Institution: University of Warwick

Unit of Assessment: B10 - Mathematical Sciences

Title of case study: Statistical modelling to develop and support adoption of an OECD test guideline for acute inhalation toxicity assessment

Period when the underpinning research was undertaken: 2005 – 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:**
Nigel Stallard	Professor of Medical Statistics	Sept 2005 – Present
Charlotte Price	Research Fellow	Sept 2005 – Aug 2010

Period when the claimed impact occurred: 2015 – 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact (indicative maximum 100 words)

Internationally harmonised recommendations for the testing of chemicals for safe use and transportation are agreed and published as test guidelines by the Organisation for Economic Co-operation and Development (OECD). Assessment of acute toxicity is one of the most common tests, requiring the use of approximately 14,000 animals per year in the UK alone**.** This includes inhalation testing, which involves exposure of animals to chemical dusts, gases or mists to assess their toxicity.

Statistical research at Warwick was vital to the adoption of a new OECD test guideline (TG433) for acute inhalation studies, an internationally agreed standard for chemical testing, which reduces the number of animals required by approximately 75% compared to the most common method, and limits animal suffering through testing at lower concentrations. In addition to animal welfare benefits, the reduction in numbers of animals used leads to significant economic savings. The new TG433 guideline applies to all 36 OECD member countries from across the world, as well as being actively enforced in the UK by the Home Office Animals in Science Regulation Unit (ASRU) in preference to other test methods.

2. Underpinning research (indicative maximum 500 words)

Regulatory acute inhalation toxicity testing of chemicals previously required testing methods which base classification on observation of death at a series of test concentrations in male and female animals as specified by Organisation for Economic Co-operation and Development (OECD) test guidelines (TG403 and TG436). There was a need to reduce the numbers of animals required and the levels of animal suffering caused by testing.

Stallard first proposed the Fixed Concentration Procedure (FCP) as an alternative method while at Reading in 2004, receiving direct funding from the OECD for this in 2006 after his move to Warwick **[G1]**. The FCP was proposed to use female animals alone with 'evident toxicity', defined as signs of toxicity that predict severe toxicity or death at higher test concentrations, as the endpoint. However, there were concerns over lack of evidence of comparability of performance with existing procedures TG403 and TG436, and with the unknown potential impact of suspected sex differences in the level of toxic effects. These concerns, together with the ill-defined nature of evident toxicity resulted in the FCP being dropped from the OECD workplan in 2007.

In 2008, the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs) invited Stallard at the University of Warwick to address the lack of evidence of comparable performance of the FCP to existing methods TG403 and TG436, and suspected sex

differences **[3.1, 3.2]**. This NC3R funded work **[G2]**, led by Stallard with additional input from Price, involved four interlinking components:

- statistical analysis of previously published experimental test data to explore potential sex differences
- the development and application of novel statistical models to enable an evaluation of the FCP in comparison with TG403 and TG436 and an assessment of the impact of sex differences on the FCP and other test methods
- a proposed modification of the FCP to remove the impact of sex differences in the level of toxic effects by initially testing very small numbers of both male and female animals
- application of the statistical models developed to compare the new proposed method with TG403 and TG436

The research demonstrated that the FCP has comparable performance to TG403 and TG436 in terms of classification accuracy, whilst requiring fewer animals (7-11 animals per test procedure compared to 30-40 animals for a standard inhalation toxicity test). Crucially, the new procedure also leads to improved animal welfare, with testing at lower concentrations leading to a reduction in toxicity related death and animal suffering, with 0-5 toxicity related deaths rather than the 20 or more toxicity related deaths required for testing of some substances with TG403 or TG436.

Working collaboratively with NC3Rs, the Warwick work formed a major part of the full evaluation of the FCP **[3.3]** leading to its reconsideration and adoption by the OECD.

3. References to the research (indicative maximum of six references) **Warwick = Bold**

All research papers were published in peer-reviewed journals

[3.1] Stallard, N., **Price, C.**, Creton, S., Indans, I., Guest, R., Griffiths, D. and Edwards, P. (2011) *A new sighting study for the fixed concentration procedure to allow for gender differences*. Human & experimental toxicology, 30 (3). pp. 239-249. doi:10.1177/0960327110370983

[3.2] Price, C., Stallard, N., Creton, S., Indans, I., Guest, R., Griffiths, D. and Edwards, P. (2011) *A statistical evaluation of the effects of gender differences in assessment of acute inhalation toxicity*. Human & experimental toxicology, 30 (3). pp. 217-238. doi:10.1177/0960327110370982

[3.3] Sewell, F., Ragan, I., Indans, I., Marczylo, T., **Stallard, N.**, Griffiths, D., Holmes, T., Smith, P. and Horgan, G. (2018) *An evaluation of the fixed concentration procedure for assessment of acute inhalation toxicity*. Regulatory Toxicology and Pharmacology, 94. pp. 22-32. doi:10.1016/j.yrtph.2018.01.001

