Quantitative assessment of nanoparticle-induced toxicity in embryonic zebrafish

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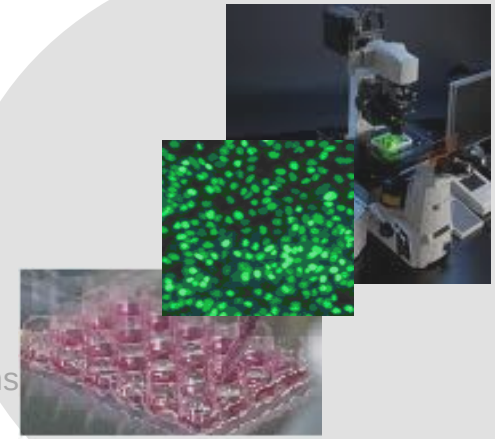
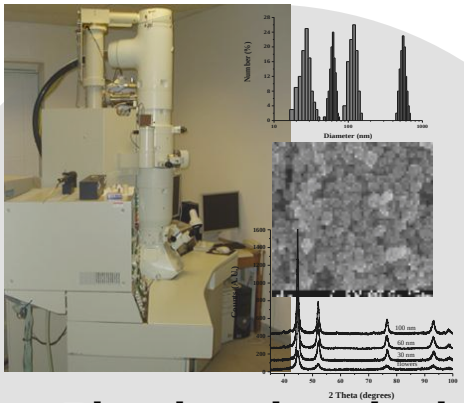
STRATEGIES FOR NANOTOXICITY ASESSMENT



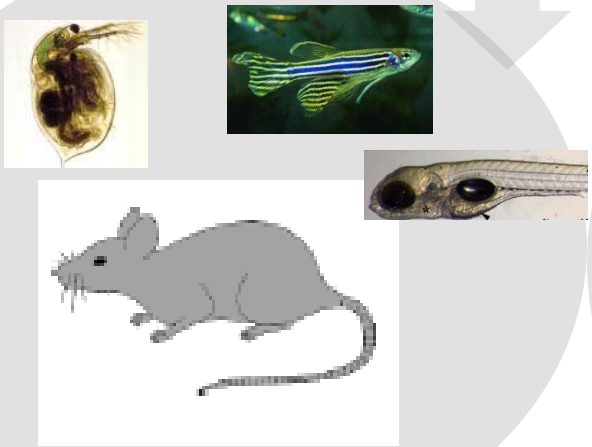
Isolated systems
Information not always transferable to in vivo full organisms

In vitro studies: cells and proteins



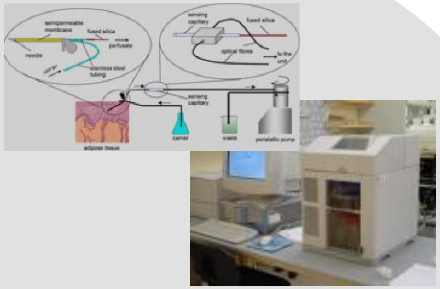
Physicochemical Characterization & Surface Analysis

Predictive models



In vivo studies

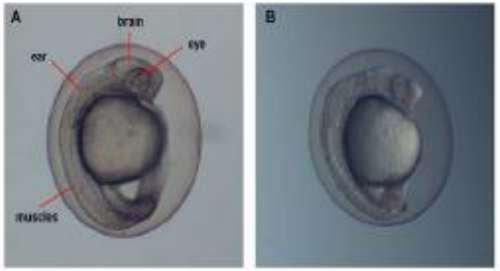
Complex, expensive, time consuming, ethical problems

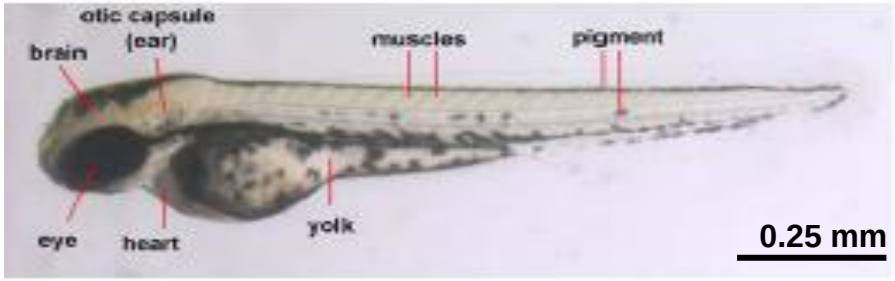


Physiology of vivo systems exposed to nano

Reduced spatial / temporal resolution (10-20 min)- real time measurement of physiological changes is difficult
Off line methods – Microdialysis – Classical measurements

In vivo Embryonic Studies



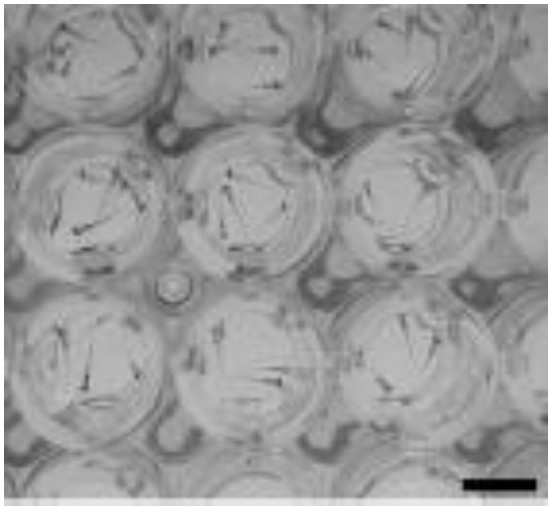


Zebrafish embryos 3 dpf with clearly defined organs

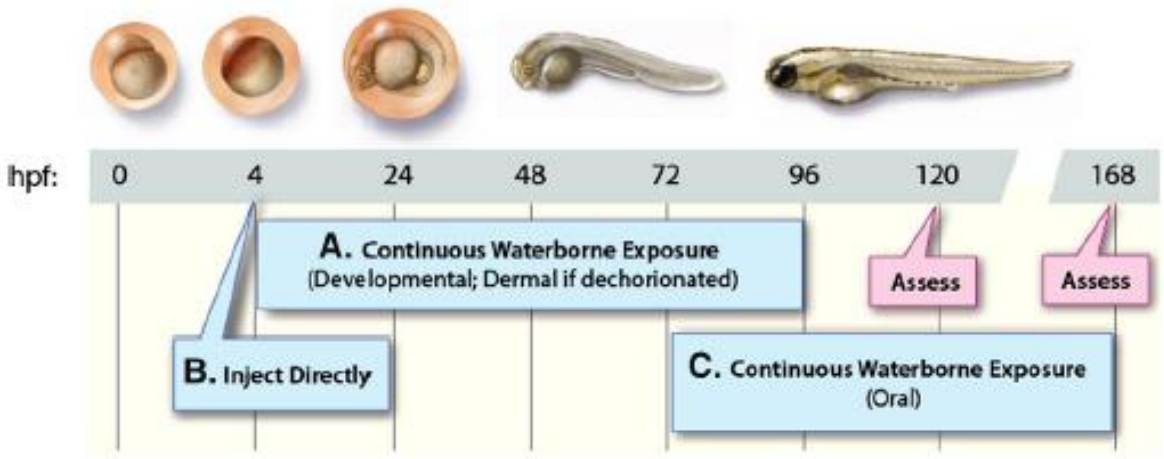
Zebrafish share the same set of genes as humans; signaling and biological processes of vertebrates

Well characterized development, optical transparency, visualization of the organs + accumulation sites of the nanoparticles

