Institution: Swansea University		
Unit of Assessment: UoA3		
Title of case study: Developing Global Nanosafety Regulatory Policy		
Period when the underpinning research was undertaken: 2007-2018		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g., job title):**	**Period(s) employed by submitting HEI:**
Professor Shareen Doak	Professor	2003-present
Professor Gareth Jenkins	Professor	1997-present
Dr. Martin Clift	Associate Professor	2015-present
Period when the claimed impact occurred: 2014-2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

Research conducted by Swansea University's *In Vitro* Toxicology Group has been pivotal to the development of standardised safety tests that facilitate nanomaterial hazard identification and risk assessment for human health. DNA damage is associated with carcinogenesis; assessing the DNA damaging capacity of a substance is a vital aspect of safety assessment for the protection of human health. Although regulatory safety tests for most chemicals are well defined, they are not appropriate for nanomaterials. This has been a substantial barrier to nanotechnology innovation worldwide, negatively impacting regulators, industry and the consumer public. Research at Swansea University (SU) has enabled current regulatory testing approaches to be re-written and has provided tailored safety testing methods for nanomaterials, ensuring the safe development of the global nanotechnology industry.

2. Underpinning research

Nanotechnology, which encompasses the manipulation of extremely small things (at the level of atoms and molecules), is widely regarded as the next global frontier in science, bringing significant economic and societal benefits through major technological breakthroughs that improve quality of life. The global nanotechnology market (nano-enabled products, nano-intermediates and nanomaterials) currently consists of ca. 3,200 companies with >10,000 employees, grossing a global turnover of >USD50,000,000,000 in 2020, with a projected growth to >USD170,000,000,000 by 2025. To maintain industry productivity and achieve this market growth, it is essential to overcome barriers preventing the widespread use and further innovative development of nanotechnologies. Specifically, the potential human safety risks posed by engineered nanomaterial (ENM) exposure are uncertain and there is a lack of a safety testing framework, as the standard methods used for chemical safety assessment are not appropriate for ENMs. Hence, the primary needs of the nanotechnology industry are:

1. A **risk assessment regulatory framework**, with effective testing strategies to support safety.
2. Effective **human and environmental hazard detection tools** to allow industry to make informed decisions on product viability and design. Effective tools must be rapid, predictive and cost effective.

Modification of the standard safety testing approach to specifically address problems associated with ENM design, has been shown by the *In Vitro* Toxicology Group to be vital in enabling evaluation of the safety of ENMs to protect human health. Indeed, IVTG-led work in providing hazard testing method adaptations have formed the basis for international regulatory changes. The adaptations described through this research have been recommended in a variety of international regulatory policy documents describing ENM safety testing and are being implemented by industry, regulatory authorities and standardization bodies.

Nano(geno)toxicology research, evaluating the DNA damaging potential of ENMs, was initiated at SU in 2007 by Shareen Doak and Gareth Jenkins who secured major research awards from both MRC (GBP475,000 **G1**) and EPSRC (GBP1,067,482 **G2**), resulting in the

work published between 2009-2012. This research demonstrated that standard safety testing systems developed to detect chemically induced DNA damage were not appropriate for evaluating ENMs and required revisions to avoid future repetition of the asbestos legacy (**R1, R2**).

In 2012, the group published recommendations on how to improve multiple aspects of the methodology underlying standard DNA damage testing systems (the micronucleus assay and Ames test) to tailor them specifically for the evaluation of ENMs (**R3**). While the group provided guidance on how to adapt the micronucleus assay to allow robust testing of ENMs, they recommended that the Ames test was not suitable at all and needed to be replaced. Furthermore, the group demonstrated successful use of a modified micronucleus test with biomedically relevant ENMs (**R4**). These published recommendations (**R3, R5**) have been widely accepted and used to adapt regulatory practice for ENMs in numerous risk assessment policy documents by a variety of international regulatory and standardisation bodies. Further work resulted in the development of newer and even more appropriate tests for evaluating nanosafety, focusing on the generation of novel cellular models for the human lung, skin and liver that better represent the complexities of the human body than traditional techniques (**R6**). Martin Clift's arrival in 2015 facilitated expansion of this work in developing more sophisticated human lung test systems that better predict human health and DNA damage impacts following ENM exposure through inhalation (**G3**).

