

Institution: University of Liverpool		
Unit of Assessment: UoA6		
Title of case study: Refined methods for handling laboratory mice to improve animal welfare, improve research data reliability and increase safety of personnel		
Period when the underpinning research was undertaken: 2006 - 2020		
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:
Prof Jane Hurst	Professor	1998 - present
Period when the claimed impact occurred: 1st August 2013 – 31st July 2020		
Is this case study continued from a case study submitted in 2014? No		
1. Summary of the impact Impact on policy, animal welfare, scientific reliability and commerce: Policy and practice on suitable handling methods for laboratory mice changed, resulting in global improvements for both laboratory mouse welfare and reliability of research/testing impacted by animal stress and anxiety. Beneficiaries: • Laboratory rodents worldwide, particularly mice; • Public concerned about (a) laboratory animal welfare, (b) lack of reliability in animal research; • Pharmaceuticals industry and academic researchers requiring (a) reliable animal responses, (b) public and government support for animal experimentation; • Policy makers, animal charities, animal technologists and trainers requiring guidance on best practice for animal handling; • International manufacturers and suppliers of laboratory animal equipment. Significance and Reach: The mouse is the most important and numerous laboratory animal used worldwide both in biomedical research and for regulatory product testing. Most facilities in the UK and major international pharma have changed practice or are currently rolling this out (more than 3,000,000 mice per annum); online best practice guidelines have been accessed by users in 107 countries. Attribution: All research was led by Prof Jane Hurst, University of Liverpool.		
2. Underpinning research The mouse is the most important and numerous laboratory animal used worldwide in industrial and academic biomedical research, and for regulatory safety testing. Routine handling is an essential component of animal maintenance and experimental use. However, handling has well known and profound effects on animal anxiety and stress (physiological and behavioural), affecting both animal welfare and the reliability of scientific data collected. Nonetheless, there had been little attempt to understand how to handle rodents to minimize such effects beyond the need to 'accustom' animals to being handled. UoL research, led by Prof Jane Hurst, provided strong scientific evidence that the tail handling method, until then universally recommended to pick up mice, induces aversion and high anxiety with little habituation on repeated handling. Hurst showed that two new handling methods that she developed (use of a handling tunnel or cupping mice on the open hand without direct physical restraint) each led to animals voluntarily approaching the handler, showing low anxiety and handling stress, and accepting physical restraint [3.1]. Using a new approach to assess voluntary interaction with the handler, in addition to application of well-established validated measures of anxiety and stress, Hurst established that responses to these different handling methods generalize across sexes, ages and strains of mice, across handlers with differing levels of prior experience, and when animals are handled in the light or dark phase of the circadian cycle [3.1]. These major differences in response to alternative methods to pick up mice had not previously been suspected.		

Hurst predicted that the refined handling methods would minimize the confounding effects of handling on research and provide more robust scientific outcomes, as well as improving the lifetime welfare of many millions of mice in laboratories. In agreement with this, she demonstrated through an NC3Rs-funded PhD studentship at UoL that the refined handling methods she developed substantially improve mouse performance in behavioural tests [3.4, 3.5]. Independent peer-reviewed studies in other laboratories in USA, Japan, EU and UK subsequently replicated Hurst's findings [3.6], providing important confirmation that positive responses to the refined handling methods generalise across laboratories and different cage types, and improve reliability in pharmacological testing. These findings further demonstrated wider positive effects on stress physiology and response to reward, known to influence a very wide range of biomedical research and testing.

Further research led by UoL focused on refining the new handling methods to allow practical implementation and replacement of tail handling in animal facilities that may house tens of thousands of mice. This study demonstrated how acclimation can be achieved through the minimal brief handling required for routine cage cleaning, resulting in substantially reduced animal fearfulness and anxiety [3.2, 3.3, 3.5]. The effectiveness of handling tunnels, requiring little or no acclimation, has made this the best practice recommendation for most situations [3.6], with provision of tunnels in the home cage giving an additional advantage for ease of handling and for nervous strains [3.2]. Collaborative research with a pharmaceuticals company (AstraZeneca plc) and cancer research organization (CRUK) showed that findings generalised when applied in other external animal units [3.3].

The underpinning research was carried out by Professor Jane Hurst from 2006 to 2019 at UoL. Professor Hurst carried out the initial work herself (2006-2010), with additional help from an undergraduate vacation student (Rebecca West, 2006) under Professor Hurst's instruction [3.1]. An NC3Rs postgraduate student (Kelly Gouveia, 2010-2014) supervised by Professor Hurst carried out further research into practical implementation of refined handling methods and impact for improving scientific outcomes [3.2-3.6].