Grants

[G1] Stallard, N. *Biostatistical analysis of draft guideline TG433 for acute inhalation toxicity studies (fixed concentration procedure)*. **Sponsor:** Organisation for Economic Cooperation and Development **Duration:** Oct 2006 – Dec 2006 **Award:** GBP4,260

[G2] Stallard, N. *Further Investigations to Support the Adoption of the Fixed Concentration Procedure for Assessing Acute Inhalation Toxicity (TG433) in the EU*. **Sponsor:** National Centre for the Replacement, Refinement and Reduction of Animals in Research **Duration:** Jul 2008 – Oct 2008 **Award:** GBP19,904

4. Details of the impact (indicative maximum 750 words)

Regulatory toxicity testing using animals remains an important part of chemical hazard identification and characterisation across the world. In the UK in 2019, 145,000 animal procedures were conducted for regulatory toxicity and safety testing with 14,000 of these used for acute toxicity assessment **[5.1]**. Warwick research has been fundamental in the development and refinement of the Fixed Concentration Procedure (FCP) for the assessment of acute inhalation toxicity leading to development and adoption of a new OECD test guideline (TG433) in June 2018 **[5.2]**. The implementation of this guideline results in a substantial reduction in the number of animals required for testing, in improved animal welfare and economic savings, and aligns the test with EU Directive 2010/63/EU which states: "*methods selected should avoid, as far as possible, death as an end-point due to the severe suffering experienced during the period before death*".

Development and adoption of a new OECD test guideline TG433

International guidelines on toxicity testing are approved by the Organisation for Economic Co-operation and Development (OECD) for its 36 member countries including UK and all EU founder countries, USA, Canada, Australia, New Zealand and Japan. As approval requires the agreement of all individual OECD member countries, overcoming this obstacle for any new guideline can be considered "*a major achievement*" **[5.3]**. One area of toxicity testing is the testing of acute inhalation toxicity for dusts, gases and mists described by OECD Guidance document GD39, which referred to specific test guidelines TG403 and TG436.

For the FCP, there were three primary concerns by the Regulators which initially prevented approval: the lack of evidence for the comparable performance of the FCP method with existing OECD guidelines TG403 and TG436, the impact of suspected sex differences in the level of toxic effects, and poorly defined nature of evident toxicity. On Warwick's contribution to guideline acceptance and the concerns above, Dr Fiona Sewell, Head of Toxicology at NC3Rs, stated: "*Statistical simulations conducted by Nigel Stallard and his team, addressed the first two areas of concern, and were vital for the final acceptance of the method by the OECD*" **[5.3]**. Further research expanding on this point was reported in Sewell *et al.* (2015) **[5.4]**, with co-authors drawn from a range of global stakeholders such as the French National Institute for Industrial Environment and Risks (INERIS), the US NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the UK Home Office.

Additionally a 2019 RSPCA report Avoiding Mortality in Animal Research and Testing **[5.5]** highlights the importance of contrasting FCP to existing methods itself **[3.1-3.2]** as a component which helped to tackle evident toxicity doubts: "*This concern [evident toxicity] surfaced repeatedly in efforts led by the UK NC3Rs to gain approval for the inhalation toxicity equivalent, the Fixed Concentration Procedure (FCP). Only extensive retrospective analysis of a large historical data set, and detailed comparison of the classifications made by the FCP with other methods succeeded in overcoming mistrust of evident toxicity and led to acceptance of the draft guideline in 2017 (OECD TG433) 13 years after its first publication*". Following publication of the work by the Warwick group, a revised FCP was proposed in a draft test guideline TG433 in 2015, and this was adopted by the OECD in 2018 **[5.1]**. The adopted test guideline includes Warwick's work **[3.1-3.2]** as two of the ten supporting citations with the diagrams illustrating how the procedure is to be conducted taken directly from one of the two research papers **[3.1]**. The overarching OECD guidance document (GD39) on inhalation toxicity testing was revised in 2018 **[5.6]** to reflect the adoption of TG433 and also to cite the Warwick papers **[3.1-3.2]**. Overall, the test is considered a "*major refinement*" on the previous methods **[5.3]**.

Improving animal welfare and reducing numbers of animals required for acute inhalation toxicity testing by changing OECD guidance

Traditionally, testing of acute inhalation toxicity has been conducted by estimating the LC_{50}, the concentration level that would lead to a death rate of 50%, following OECD test guideline TG403 or TG436. Depending on the initial concentration used, a standard LC_{50} test requires testing of 30-40 animals **[5.3]**, typically with at least a quarter of these exposed to lethal concentration levels.

