Institution: University of Plymouth

Unit of Assessment: UoA6 Agriculture, Veterinary and Food Science

Title of case study: The Safety of Nanotechnology in Fisheries and Aquaculture

1. Summary of the impact

Research on the environmental safety and toxicity of nanomaterials in fishes has had a global impact across both government and industry contributing to:

(i) Consensus building on biological effects allowing regulatory agencies/governments to make proper decisions on the hazard of nanomaterials to farmed fish and wildlife.

(ii) Critical evaluation of the internationally agreed *process* of toxicity testing to determine whether the current legislative test methods are fit for purpose and acceptable to the aquaculture industry.

(iii) Identification of national/international research priorities and policies via work with the OECD and the US Government.

(iv) Influencing government policy to support training and information for industry.

2. Underpinning research

The research programme underpinning this case study has focussed on 'fact finding' on nanoparticle toxicity (hazard assessment), the fundamental biological mechanisms involved, and especially on reporting 'no effect data' (negative results), so that regulators and Government(s) can determine safe/allowable levels of nanomaterials in the environment. This team led by Professor Richard Handy has been supported by several NERC grants, EU FP7 projects (NanoImpactNet, MARINA), an RCUK Fellowship (Henry, 2008-2013), and studentships from NERC (Ramsden, 2009-2012) and the Iraq Ministry for Higher Education (Al-Bairuty, Al-Jubory 2009-2013). Key post-doctoral staff included Boyle (2009-2012) and Shaw (2006-present, currently on EU FP7 MARINA).

The research includes first reports on changes in locomotion/behaviour and brain injury (Boyle et al., 2012; Al-Bairuty et al., 2012), the first proper dietary uptake study in fishes (Ramsden et al., 2009), reports of no effect levels for dietary exposure (Fraser et al., 2010), and experiments comparing the toxicity of nano-forms of metals with dissolved metals (Shaw et al., 2012). The latter two issues are especially important. The absence of effects is sometimes not considered academically interesting, but such information is absolutely vital for establishing safe levels in the environment/food chain. Comparisons with existing substances (dissolved metals) are also critical for regulatory decision making in order to know whether nanomaterials can be captured by existing legislation or require policy development.

Our research set out hypotheses on bioavailability/absorption mechanisms (Handy et al., 2008b) and tested these experimentally, including the first paper on titanium dioxide absorption across the gut (Al-Jubory and Handy, 2012). Together the findings provide a rational basis for considering key triggers in environmental risk assessment such as persistence and bioaccumulation potential. They also provide information on the human health risk (e.g., brain injury), and on food chain hazards to humans through agricultural usage.

Our dietary exposure studies have shown that fish will readily eat food containing nanomaterials and will grow normally (a manageable hazard for the aquaculture industry), and most nanomaterials so far tested do not accumulate in the edible muscle, meaning a low risk of exposure for humans eating fish. In the previous absence of approved methods for detecting nanomaterials in fish/shellfish our research has developed new methods for routine use in the food and agriculture industries (Patent application No: 1207745.9, May 2012 for the detection of TiO_2).

One major obstacle to the safe, responsible innovation of nanotechnology in agriculture is the validation of regulatory tests; without these tests companies cannot register their nano-products in the EU or USA. Our research has worked on validating individual regulatory ecotoxicity tests, providing the evidence base and recommendations for altering the overarching testing strategy in Europe, and has provided the tools that Government(s)/industry urgently need for risk assessment. We have led several international working groups on test methods for nanoparticles (Handy et al., 2012a; 2012b), identified conceptual flaws in current bioaccumulation tests (Handy et al., 2012a), and provided a critique of terrestrial tests that support agricultural usage. We have identified that most of the current agrifood testing protocols need modifications to work with nanomaterials. We continue to address these issues in Europe.

3. References to the research (Plymouth authors in **bold**)

1. **Boyle, D., Al-Bairuty, G. A., Ramsden, C. S., Sloman, K. A., Henry, T. B.** and **Handy, R. D.** (2013) Subtle alterations in swimming speed distributions of rainbow trout exposed to titanium dioxide nanoparticles are associated with gill rather than brain injury. *Aquatic Toxicology*, 126, 116– 127.
Impact factor, 4.225; 3/94 in the SJR ranking of all toxicology journals.

2**. Al-Jubory, A. R**. and **Handy, R. D.** (2012) Uptake of titanium from TiO_2 nanoparticle exposure in the isolated perfused intestine of rainbow trout: nystatin, vanadate, and novel CO_2-sensitive components. *Nanotoxicology*, in press (posted online Oct. 2012).
Impact factor 7.84; 13/94 in the SJR ranking of all toxicology journals.