3. References to the research

3.1 **Hurst JL and West RS** (2010) Taming anxiety in laboratory mice. *Nature Methods* 7(10), 825-826. [DOI:10.1038/NMETH.1500](https://doi.org/10.1038/NMETH.1500)

Awarded NC3Rs 2010 Prize (sponsored by GSK) for best original contribution to scientific and technological advances in 3Rs within last two years. Paper recommended by Faculty 1000. Online attention in 98th percentile of 113,998 tracked articles of similar age in all journals [Nature Methods Oct 2020]. **384 citations** [Google Scholar Feb 2021]

3.2 **Gouveia K and Hurst JL** (2013) Reducing mouse anxiety during handling: effect of experience with handling tunnels. *PLOS ONE* 8, e66401. [DOI:10.1371/journal.pone.0066401](https://doi.org/10.1371/journal.pone.0066401) **110 citations** [Google Scholar Feb 2021]

3.3 **Gouveia K** (2015) Taming anxiety and variation in laboratory mice. PhD thesis, University of Liverpool. Supervised by Jane Hurst.

3.4 **Gouveia K and Hurst JL** (2017) Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. *Scientific Reports*, 7: 44999. [DOI:10.1038/srep44999](https://doi.org/10.1038/srep44999). **75 citations** [Google Scholar Feb 2021]

3.5 **Gouveia K and Hurst JL** (2019) Improving the practicality of using non-aversive handling methods to reduce background stress and anxiety in laboratory mice. *Scientific Reports*, 9:20305. [DOI:10.1038/s41598-019-56860-7](https://doi.org/10.1038/s41598-019-56860-7). **10 citations** [Google Scholar Feb 2021]

3.6 <https://www.nc3rs.org.uk/how-to-pick-up-a-mouse>, website active since September 2016, includes a video tutorial and instructional clips, information poster for animal units, and FAQs authored by **Gouveia K, Waters J & Hurst JL**, and a webinar by **Hurst JL**. Research page shows multiple papers confirming original findings and repeatability across laboratories. **Landing page is 13th most popular page on NC3Rs website since September 2016: 35,290 unique page views from 107 countries** [31st July 2020].

4. Details of the impact

Prior to Prof Hurst's research, grasping the tail between fingers or by forceps was the method universally used to pick up laboratory mice worldwide and recommended in all guidelines. Refined handling methods that she developed were not in use in other laboratories. After initial publication in 2010, uptake of the refined methods was low and tail handling continued as the global standard. To promote impact, Hurst gained a BBSRC Sparking Impact award to create training resources (2013-2014) and partnered with UK National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) to establish a freely available online training hub on mouse handling (live since September 2016). Hurst's team have also delivered more than 80 invited presentations and/or tutorial demonstrations across 12 countries to widen awareness.

External recognition confirming high level of impact:

The substantial impact of this research on laboratory rodent welfare and improving scientific reliability of animal models has been recognized through several prestigious national and international awards. Prof Hurst was awarded the NC3Rs 2010 international prize for the most significant 3Rs advance in medical, biological or veterinary sciences; the Swiss Laboratory Science Association 2019 international prize for Refinement; and an OBE (2020 New Years Honours) for services to animal welfare [5.1]. Working with Hurst at UoL, animal care technician John Waters has provided practical advice and training resources to animal caretakers to encourage implementation in animal facilities. His highly effective role in promoting uptake has been recognized through the Andrew Blake Tribute Award 2017, the AAALAC International Fellowship 2019, and the Times Higher Education Outstanding Technician of the Year 2020 award (for contribution in 2018-2019) [5.1].

Impact on policy and animal welfare:

Research from UoL and subsequently from other laboratories has confirmed that replacement of tail handling with the UoL refined handling methods substantially reduces aversion, stress and anxiety in laboratory mice, with additional benefits for physiological health, avoidance of depression-like behaviour and improved reliability of scientific data in mouse models influenced by background stress [5.2, 5.6]. The UoL refined handling methods are now strongly promoted as best practice for mouse handling by the NC3Rs, the UK authority for providing guidance on design and implementation of animal research to industry and academia nationally and internationally. This has been widely advertised to users (scientists, veterinary and other care staff, policy makers, students) through NC3Rs Newsletters, meetings and the NC3Rs website since 2016, with online advice accessed by users in 107 countries [5.2, 5.6]. NC3Rs Chief Executive states that Prof Hurst's research *"has led to the largest improvement in laboratory mouse welfare in a decade"* [5.6]. She confirms that the research findings both *"have welfare implications for millions of mice worldwide"* and *"can affect the reliability and therefore quality of animal research"* in this most commonly used species. Major 3Rs centres in other countries also promote the use of Hurst's refined handling methods in place of tail handling, referring users to her scientific evidence and online NC3Rs mouse handling hub for training resources. This includes the influential North American 3Rs Collaborative (NA3RsC), Norecopa (Norway's 3Rs centre accessed by countries across Europe), the Danish 3R-Center, the Swiss 3R Competence Centre, and Charité 3R [5.2]. An independent online survey of those working with laboratory mice in Sep 2018 – Jan 2019 (390 respondents from 27 countries) found that >80% of respondents were convinced that tunnel handling improves animal welfare, and >70% were convinced it improves scientific outcomes [5.4]. In an independent survey of 107 participants on three courses on mouse handling in Berlin in Nov-Dec 2019, almost every respondent agreed that tunnel and cup handling improve mouse well-being (99%) and the human-animal relationship (96%) [5.4].

To promote rapid uptake of Hurst's refined methods to replace tail handling and ensure staff were given appropriate support to achieve this, the UK Home Office Animals in Science Regulation Unit (ASRU) implemented a formal review on mouse handling across all licensed establishments in the UK using laboratory mice in early 2019 [5.3].

Impact on uptake of refined methods:

An independent online survey in Sep 2018 – Jan 2019 (390 respondents from 27 countries, 30% from UK, 30% non-academic) found that 61% of respondents working with mice were using Hurst's refined methods by the survey date [5.4]. This survey also indicated that >80% of respondents who had heard of the methods were using them.

A UK Home Office ASRU survey of all UK licensed establishments using mice (industry, academia) in the first half of 2019 found that 68% of UK establishments were using the new handling methods, with 59% using these methods exclusively, completely replacing picking up mice by the tail. Those still using tail handling were in the process of introducing refined handling methods [5.3]. Interviews with animal care staff and training officers (conducted independently by NC3Rs) confirm that mice are *"less anxious"* and *"more calm and relaxed"* during procedures, *"aggressive mice are a lot calmer"* and *"non-aversive handling methods are essential for jumpy strains"*. Users state *"I can spot health issues earlier because [animals] display more natural behaviours"*, *"you don't get bitten as often"*, *"you can much easier get mice out of the cage with a tunnel"*, *"it creates that connection between the human and the animal"* and *"it only took me a week to learn how to do the tube handling and cupping, and now it's as fast as tail handling"* [5.5]. The very rapid increase in uptake from 2016 to early 2019 combined with user satisfaction with the refined methods predict an even higher uptake by July 2020.

The biopharmaceuticals industry is a major user of laboratory mice, sensitive to the economic value of gaining reliable data from animal models and the ethical requirement to ensure the highest standards of animal welfare. For example, the multinational Novo Nordisk replaced tail handling with Hurst's refined methods across all their animal facilities globally (Denmark, China, USA) in 2018-2019. Not only has this improved welfare for approximately 37,000 mice used per year, but it has also led to a noted improvement in job satisfaction among animal caretakers and wider improvements in handling other species. The Novo Nordisk Vice President of Laboratory Animal Science states *"This change would not have happened without the research that Professor Jane Hurst published from 2010 onwards. Her persistence, offers of help, and partnering with NC3Rs to provide training resources, have been instrumental in driving this important refinement"* [5.7]. AstraZeneca plc rolled out the refined methods simultaneously across all five of their internal animal facilities in the UK, Sweden and USA. Hurst presented current scientific evidence and a Q&A session to all sites at the launch of this major initiative (May 2018), with Hurst and Waters visiting UK and USA facilities to provide advice. Implementation has benefited approximately 121,000 mice used per year. The Director of Animal Science and Technologies at AstraZeneca confirms that without Professor Hurst's research and a robust evidence base for the new methods *"we would have been unaware of the aversion caused to mice by picking them up by the tail"*. This has provided benefits for animal welfare, efficiency, staff satisfaction and reduced variability in scientific testing. For example, at the Swedish site, *"tube handling and cupping have improved behavioural tests that are part of the phenotyping screens we conduct"*. They report that *"the methods do not take any extra time and reduce signs of stress (such as defaecation)"*, *"mice are easier to handle"*, *"staff enjoy the methods for handling"*, and *"having mice that are less anxious and therefore more consistently calm means that there is less variability in the scientific output"* [5.8].

