

The Development and Implementation of Safer-by-Design Strategies for Nanomaterials through a High Throughput Screening Approach

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Objectives:

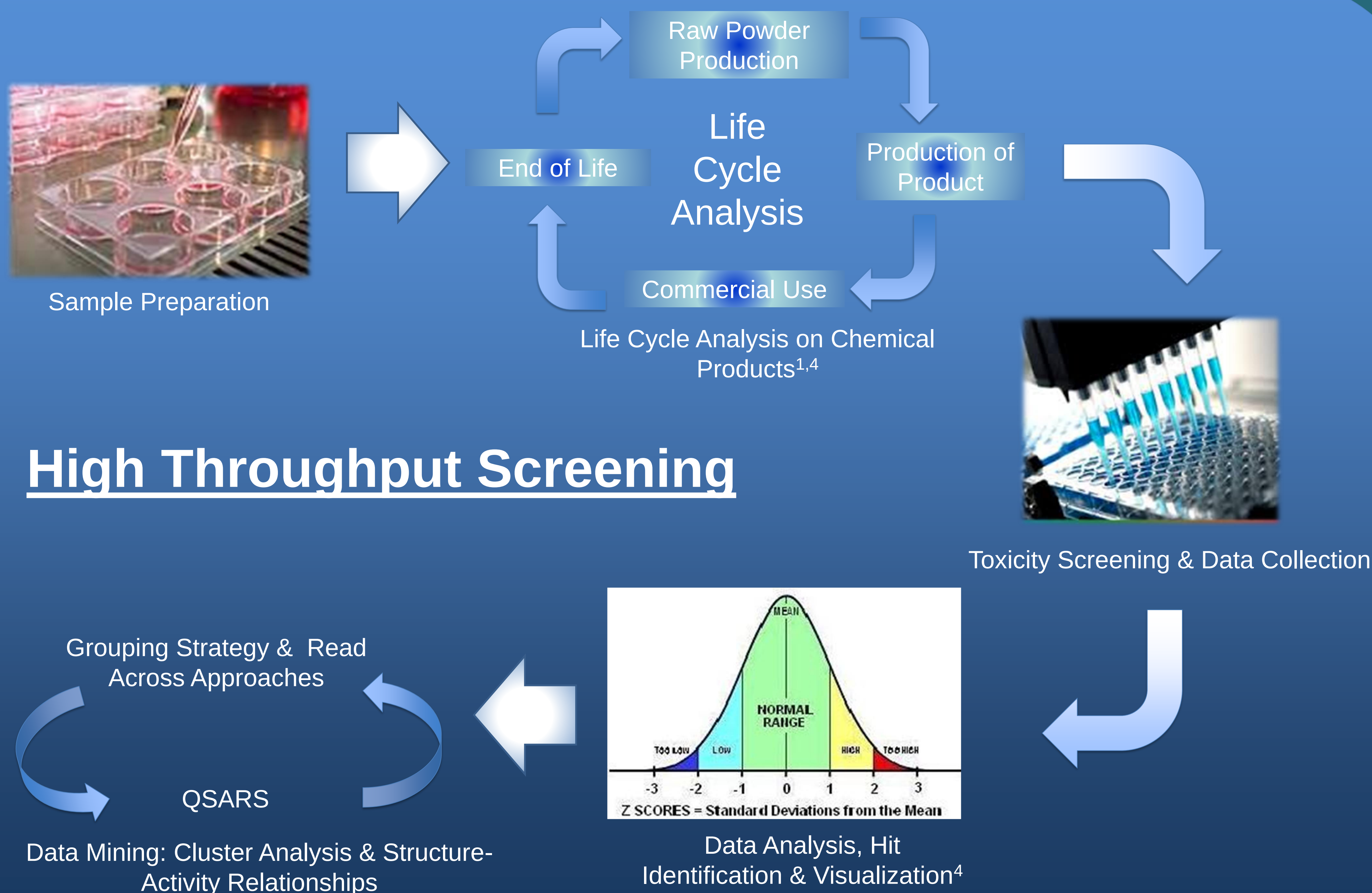
- Develop a high throughput screening approach (HTS) for testing *in vitro* toxicity thresholds for nanomaterials (NMs) using *Mytilus edulis* hemocytes
- Test NMs from industrial partners for safer-by-design and life cycle analysis

