National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

CRACK IT Review 2019

Pioneering Better Science

About the NC3Rs

The NC3Rs is a Government-backed organisation that works with scientists and organisations across the bioscience sector to discover and implement new technologies and approaches that replace, reduce and refine the use of animals in research. It has an annual budget of £10M, with core funding provided via the Medical Research Council (MRC) and the Biotechnology and Biological Sciences Research Council (BBSRC), as part of UK Research and Innovation (UKRI).

Further information can be found at www.nc3rs.org.uk

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Foreword

CRACK IT is the NC3Rs' open innovation programme.

Launched in 2011, CRACK IT is designed to support 3Rs-focused R&D by enabling and funding collaborations between big business, academia and the SME sector.

The goal is simple – to deliver ready-to-use products and services for industry and the biosciences sector to replace, reduce and refine their use of animals.

CRACK IT consists of two parts: CRACK IT Challenges, a novel 3Rs funding competition for collaborative R&D, and CRACK IT Solutions, a technology partnering hub designed to accelerate the translation of technologies with 3Rs potential. To date we have committed almost £30M through the CRACK IT programme (including almost £9M secured from partner organisations); worked with 23 major companies from the pharmaceutical, chemical, agrochemical and consumer product sectors; supported the formation or growth of 68 SMEs; and delivered 12 new 3Rs products and services for industrial and academic end-users that are better, cheaper or quicker than existing approaches.

In this review we describe the outputs and impacts of CRACK IT Challenges and Solutions, highlighting the unique opportunities that CRACK IT provides industry, academics and SMEs, and the lessons we have learnt from leading this ambitious programme. The case studies describe CRACK IT Challenges and Solutions in practice including the prototypes, products and services developed, and their 3Rs impacts.

We have shown, in a short period, how open innovation can benefit the 3Rs. We have established CRACK IT as a strong brand and this has enabled the NC3Rs to work with leading multi-national organisations, innovative SMEs, and first-class academics. There are benefits for all of the participants involved: industry gets early access to scientific and technological innovations emerging from the science base, and an end-product or service which meets their needs; academics have a pathway for exploiting their research and new opportunities for collaboration and funding; and SMEs are provided with a new market and customer base to expand.

