Metal nanoparticle (NP) toxicity testing *in vitro* and questions surrounding the oxidative stress paradigm.

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The *in vitro* system is the preferred approach to identify the major mechanisms of toxicity of nanomaterials, to facilitate hazard ranking and to prioritise for *in vivo* testing. It is recommended that testing should be based on scientific paradigms to allow screening of multiple toxicants. Oxidative stress as a valid test paradigm to compare NP toxicity was first proposed by Xia et al. 2006. In this paradigm particle-induced reactive oxygen species (ROS) production creates a redox imbalance overwhelming cellular antioxidant defence and leads to oxidative injury, pro-inflammatory responses and ultimately cytotoxic effects. The exact mechanism of ROS elicitation is difficult to elucidate, they may be directly produced by NPs themselves or as a result of mitochondrial dysfunction caused by physical disruption.

Some metal nanoparticles are susceptible to spontaneous ROS generation as they are redox active. The release of ions also influences redox homeostasis and ion balance which can lead to oxidative stress. Metal nanoparticle dissolution introduces increased complexity and a debate surrounds whether metal nanoparticle toxicity is governed by the release of toxic concentrations of ions or is nanoparticulate-specific. Using redox active copper nanoparticles (CuNPs) we have investigated the role played by ions and their contribution to ROS elicitation in toxic effects (Song et al. 2012).

There is also ROS-independent pathways of cellular toxicity induced by nanoparticles. For an array of ZnO nanoparticles employed in our studies ROS production did not contribute to toxicity (Fernández - Cruz et al. 2013). Moreover it is unclear whether oxidative stress associated with nanomaterials is the direct cause of cytotoxicity or a secondary effect of cellular insult. Using a co-incubation strategy liver cells were challenged with high ROS levels following CuNP exposure, while at the same time the aryl hydrocarbon receptor (AhR) gene battery of cellular antioxidant defence was activated by the ligand ß-Naphthoflavone. Activation of phase I cytochrome P450`s (CYP1A1), phase II detoxification enzymes (GST's) and antioxidants (GSH) resulted in complete abrogation of oxidative insult and redox homeostasis but did not result in a concurrent reduction in cytotoxicity. Results from this study point to non-oxidative stress-mediated cellular damage resulting from nanomaterials direct physical perturbation of cellular structures. This has important implications for using oxidative stress as a paradigm in screening for nanoparticle toxicity and hazard assessment.

References

Xia, T., Kovochich, M., Brant, J., Hotze, M., Sempf, J., Oberley, T., Sioutas, C., Yeh, J.I., Wiesner, M.R., Nel, A.E. (2006) Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Lett.* 6(8), 1794-807.

Song, L., Connolly, M., Fernández-Cruz, M.L., Vijver, M.G., Fernández, M., Conde, E., de Snoo, G.R., Peijnenburg, W.J., Navas, J.M. (2013) Species-specific toxicity of copper nanoparticles among mammalian and piscine cell lines. *Nanotoxicology* Early Online, 1-11.

Fernández-Cruz,M.L, Lammel,T., Connolly, M., Conde,E., Barrado, A.I., Derick, S.,Perez, Y., Fernandez, M., Furger,C., Navas, J.M. (2013) Comparative cytotoxicity induced by bulk and nanoparticulated ZnO in the fish and human hepatoma cell lines PLHC-1 and Hep G2. *Nanotoxicology* 7(5), 935–952.