Impact on training:

NC3Rs' website provides the main source of 3Rs information in the UK, with a strong global reach. A resource hub on best practice in mouse handling based on Hurst's refined methods has been one of the 'top level' hubs under 3Rs resources since 2016 [5.2]. This hub features a video tutorial (14,390 views) and short training clips (17,565 views, 871 downloads) produced by Hurst's team, a webinar by Hurst (5,453 views) and short video interview, a summary of the evidence base, FAQs and tips for implementation. The hub landing page is the 13th most popular page on NC3Rs website since initiation, receiving 35,290 unique views (62,449 unique views totalled across mouse handling pages); users are from 107 countries (22.3% UK, 20.4% USA). NC3Rs have sent out 4,015 instructional posters for animal facilities to 415 institutions in 56 countries, now translated into Chinese, French and German [5.2,5.6].

NC3Rs workshops for institutional trainers and Named Training & Competence Officers (NTCOs) were attended by 80 delegates each in 2017 and 2018, both featuring plenary presentations from UoL's Hurst and Waters. Many institutions now provide their own internal training [5.5], with training also given in UK modular training courses required to gain personal and project licences e.g. The Learning Curve have trained 180-250 students per annum in these methods since 2017; Red Kite Veterinary Consultants trained 246 candidates March 2017- Nov 2019 [5.9]. Refined mouse handling was a central topic for the Sixth UK-China Seminar on Animal Welfare and Ethics in May 2019 to promote China's alignment with international standards. The Swiss Laboratory Animal Science Association provides training in the refined methods in CPD training courses, and Hurst's team have delivered over 80 presentations and tutorials across 12 countries.

Impact on provision of new commercial products:

The new cupping method does not require any equipment, as tunnel handling can use cardboard tubes already available as enrichment resources. However, purpose-designed clear autoclavable handling tubes provide a new product supplied by Datesand Ltd (UK), LBS Biotechnology (UK), Braintree Scientific Inc (USA) and SerLab (France) from 2016 onwards. Datesand Ltd have sold 84,993 tunnels from 2018-2020 (60% UK, 40% international). Customers were from 15 countries (54% industry, 46% academic sector) [5.10].

5. Sources to corroborate the impact

Sources of information in the public domain

5.1 Prizes arising from the research and provision of training resources to promote uptake. NC3Rs 2010 Prize; Swiss Laboratory Animal Science Association (SVG) 2019 Refinement Prize; OBE 2020 New Year Honours; Andrew Blake Tribute Award 2017; AAALAC International 2019 Fellowship; Times Higher Education Outstanding Technician of the Year 2020 shortlist.

5.2 Policy changes to promote new handling methods as best practice. NC3Rs website; North American 3Rs Collaborative website; Norecopa website; Swiss 3R Competence Center resource hub; Charité 3R toolbox; Danish 3R-Center; Procedures with Care training website.

5.3 Promotion of refined handling methods by UK Home Office ASRU and evidence of uptake across UK facilities. October 2018 Establishment Licence Holder Newsletter; update by ASRU Chief Inspector at LASA Annual Conference (November 2019).

5.4 Independent surveys of impact and uptake of refined methods.

Henderson LJ, Smulders TV & Roughan JV (2020) *PLOS ONE* 15(4):e0231454, DOI:10.1371/journal.pone.0231454.

Hohlbaum K, Humpenöder M, Nagel-Riedasch S, Ullman K & Thone-Reineke C (2020) *ALTEX* 37(4), 671-4, DOI:10.14573/altex.2005121.

5.5 Interviews of trainers and animal care staff that have implemented refined methods in animal facilities.

Individual users / beneficiaries to corroborate claims

5.6 Testimonial from NC3Rs Chief Executive

5.7 Testimonial from Novo Nordisk Vice President of Laboratory Animal Science.

5.8 Testimonial from AstraZeneca plc Director of Laboratory Animal Science

5.9 The Learning Curve and Red Kite Veterinary Consultants Ltd both provide approved modular training courses (an approved course is compulsory for animal technologists, *in-vivo* scientists and support staff working under the Animals (Scientific Procedures) Act in UK).

5.10 Tunnel sales: Datesand Ltd catalogue, referencing UoL research. Managing Director can corroborate sales figures.