International recognition of this hazard assessment research is reflected by the award in 2018 of a Horizon 2020 (H2020) European Commission funded project "PATROLS" (**G4**) valued at EUR12.700,000 PATROLS is coordinated by Doak and consists of 23 partners located in Europe, Canada, the US, Japan and Korea. PATROLS includes representation from key beneficiaries including government, regulatory and industrial bodies.

3. References to the research) ***Names provided in bold indicate authors of this document.**

The research from the *In Vitro* Toxicology Group has led to more than 70 peer-reviewed publications in leading journals in the field, been cited over 1600 times and has led to over GBP32,000,000 in research grant income from the European Commission, research councils and industry.

R1. Singh N, Manshian B, **Jenkins GJS**, Griffiths S, Williams P, Maffeis TG, Wright CJ, **Doak SH** (2009). Nanogenotoxicology: the DNA damaging potential of engineered nanomaterials. *Biomaterials*, 30, 3891-3914.
This seminal paper filled a gap in our current understanding of nanogenotoxicology testing and provided specific recommendations that addressed the substantial limitations in our knowledge within the field, at that time. Its publication led to Doak being invited onto national and international committees concerned with nanomaterial safety, including the Genetic Toxicology Technical Committee (GTTC) of the International Life Sciences Institute (ILSI, USA) and the UK Government Committee on Mutagenicity (COM). This output was a key publication linked to the award of an MRC (GBP475,000.00) grant.

R2. **Doak SH**, Griffiths SM, Manshian B, Singh N, Williams PM, Brown AP, **Jenkins GJS** (2009). Confounding experimental considerations in nano(geno)toxicology. *Mutagenesis*, 24, 285-293.
This paper provided further recommendations on how the field should move forward, underpinned by new data to highlight where there were experimental design problems specifically associated with nanomaterial physico-chemical behaviour when undertaking hazard assessment tests. This paper, together with **R1**, resulted in Doak being invited to sit on the GTTC and COM committees. This output was a key publication linked to the award of an MRC (GBP475,000) grant.

R3. **Doak SH**, Manshian B, **Jenkins GJS**, Singh N (2012). In vitro genotoxicity testing strategy for nanomaterials and the adaptation of current OECD guidelines. *Mutation Research*, 745, 104-111.
This paper, for the first time in the field, specifically outlined which global regulatory test systems were appropriate for ENM DNA damage testing and the method adaptations needed. The recommendations and guidance provided in this paper have been adopted internationally. The paper underpins the changes to regulatory practice and consequently has been cited in all the sources to corroborate the impact. The paper was also a key output that led to the successful award of the H2020 PATROLS project, which is coordinated by Doak.

R4. Singh N, **Jenkins GJS**, Nelson BC, Marquis BJ, Maffeis TGG, Brown AP, Williams PM, Wright CJ, **Doak SH** (2012). The role of iron redox state in the genotoxicity of ultrafine superparamagnetic iron oxide nanoparticles. *Biomaterials*, 33, 163-170.
Submitted to REF2014, this output contains a significant portion of the data generated through the successfully awarded MRC grant (GBP475,000). The paper demonstrates the application of the genotoxicity testing systems developed through **R1-R3**. It illustrates how the adapted genotoxicity testing system can be applied to evaluate and understand the DNA damaging potential of ENMs in a robust and reliable manner. The manuscript considers iron oxide nanomaterials destined for biomedical uses as the test material and illustrates how the physico-chemical features of the material strongly influence its safety.

R5. Elespuru RK, Pfuhler S, Aardema M, Chen T, **Doak SH**, Doherty A, Farabaugh C, Kenny J, Ouedraogo G, Mahadevan B, Moore M, Tanir J, Manjanatha MGM, Stankowski LF (2018) Genotoxicity assessment of nanomaterials: recommendations on best practices, assays and methods. *Toxicological Sciences*, 164, 391-416.
This seminal paper is the result of Doak's role in the GTTC committee; it is the first to consider the full suite of regulatory genotoxicity testing methods and the manner in which they can be used to assess nanomaterial safety. It is co-authored by experts from industry, regulators and academics, located across three continents. This paper led to Doak being invited to present the analysis outcomes to several government public health organisations e.g., Public Health England (UK), National Institute for Public Health and the Environment (Netherlands), and in Australia at the Food Standards Australia New Zealand (FSANZ), Therapeutic Goods Administration (TGA) and National Industrial Chemicals Notification and Assessment Scheme (NICNAS).