Cytotoxicity and screening: organ defects, early drug discovery,

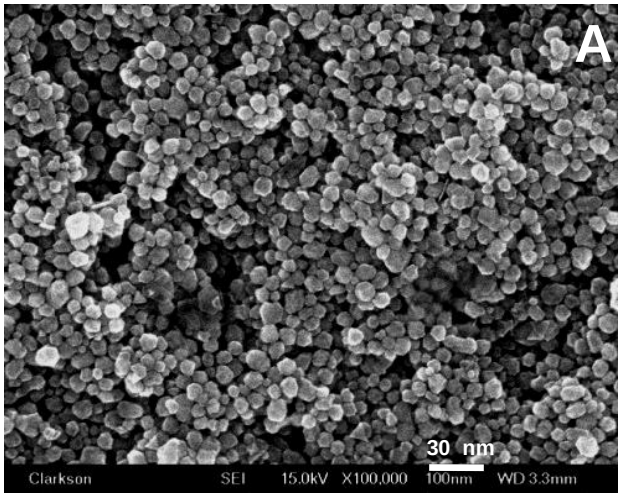


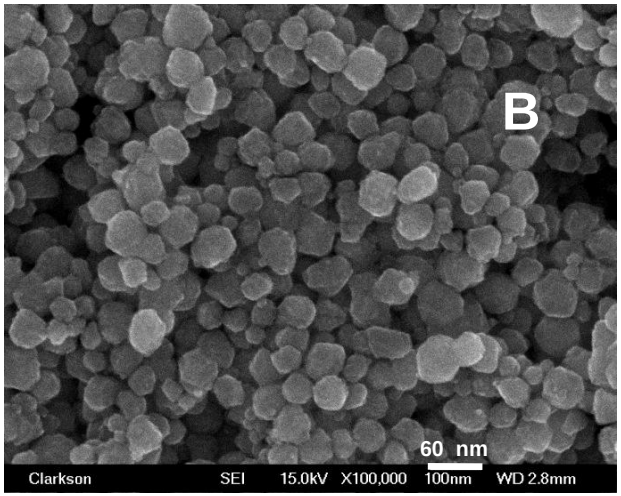
7 dpf in a 96 well-plate

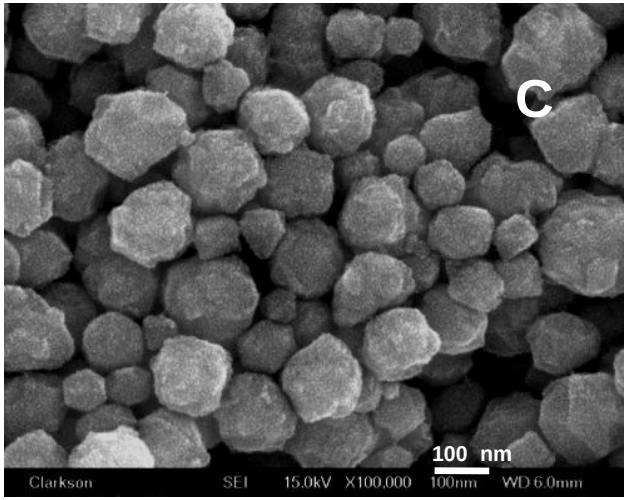


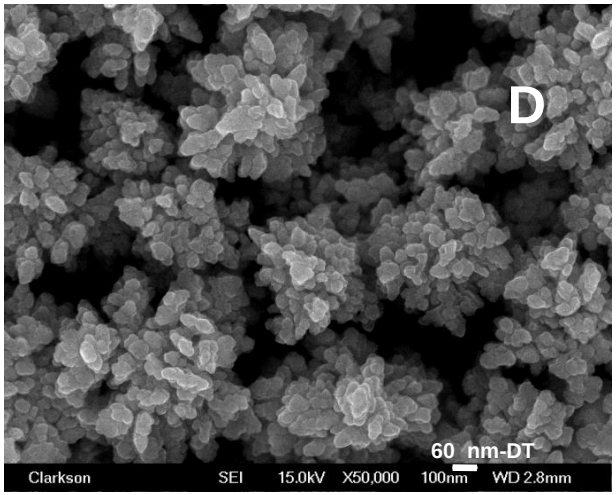
Barros, T.P., Alderton, W.K., Reynolds, H.M., Berghmans, S., ***J. Pharmacology***, **2008**, 154, 1400
Fako, V.E., Furgeson, D.Y., ***Advanced Drug Delivery Reviews***, 61, **2009**, 478-486.

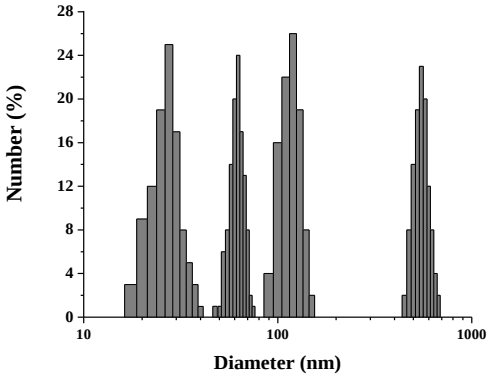
Ni nanoparticles: Is the toxic effect size/shape dependent ?







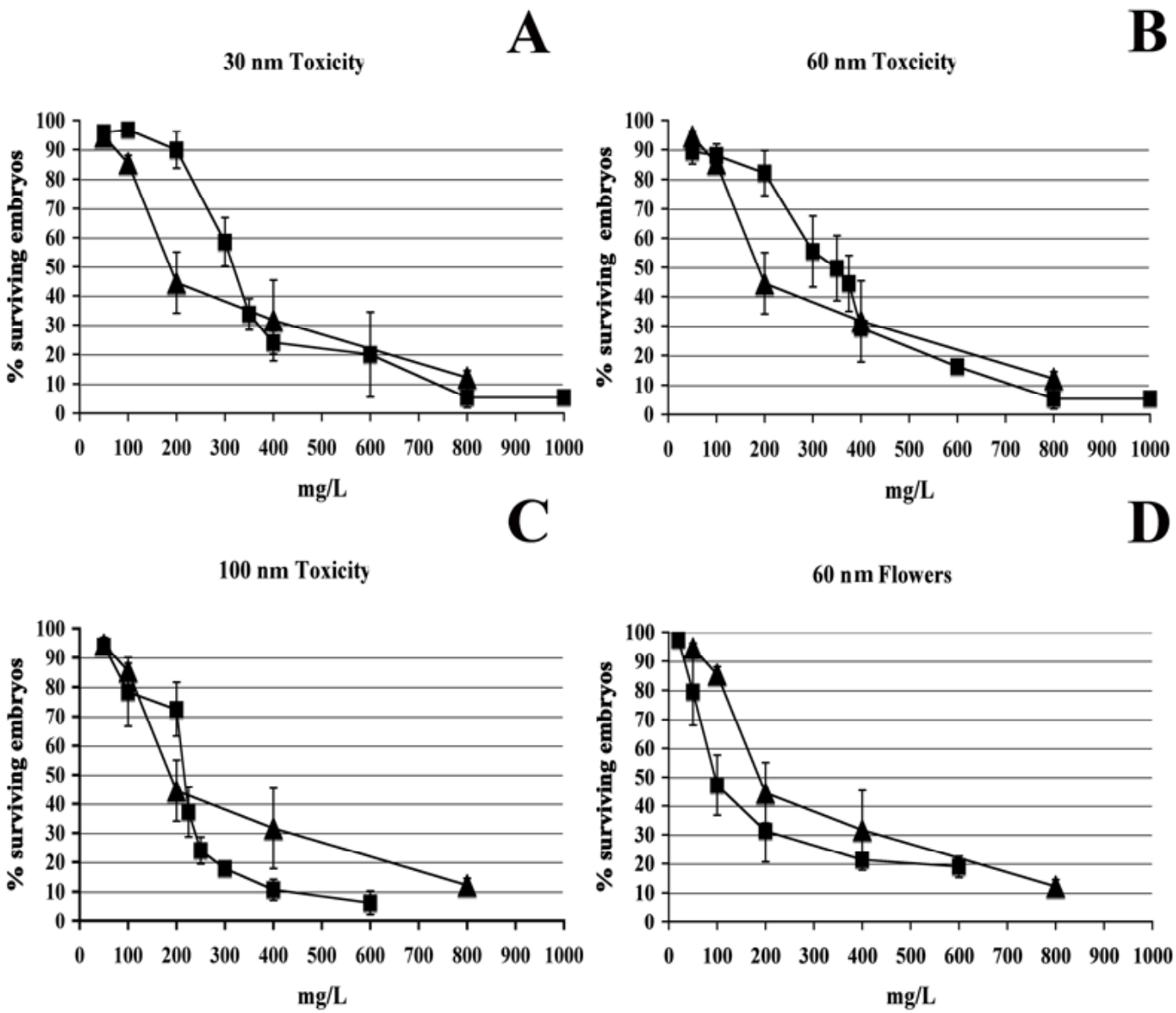




Nickel has previously shown to be toxic but the exposure studies have mainly been done with aqueous nickel

C. Ispas, D. Andreescu, A. Patel, D. Goia, S. Andreescu, K. Wallace, ***Environmental Science and Technology***, 43, 16, **2009,** 6349-6356.

