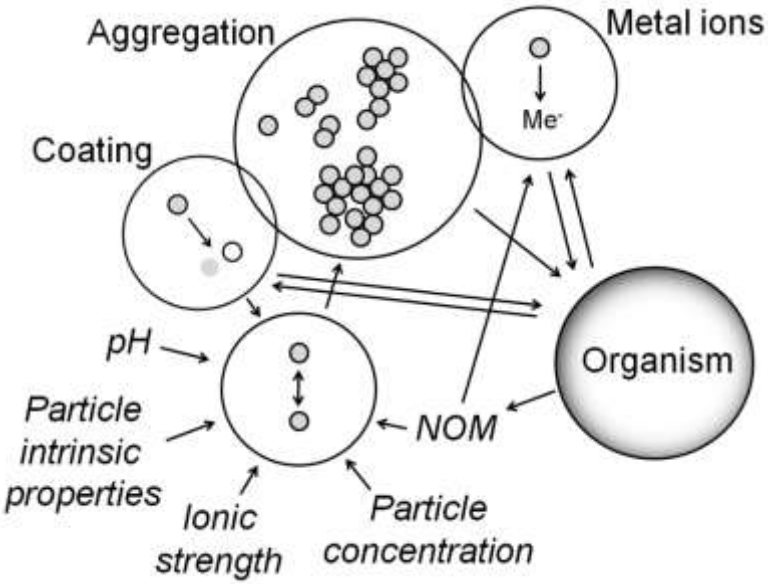
- The TEST SYSTEM is a crucial factor!
  - No control over the test system → no control over the results → regulatory adequacy of data ↓
  - This is true for all chemicals, but MN poses specific challenges



Criteria for reliable, reproducible and flexible algal toxicity testing	PLATEFORM	Traditional setups	
		Erlenmeyer flasks	Microtiter plate
Allows testing at fixed CO_2 concentration in vial headspace (e.g. for volatile substances)	Yes	Yes	No
Versatile biomass determination methods	Yes	Yes	No
Versatile test container material	Yes	No	No
Homogenous light, pH and temperature conditions in and between vials	Yes	No	No
Sufficient replicate volume to allow sampling for exposure analysis	Yes	Yes	No
Appropriate for high-throughput screening (i.e. large number of replicates per m^2)	Yes	No	Yes

Before, during and after

- ~~control~~ and describe…



Effect = f(conc|(time, organism, media) …?

Controlled exposure is difficult!

Characterization is needed!!

ENVIRONMENTAL Science & Technology

Article

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A Multimethod Approach for Investigating Algal Toxicity of Platinum Nanoparticles

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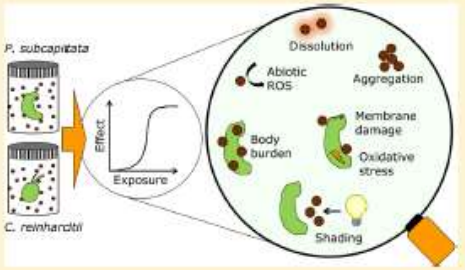
Supporting Information

ABSTRACT: The ecotoxicity of platinum nanoparticles (PtNPs) widely used in for example automotive catalytic converters, is largely unknown. This study employs various characterization techniques and toxicity end points to investigate PtNP toxicity toward the green microalgae *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii*. Growth rate inhibition occurred in standard ISO tests (EC_{50} values of 15–200 mg Pt/L), but also in a double-vial setup, separating cells from PtNPs, thus demonstrating shading as an important artifact for PtNP toxicity. Negligible membrane damage, but substantial oxidative stress was detected at 0.1–80 mg Pt/L in both algal species using flow cytometry. PtNPs caused growth rate inhibition and oxidative stress in *P. subcapitata*, beyond what was accounted for by dissolved Pt, indicating NP-specific toxicity of PtNPs. Overall, *P. subcapitata* was found to be more sensitive toward PtNPs and higher body burdens were measured in this species, possibly due to a favored binding of Pt to the polysaccharide-rich cell wall of this algal species. This study highlights the importance of using multimethod approaches in nanoecotoxicological studies to elucidate toxicity mechanisms, influence of NP-interactions with media/organisms, and ultimately to identify artifacts and appropriate end points for NP-ecotoxicity testing.



