SAMTEFONE

Standardized Analysis of Morphology & Toxicology Effects in Freshwater Organisms to Nano-carrier Exposure

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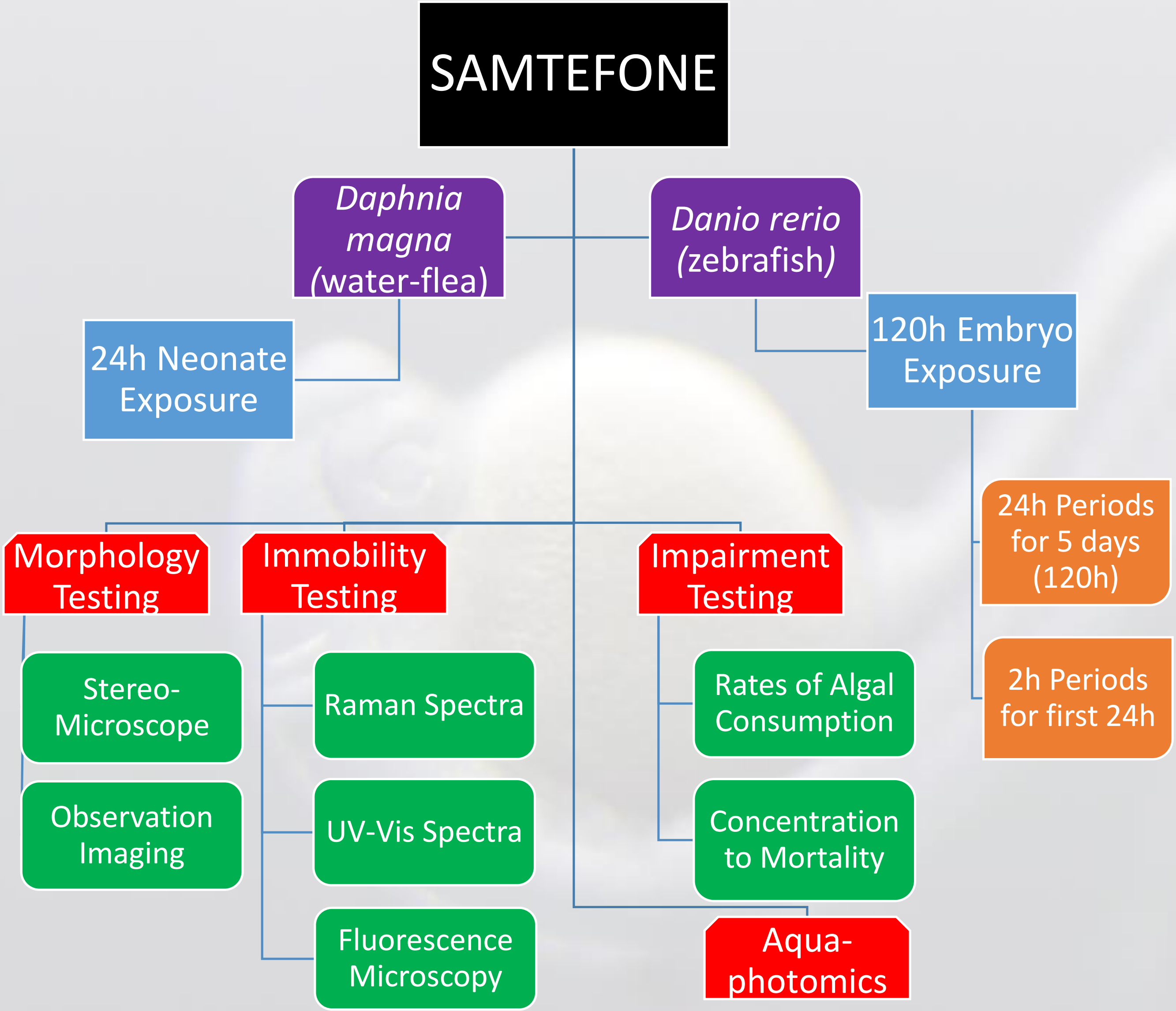
2015/2016 Research

Commencement: 1st October 2015

SAMTEFONE Research Plan/Objectives:



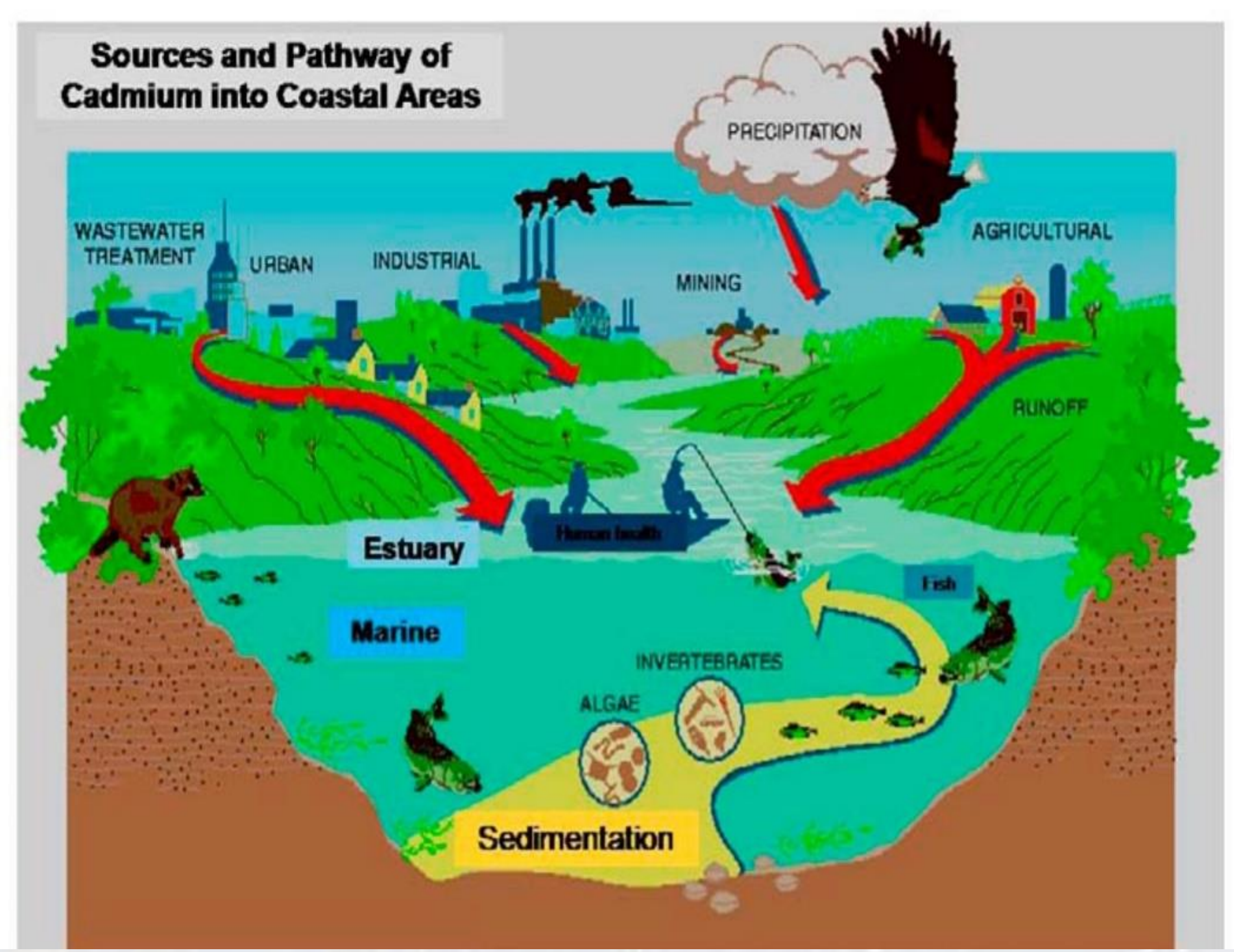
Objectives:

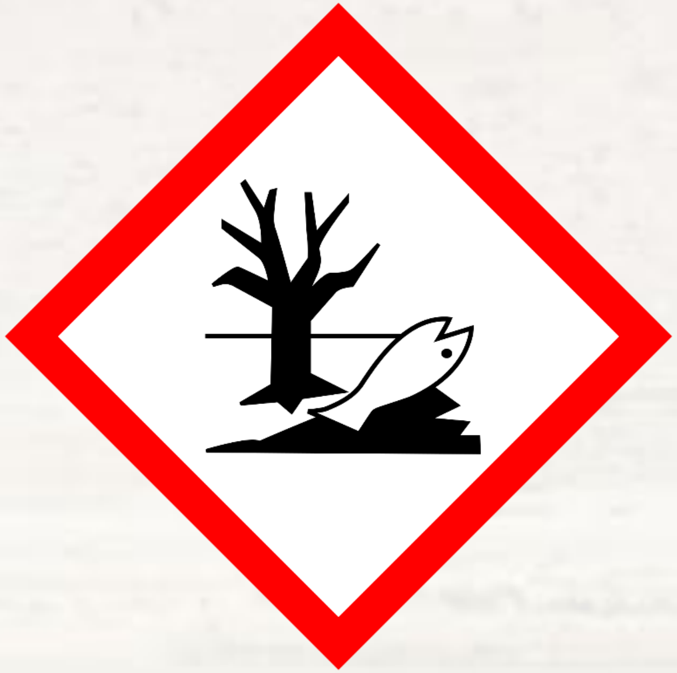
1. Produce a standardized method to test nano-toxicology under replicated environment conditions

2. Determine whether the techniques developed can be transferred to determine toxicological or morphological risks of other nano-carriers (zeolites, C60, etc.)