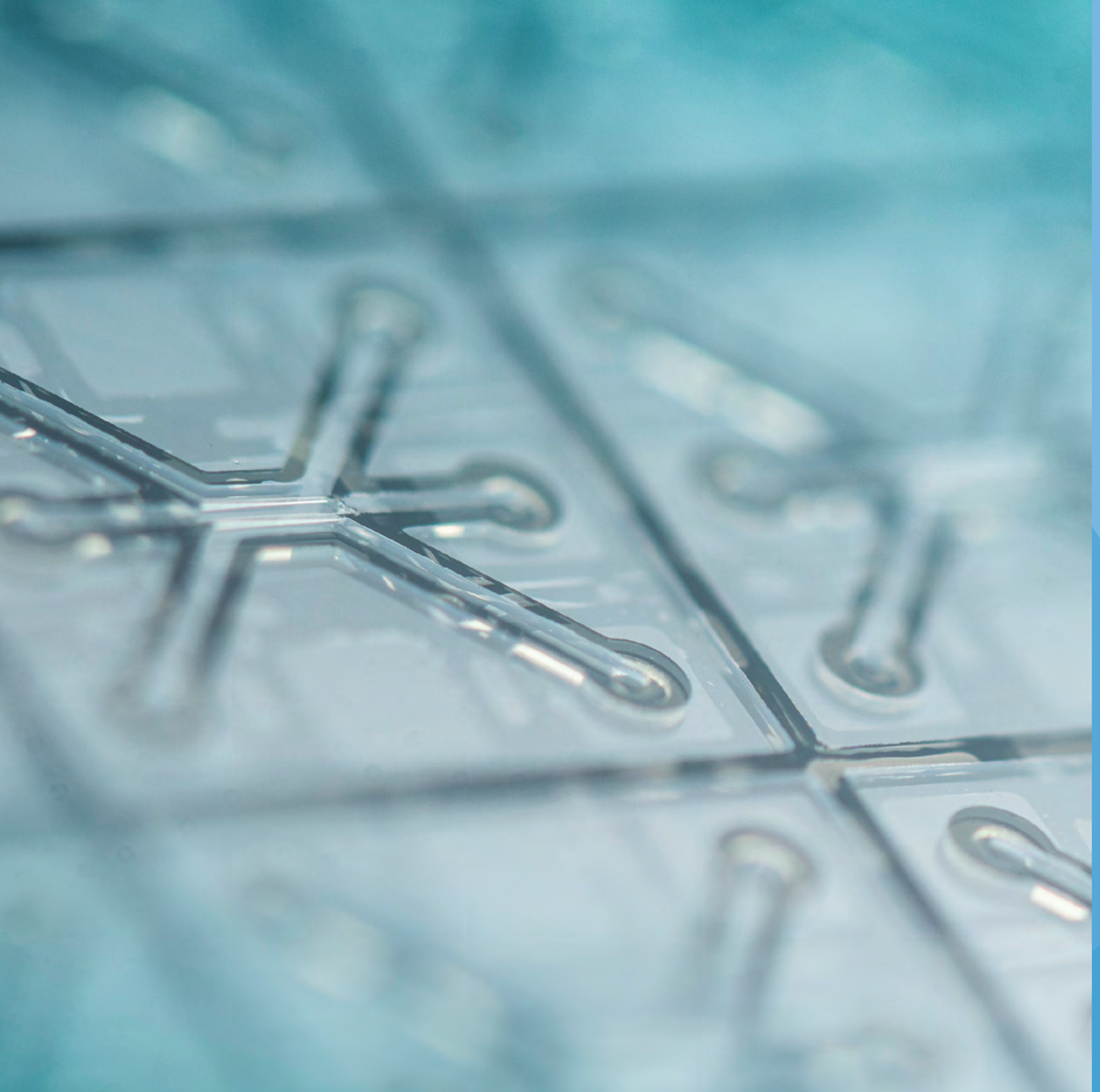
CRACK IT has been effective at testing the 3Rs benefits of emerging technologies in real-world settings, and adding to the knowledge base with nearly 80 papers[1] published on the associated R&D. We have established a strong track record to build on.

Our plans include launching mega-Challenges. These will require more collaborative R&D than we can currently support with the £1M maximum budget per Challenge. Forming new alliances will be essential. Aligned with this will be a new focus on technologies such as artificial intelligence, where the 3Rs potential has yet to be fully explored.

Dr Vicky Robinson CBE, Chief Executive

Professor Stephen Holgate CBE, Board Chairman

[1] Publications described in the case studies are listed in Annex 1.



Introduction

Figure 1: CRACK IT Challenges competition process

CRACK IT Challenges

Through the CRACK IT Challenges funding competition, we have responded directly to major issues facing industry and the bioscience sector related to the use of animals. These include bottlenecks in drug and chemical development caused by poorly predictive *in vivo* models, and new therapeutic areas such as gene and cell therapies where the regulatory landscape for preclinical testing is still evolving.

We have committed £29.1M for CRACK IT Challenges[2]. Of this, £8.76M (30%) has been secured from external sources. This includes £6.55M as core funding for the competition from Innovate UK (£3.5M), the MRC (£3M), and Unilever (£50k); and £2.21M ring-fenced for specific Challenges from Alzheimer's Research UK (£0.35M), Defra (£0.5M), Dstl (£100k), EPSRC (£0.83M), and Versus Arthritis (£0.43M).

[2] This includes the budget for the 2019 Challenges.

The funding competition

The process for CRACK IT Challenges is shown in Figure 1 (p6). Topics for potential Challenges are identified through the NC3Rs networks, ongoing collaborations with industry, and an annual open call. Challenge proposals are independently reviewed with the final selection based on the projected 3Rs and commercial impacts, and the budget required. Specific deliverables for the Challenges are developed in collaboration with one or more sponsoring organisations (referred to as the Sponsors). Depending on the level of R&D required, Challenges can be single phase with contracts of up to £100k for 12 months, or two-phase with contracts of up to £1M for 36 months. Two-phase Challenges are run using the Small Business Research Initiative processes provided by Innovate UK. Contracts for both single and two-phase Challenges are run in open competition, with EU-based establishments eligible to apply[3].