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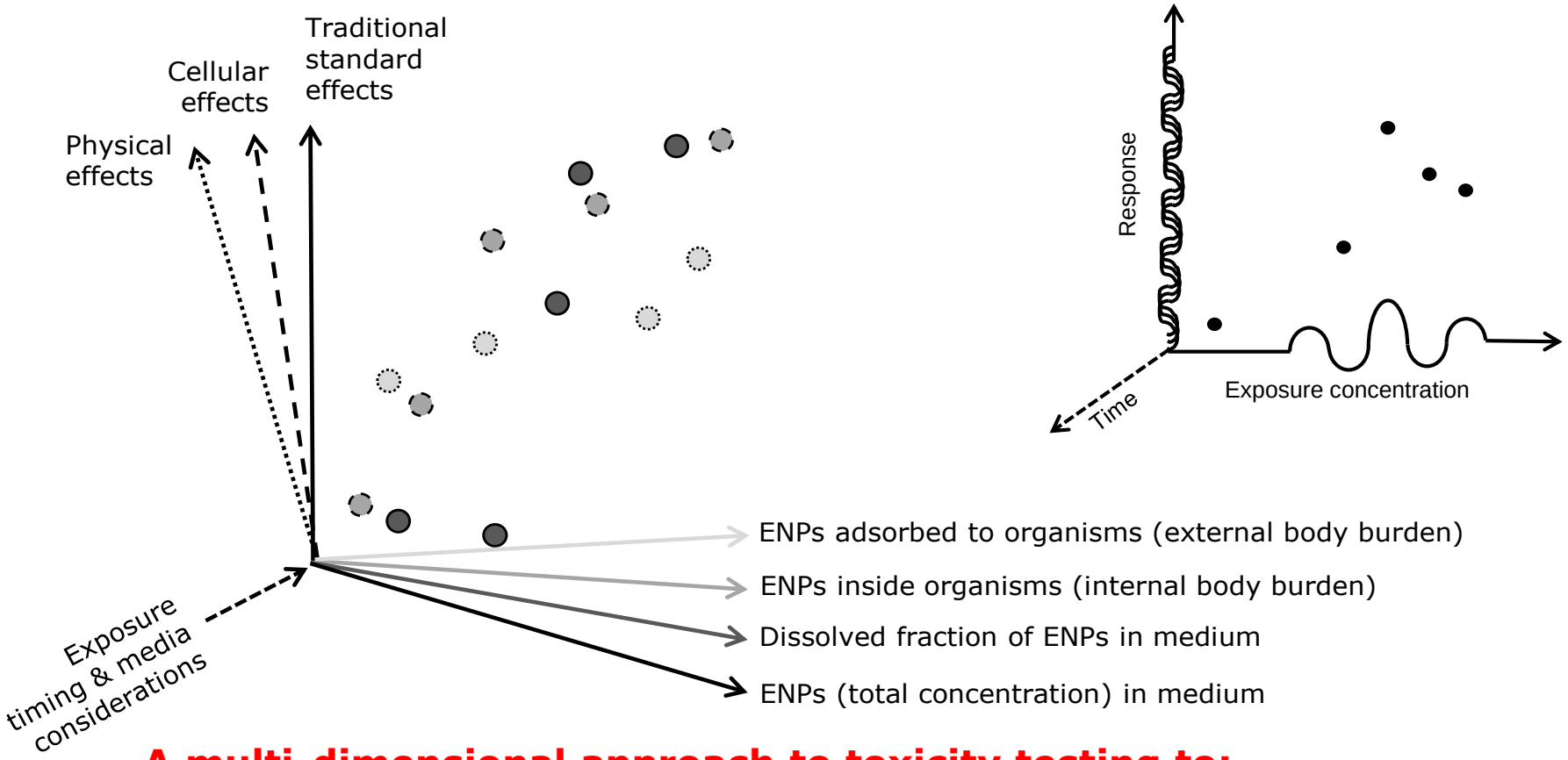
Controlled exposure AND characterization is needed for reliable data interpretation