3. Examine if the confirmed techniques can identify nanomaterials without conducted invasive procedures (i.e. dissection, fixation, etc.) to prevent complications in the analytical process

Ecotoxicology



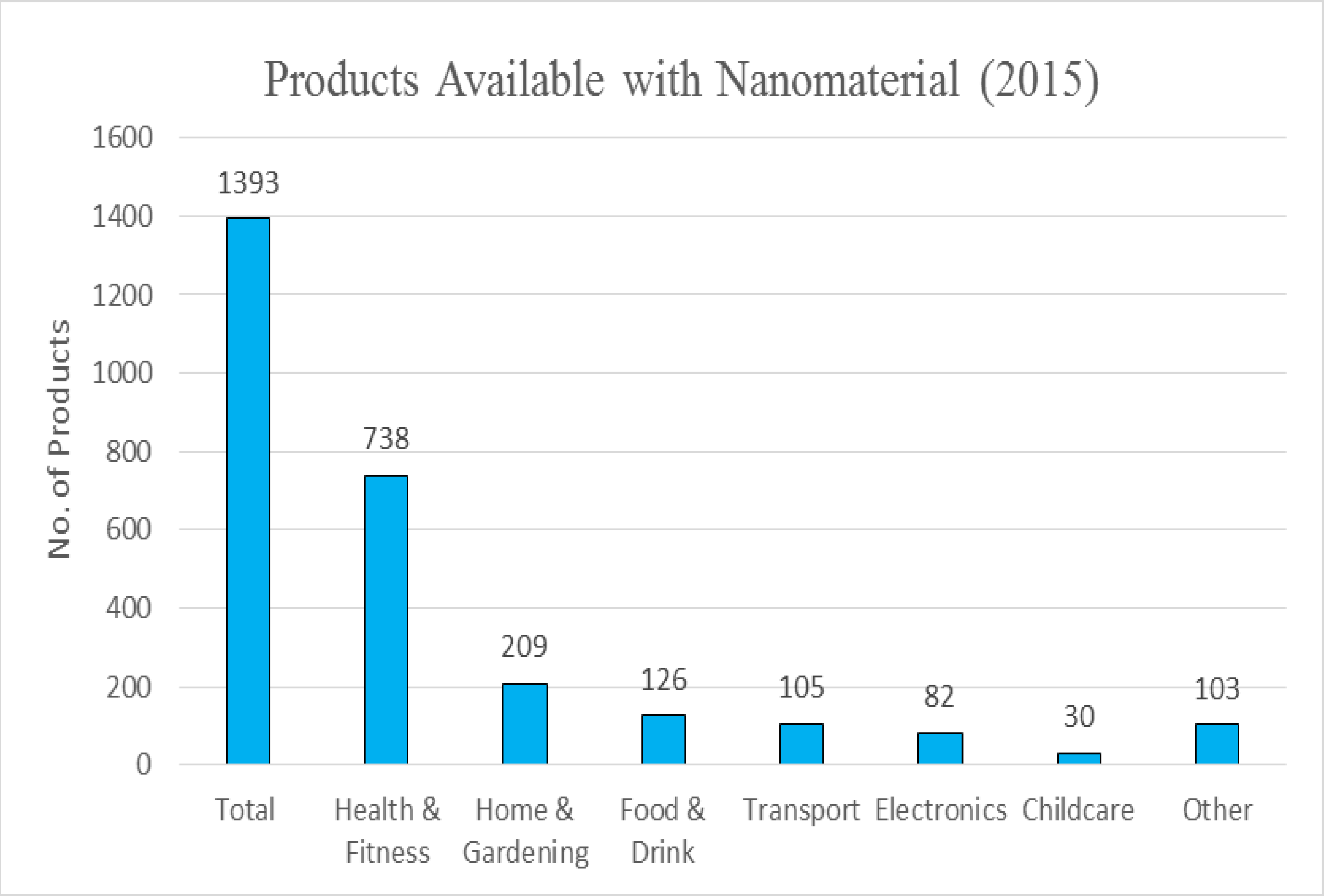


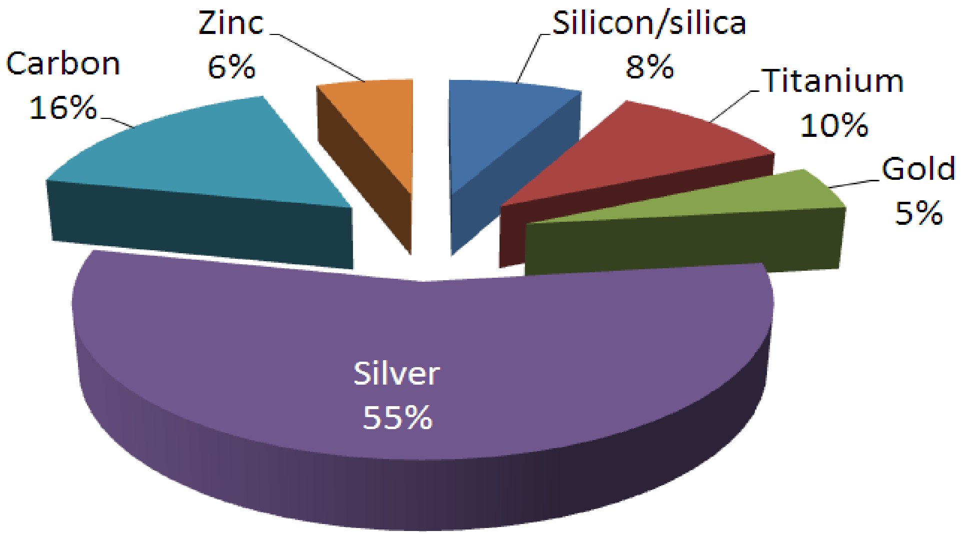
Breakdown of transport routes of potential environmental toxins into the environment

A variety of chemical substances have been distinguished as exotoxins:

- Pesticides & Fertilisers
- Polychlorinated Biphenyls (temp. resistant)
- Volatiles Solvents
- Silicate fibrous crystals (fiberglass)
- Heavy metals
- Reactive ions (chlorine, dioxins)

Nano-particles Applied to Products





Graphene based tennis racquet

stronger, lightweight and stable nanomaterial is contained, no leaching

Nano silver based milk bottle

Claims antibacterial nanomaterial leaches, safe???