R6. Wills JW, Hondow N, Thomas AD, Chapman KE, Fish D, Maffeis TG, Penny MW, Brown RA, **Jenkins GJS**, Brown AP, White PA, **Doak SH** (2016). Genetic toxicity assessment of engineered nanoparticles using a 3D in vitro skin model (EpiDerm™). *Particle Fibre Toxicology*, 13, 50.
This paper is a strong example of where the group have developed and adapted novel methods utilising advanced cell culture models to suit nanomaterial hazard assessment. It was a key paper that led to the award of PATROLS and more recently awarded H2020 grants. Additionally, this paper resulted in Doak's being invited to present this work at the quadrennial International Workshop on Genotoxicity Testing (IWGT), Japan, Nov 2017.

Examples of grant income stemming from research:

G1. MRC - GBP475,000 April 2008-Nov 2011. **PI-Doak**, CoI Jenkins. Understanding the Genotoxic Potential of Ultra-Fine Superparamagnetic Iron Oxide Nanoparticles.
G2. EPSRC - GBP1,067,482 Oct 2009-Oct 2013. **CoI-Doak.** Nanoparticle Cytometrics: A Quantitative Analysis of the Toxic Effect of Nanoparticles.
G3. Unilever - GBP90,000 March 2017-Feb 2020. **PI-Clift**, CoI-Doak. An Advanced Multi-Cellular and Dynamic Flow Model of the Human Alveolar Airway to Study the Impact of Inhaled Particulate.

G4. European Commission H2020: PATROLS – GBP12,700,000 Oct 2017-March 2021. **PI-Doak,** CoI-Jenkins & Clift (SU are the lead; 24 partners from 14 different countries worldwide).

4. Details of the impact

The work of SU's *In Vitro* Toxicology Group has been pivotal in the development of standardised safety tests for nanomaterial hazard identification and risk assessment to protect human health. Beneficiaries include 1) regulators and policymakers who use the research to formulate a nanomaterials regulatory framework (see Fig. 1 for global reach); 2) the nanotechnology industries who need to protect their workforces and develop nano-products that are safe and accepted by society; and 3) the consumer public, with safer nanomaterial enabled products available to them.

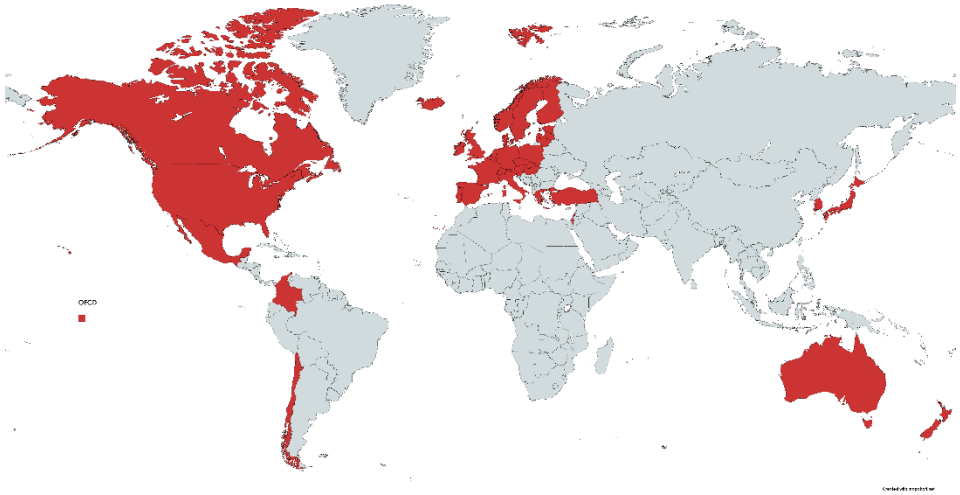

Figure 1 Global reach of regulatory policies influenced by Doak's research

Regulatory and Policy Impact

The tailored adaptation of testing approaches for nanomaterial safety evaluation has had global impact. SU led research (**R2, R3, R5**) has been widely utilised in international risk assessment policy documents to adapt the DNA damage testing methodology to include appropriate measures for evaluating the safety of nanomaterials. Organisations benefitting from this research include the:

- **Organisation for Economic Co-Operation and Development** (OECD) (**C1**; 2014),
- **European Chemicals Agency** (ECHA) (**C2**; 2013-2017),
- **European Food Safety Authority** (EFSA) (**C3**; 2011-2018),
- **Australian Pesticides & Veterinary Medicines Authority** (APVMA) (**C5**; 2015).

