Investigations into the bioavailability of manufactured nanoparticles in fish

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

....(Rhys Goodhead)

Abstract

The field of nanotoxicology has emerged as a discipline in parallel with the rapid expansion of nanotechnology and the use of nanomaterials in modern life. Assessing the potential impacts of manufactured nanoparticles (MNPs) on the environment and human health is critical to the sustainable development of the nano-industry. Current knowledge on the ecological implications of nanotoxicology has major uncertainties surrounding the fate and behaviour of nanomaterials in the exposure environment. Bioavailability, uptake and partitioning of nanomaterials to organisms are key determinates to toxicity, yet these foundations of basic data are only now just starting to emerge in any useful and coherent manner for aquatic animals. This thesis work set out to address this gap in knowledge and further our understanding of these important principles for fish.

In an attempt to develop a high through-put screening system for toxicity of MNPs, studies assessing the utility of primary isolated rainbow trout (*Oncorhynchus mykiss*) hepatocytes found they showed very limited responses to a range of MNPs. There was a lack of any evidence for either lipid peroxidation or xenobiotic detoxification activity. In these studies isolated trout hepatocytes were found to be unresponsive to the induction of these biological responses after exposure to positive controls. The findings demonstrated that the MNPs tested showed low toxicity generally and that fish hepatocytes do not provide a useful system for the screening of potential toxic effects of MNPs. In this cell culture work, coherent anti-Stokes Raman scattering (CARS) microscopy was applied to demonstrate that the particles supplied in the culture medium did cross the cell membrane and enter into the exposed cells.

In the second phase of the work in this thesis CARS was investigated as an experimental technique for tracing a wide range of metal and metal oxide MNPs into cells and tissues. CARS was applied to evaluate initial detection of different MNPs and investigate the imaging capability on a range of cells, tissues and organisms. Finally, CARS was applied to assess localisation ability of MNPs within biological matrices. MNPs were shown to be taken into trout hepatocytes using a 3D reconstruction to determine the origin of the MNP signal within the cell. Uptake of MNPs was established into trout gill and kidney tissue, *corophium* and *daphnia* species and were shown to have different partitioning in zebrafish embryos. In summary CARS showed great potential for tracing particle uptake and bio-distribution both *in vitro* and *in vivo*. Particular benefits include imaging MNPs in living organisms, without the need for labelling or fixing the material. Limitations of the CARS technique are also discussed.

In chapter 4, the consequences of the presence of natural organic matter (NOM) were investigated on the uptake of MNPs into fish. Carp (*Cyprinus carpio*) were exposed to cerium dioxide (CeO_2) MNPs in combination with NOM over 28 days. Elevated levels of uptake of cerium were measured in the brain, gill and kidney tissue by induction coupled plasma mass spectroscopy (ICP-MS) for fish exposed to 50 µg/l CeO_2 MNPs in combination with 250 µg/l of NOM. There were no such effects of the NOM enhancing uptake for the bulk CeO_2 particles. Detailed studies on the behaviour of the CeO_2 MNPs in the exposure medium demonstrated the highly complex and dynamic nature of the interactions with NOM. This study discusses some of the difficulties in the techniques, analysis and interpretation of data derived from studies of this nature. The finding that NOM may enhance MNP uptake presents a potential issue for current risk assessment criteria for MNPs that do not consider natural conditions.

The final experimental chapter considered maternal transfer as a potentially important route for exposure of embryos and early life stage animals to MNPs in live bearing animals. In this work guppies (*Poecilia reticulata)* were exposed to 7 nm silver citrate stabilised particles and citrate stabilised bulk sized particles, dosed via the diet for a full gestation cycle. Maternal transfer of Ag to the larvae was significantly higher for the nanoparticulate treatment compared with the bulk and control treatments and larval burden was significantly higher compared with the maternal sires. However, there was no impact of Ag on larval survival, birth weights, or on indices of body condition in the exposed adults. The enhanced uptake of nano Ag compared to bulk Ag particles into the guppy offspring emphasises the potential for exposure to sensitive early life stages of organisms, which to date has not been widely considered and suggests greater research is needed in this area.