The Challenge briefs are widely promoted across the bioscience sector and prior to the application deadline, we host an event that allows potential applicants to meet the Sponsors and initiate collaborations with other scientists. Applications are subsequently assessed by an expert Panel convened specifically for each Challenge, and an additional Dragons' Den-style interview for single phase Challenges and Phase 2 funding. Payments to successful applicants (referred to as Contractors) are milestone-driven.

The Sponsors

Sponsors play a critical role in identifying and developing the Challenge, assessing applications for funding, and providing in-kind contributions (or funding) as part of the collaboration. In-kind contributions are tailored to the specific requirements of the Challenge and include access to data, equipment, expertise or compounds; conducting in-house validation studies; and providing dedicated scientific and technical staff time.

The active engagement of the Sponsors and the provision of in-kind contributions are pivotal to the success of the Challenges, providing a unique opportunity for academics and SMEs to work with new partners and access resources that they would not otherwise be able to. Sponsors also participate in the quarterly review meetings with the Contractors and the NC3Rs to review progress, experimental findings and next steps, enabling them to shape the products and services developed so that they can ultimately be deployed to meet their business needs.

To date there have been 27 Sponsors. Of these 17 (63%) are from the pharmaceutical sector, four (15%) from the chemical, agrochemical and personal care industries, and six (22%) from academic, government and charitable organisations. Thirteen of the Sponsors have sponsored more than one Challenge. A list of Challenges and Sponsors is in Annex 2.

The Challenges

We have launched 34 Challenges. Of these, seven are single phase and 27 are two-phase. Most of the Challenges (68%) focus on replacing animal use particularly for drug efficacy studies or drug/chemical safety assessments. These Challenges largely require the development of complex cellular and microphysiological systems or *in silico* modelling. The remaining 11 (32%) focus on refining animal use to minimise pain and suffering and improve the quality of the information obtained from *in vivo* studies. These Challenges largely require the development of hardware that allows animal models to be characterised and monitored in more detail than existing approaches permit.

[3] Academic, public organisations and SMEs.

The Contractors

Contracts have been awarded for 27 of the 34 Challenges launched. There have been a total of 85 contracts[4], 17 to teams based at single institutions and 68 to consortia involving multiple organisations. Of the contracts, 55 (65%) have been awarded to UK-based organisations and 30 (35%) to those located elsewhere in the EU. Awards are made to a Lead Contractor – over half (52%) of the contracts awarded for single phase Challenges, or in Phase 2, are to SMEs. Many of the Challenges require a multi-disciplinary approach and the competition has provided an effective mechanism for engaging experts in areas such as electronic engineering, software development and mathematics as well as biologists with expertise in stem cells and complex tissue models.

Prototypes, products and services

Nineteen of the 27 Challenges where contracts were awarded have been completed, and 18 (95%) have delivered a working prototype. Of these 11 (61%) have led to new products or services, with four companies being established. The remaining seven (39%) prototypes require further development work and three of the Contractors have secured additional funding for this.