HTS & Nanomaterials

Rapidly developing technologies makes it difficult to screen all NMs in a realistic timeframe

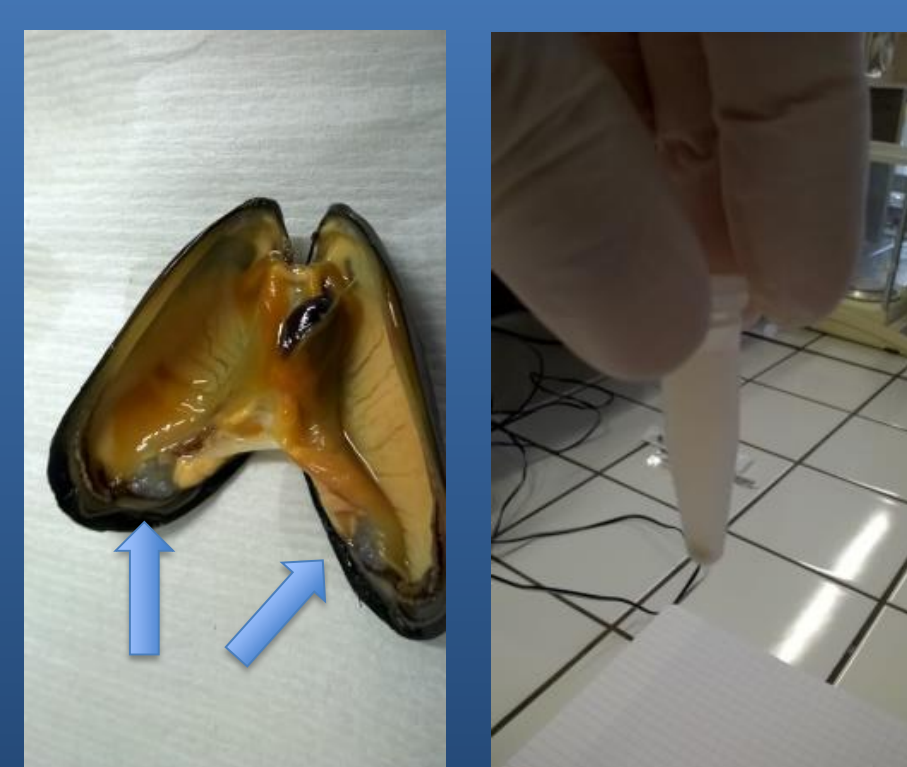
Use of HTS to establish thresholds of NMs toxicity for the development of a Regulatory framework⁴