Collectively, the studies conducted in this thesis contribute to the science base of nanotoxicology, specifically in areas where data are especially lacking and with a focus on bioavailability. These studies have identified that fish hepatocytes do not offer an effective screen for MNPs, and the data produced further suggests that the MNPs tested are not toxic in that form. Working with CARS I have helped advance the understanding on its utility for nanotoxicology studies, with regards to its application and limitation for uptake analyses. The study of MNPs in combination with NOM has identified the fundamental change that real life exposure scenarios may instigate for toxicity assessments of MNPs, with significant impact on risk assessment criteria. Finally, I've established that maternal transfer is an exposure route for MNPs that requires further study, with evidence of transfer to sensitive life stages in a non-mammalian system.

Acknowledgements

Ahh the acknowledgements the one place I can be a verbose as I choose!

There are so many people who have made this PhD both a fascinating and hugely enjoyable experience. The paths of numerous people have been crossed, yet despite the brevity of some, they still made lasting impression on me. I will certainly look back upon this time with great fondness.

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A good lab group also helps the world go round, so my thanks to those who have had to deal with my singing and capers around the office and aquarium. Special mentions to Toby and his terrible taste in music, Charlie, Greg and Tamsyn for all the sport, you can never have too much and it has helped keep me sane on occasion. Marta, Okhyun, Amy, for your friendship and just generally putting up with me, Emma for bat nights and Viv for dipper days, Tess for understanding the nano problem. To those who left our shores for Australia, you have never really left. You cannot have a lab without technical support and in Jan and Mike we are very fortunate, thanks for helping me with my varied demands, often at short notice, you always came up trumps. There are of course many many others I could thank from over the four years, perhaps unknowingly you have all helped in some ways either as a support group or to share the good times with.

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CONTENTS

Scown, T. M., Goodhead, R. M., Johnston, B. D., Moger, J., Baalousha, M., Lead, J. R., van Aerle, R., Iguchi, T., and Tyler, C. R. (2010). Assessment of cultured fish hepatocytes for studying cellular uptake and (eco)toxicity of nanoparticles. Environmental Chemistry ***7****, 36-49.*

LIST OF (NON RESEARCH PAPER) FIGURES

RESEARCH PAPERS AND AUTHOR'S DECLARATION

Research paper 1. Scown, T. M., Goodhead, R. M., Johnston, B. D., Moger, J., Baalousha, M., Lead, J. R., van Aerle, R., Iguchi, T., and Tyler, C. R. (2010). Assessment of cultured fish hepatocytes for studying cellular uptake and (eco)toxicity of nanoparticles. Environmental Chemistry **7**, 36-49.

Research paper 2. Goodhead, R. M., Moger, J., Fabrega, J., Scown, T.M., Galloway, T., and Tyler, C.R. Tracing Engineered Nanoparticles in Biological Tissues using Coherent Anti-strokes Raman Scattering – A Critical Review. *Manuscript in preparation.*

Research paper 3. Rhys M. Goodhead, Blair D. Johnston, Paula A. Cole, Mohammed Baalousha, David Hodgson Taisen Iguchi, Jamie R. Lead, Charles R. Tyler. Natural organic matter affects bioavailability of cerium oxide nanomaterials to fish. *Manuscript in preparation.*

Research paper 4. R Goodhead, I. Romar., D Croft, T. Iguchi, J.R. Lead, C. R. Tyler. Silver nanoparticles show enhanced maternal transfer compared with larger silver particles when dosed in the diet of a live bearing fish species *Poecilia reticulata. Manuscript in preparation*