Lessons learnt

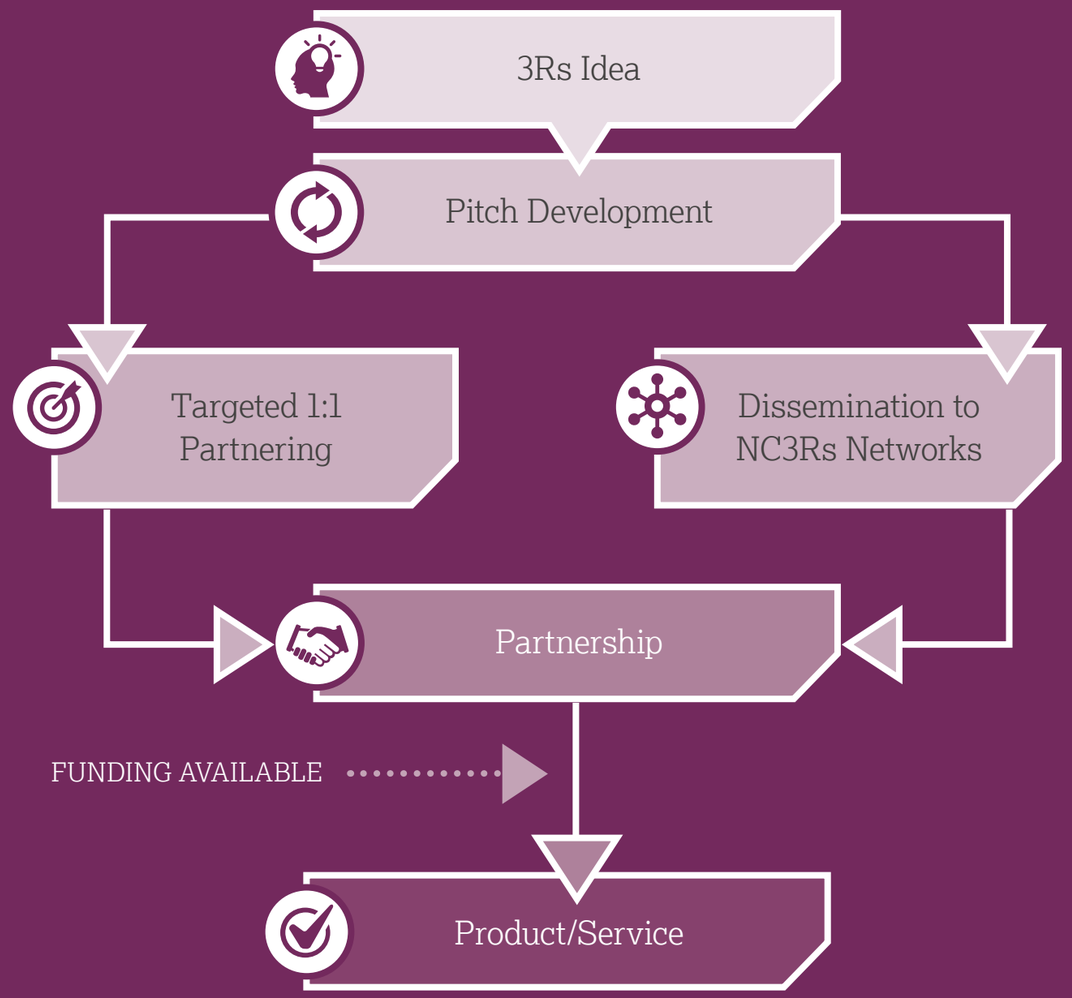
CRACK IT Challenges is an ambitious programme with inherent risks relating to the complexity of the scientific and technical problems we aim to solve. Based on our experience, we have evolved how we manage CRACK IT focusing on de-risking projects, supporting the next steps for commercialisation, and building confidence in the products and services delivered to maximise their 3Rs impacts.