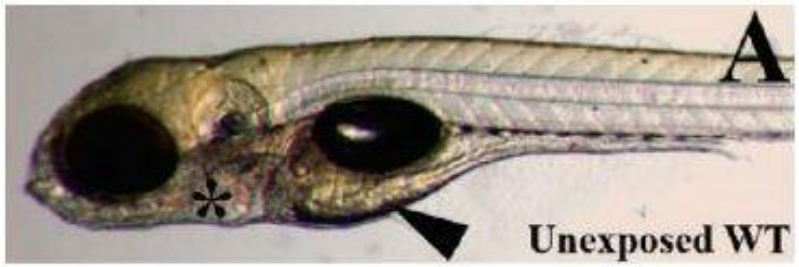
Degree of lethality function of Ni NPs concentration and dimensions

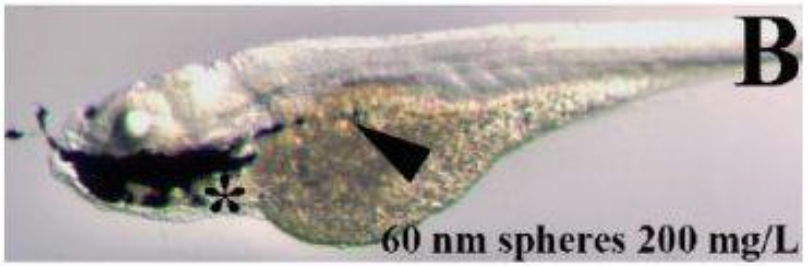


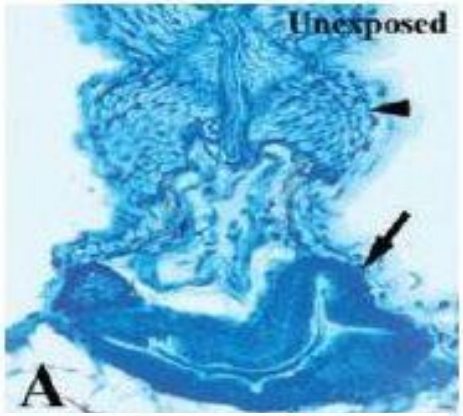
N=25 embryos ± standard error (n= 3-6)

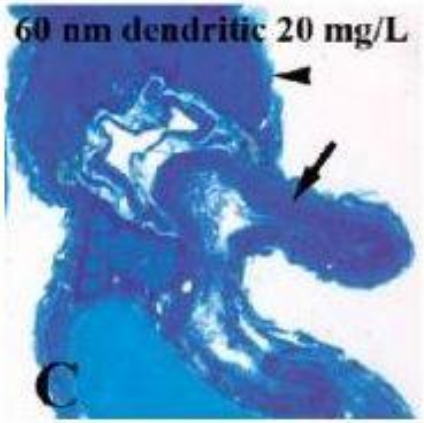
C. Ispas, D. Andreescu, A. Patel, D. Goia, S. Andreescu, K. Wallace, ***Environmental Science and Technology***, 43, 16, **2009,** 6349-6356.

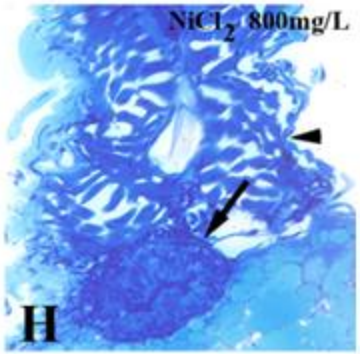
Nanoparticles & soluble Ni generate different intestinal defects

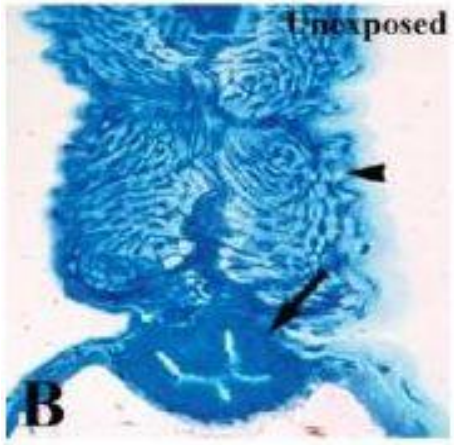


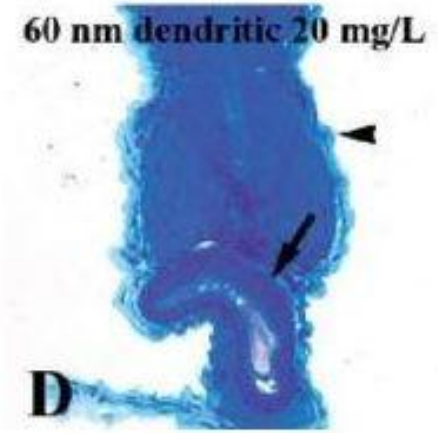












Ispas C. et al., Env. Sci. & Technol., 2009.

- Skeletal muscle fibers have become separated at concentrations in the 800 mg/L range for embryos exposed to nanoparticles
- No intestinal defect with soluble nickel

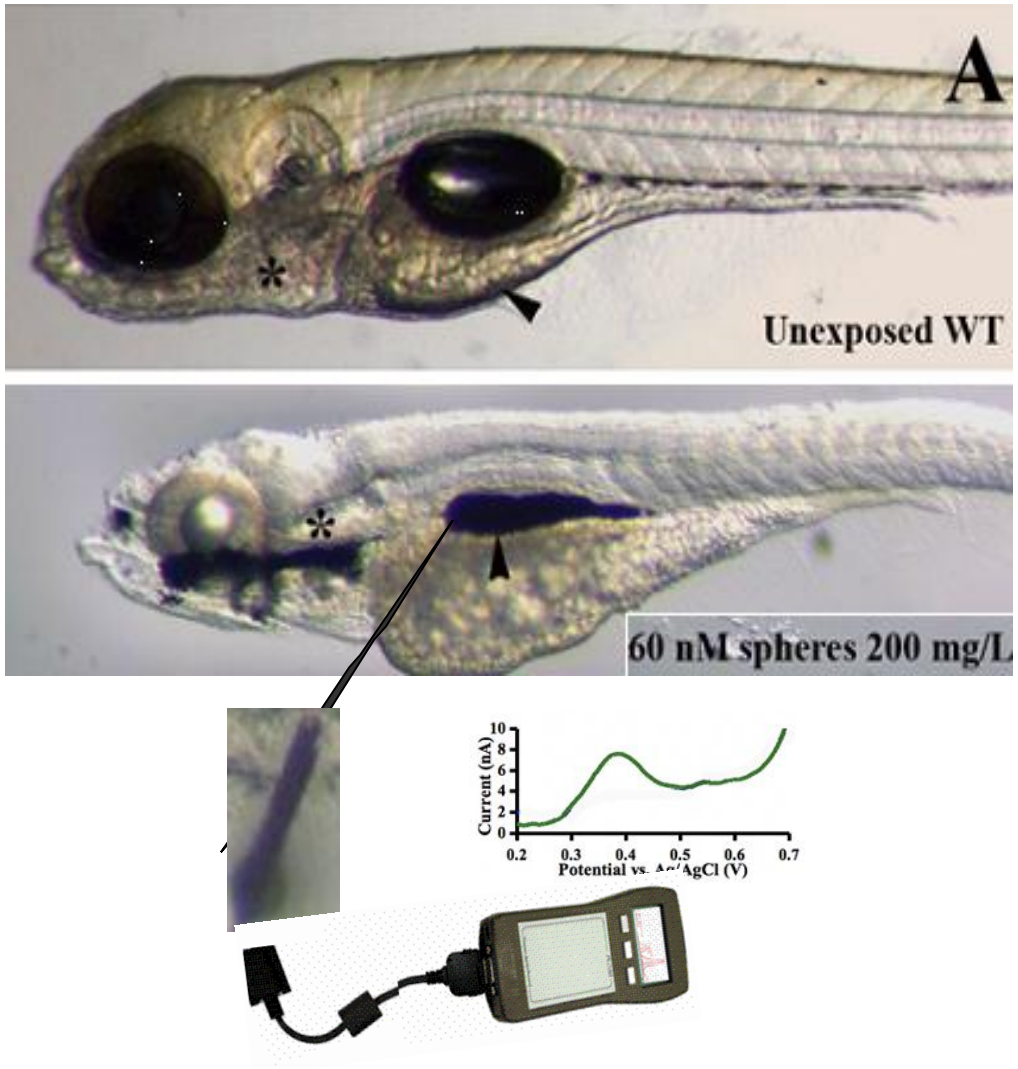
NPs induced Toxicity at Tissue and Organ Levels

NPs in contact with tissues can induce an inflammatory / oxidative response in situ

Effect of NPs can vary with the composition, size, shape and exposure time of the material