Statement: I, Rhys Goodhead, was involved in the following parts of the presented papers: I planned and carried out the hepatocyte isolations, exposures, LDH, TBARS and GST assays in **paper 1** and co-wrote **paper 1** with Tessa Scown. I was responsible for the CARS microscopy and GST work and Tessa Scown carried out the TBARS and LDH assays. Equal contribution was made towards cell isolation and exposures. Charles Tyler and Julian Moger contributed to the design of **paper 2.** I carried out all of the zebrafish exposure and imaging work and assisted Julian Moger with the imaging of the invertebrates for **paper 2**. Julian Moger imaged the gill and worm tissue and I played the lead role in writing this paper. Blair Johnstone, Jamie Lead, Mohamed Baalousha and Charles Tyler all had significant contributions towards the study design for **paper 3.** Blair Johnston and I carried out the preliminary experimental work for paper 3 and I was responsible for the subsequent follow up exposure. All authors contributed some part towards the writing of **paper 3** however I was the predominant contributor. I prepared all of the samples for ICP-MS analysis in **papers 3** and **4**. I played the leading role in planning, designing, implementing and writing **paper 4** with significant contribution from Charles Tyler and technical support from Victoria Jennings. Isabel Romar synthesized the silver nanoparticles for **paper 4**. Nanoparticle characterisation work for **paper 1-4** was carried out by Mohamed Baalousha, Jamie Lead, Paula Cole and ICP-MS measurements take by Stephen Baker. All papers had large editing contributions from Charles Tyler.

The published versions of papers 1, is included in the appendix.

Other publications completed during this PhD included;

Goodhead, R. M., and Tyler, C. R. (2009). Endocrine-Disrupting Chemicals and Their Environmental Impacts. In Organic Pollutants - An Ecotoxicological Perspective (C. H. Walker, Ed.), pp. 265-292. CRC Press.

Tyler, C.R. and Goodhead, R. M. (2010). Impact of hormone-disrupting chemicals on wildlife. In Silent Summer: The State of Wildlife in Britain and Ireland (Maclean, N Ed.), pp. 125-140. Cambridge University Press.

LIST OF GENERAL ABBREVIATIONS

Ag^+	Silver ion
Ag	Silver
Al_2O_3	Aluminium oxide
Au	Gold
BSA	Bovine serum albumin
C_{60}	Buckminsterfullerene (fullerene)
CARS	Coherent anti-Stokes Raman scattering
CeO_2	Cerium dioxide
CNT	Carbon nanotube
DLS	Dynamic Light Scattering
DLVO	Derjaguin & Landau, Verwey and Overbeek Theory
DOC	Dissolved organic carbon
DWCNT	Double-walled carbon nanotube
E-CARS	Epi-detected CARS
ENMs	Engineered nanomaterials
ENPs	Engineered nanoparticles
FA	Fulvic acid
F-CARS	Forwards detected CARS
Fe_2O_3	Iron oxide
GSH	Glutathione
GST	Glutathione-S-transferase
HA	Humic acid
hpf	Hour post fertilization
ICP-OES	Inductively coupled plasma – optical emission spectrometry
ICP-MS	Inductively coupled plasma – mass spectrometry
IR	Infra-red
LC_{50}	Median lethal concentration
LDH	Lactate dehydrogenase
MNPs	Manufactured nanoparticles
MRI	Magnetic Resonance Imaging
MWCNT	Multi-walled carbon nanotube
NOEC	No observable effect concentration
NOM	Natural organic matter
NMs	Nanomaterials
PEG	Polyethylene glycol
ROS	Reactive oxygen species
STM	Scanning tunnelling microscope
STW	Sewage treatment works
SWCNT	Single-walled carbon nanotube
TBARS	Thiobarbituric acid reactive substances
TEM	Transmission electron microscopy
THF	Tetrahydrofuran
TiO_2	Titanium dioxide
UFPs	Ultrafine particles
UV	Ultraviolet (also UVA and UVB)
VTG	Vitellogenin
WWTWs	Wastewater treatment works
ZnO	Zinc oxide

LIST OF SPECIES

Arenicola marina	Lugworm
Carassius auratus	Goldfish
Corophium velator	mud shrimp
Cyprinodon variegatus	Sheepshead minnow
Cyprinus carpio	European carp
Danio rerio	Zebrafish
Daphnia magna	Water flea
Esox lucius	Pike
Micropterus salmoides	Largemouth bass
Oncorhynchus mykiss	Rainbow trout
Oryzias latipes	See-through medaka
Perca fluviatilis	*Perch*
Pimephales promelas	Fathead minnow
Poecilia reticulata	Guppy