Source: www.nanotechproject.org

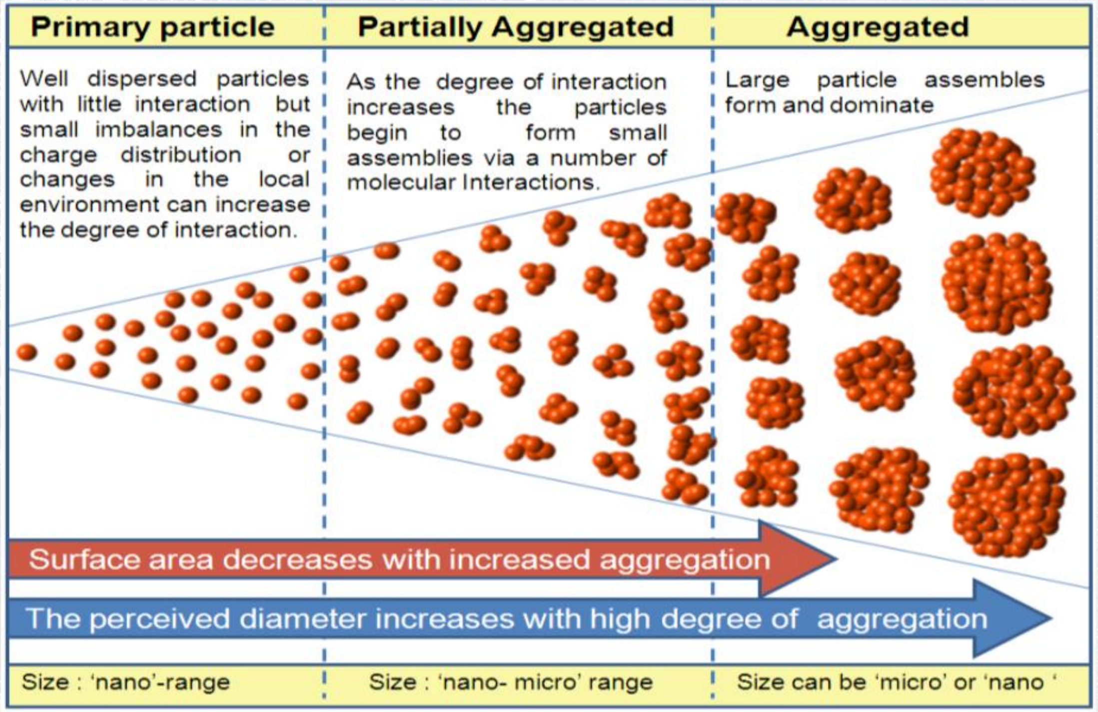
Nano-Ecotoxicology

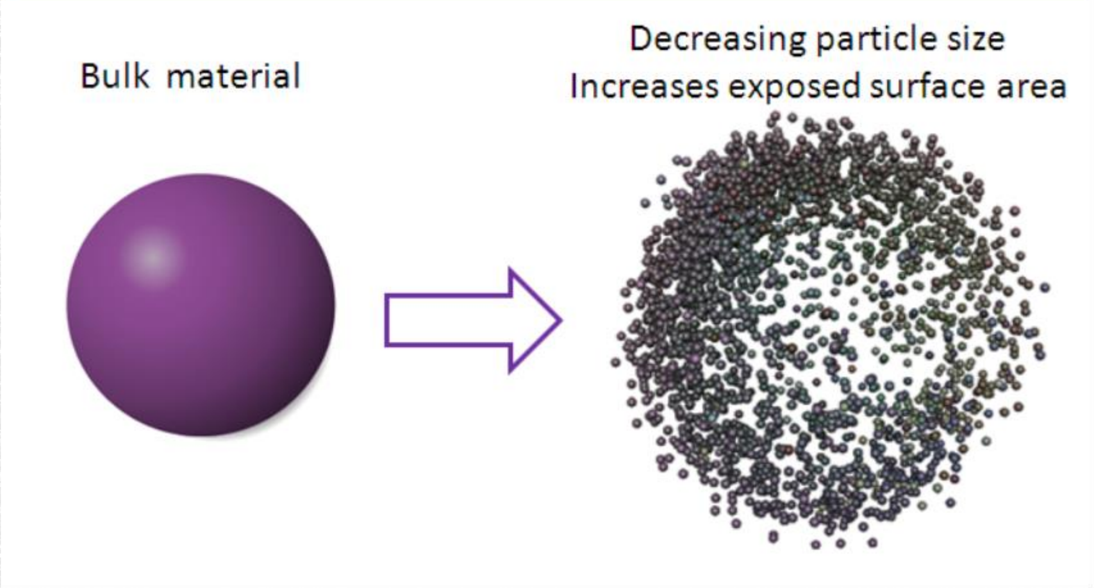
Ecotoxicology

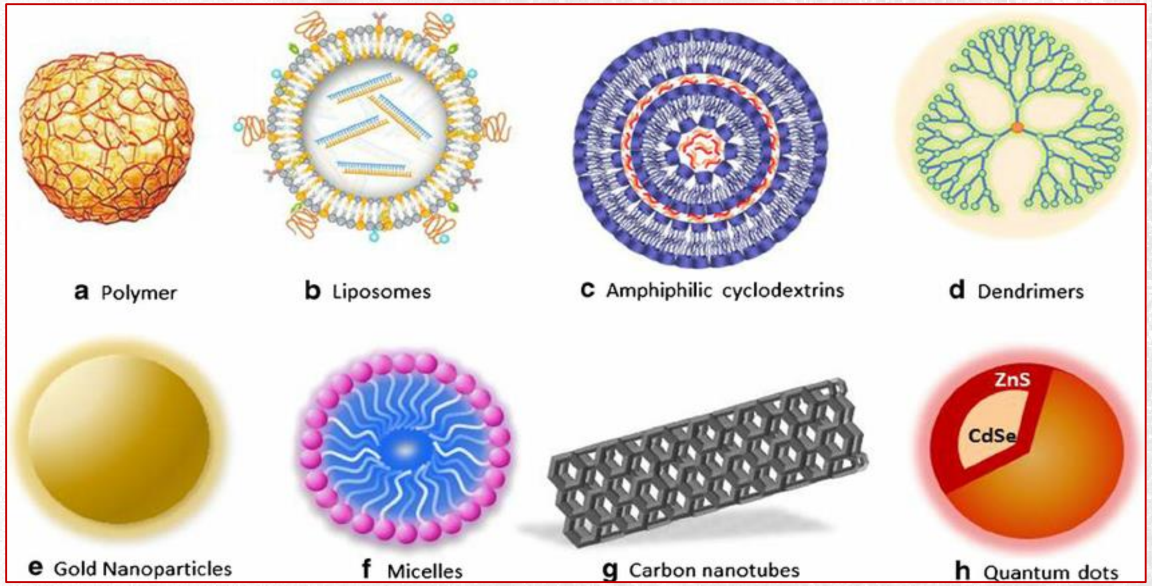
- Examines direct toxic effect of chemical substance on the environment
- Toxicity dependant of chemical concentration

Nano-Ecotoxicology

- Examines alternate routes of toxicity of nanoparticle compared to bulk chemical in the environment
- Toxicity dependant on structural properties and integrity of the nanoparticles in the environment



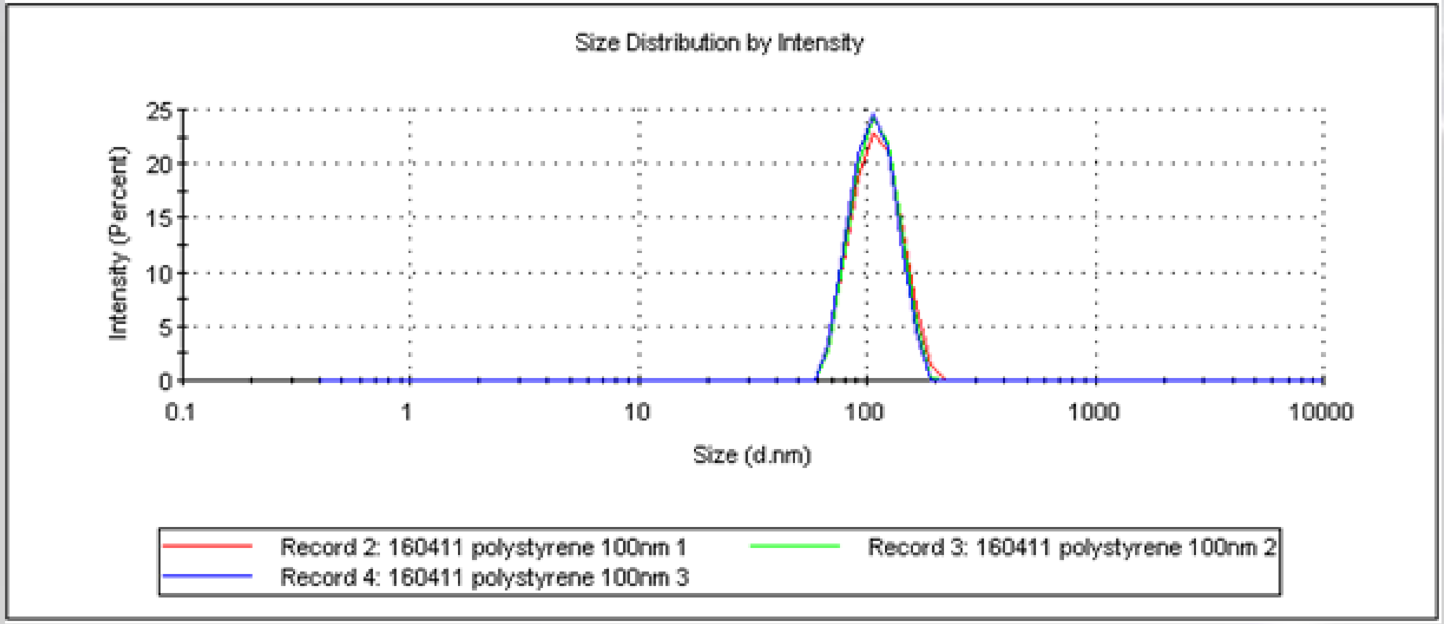


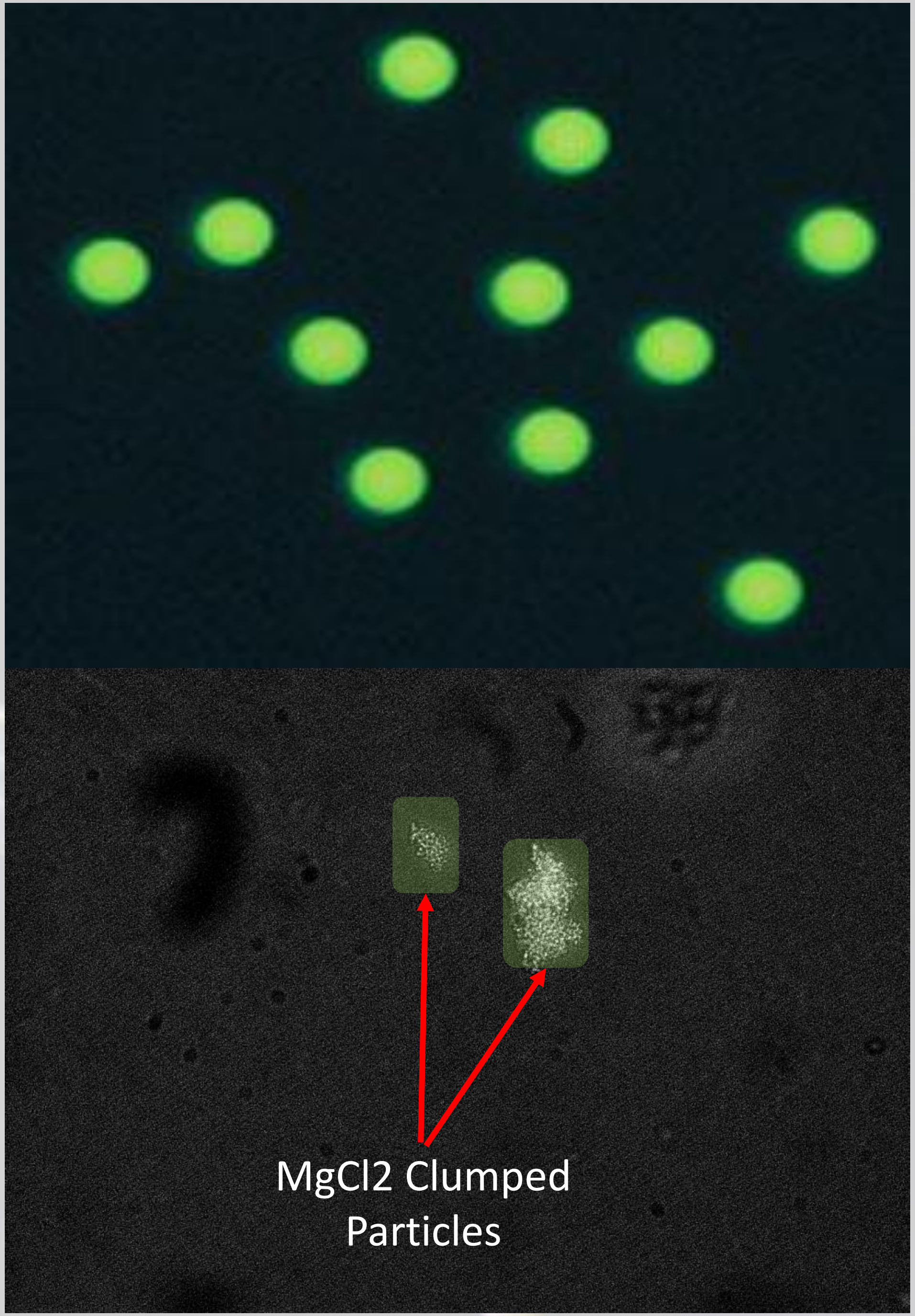


Polystyrene Nano-Beads

Firefli Polystyrene ~100nm Nano-beads in DI water with fluorescein dye (508nm green) integrated within the styrene chains to avoid dye leaching

Ideal test particle:
- Relatively non-toxic in bulk form
- Good structural integrity
- Low size discrepancy range





Analytical Techniques

Microscopy:

Stereo-microscopy

Coulter Particle Counter

Cell Observation (Fluorescence) Microscopy

Spectroscopy:

Raman Spectroscopy:

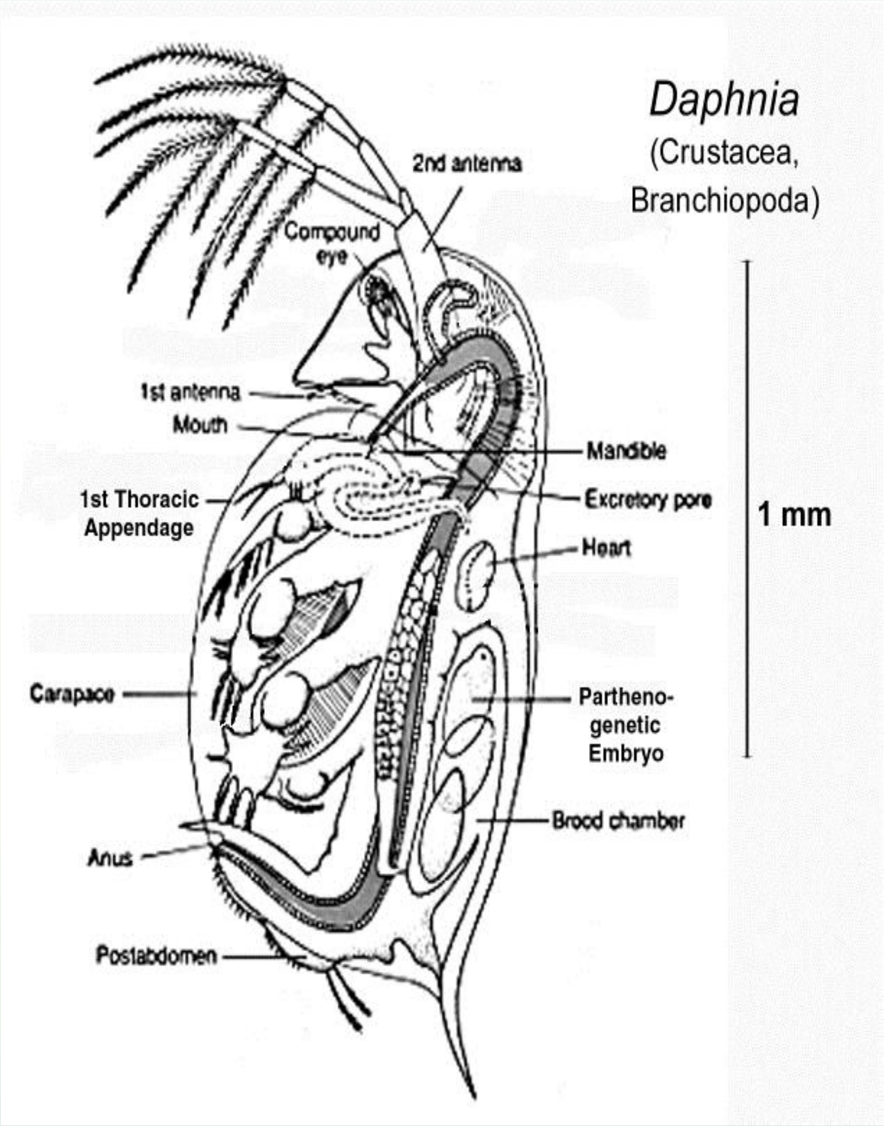
- Applied a monochromatic laser light onto samples, and analyses inelastic “Raman” scattering frequencies are “fingerprints” to bonds in the sample molecule

UV-Vis Cell Plate Spectroscopy:

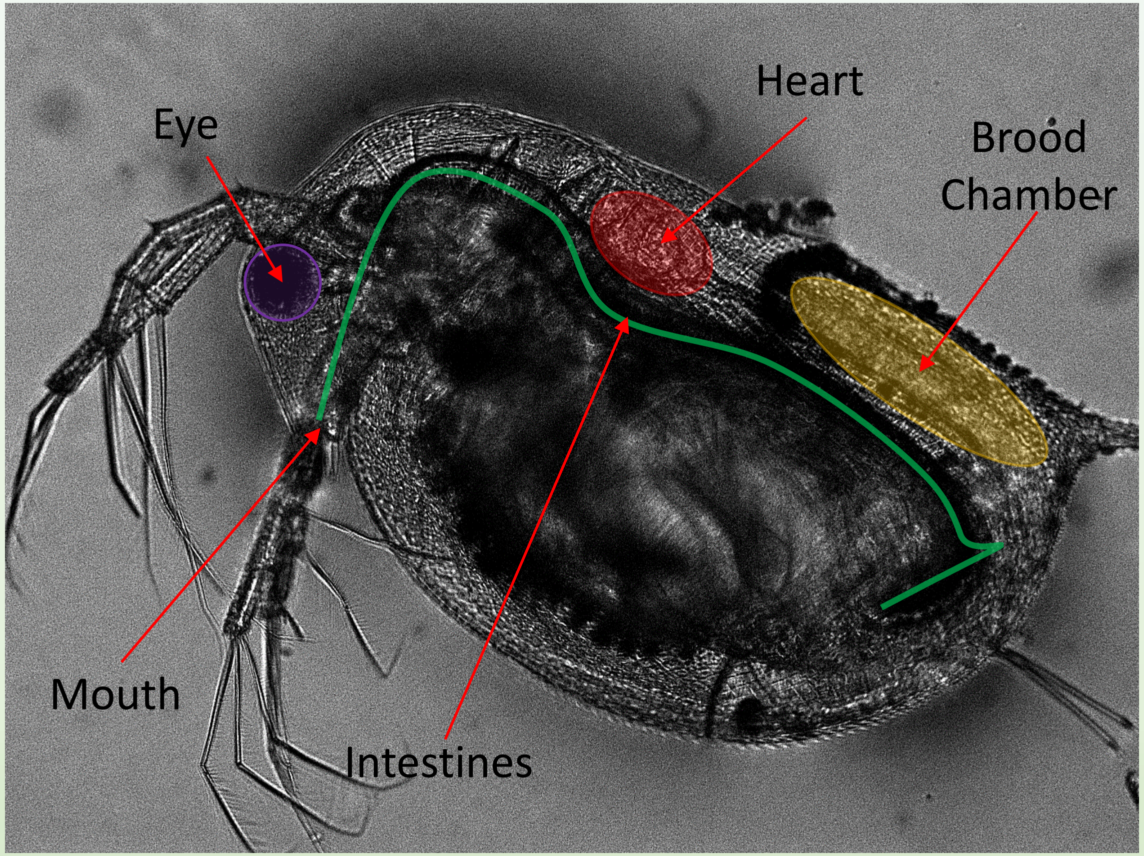
- Introduces UV-Vis light (190nm – 750nm) to samples to induce light intensity loss within bonds to provide quantitative analysis on analytes within sample

Daphnia magna Testing:

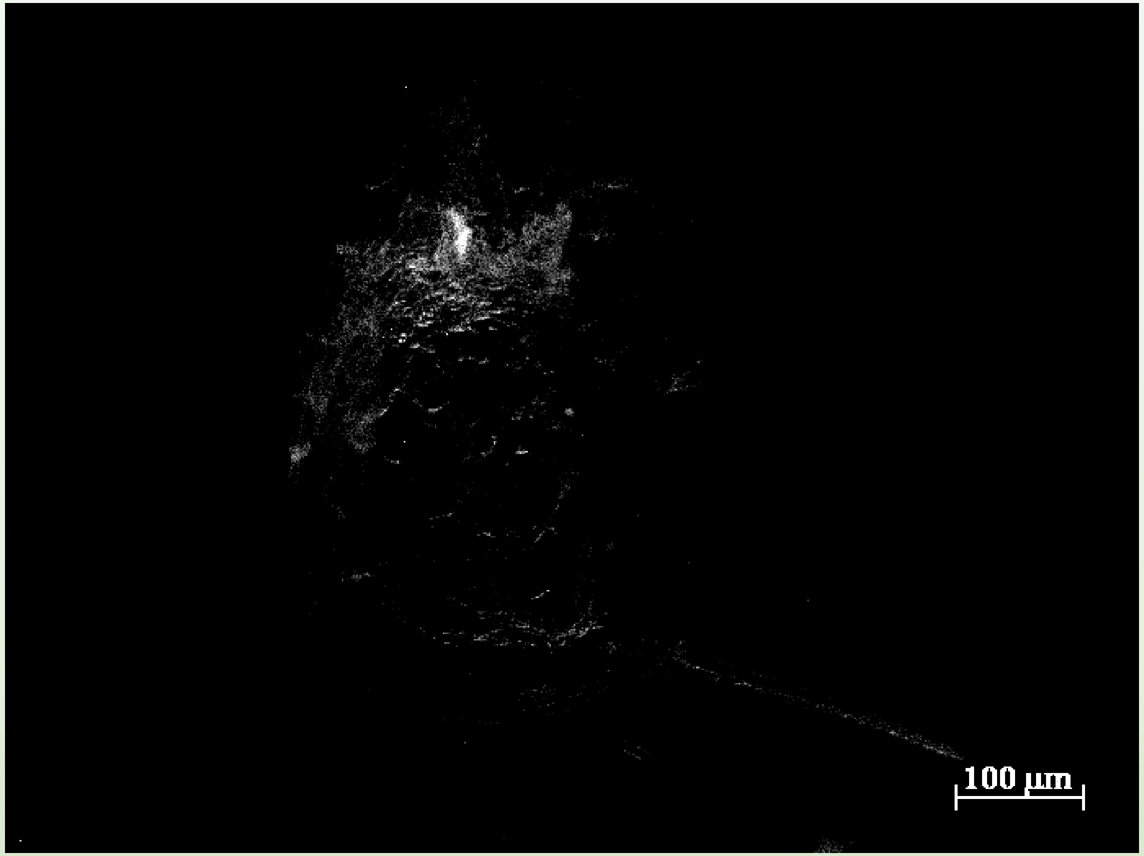
- Initial testing would utilize and optimize a standardized testing methods from analysing fluorescent polystyrene nano-spheres will be tested on *Daphnia magna* (freshwater fleas)
- Testing based on the OECD Testing Chemical Guidelines No. 202 for Immobility/Mortality



Daphnia magna Optical Analysis:



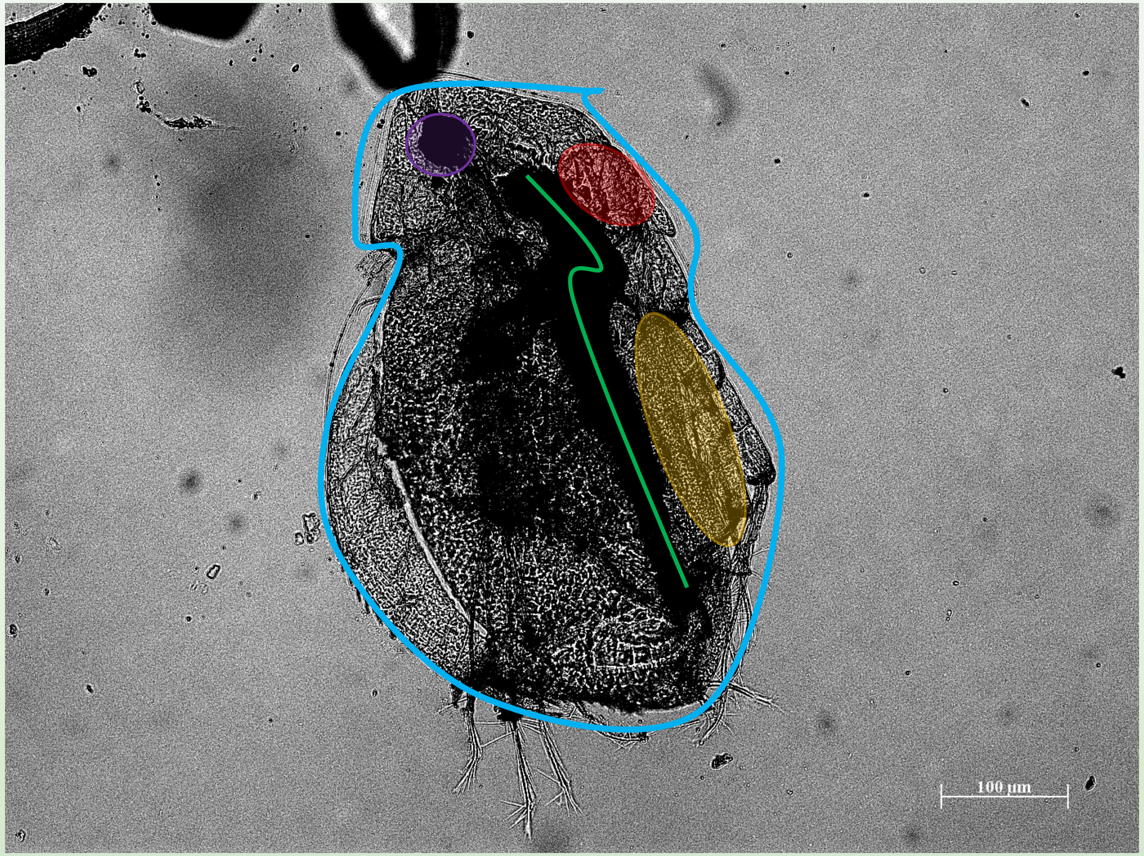
Daphnia magna - Light Microscope (3.7ms)



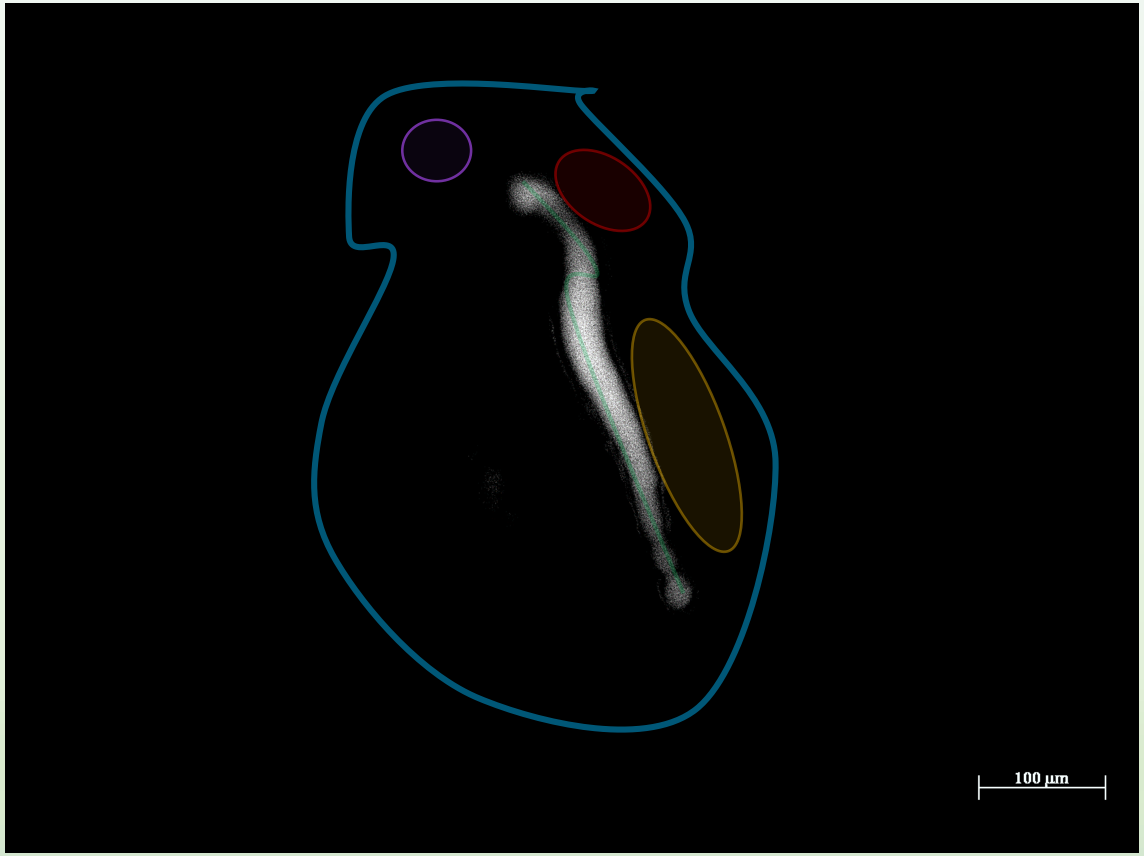
Daphnia magna – 489nm Fluorescence (93.3ms)

Daphnia magna Optical Analysis:

Initial Fluorescent Nano-polystyrene Testing Comparison Images



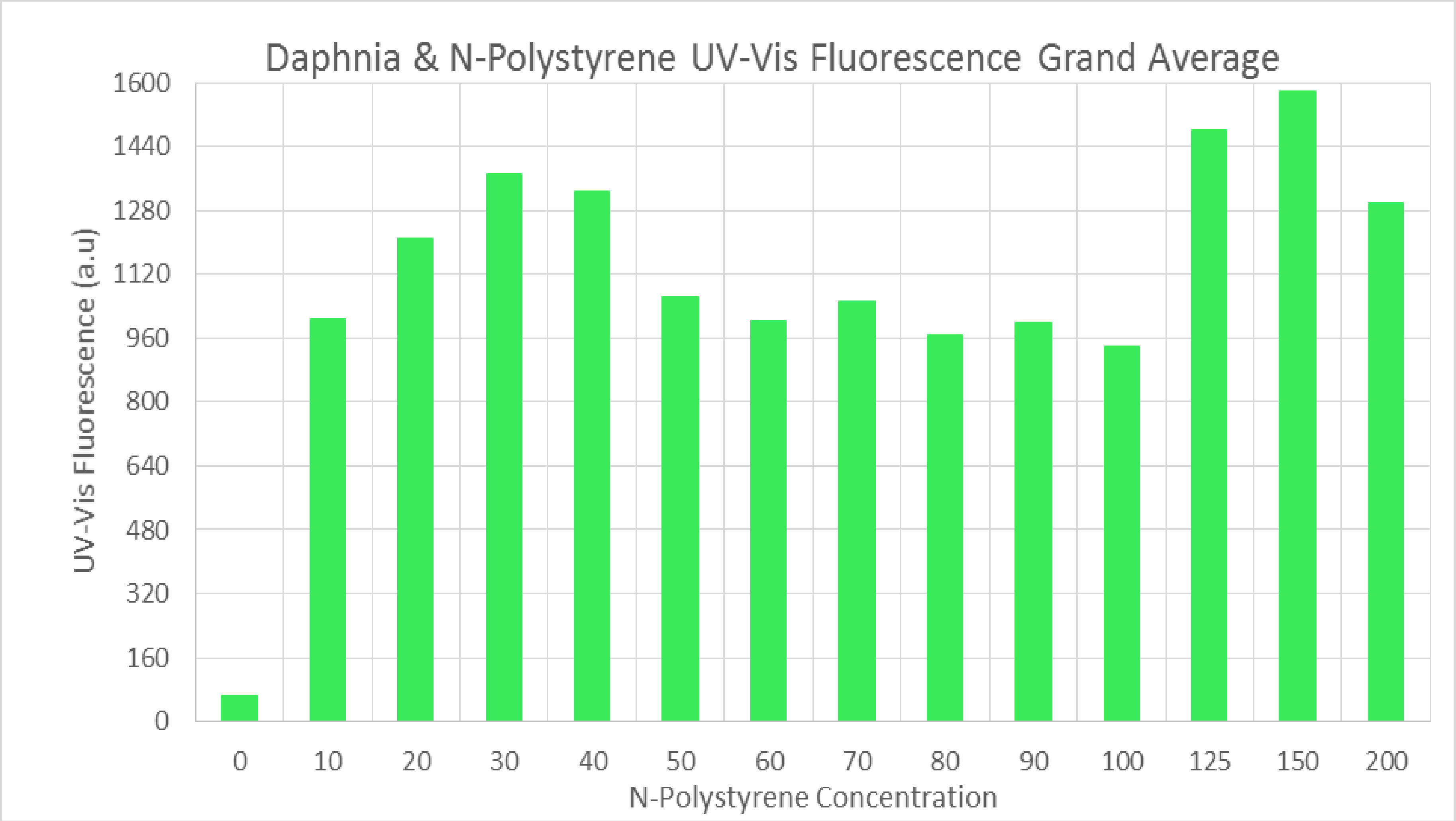
Daphnia magna White-light Analysis 3.7ms



Daphnia magna 489nm Excitation 3.7ms (60ppm N-Polystyrene)