Effect of NPs – **low concentration exposure**:
mortality and developmental abnormalities, morphological malformations, behavioral abnormalities

Mechanism of cytotoxicity : inflammatory response, release of reactive oxygen species:
determine markers for inflammation and oxidative stress at the accumulation site – use implantable microelectrodes and perform electrochemical measurements at organ levels



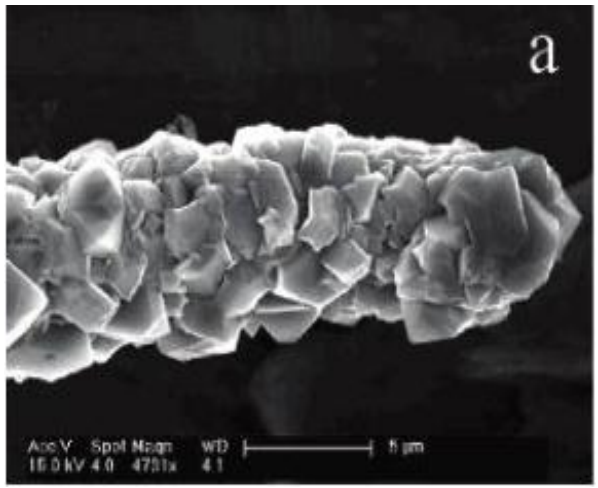
- Tissue response at the accumulation site
- Dissolution
- Study of inflammation
- Oxidative stress
- Neurological damage

IN VIVO ELECTROCHEMICAL STUDIES AT ORGAN LEVELS AT THE NANOPARTICLE ACCUMULATION SITE

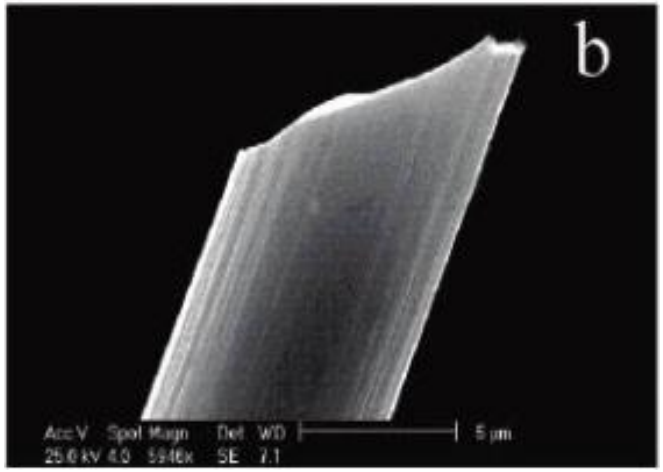
In vivo – implantable
High spatial/temporal resolution at precise locations – particular organs
High sensitivity
Study biochemical events for low concentration exposure

MICROELECTRODES

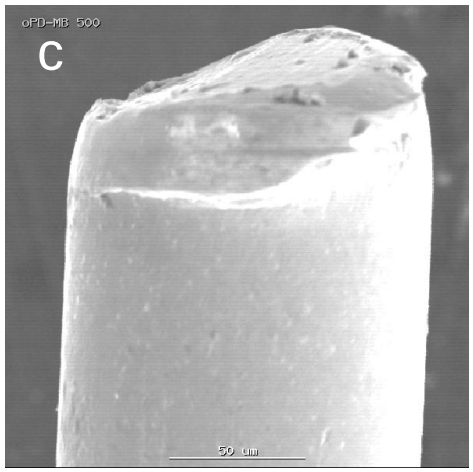
Boron-doped diamond wire

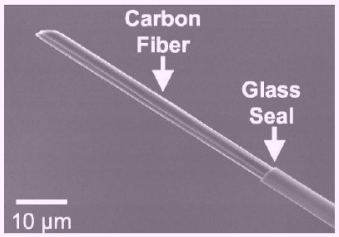


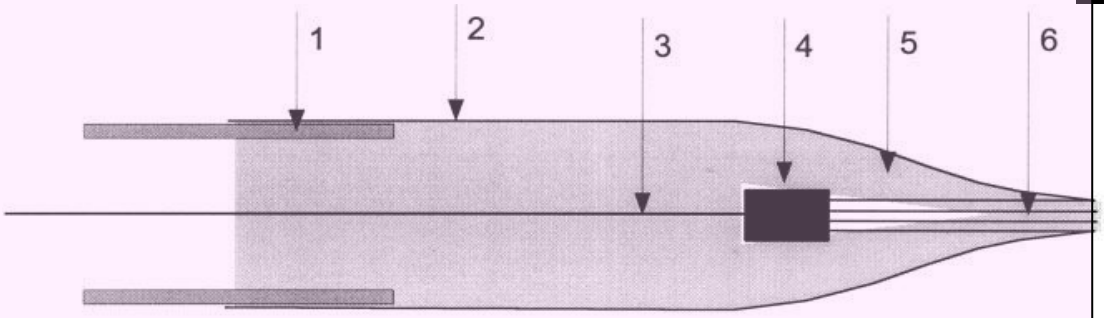
Carbon Fiber - 5 µm



Pt wire – 50 µm







1. Polyurethane catheter 4. Sody Escil resin
2. Pulled glass capillary tube 5. Eccobond 27 epoxy
3. Insulated copper wire 6. Carbon fibers bundle

Njagi, J., Elrichman, J, Aston, J.W., Leiter, J.C., Andreescu, S. **Sens. Actuat. B, 2009**.
J. Njagi, M.S. Ball, M. Bright, K. Wallace, S,. Andreescu, **Anal. Chem. 2010**, 82 (5), 1822-1830.
Suziki A.; Ivandini T. A.; Yoshimi K.; et al. **Anal. Chem. 2007**, 79, 8608-8615.

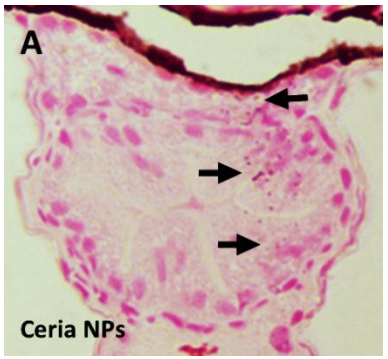
- Sensor response dependent on sensor and sample size !
- Diffusion characteristics, chemical / biochemical reactions vary between macroscopic and ultramicroscopic devices

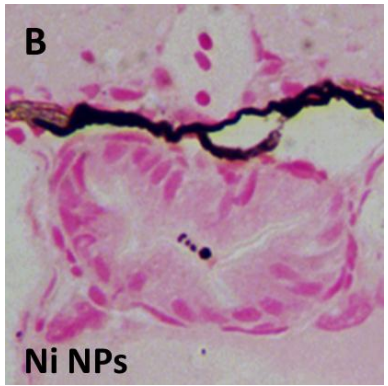
ELECTROCHEMICAL ASESSMENT OF NANOPARTICLE INDUCED TOXICIY

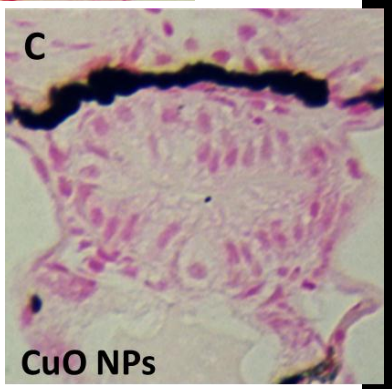
1. In vivo assessment of intestinal serotonin neurotransmission in embryonic zebrafish - Electrochemical measurements in live embryos