- De-risking projects: In 2012, we introduced a two-phase process that allows up to three contracts to be awarded per Challenge for a six-month period. These Phase 1 contracts, of up to £100k each, allow proof-of-concept work to be conducted prior to the selection of one Contractor for Phase 2. This means that we can consider and test novel and different approaches early on before a final decision, and subsequent larger financial and resource investment, is made. Contractors that do not get Phase 2 funding have still had an opportunity to test the viability of their proposal and to receive expert advice on its potential.
- Supporting the next steps for commercialisation: CRACK IT Challenges has a high return on the number of prototypes, products and services that it delivers. Nevertheless, Contractors often face major barriers to commercialisation that include securing investment to grow their business and building a customer base. To help address this, we established the CRACK IT Advisory Panel[5]. Made up of industry experts and entrepreneurs, the Panel's members give dedicated advice and guidance to Contractors to help develop and support their commercialisation plans. The Panel also oversees the Business Growth Scheme. This was introduced in 2017 to provide awards of up to £50k for Challenges close to completion enabling Contractors to undertake validation studies to build customer confidence, invest in manufacturing and scale-up, and explore other business development activities. One award has been made to date.
- Building confidence to deliver 3Rs impacts: The focus on the end-users' requirements has ensured that we have delivered in a short time-frame 3Rs products and services that are already being deployed. It is clear, however, that we need to do more to support the wider uptake beyond the Sponsors and importantly to build confidence in these new products and services to incentivise others to shift from their existing practices. We have taken a "hands-on" approach. We have launched a new CRACK IT website – the Innovation Platform – to showcase the products and services delivered by CRACK IT Challenges to help raise awareness. We have also organised cross-company validation studies to provide companies with a low barrier entry point for exploring some of the new products, and funded projects through other NC3Rs schemes to demonstrate the feasibility of using the products in different research settings.

[4] This includes contracts for single phase Challenges, and Phases 1 and 2 for two-phase Challenges.

[5] Membership of the CRACK IT Advisory Panel and its terms of reference can be found in Annex 3.

Figure 2: CRACK IT Solutions process



CRACK IT Solutions

The 3Rs impacts of new technologies are not always fully exploited. This is because in many cases, academics and SMEs may not have recognised that their technology could be relevant to the 3Rs, or where they have, they may not have access to the networks, expertise or investment to allow further development. CRACK IT Solutions is a technology partnering hub designed to address this by accelerating the commercial readiness of technologies with 3Rs potential.

CRACK IT Solutions provide a route for academics and SMEs to showcase technologies with 3Rs potential (referred to as “Solutions”) to the wider scientific community, helping to identify new partners and customers to use, develop, and validate the technology. Importantly, it also provides a gateway for industry and others to horizon scan for new collaborators and technologies that support their business.

The process

The process for CRACK IT Solutions is described in Figure 2 (p12). Technology developers contact the NC3Rs through the Innovation Platform website. Proposals are reviewed by the CRACK IT Advisory Panel to assess the 3Rs potential of the technology and subject to this, we help the technology developer prepare a "pitch". This describes the background and status of the technology, its 3Rs potential, and what the Solution provider is seeking in terms of collaborators. The pitch is then disseminated through the Innovation Platform and the NC3Rs networks.

We have showcased 57 Solutions from 23 academics, one government-funded organisation and 33 SMEs. Of these, 43 (75%) of the Solutions focus on technologies with replacement potential, 11 (20%) on refinement and three (5%) on reduction. More than 240 new "contacts"[6] have been made as a result of CRACK IT Solutions.

Seed funding for partnerships

EU-based organisations that form partnerships through CRACK IT Solutions are eligible for funding of up to £50k over 12 months to allow for proof-of-concept work. To date, 21 awards totalling £0.76M have been made, with an additional £0.35M leveraged through the partnerships. The funding has supported 14 collaborations with an SME partner, with three of these involving the SME working with a major company. Just over half (11) of the awards have resulted in the technology being taken up by other groups or additional funding being secured for further technology development. The majority (70%) of the awards focus on replacement. A list of the awards made is in Annex 4.

Lessons learnt

CRACK IT Solutions has been an effective mechanism for brokering and funding collaborations allowing technologies to be transferred between organisations and tested and developed for wider exploitation. Some of the proof-of-concept work required to underpin these collaborations is complex or time-consuming. Recognising this, in 2017 we increased the amount of seed funding available for collaborative projects to £50k (from £30k) per Solution. There is a steady pipeline of Solutions being provided for showcasing, nevertheless, there is more that we can do to encourage technology developers, including researchers funded by the NC3Rs, to use the Innovation Platform website to maximise the impacts of their work for 3Rs benefits. Broadening our outreach is essential and we need to place greater emphasis on helping university technology transfer offices horizon scan for 3Rs-relevant technologies. The Regional Programme Managers that we have appointed are well-placed to facilitate this.