3. **Shaw, B. J., Al-Bairuty, G. and Handy, R. D.** (2012) Effects of waterborne copper nanoparticles and copper sulphate on rainbow trout, (*Oncorhynchus mykiss*): Physiology and accumulation. *Aquatic Toxicology*, 116-117, 90-101.
Impact factor, 4.225; 3/94 in the SJR ranking of all toxicology journals.

4. **Fraser, T. W. K., Reinardy, H. C., Shaw, B. J., Henry, T. B., Handy, R. D.** (2011) Dietary toxicity of single-walled carbon nanotubes and fullerenes (C60) in rainbow trout (*Oncorhynchus mykiss*). *Nanotoxicology*, 5 (1), 98-108.
Impact factor 7.84; 13/94 in the SJR ranking of all toxicology journals.

5. **Ramsden, C. S., Smith, T. J., Shaw, B. J., and Handy, R. D.** (2009) Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (*Oncorhynchus mykiss*): No effect on growth, but subtle biochemical disturbances in the brain. *Ecotoxicology*: 18, 939-951.
Impact factor 3.185; 10/94 in the SJR ranking of all toxicology journals.

6. **Handy, R. D.,** Cornelis, G., Fernandes, T., Tsyusko, O., Decho, A., Sabo-Attwood, T., Metcalfe, C., Steevens, J. A., Klaine, S. J., Koelmans, A. A. and Horne, N. (2012a) Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. *Environmental Toxicology & Chemistry*, 31, 15-31.
Impact factor 2.847; 5/94 in the SJR ranking of all toxicology journals.

4. Details of the impact

The impact of our research since 2008 has been to highlight the hazards built on the substantial impact that had become apparent from 2006 and the effect of using the emergent technology of nano-ecotoxicology in agricultural systems. Prior to 2006 there was no cohesive international effort on the environmental hazards of nanomaterials. Our impact has been to help establish a new scientific community on nano-ecotoxicology/chemistry involving government, industry, consultancy and academia; building and developing a consensus view on hazard, fate, and effects. An early consensus report was deliberately published independently, i.e., not from any one governmental regulatory agency or industry, as an unbiased view from the grass-roots scientific community. Through our invitation by the Office of the President of the United States to act as an external advisor to the US National Nanoscience (NNI) funding initiative, we were able to influence the European Commission in establishing the NanoSafety Cluster, and Handy was instrumental in setting up the US-EU ''cores of research' aimed at international data sharing. A further consensus report involving the SETAC Nano-advisory group was published in 2012.

As a result of these seminal reports and the emergence of the new scientific discipline of nano-ecotoxicology, we were invited to prepare a report for DEFRA on test methods for nanomaterials that set out the validation problems around testing nanomaterials. This showed that the UK/EU hazard assessment strategy urgently needed modification, and was subsequently used by DEFRA to argue the case for testing strategies with the EU Working Party on Manufactured Nanomaterials (WPMN). As a result, the international community has agreed that there is a problem with testing methods and an international sponsorship programme of testing by the OECD was commissioned involving members of the International Organisation for Standardization (ISO).Our impact has included OECD guidance documents. This reported in 2012. In the UK, this work and a paper by Owen and Handy (2007) raised nanoscience as a priority research funding issue; leading to a

National Nanoscience call from NERC led by Owen, and with Handy on the UK task force. Subsequently, the testing method issues were included in the Framework 7 call in Europe. Our research and consensus-building report was used in a 2010 Report by the Australian Department of the Environment, Water, Heritage and the Arts;. and in Reports from the Royal Commission on Environmental Pollution (2008),) and the House of Lords Science and Technology Committee (2010).

The regulatory testing activities that have emerged from this work are directed at Governments across the EU and North America who are required to implement the regulatory process enabling the registration of new products, and industry now has official guidance on how to collect data to support their product registrations. This is an absolute requirement as registration of new products (such as nano crop protection products) cannot proceed without the appropriate testing dossier. Specifically, our research and testing methods have been used in a Report by the Dutch Food and Environmental Safety Agency (2009) and in official guidance documents on test methods from the OECD, and our expertise has led to our direct involvement in writing regulatory guidelines (OECD, 2010; 2012). These documents are shared in the US by ISO, and so the impact of our knowledge is global across both government and industry. This application of our technical knowledge is on-going at the OECD, and we are providing technical advice to several sub-committees (SG3, SG7) and on specific materials (OECD programme for TiO_2). The above regulatory activities disseminate information to industry through WPMN documents from the OECD, and from our reports to DEFRA locally in the UK.