Implementation of safer-by-design approach through life cycle analysis

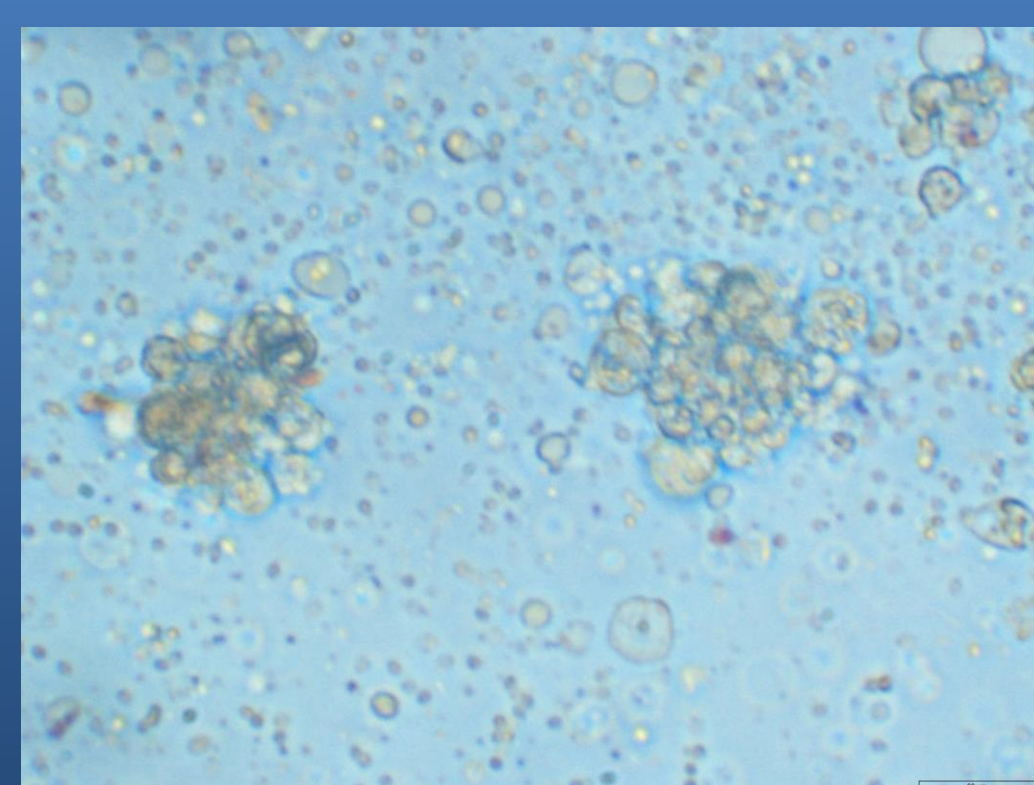


Material and methods

Developing an HTS Strategy on *M. edulis* Hemocytes



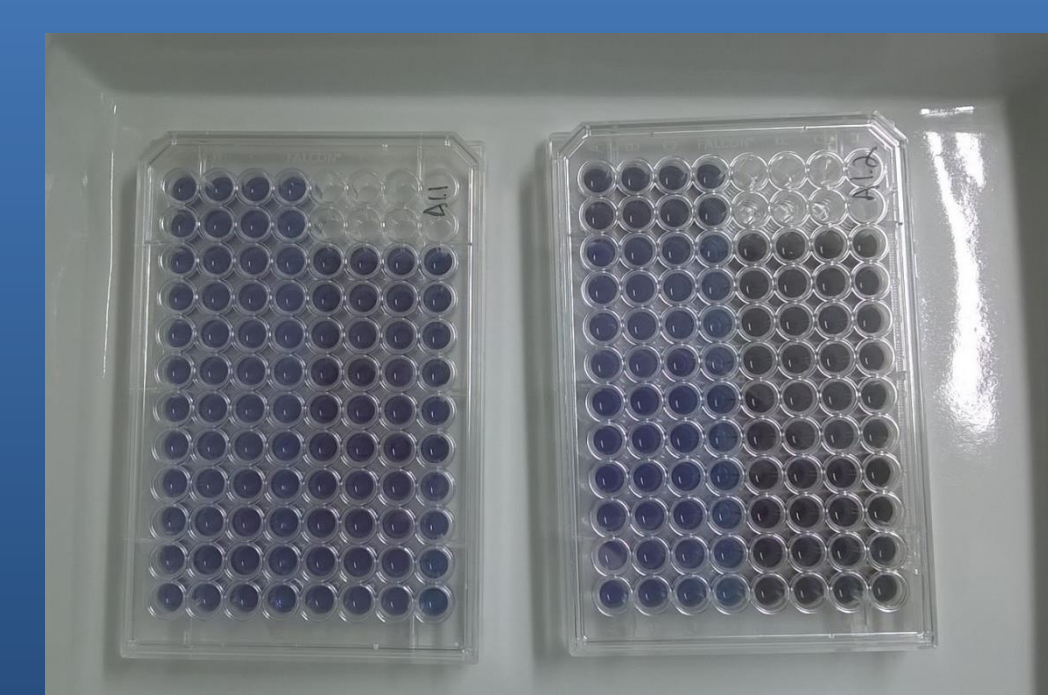
Hemolymph Extraction from the *M. edulis* Posterior Adductor Mussel



Establish and Maintain a Mixed Primary Cell Culture on Hemocytes for 24 Hours^{2, 3}



24 Hour exposure to NMs (.001µg/mL – 200µg/mL)



In vitro Toxicity: Cellular Viability, Immune Response, Oxidative Stress & Apoptosis³

Results

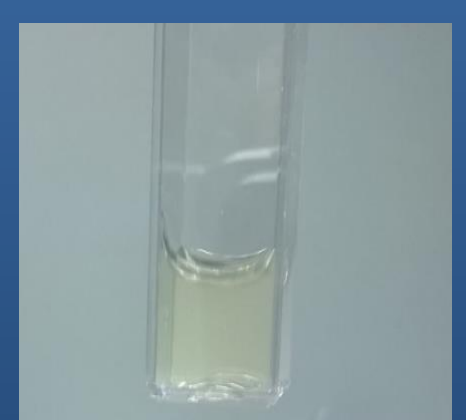
Dynamic Light Scattering (DLS) in culture medium

NMs Currently Tested

- SiC99 (Silicon Carbide)
 - 35 & 75nm powder preparation



- SiQC99 (Carbon Coated Silicon)
 - 40 & 75nm powder preparation



- Differences in size distribution of NMs depending on the concentrations tested (impact of complex culture media and sedimentation?)