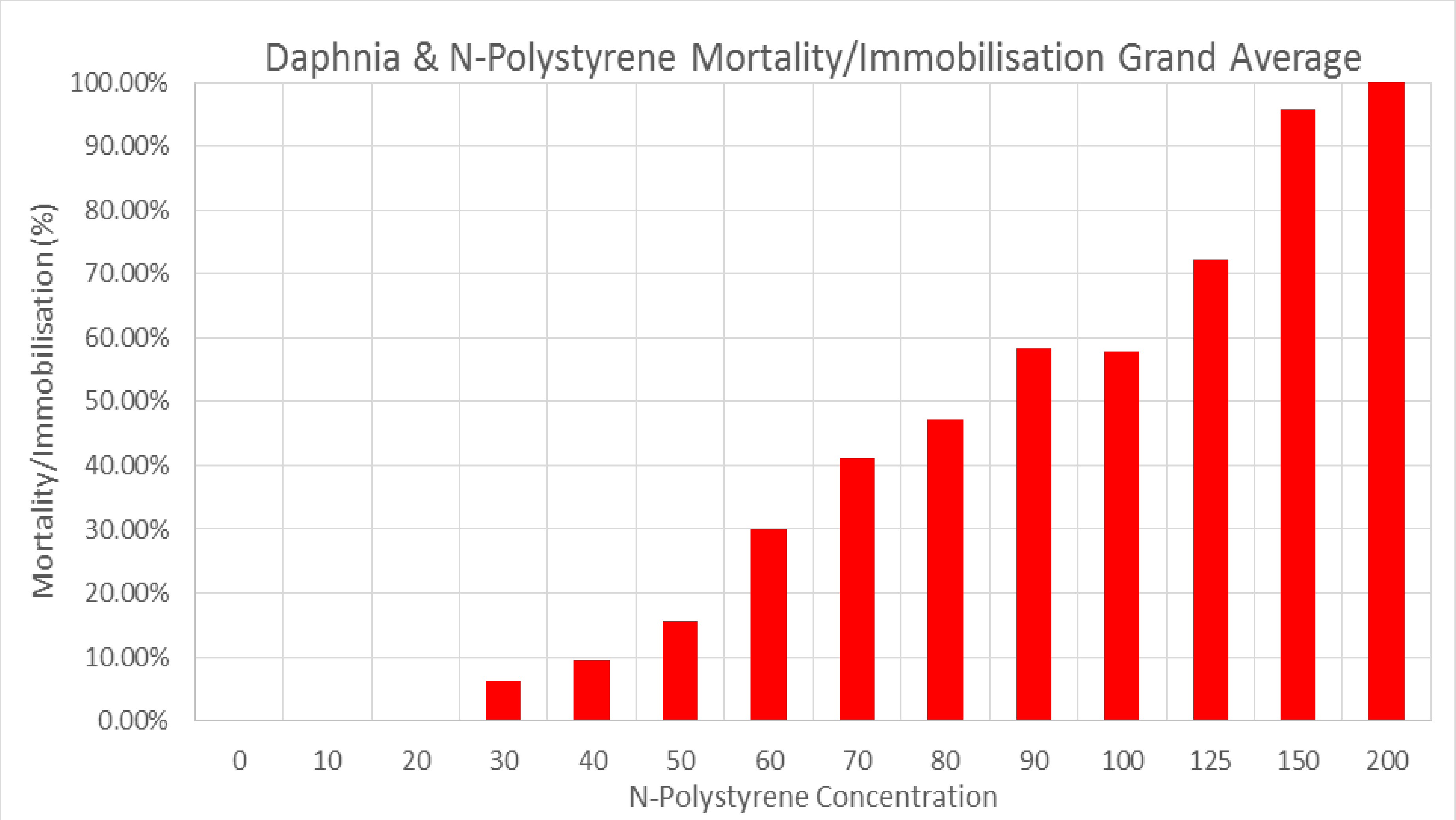
Daphnia m. UV-Vis Fluorescence Analysis:

- 550 *Daphnia magna* run
- 13 N-polystyrene Conc. & 1 control per test
- Checked using 489nm excitation, with 508nm emission
- Each concentration scanned three times

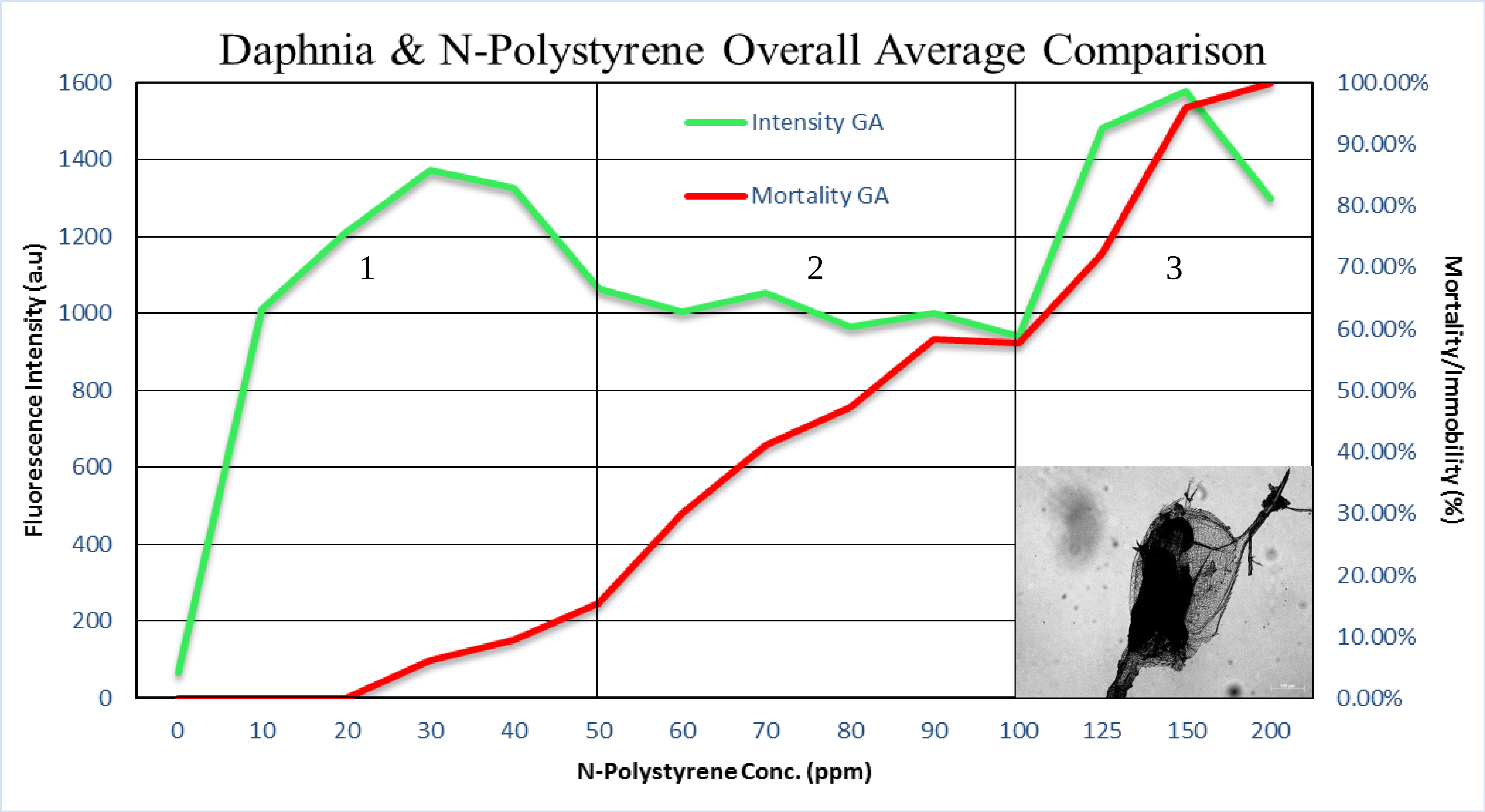


Daphnia m. Mortality/Immobilisation Analysis:

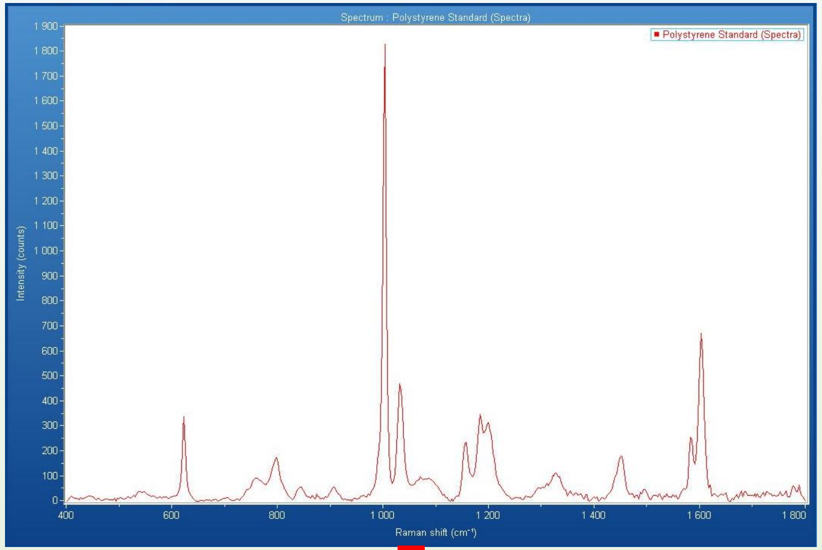
- 550 *Daphnia magna* run
- 13 N-polystyrene Conc. & 1 control per test
- Run using regulations under OECD Testing for Chemicals No. 202
- Daphnia either dead or sufficiently immobilised counted as affected

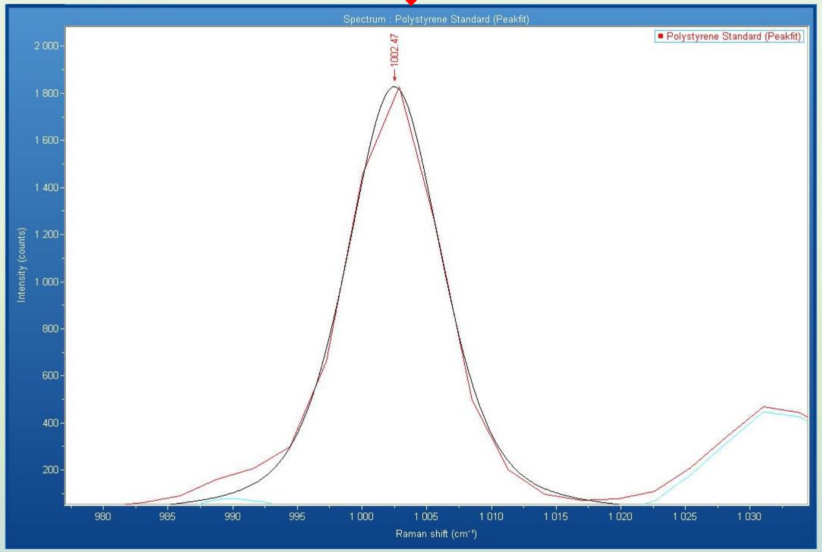


Daphnia magna Immobilisation Analysis:

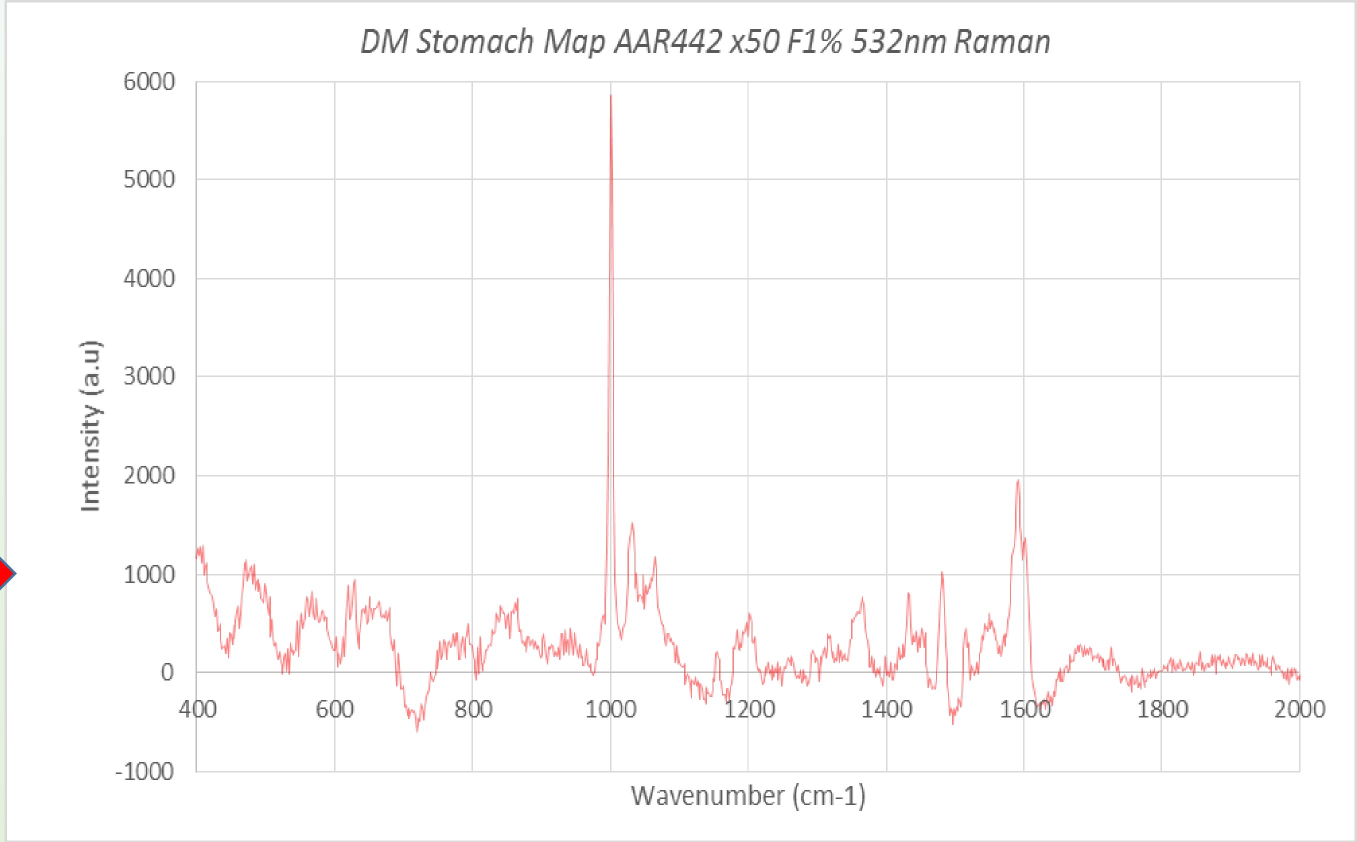


Daphnia magna Raman Analysis (Cont.):





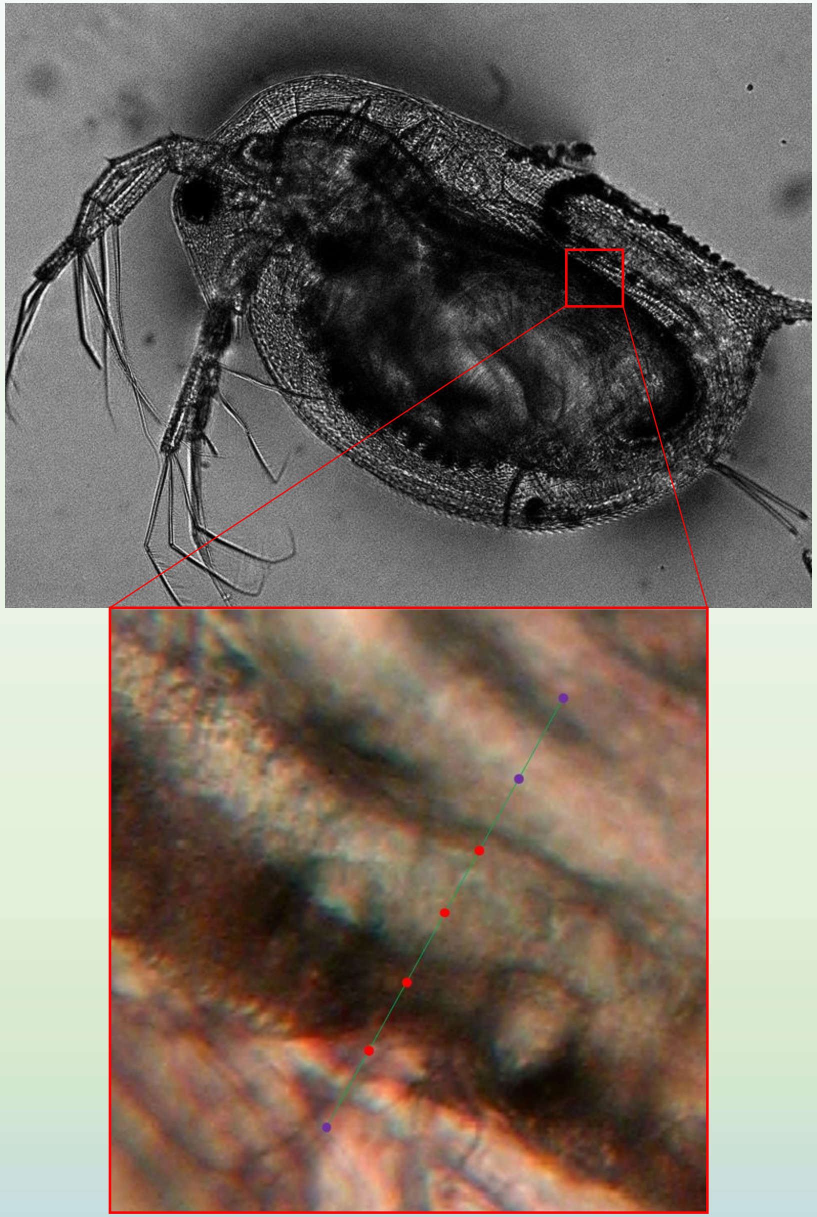
Polystyrene Standard (Spectra/ Key Peak)

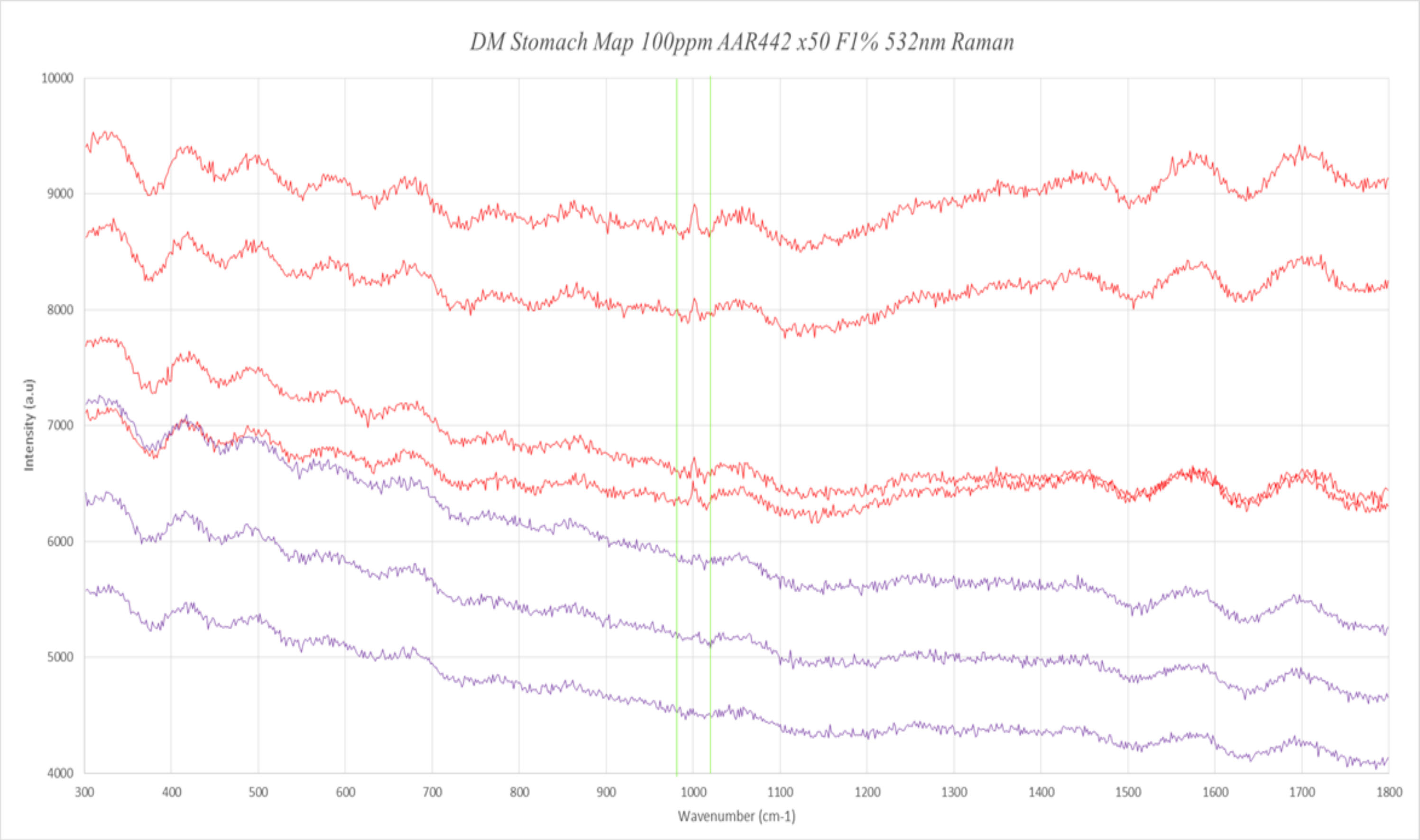


Raman spectra analysis of *Daphnia magna* samples with N-Polystyrene produced distinct peaks at ~1000cm^{-1} & ~1580cm^{-1}