[6] Contacts refer to individuals who have contacted the Solution provider to find out more about the technology, to access it, or to collaborate.

CRACK IT Challenges Case Studies

Cognition: Wireless EEG recording in mice

We have funded the development of an ultra-lightweight wireless electroencephalography (EEG) recording device, called TaiNi, with animal welfare and technical advantages that outperform other available systems used in mice.

Challenge Contractor:
Professor Esther Rodriguez-Villegas

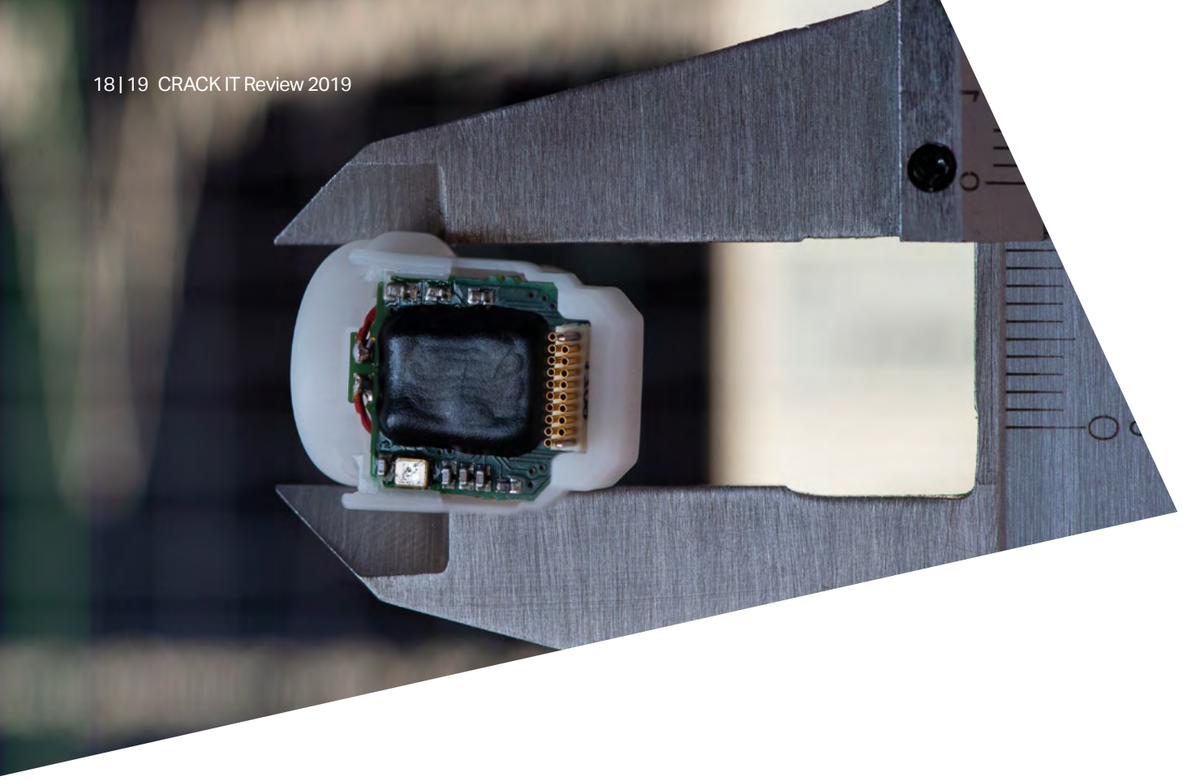
Organisation:
Imperial College London

Start date:
2012

Duration:
3 years

Amount:
£0.5M

Sponsor:
Eli Lilly



The Challenge

EEG recordings are conducted in mice to understand neural activity and how it relates to specific behaviours or cognitive tasks (e.g. memory, learning and decision-making) that are relevant to understanding brain disorders such as schizophrenia and Alzheimer's disease. Studies typically involve the use of tethered recording systems which restrict the mouse's movement and the testing paradigms they can be used in. Alternative wireless recording devices are available, but even the state-of-the-art are heavy for the mice to carry for prolonged periods (equivalent to a human carrying a 6kg weight on their head) and maintaining an upright head posture can be difficult for the animals. The devices also have a limited battery life of around four hours.