An international programme to develop the OECD guidance for nanomaterial-specific DNA damage testing methods was initiated in Canada in 2013, involving 16 countries and published in 2014. Doak, an independent expert advisor for the OECD's Working Party on Manufactured Nanomaterials (WPMN), was the Plenary Speaker and was centrally involved in discussions that led to an open-access OECD report with recommendations on nano-specific DNA damage testing approaches for international regulatory approval processes (**C1**). Doak and Jenkins were key contributors to OECD meetings on adaptation of the *In Vitro* Micronucleus Assay for Testing Nanomaterials in 2014 (France; 14 participating countries) and 2019 (Italy; 10 participating countries). Doak also attended the 2018 WPMN and advised on accelerated use of new methods to support regulatory needs. Doak's work in relation to other regulatory policy document changes (**C2 ECHA; C3, EFSA; C4, APVMA)** has been applied through similar mechanisms to those described above for the OECD.

The Principal Administrator for the OECD in 2017 stated: "*The impact of this work on both global regulatory policy and industrial practice is significant as it will support current regulatory*

frameworks that underpin the development and implementation of an effective, proportionate and acceptable future regulatory framework for nanosafety, including REACH, medical device and cosmetic regulations. This in turn will enable the nanotechnology industry to advance, promoting responsible economic growth." **(C5)**

Doak and Jenkins have participated in several policy-shaping regulatory committees, such as the UK Government Committee on Mutagenicity (COM), serving from 2013-present and 2009-2019 respectively. In 2010 Doak was invited to join both the GTTC and the European Commissions' Scientific Committee on Consumer Safety (SCCS) Working Group on Nanomaterials in Cosmetics. Through these activities, the group's research has been applied to facilitate regulatory decision making in nanomaterial safety dossiers produced by:

- The European Commissions' SCCS (**C6**, **C7**; 2015-2019).
- Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (**C8**; 2014).
- The evaluation of nano-carbon black and nano-enabled medical devices (**C9**; 2017).

In 2017, Doak was invited to lead the GTTC Nanomaterials Working Group (alongside a US Food and Drug Administration, FDA, representative) and is a member of the GTTC Steering Team. Her role in these committees has resulted in changes to both attitude and practice in terms of nanomaterial safety regulation.

Industrial and consumer public impact

Industry has benefitted through the provision of robust ENM safety testing methods, increasing the confidence of regulators in approving ENM products. Multinational, national and local SME companies have benefited from the provision of research expertise and guidance. This has enabled companies to be in a position to file nano-related Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulatory submissions and is exemplified by the Health & Safety manager of OCSiAl, who stated: "*the knowledge-sharing and guidance provided by Swansea University has allowed us to proceed with the registration of our project under REACH, minimising the costs for compliance with potential regulatory classifications" (***C10).** The consumer public also benefit from safer nanomaterial enabled products produced by, for example, Royal Mint, Unilever, Eurometeux, Knauf Insulation, Iron Oxide REACH Consortium, and the Titanium Dioxide Industry Consortium, who have all been provided with both testing guidance and provision of experimental data (2014-2020).

5. Sources to corroborate the impact

C1. OECD document: Genotoxicity of Manufactured Nanomaterials: Report of the OECD Expert Meeting. Series on the Safety of Manufactured Nanomaterials No. 43 ENV/JM/MONO(2014)34; 03-Dec-2014 (p11-13).
C2. ECHA's Guidance on information requirements and chemical safety assessment: Appendix R7-1 for nanomaterials applicable to Chapter R7a Endpoint specific guidance (May 2017; p62).
C3. EFSA document: Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. May 2011, and in the subsequent 2018 updated document (p42-43).
C4. Australian Government; Australian Pesticides & Veterinary Medicines Authority. Nanotechnologies for pesticides and veterinary medicines: regulatory considerations (final report), July 2015 (p120).
C5. Testimonial from Principle Administrator for the OECD.
C6. European Commission's SCCS "Opinion on Carbon Black (nano-form)" (2015; p41,62).
C7. European Commission's SCCS "Guidance on the Safety Assessment of Nanomaterials in Cosmetics" (2019; p50,53-54).
C8. European Commission's SCENIHR: Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices. 2015 (p32,37).
C9. ISO/TR 10993-22:2017(en) Biological evaluation of medical devices-Part 22: Guidance on nanomaterials, 2017 (https://www.iso.org/standard/65918.html).
C10. Testimonial from Health & Safety Lead Manager, OCSiAl.