2. Oxidative stress and inflammation – assessment of NO and superoxide

3. Predictive tools for assessing surface reactivity in vitro

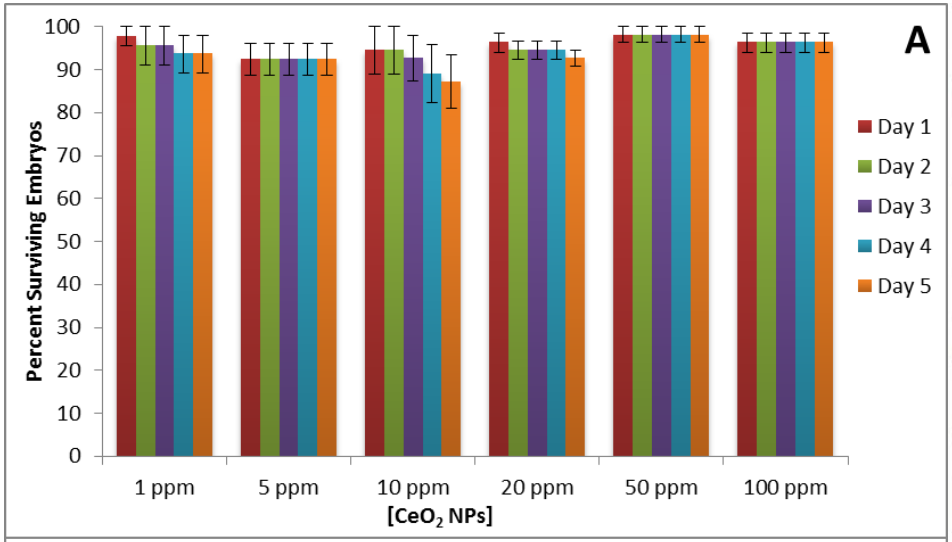


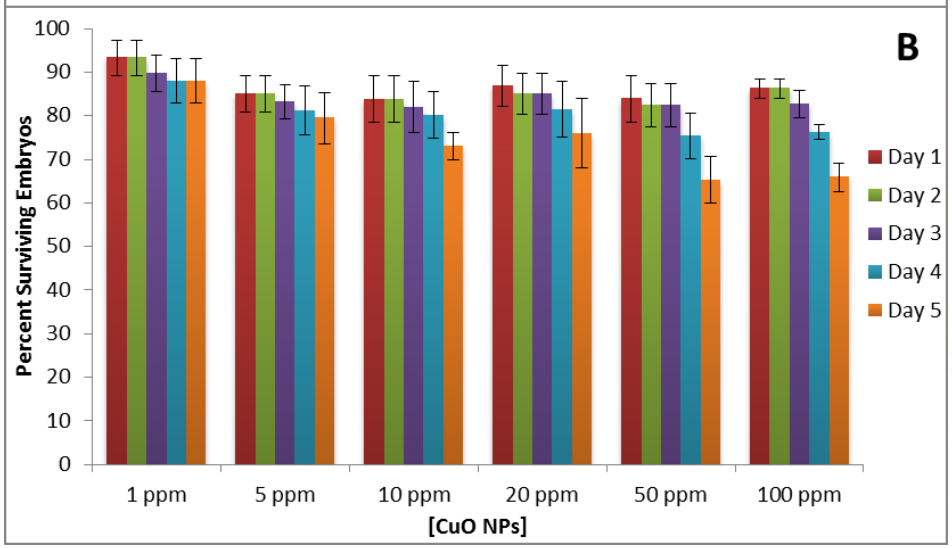


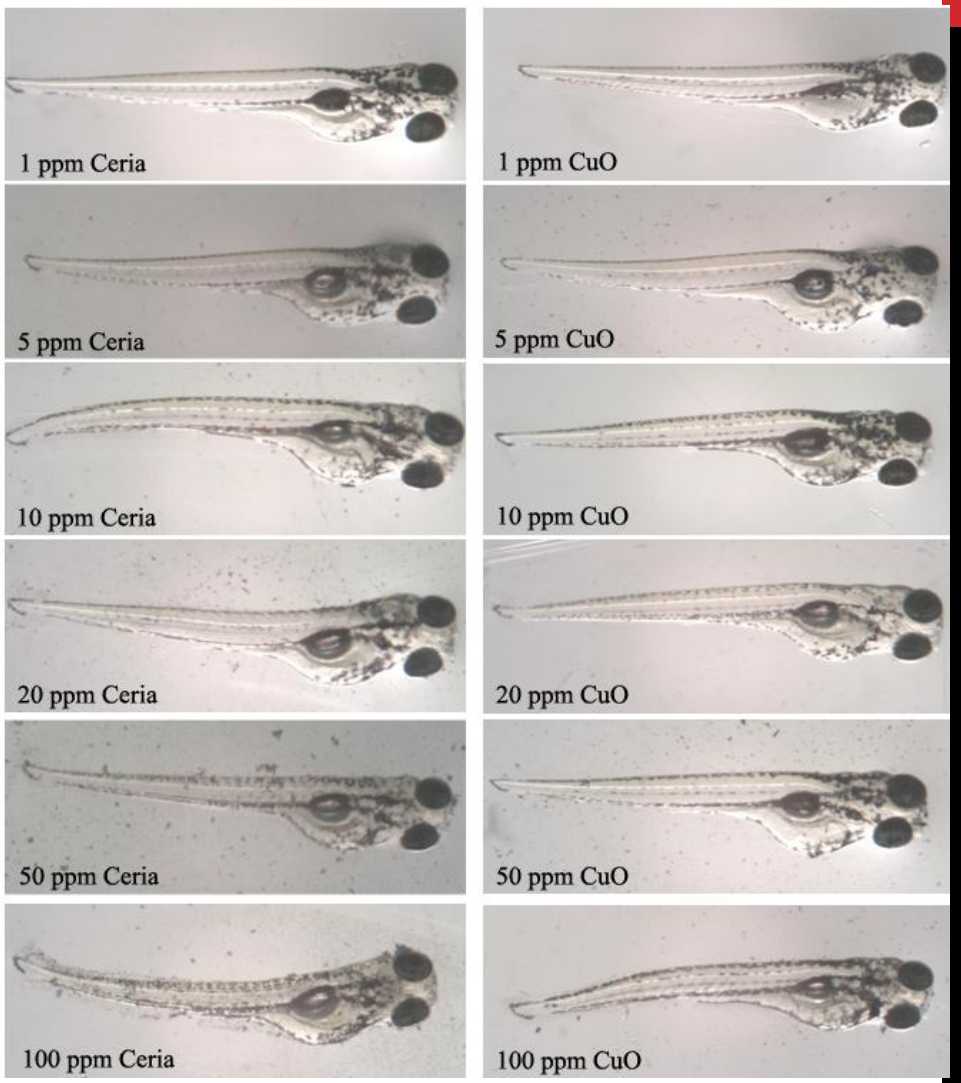


Ni/NiO / CeO2 / CuO NPs 1-100 ppm

VIABILITY OF NPS ON EMBRYOS

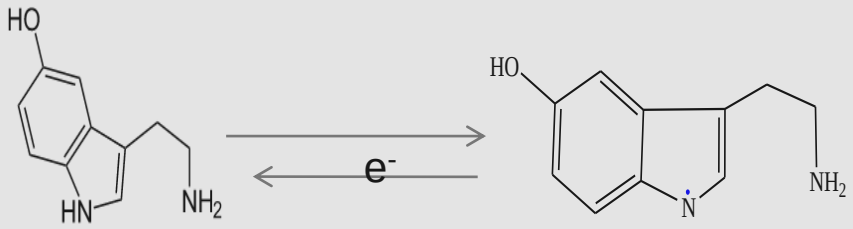






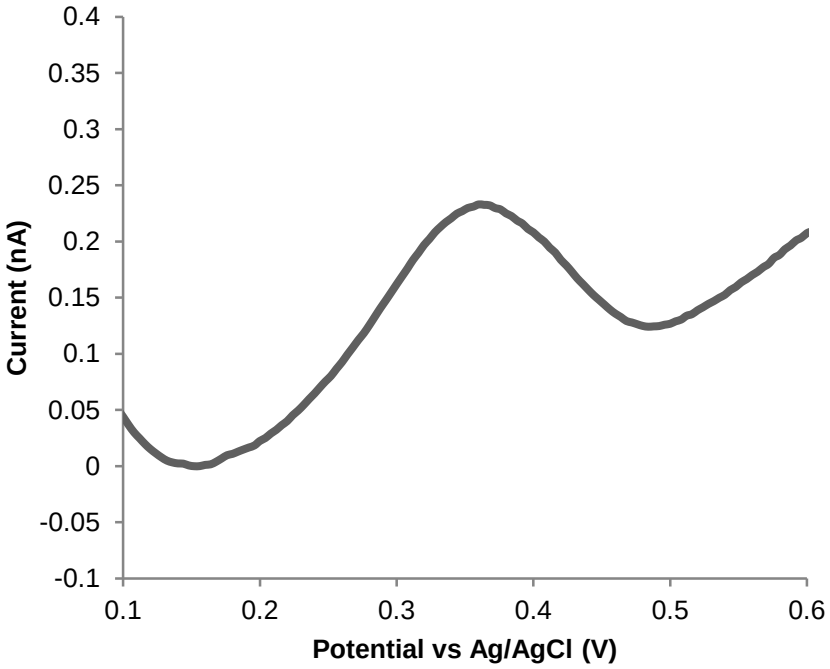
Özel, R.E. Wallace, K. Andreescu, S., **Environmental Sciences: Nano** , 2013.