Polystyrene has two key peaks:
1002.5cm^{-1} aromatic C-C breathing mode
1583.1cm^{-1} vinyl C=C breathing mode signal

Daphnia magna Raman Analysis:

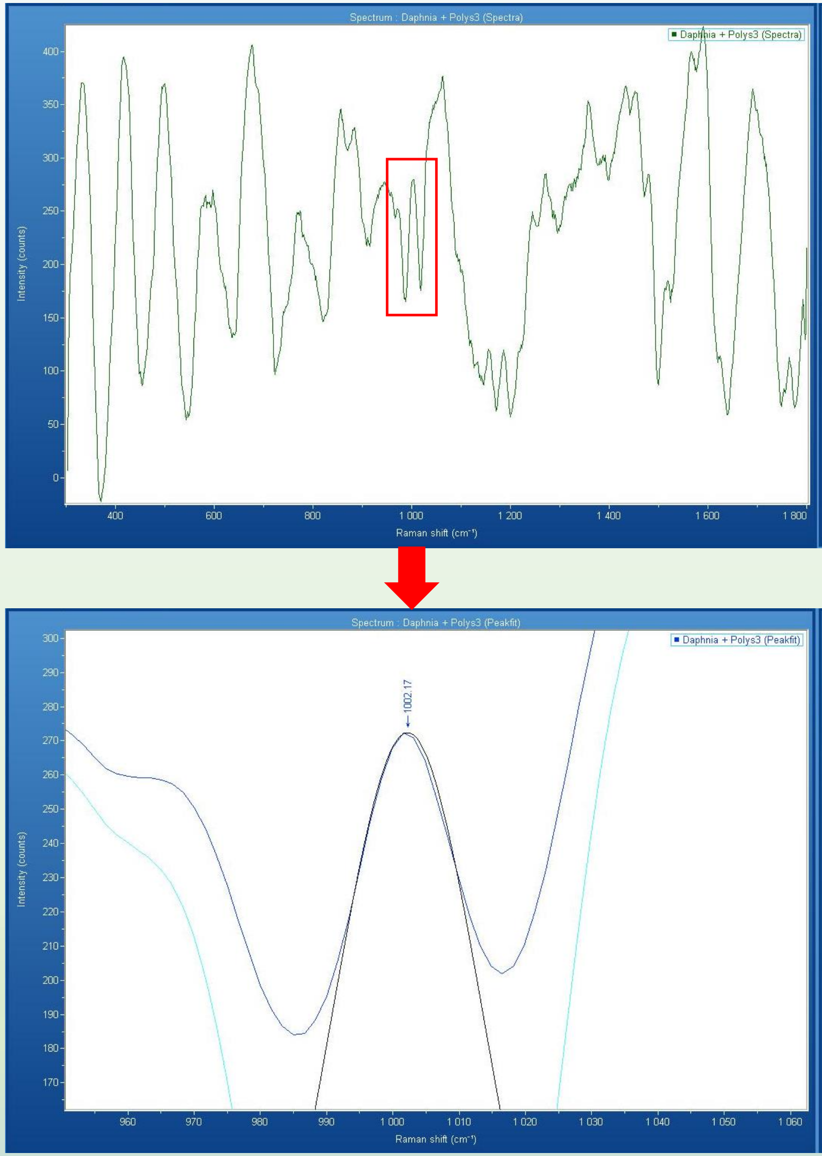




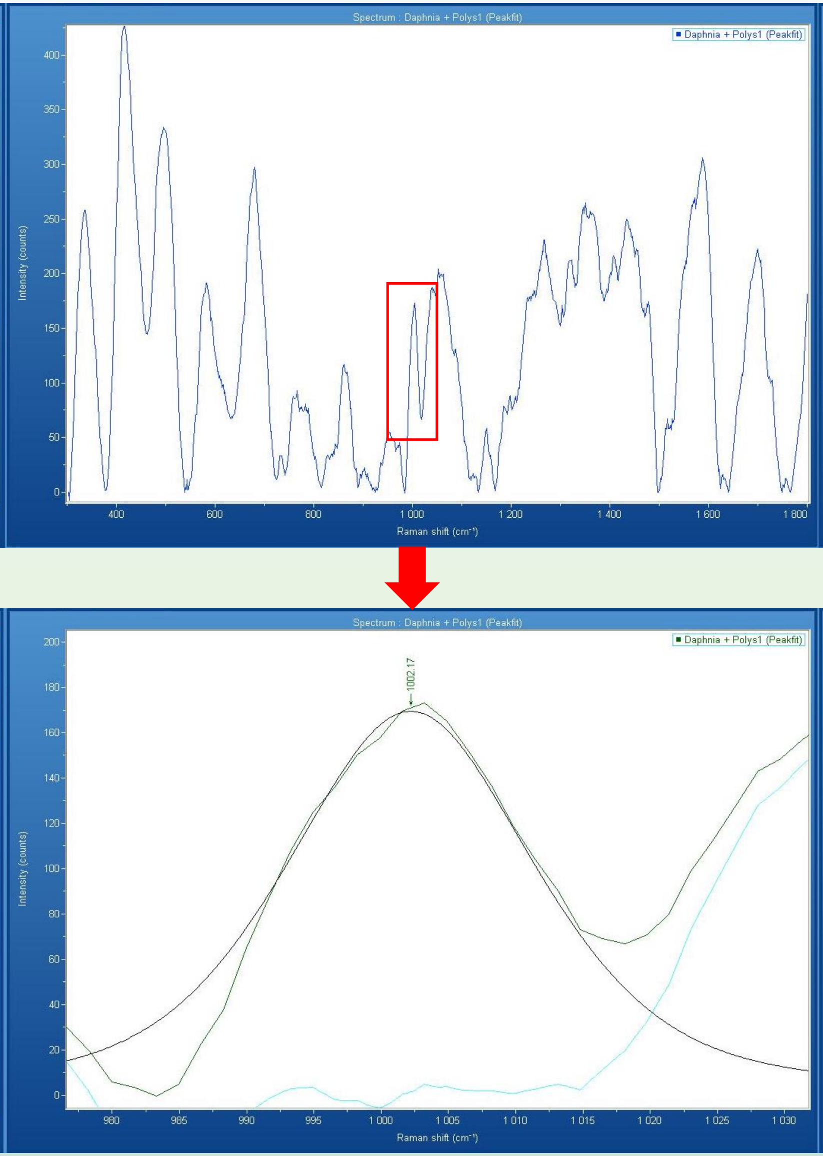
Daphnia Raman Spectra Line Map

(*Red Spectra* = *Daphnia Intestines*)

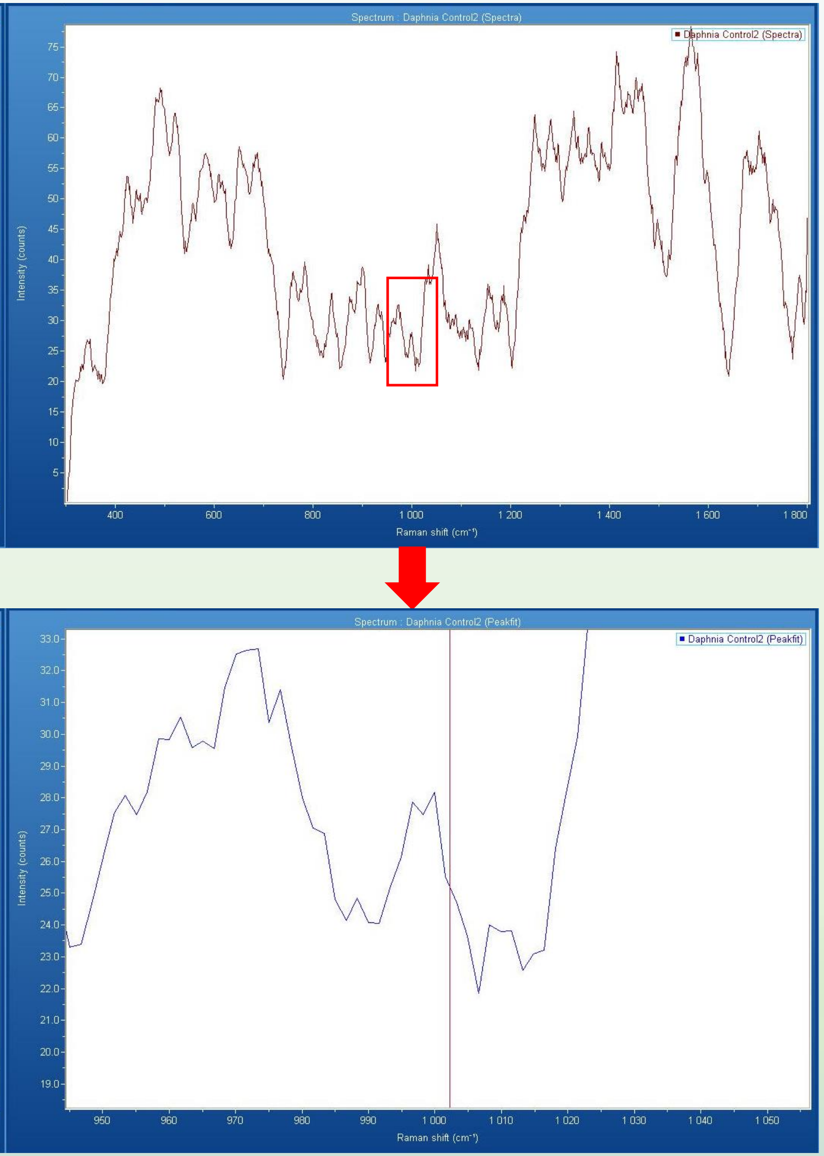
Daphnia magna Raman Analysis (Cont.):



Daphnia Intestine & N-Polystyrene



Daphnia Intestine & N-Polystyrene 2



Daphnia Intestine Control

Conclusion

- Literature review suggests a lack of research into effective toxicology analysis of nano-particles within the environment
- Current use of fluorescence & cell observer imaging distinguished emission from polystyrene nano-spheres absorption routes within *Daphnia magna*
- UV-Vis spectra compared to mortality/immobility testing showed a three part breakdown of effects from increasing the N-polystyrene concentration
- Raman spectra on Daphnia intestines provided discernible signals not present within control sample that compare identically with polystyrene Raman signals
- Plans to expand onto alternate freshwater species to confirm technique applications