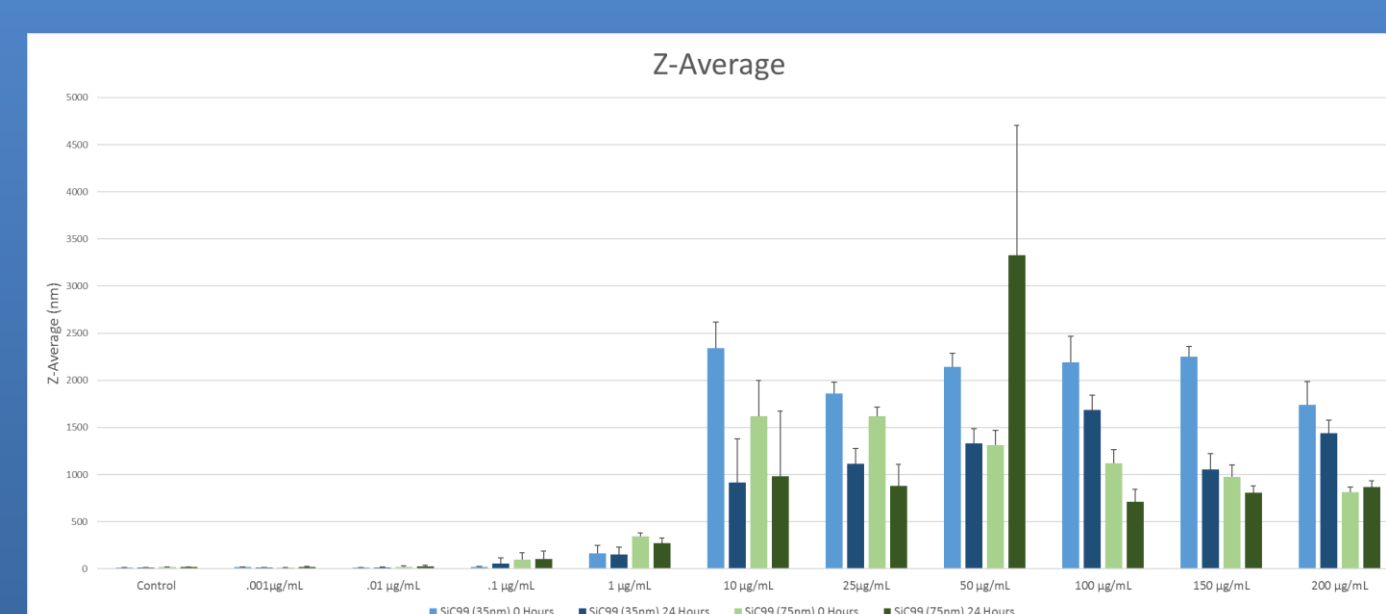


Figure 1: Z-average (nm) of nanomaterials in culture media (0 & 24 hours)