The FCP, based on **[3.2]**, and adopted by OECD from June 2018 on the basis of Warwick work **[3.1-3.2]**, leads to animal welfare and use improvements. First, the number of animals required is reduced to 7-11, a decrease of approximately 75% compared to the 30-40 animals used in a typical LC_{50} test **[5.3]**. Second, the use of the endpoint of 'evident toxicity', defined as signs of toxicity that predict severe toxicity or death at higher test concentrations, rather than death, leads to testing at lower concentration levels. This leads to improved animal welfare compared to the other test methods, with 0-5 animals exposed to lethal concentrations **[3.1]**.

Regarding the benefits of the new procedure, Dr Sewell stated, "*Acceptance of this test guideline has the potential to reduce the number of animals used for the classification of chemicals worldwide, whilst simultaneously reducing the levels of suffering, through the use of evident toxicity in place of death as an endpoint*" **[5.3].**

The work by Stallard **[3.1-3.2]** was also included as two of the six publications cited as examples of progress towards developing the 3Rs (Reduction, Refinement and Replacement of animals in research) by signatory organisations to the Concordat on Openness on Animal Research in the UK in their 2015 Report **[5.7]**.

On the current state of inhalation testing in the UK, Dr Fiona Sewell explained, "*In the UK, the Home Office Animals in Science Regulation Unit (ASRU) is now actively enforcing the FCP in preference to the other accepted approaches*", which reflects a substantiative nationwide shift in policy. While global harmonisation for FCP is still ongoing, the NC3Rs are "*working with the UK's representatives to the OECD to build a case to remove the two existing Test Guidelines that use death as an endpoint so that the only accepted OECD method is the FCP*" **[5.3]**

Regarding the journey towards acceptance, the Chief Executive of the NC3Rs stated that *"The new OECD test guideline for inhalation toxicity has massive 3Rs benefits. The route to acceptance has been a difficult one even when we felt the data was water-tight. I am extremely proud of the work and commitment that the NC3Rs team and others in the UK, including our OECD national coordinator have put into making this method with its benefits for animal welfare a reality."* **[5.8]**

Economic benefits from reducing animal numbers required for acute inhalation toxicity testing by changing OECD guidance

In addition to animal welfare improvements, the reduction in animal numbers required in TG433 leads to an economic benefit. The average cost of acute inhalation testing for a substance using the conventional method is estimated by US Environment Protection Agency to be over USD32,000 **[5.9]**. The reduction in the number of animals by around 75% (7-11 compared to 30-40) would therefore lead to considerable economic savings.

5. Sources to corroborate the impact (indicative maximum of 10 references)

[5.1] Annual Statistics of Scientific Procedures on Living Animals, Great Britain 2019: table 7.4 (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/900515/annual-statistics-scientific-procedures-living-animals-2019-tables.ods)

[5.2] OECD Test Guideline TG433 adopted June 2018 https://www.oecd-ilibrary.org/test-no-433-acute-inhalation-toxicity-fixed-concentration-procedure_5jfmcl7qq1lt.pdf?itemId=%2Fcontent%2Fpublication%2F9789264284166-en&mimeType=pdf

[5.3] Statement from Head of Toxicology, N3CRs

[5.4] Sewell, F., Ragan, I., Marczylo, T., Anderson, B., Braun, A., Casey, W., Dennison, N., Griffiths, D., Guest, R., Holmes, T., van Huygevoort, T., Indans, I., Kenny, T., Kojima, H., Lee, K., Prieto, P., Smith, P., Smedley, J., Stokes, W. S., Wnorowski, G. and Horgan, G. (2015) *A global initiative to refine acute inhalation studies through the use of 'evident toxicity' as an*

endpoint: Towards adoption of the fixed concentration procedure. Regulatory Toxicology and Pharmacology, 73 (3). pp. 770-779. doi:10.1016/j.yrtph.2015.10.018

[5.5] Hawkins et al (2019) Avoiding mortality in animal research and testing. RSPCA. ISBN 978-0-901098-17-7. https://www.researchgate.net/publication/331023498_Avoiding_mortality_in_animal_research_and_testing/download

[5.6] OECD Guidance document on inhalation toxicity studies GD39 (Second edition) (2018) https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en

[5.7] Concordat on openness on animal research in the UK Annual report 2015. Understanding Animal Research. http://www.understandinganimalresearch.org.uk/files/9214/4319/6363/UAR_Concordat_Report_2015.pdf

[5.8] NC3Rs press release about new guideline (May 2017) https://www.nc3rs.org.uk/news/finally-new-test-guideline-refined-acute-inhalation-studies

[5.9] Cost Estimates of Studies Required for Pesticide Registration July 2019. US Environmental Protection Agency. https://www.epa.gov/sites/production/files/2019-07/documents/studies-cost-estimates-jul-2019.pdf