1. MEASUREMENT OF INTESTINAL 5-HT IN ZEBRAFISH EMBRYOS



Serotonin

- 5-HT is associated with regulation of mood, some cognitive functions including memory and learning, sleep, appetite and muscle contraction
- Abnormal levels have been implicated in diseases such as Celiac and irritable bowel syndrome



Middle segment of intestine is used for implantation

•In vivo [5-HT] = 30.8 (±3.4)nM.

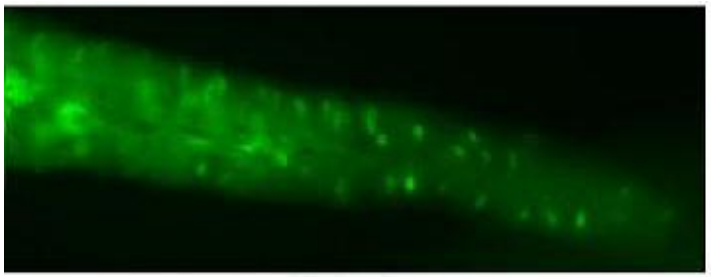
Özel, R. E.; Wallace, K. N.; Andreescu, S. *Anal Chim Acta* **2011**, *695*, 89.
J. Njagi, M.S. Ball, M. Bright, K. Wallace, S,. Andreescu, ***Anal. Chem***., 82 (5), **2010**, 1822-1830.

EFFECT OF NP (50 PPM) EXPOSURE ON 5-HT

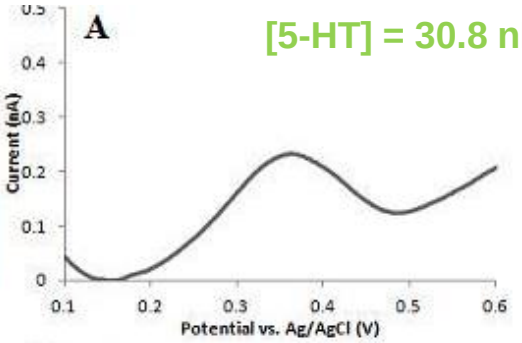
FLUORESCENCE VS. ELECTROCHEMISTRY

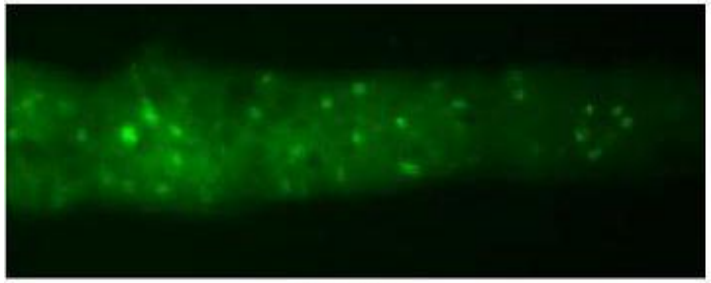
Effects are concentration dependent

Electrochemical detection of 5-HT in the presence of xeno-material provides quantitative information on their neurotoxic properties.

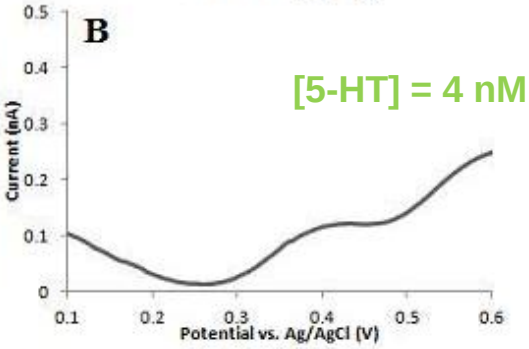


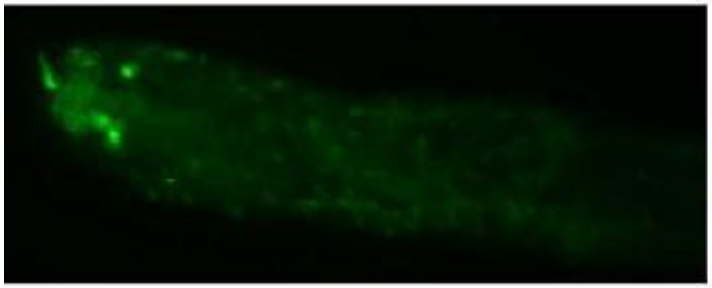
Control



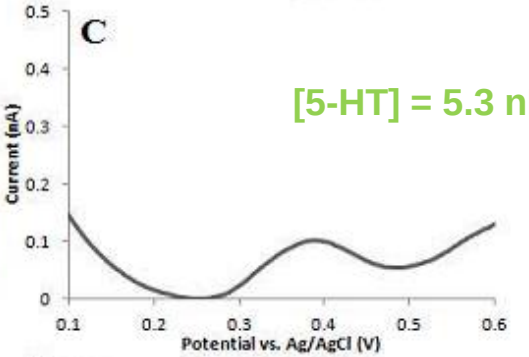


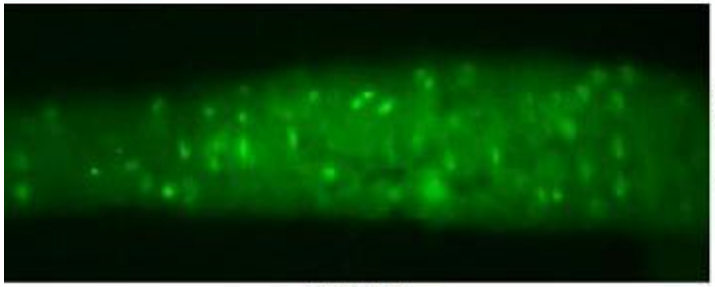
Ni NPs



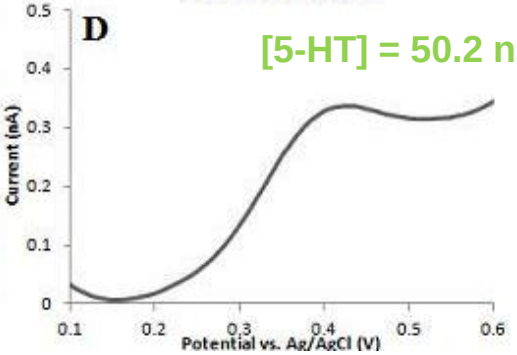


CeO_2 NPs





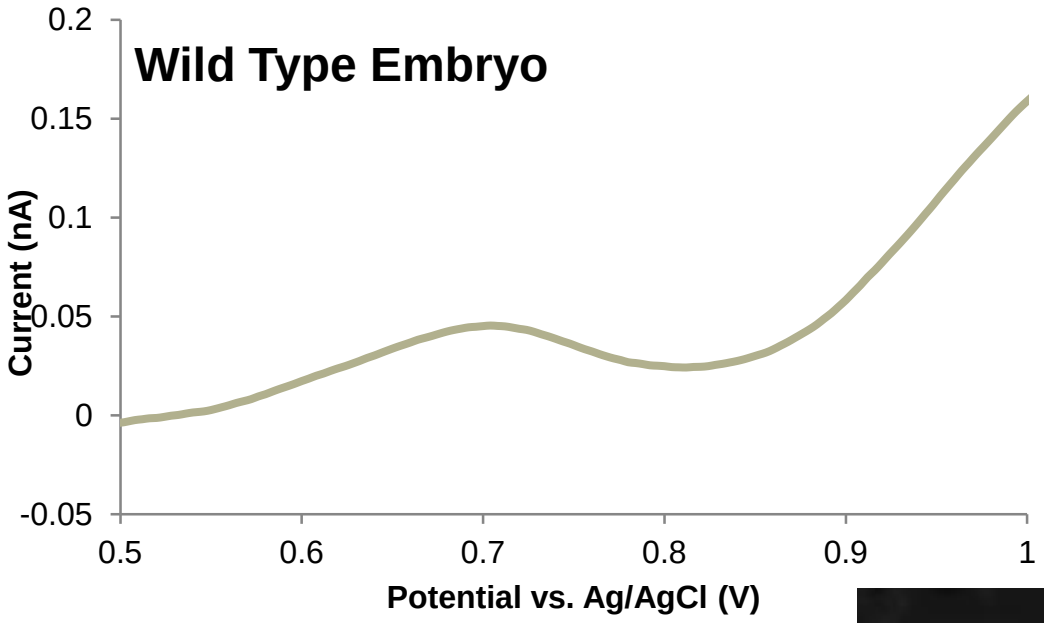
CuO NPs



Özel, R.E. Hayat, A., Wallace, K. Andreescu, S., **RSC Advances**, 2013.