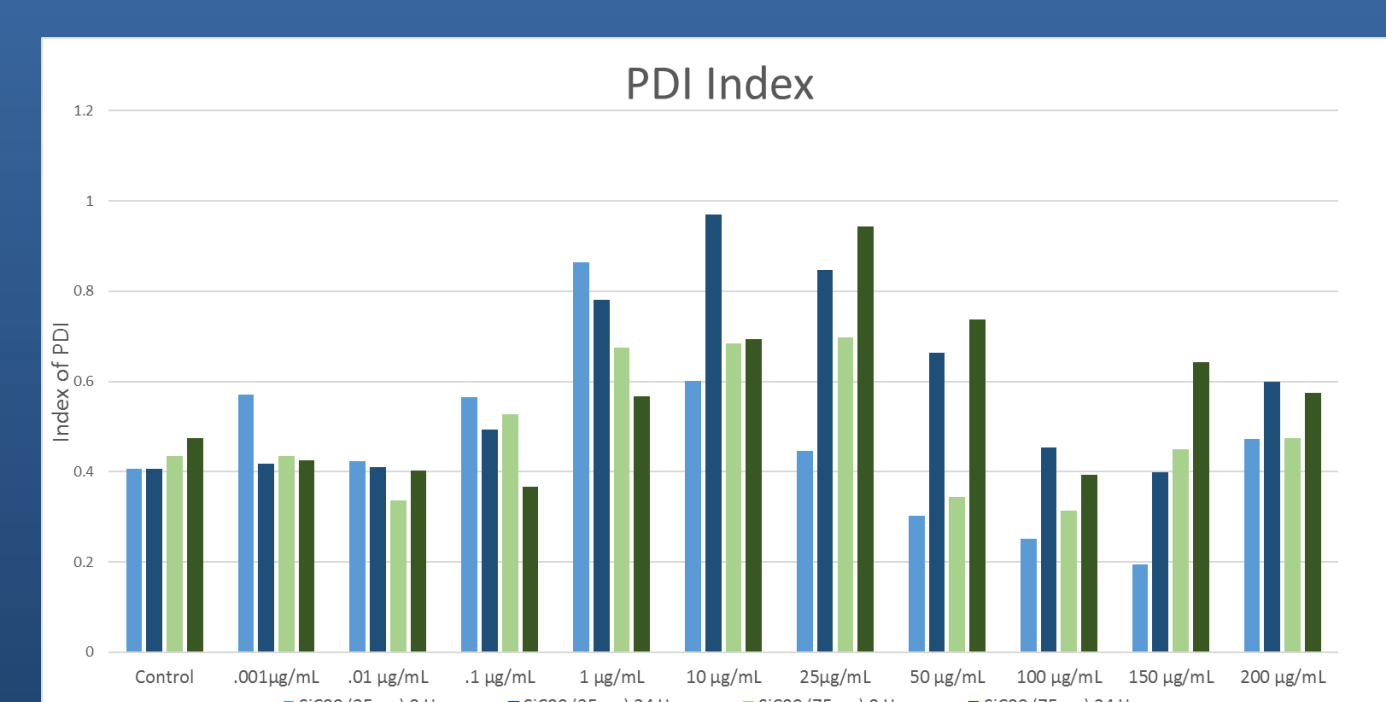


Figure 2: Polydispersion Index of nanomaterials in culture media (0 & 24 hours)

Cellular Viability Profiles (Alamar Blue)

LC50 Analysis:

- SiC99 35nm: >200µg/mL
- SiC99 75nm: 30.89 µg/mL
- SiQC99 40nm: 25.68 µg/mL
- SiQC99 75nm: 11.01 µg/mL