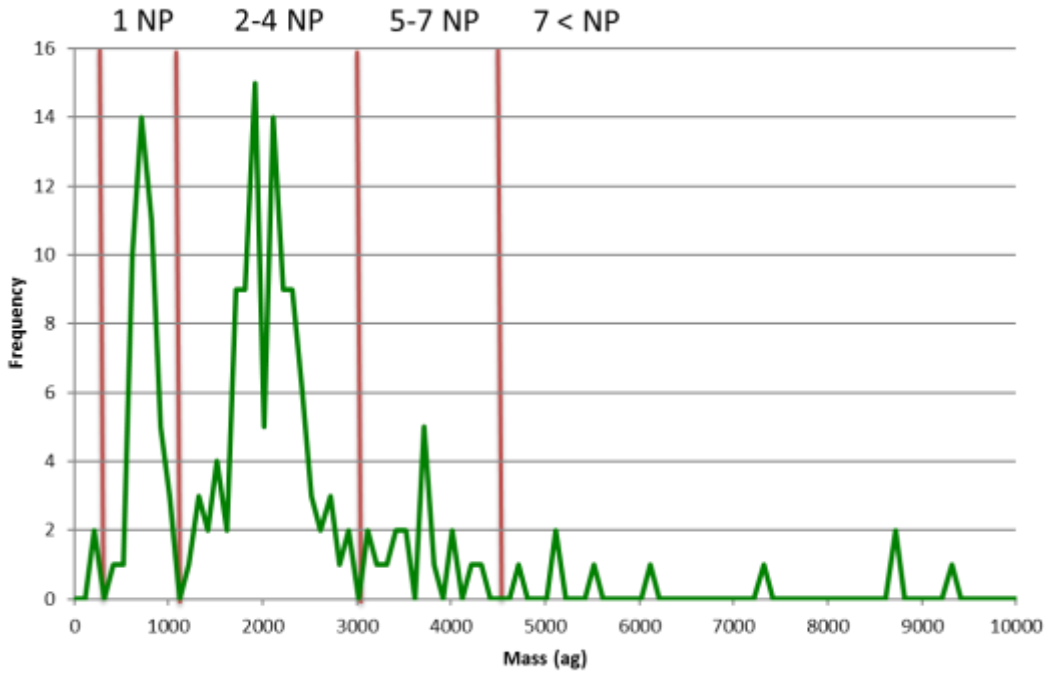


A multi-dimensional approach to toxicity testing to:

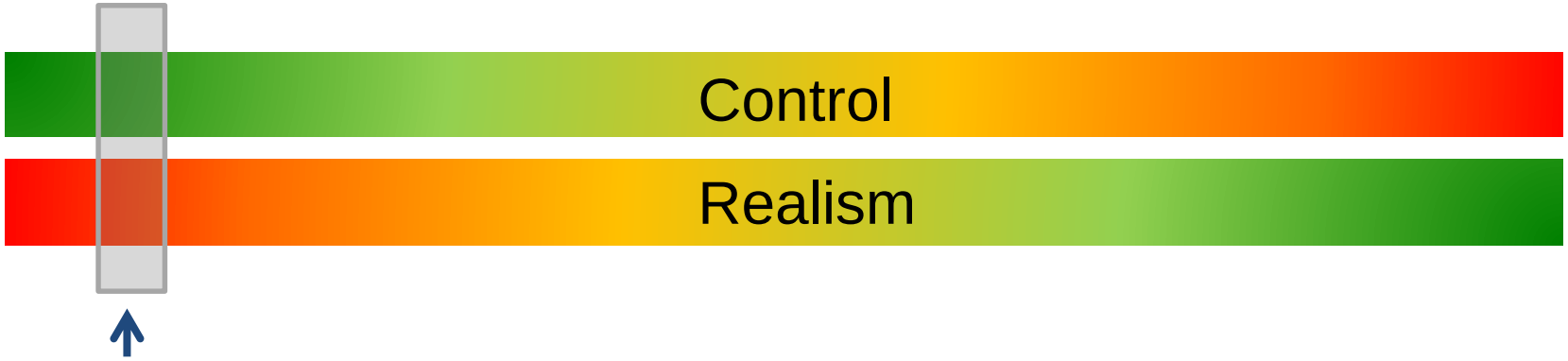
- Improve our understanding of the concentration-response data, and
- Identify potential ENP-specific effect mechanisms

Single cell – ICP-MS

- Limit of detection in the femtogram range (10^-15g)
  - Enables number of nanoparticles on individual cell level



Conclusion



Describe
Characterize
Measure

Control

Standard tests for addressing control
Time & organism interaction with NP
Keep within one NP regime (& mono-disperse?)

Describe

Characterization during incubation
(in parallel…)
Realism – when can we extrapolate?
Insights in NP specific effects

Thanks for your attention

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- WP5 partners (Leiden University, Heriot-Watt University and Exeter University)
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