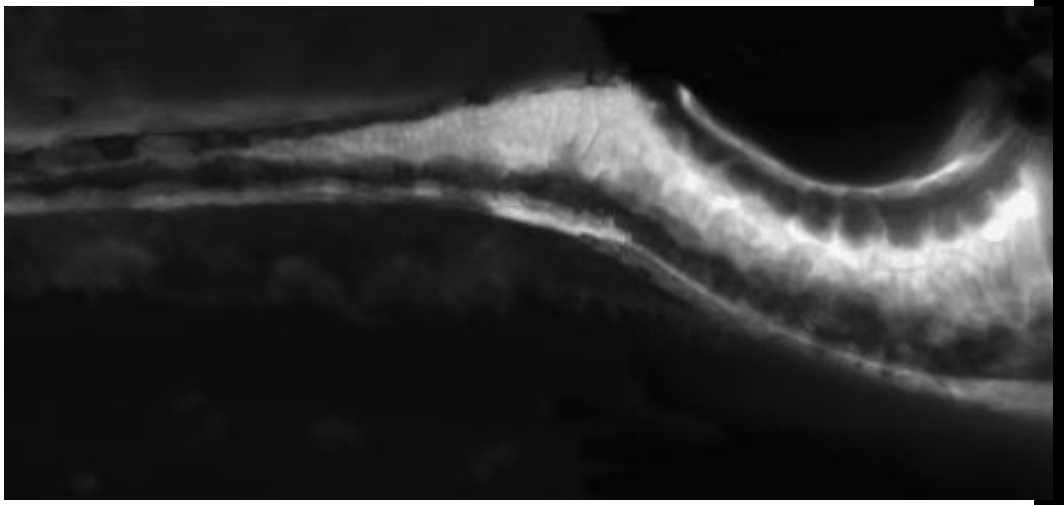
Özel, R.E. Wallace, K. Andreescu, S., **Environmental Sciences: Nano** , 2013.

2. INTESTINAL NO AS A MARKER OF NP-INDUCED OXIDATIVE STRESS AND INFLAMMATION



$$[NO]_{Wild\ Type} = 0.79\ (\pm 0.15)\ \mu M$$

- Cross-validation of NO sensor has been done by fluorescence microscopy utilizing DAF-DA-FM, a specific probe for nitric oxide.



Özel R.E. Alkasir, R., Ray, K., Wallace, K., Andreescu, S., **Small**, 2013, in press.

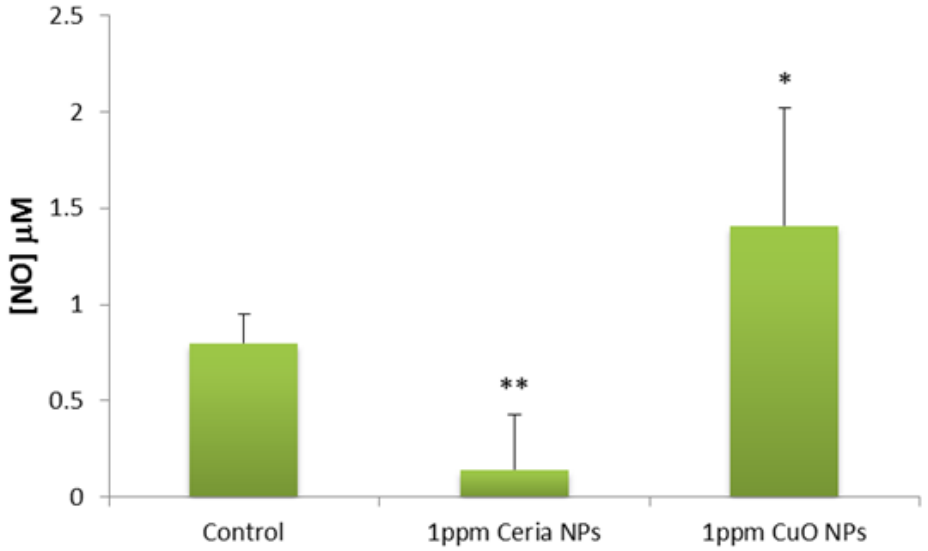
Effect of NPs on intestinal NO

Exposure to NPs induced changes in physiological NO – detected electrochemically within the intestine of live embryonic zebrafish.

CuO NPs were found to trigger inflammatory response even at low-level exposure

Ceria NPs affected the levels of NO in a dose-dependent manner;

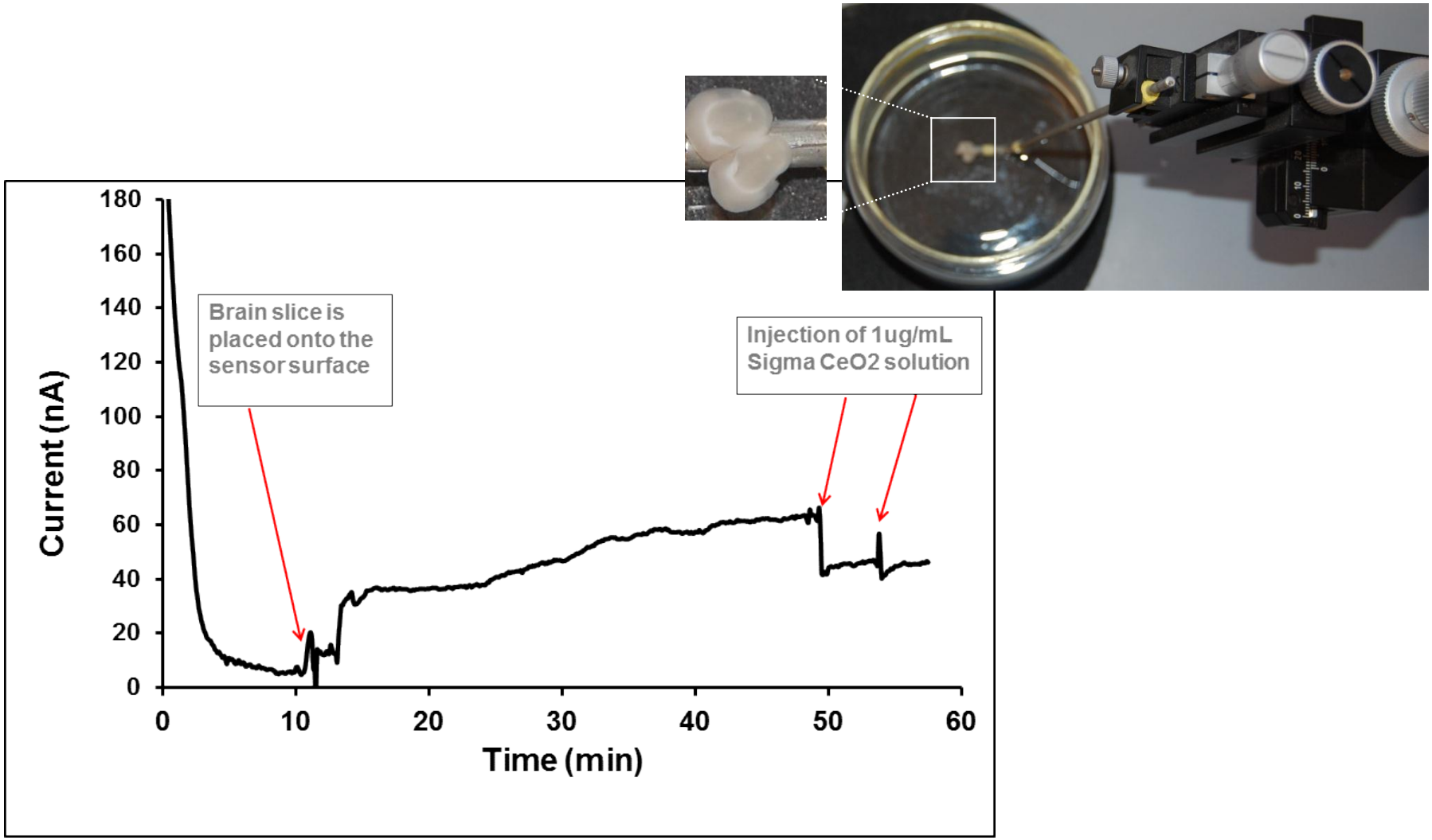
- Low concentrations: act as inorganic antioxidant
- High concentrations: triggers inflammatory response



Özel R.E. Alkasir, R., Ray, K., Wallace, K., Andreescu, S., **Small**, 2013, in press.