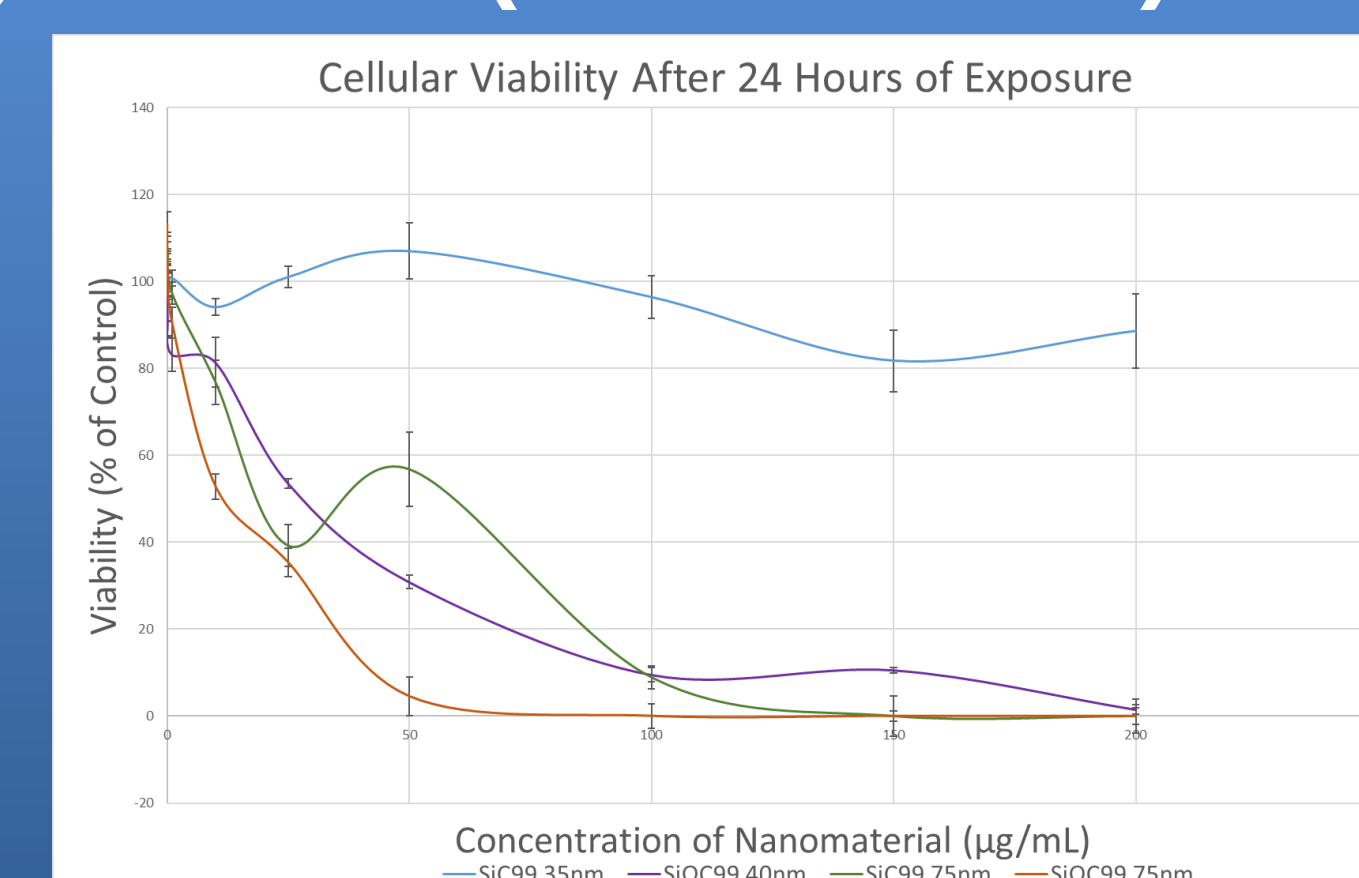


Figure 3: Dose Response (% of control) of hemocyte cells exposed to NMs (.001µg/mL – 200µg/mL)

- No effect on hemocyte viability for SiC99 35 nm
- Differences in LC50 depending on the type and size of NMs

Conclusion and perspectives

- HTS can quickly screen a large number of nanomaterials across multiple cellular pathways with various doses and durations of exposure
- HTS allows identifying nanomaterials with high environmental risk for comprehensive testing
- Further development of the HTS database on biomarkers for oxidative stress, cytotoxicity, immune response and genotoxicity
- Additional Nanomaterials from partners within the NanoReg II project will be integrated into the HTS platform developed
- Results on *in vitro* assays will be validated through *in vivo* exposures

¹ Arts J., Hado M., Irfan M.A., Keene A., Kreiling R., Lyon D., Maier M., Michel K., Petry T., Sauer U., Warheit D., Wiench K., Wohleben W., Landsiedel R. A decision-making framework for the grouping and testing of nanomaterials (DF4nanogrouping). Regulatory toxicology and pharmacology. S1-27, 71.

² Cao A., Mercado L., Ramos-Martinez J.C., Barcia R. 2003. Primary cultures of hemocytes from *Mytilus galloprovincialis* link: expression of IK-2Rα subunit. Aquaculture. 108, 216.

³ Katsumi A., Arostegui I., Valsami-Jones E., Cajaraville M. Cytotoxicity of Au, Zn and SiO₂ nps using *in vitro* assays with mussel hemocytes and gill cells: relevance of size, shape and additives. Nanotoxicology. 1-9.

⁴ Nel A., Xia T., Meng H., Wang X., Lin S., Ji Z. Zhang H. 2013. Nanomaterial toxicity testing in the 21st century: use of a predictive toxicological approach and high throughput screening. Acc Chem Res. 607-621 (3)46.