One concern identified by us was a UK/EU skills shortage to support industrial growth of the agri-food/chemicals nanotechnology sectors and we were commissioned by DEFRA to write a report on the skills gap (Handy et al., 2009). This has had a wide impact on training policy in the UK. Our findings were presented to the Science Minister (David Willets MP) at the Department of Business, Innovation and Skills (BIS) meeting on 11th May 2011, and then in a closed meeting with the Chief Scientist's Committee. Evidence on hazards has also been presented to the UK Government, House of Commons, Science and Technology Committee with respect to food safety (via Prof Stephen Holgate). This further informed discussions with DEFRA on training and the Life Science Action Plan in the UK, so that training in nanoscience is now included (ACHS, 2011). Our research also had impact with DEFRA's Advisory Committee on Hazardous Substances, where we contributed evidence to decisions on allowing the granting of authorisation for the first commercial use of nano products for aquaculture in the UK. Our research also continues to be used more widely in providing advice relevant to the fishing industry's use of nutrients.

5. Sources to corroborate the impact

1. *Use of our consensus building reports and primary research for setting the opinion of the Australian agency responsible for environmental protection (Note Handy et al 2012a above is also a consensus report involving the SETAC Nano-advisory group):*

    G.E. Batley and M.J. McLaughlin (2010) Fate of Manufactured Nanomaterials in the Australian Environment. CSIRO Niche Manufacturing Flagship Report, March 2010, Prepared for the Department of the Environment, Water, Heritage and the Arts, CSIRO, Australia. Available at: http://www.environment.gov.au/.../pubs/manufactured-nanomaterials.doc

2. *Technical documents on test methods and how to make them work for nanomaterials that provide advice to Governments and to the European Commission, and is the guidance document that industry will follow:*

    a. An Assessment of Regulatory Testing Strategies and Methods for Characterizing the Ecotoxicological Hazards of Nanomaterials. Final Report DOI: 10.1007/s10646-008-0215-z, Defra, London, UK. Available at: http://randd.defra.gov.uk/Document.aspx?Document=CB01097_6262_FRP.pdf [evidence of regulatory impact at national level]
    b. OECD (2012) Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials. OECD Environment, Health and Safety Publications Series on the Safety of Manufactured Nanomaterials, No. 36, ENV/JM/MONO(2012)40, 18th

December 2012, Organisation for Economic Co-operation and Development, Paris. [evidence of regulatory impact at international level]

3. *Use of our research data in House of Lords report on food safety:*
House of Lords (2010) Science and Technology Committee, 1st Report of Session 2009–10. Nanotechnologies and Food. Volume I: Report published by the Authority of the House of Lords. Available at: http://www.publications.parliament.uk/pa/ld200910/ldselect/ldsctech/22/22i.pdf

4. *Use of our data by the Royal Commission on Environmental Pollution:*
Lawton (2008) Novel Materials in the Environment: The case of nanotechnology. Presented to Parliament by Command of Her Majesty, November 2008. Available at: http://www.official-documents.gov.uk/document/cm74/7468/7468.pdf

5*. Use of our data on hazard and testing policy for agriculture/food security by the Food and Agriculture Organisation/World Health Organisation, and by the Dutch Food and Environmental Safety Agency, RIVM:*

a. FAO (2009) FAO/WHO Expert Meeting on the Application of Nanotechnologies in the Food and Agriculture Sectors: Potential Food Safety Implications. FAO and WHO press, Rome. Available at: http://www.evira.fi/attachments/elintarvikkeet/elintarviketietoa/fao_who_nano_expert_meeting_report_final__2_.pdf
b. RIVM (2009) Nanotechnology in perspective. Risks to man and the environment Editors: M. van Zijverden and A.J.A.M. Sips Report 601785003/2009. Available at: http://rivm.nl/bibliotheek/rapporten/601785003.pdf

6. *Use of our data by the Woodrow Wilson Centre in the USA (an NGO):*
Luoma, S. (2008) Silver nanotechnologies and the environment. Old problems or new challenges. Woodrow Wilson International Centre for Scholars. Available at: http://www.nanotechproject.org/process/assets/files/7036/nano_pen_15_final.pdf

7. *Use of our test method report and consensus reviews by the US Environmental Protection Agency:*
US EPA (2009) International Perspectives on Environmental Nanotechnology - Applications and Implications (EPA 905/R-09/032 November 2009). Available at: http://el.erdc.usace.army.mil/nano/pdfs/1446.pdf

8. *Evidence of dissemination and impact on training needs and skills gaps at national level.*
Advisory Committee on Hazardous Substances (ACHS) Fifteenth Annual Report 2011. Defra, United Kingdom. http://www.defra.gov.uk/achs/files/Annual-report-2011_final.pdf
Note, this reference is evidence of use/impact of the following report: Handy, R. D., Maycock, D. and Jha, A. N. (2009) An Evaluation of the UK Skills Base for Toxicologists and Ecotoxicologists, with Focus on Current and Future Requirements, Particularly with Regard to the Skills Required for the Hazard Assessment of Chemical Substances including Nanomaterials. Peer reviewed report to Defra. Available at: http://randd.defra.gov.uk/Document.aspx?Document=CB0419_8185_FRP.pdf