Real time monitoring of superoxide and NO radicals in a brain slice model using an electrochemical superoxide microsensor

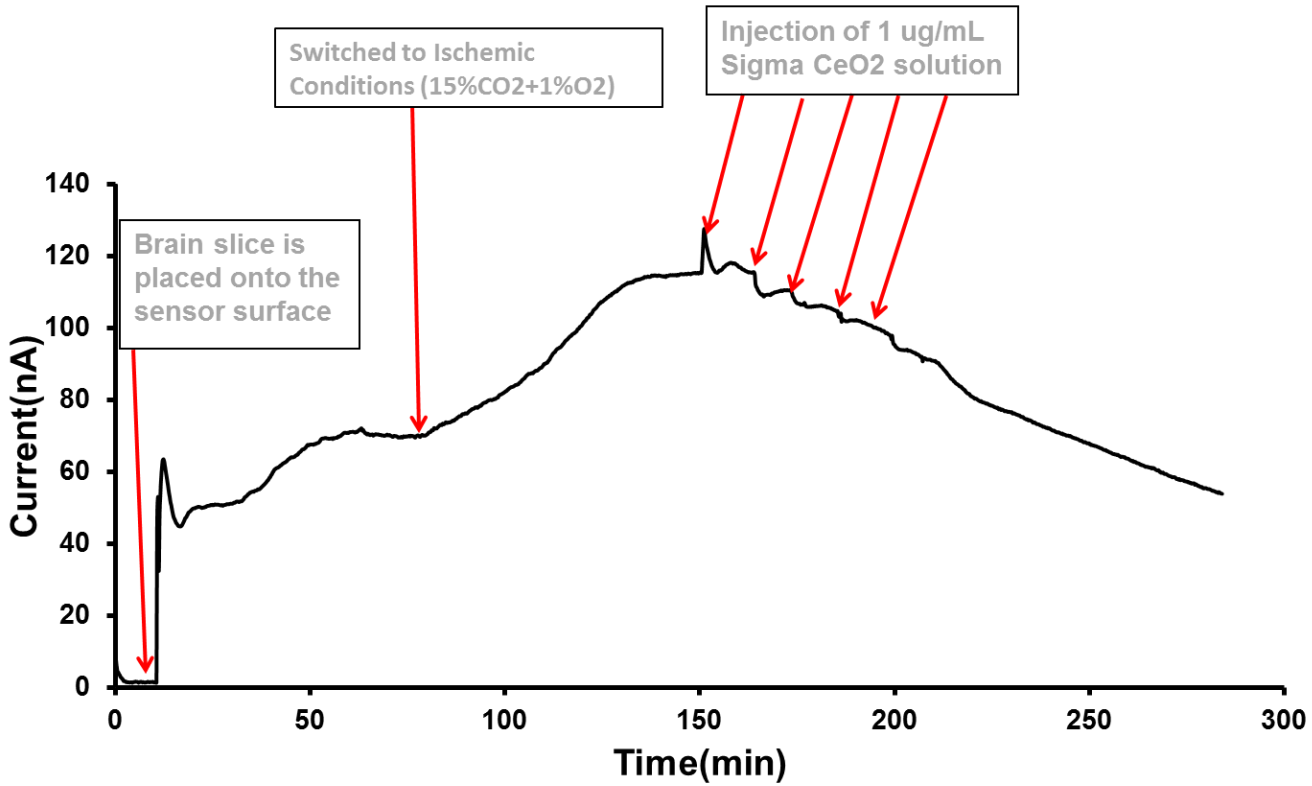
Effect of ceria on $O2^{\bullet -}$ in Control Slice



Ganesana, M., Erlichman, J., Andreescu, S., **Free Radicals Biology and Medicine**, 17;53(12):2240-2249, 2012.
Njagi, J., Erlichman, J., Leiter, JL. Andreescu, S., **Sensors and Actuators**, 2012.

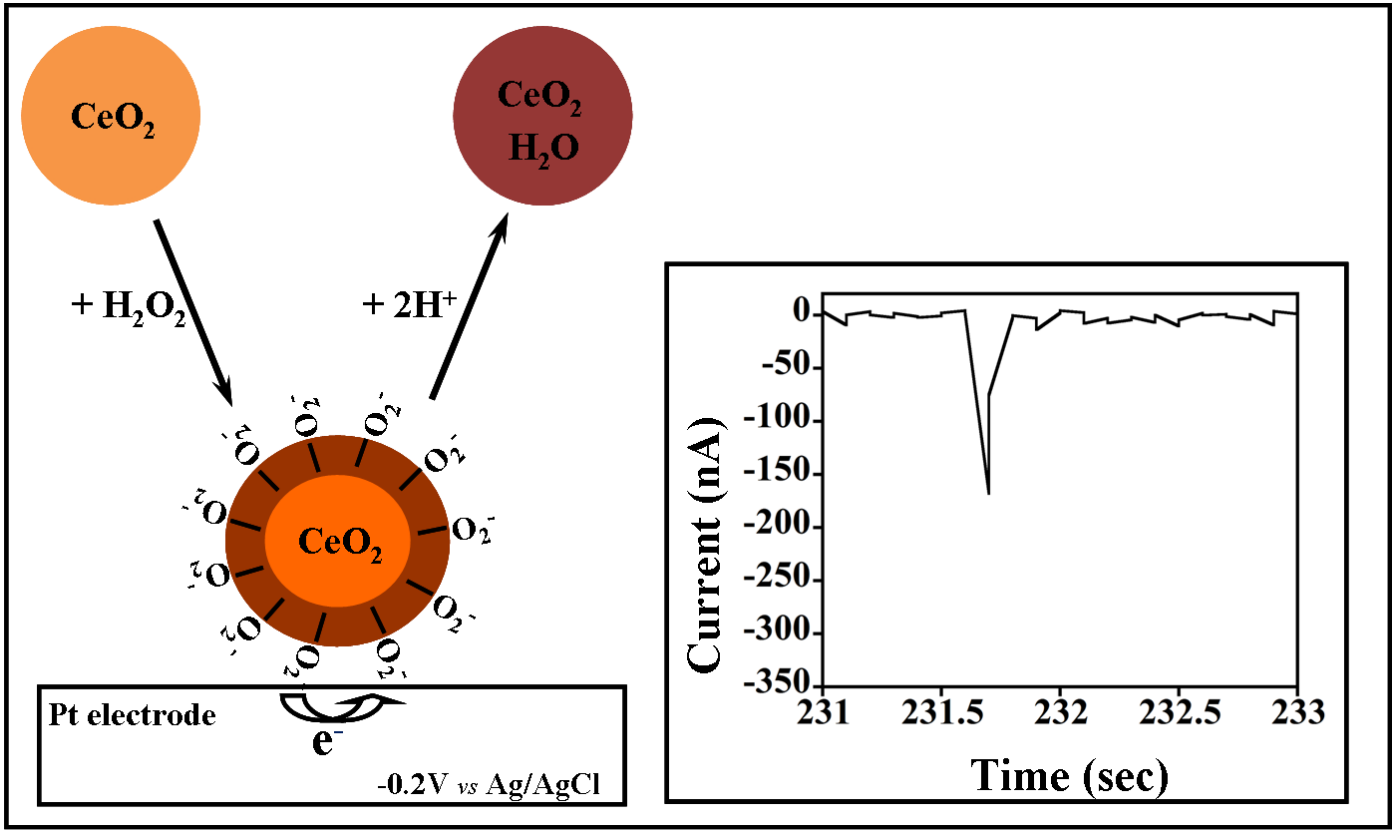
Real time monitoring of superoxide

Effect of ceria on $O_2^{\bullet -}$ in ischemic brain



Ganesana, M., Erlichman, J., Andreescu, S., **Free Radicals Biology and Medicine**, 2012.

3. ELECTROCHEMISTRY AS PREDICTIVE TOOLS OF OXIDATIVE RESPONSE



- Evaluate redox induced changes of ceria nanoparticles asssociated with oxidative damage by nanoparticle collision at microelectrodes
- Screening approach to assess surface reactivity and ability of NPs to generate or inactivate free radicals (oxidant / prooxidant)

Sardesai, N., Andreescu, D., Andreescu, S., ***J. Am. Chem. Soc***, ASAP, 2013.

Conclusion

Micro- electrochemical devices - useful tools for studying mechanisms of NPs induced toxicity

- ✓ neurotoxicity
- ✓ oxidative stress, ROS, O2-, NO
- ✓ predictive tool of particle reactivity

Potentially the results could be applied to humans and serve as a general guideline for assessing exposure risks and benefits (nanomedicine) of NPs

ACKNOWLEDGEMENTS

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