To address the animal welfare and technical challenges associated with existing recording systems, Lilly posed the Cognition Challenge to develop an ultra-lightweight EEG recording device for use in awake, freely moving mice in a range of behavioural tests.

The product

The Cognition Challenge was awarded to Professor Esther Rodriguez-Villegas, an expert in low power electronics at Imperial College London. By re-designing the wireless circuitry and focusing on energy efficiency, Esther and her team of engineers worked with Lilly scientists to develop and test a device called TaiNi.

TaiNi weighs just 1.5g (including the battery). It is capable of 72 hours recording from 16 channels and of capturing local field and action potentials from mice performing a range of tasks, with data synchronisation to behaviour with sub-second precision.

3Rs and scientific benefits

Mice wearing the TaiNi device can move freely, exhibit natural behaviours and, unlike with other wireless and tethered systems, can be socially housed. This has animal welfare benefits as well as providing the potential to conduct for the first-time electrophysiology on socially interacting animals. The TaiNi device is lighter than other commercially available wireless devices or tethered recording systems. In studies of spatial working memory using an automated T-maze task, the Lilly scientists compared the number of trials mice completed when wearing a commercially available 4g wireless device, the TaiNi device, or no transmitter. Mice wearing the TaiNi device perform only 6.5% (mean of 47 trials per mouse) fewer trials compared with animals without a transmitter, whereas those with the heavier device perform 35.9% (mean of 31 trials per mouse) fewer trials, illustrating the impact device weight has on the mice. The ability to complete additional trials increases the statistical power and provides the opportunity to reduce the number of animals required for a given experiment.

The improved battery life offered by the TaiNi device – approximately 17 times longer than competitor products – means that mice do not have to be handled as frequently to change the battery (or to untwist the tethered systems). Handling is known to affect mouse welfare and the reliability of behavioural data and the Lilly scientists have shown that using the TaiNi device they can record for long periods of time without disturbing the mice, including over multiple diurnal cycles. These technical improvements have already allowed the identification of the novel phenomena of discrete categories of 140–220 Hz ripple-oscillations that are thought to play a critical role in long-term memory.

Wider impacts

Esther and her team have established a new spin-out company called TainiTec to commercialise the TaiNi device. Since its formation in 2017, they have built a customer base which includes academic research laboratories and pharmaceutical companies. With a £50k award from the NC3Rs Business Growth Scheme the team are working on optimising the production process to ensure that they can meet current demand for the device from European-based organisations and ultimately expand into the US market.

Lilly are working with Esther to apply TaiNi across their drug discovery pipeline. They have so far deployed 16 devices to support a variety of research projects and are investing in new infrastructure, including computer hardware and the re-design of experimental suites, to accommodate their switch from less refined recording devices. TaiNi has been promoted by Lilly to other pharmaceutical companies and collaborators, including through the EU Innovative Medicines Initiative project, PRISM. In 2018, the NC3Rs awarded £75k to a team at the University of Exeter to test the use of the TaiNi device for a contextual memory task, in a collaboration with Lilly scientists to ensure the transfer of skills and experience.

The development and validation of TaiNi was published in *Scientific Reports* in 2017. In 2018, Esther was awarded the Imperial College London Provost's Award for Excellence in Animal Research and the AAALAC/IQ Consortium Global 3Rs Award for Europe.

Sponsor in-kind contributions

Lilly provided a range of in-kind contributions. This included in-house testing of prototype devices in real-world experimental settings, scientific validation studies to build confidence in the device, changes in infrastructure to accommodate the experimental set-up for the device, and the provision of computing hardware for data capture and analytics from the TaiNi device. Lilly also provided 0.75 of a full-time equivalent (scientific and engineering staff) per year and a total financial contribution of £0.15M to support the prototype evaluation.

Rodent Big and Little Brother: Home cage monitoring of rats and mice

We have funded the creation of a system that enables continuous monitoring of group-housed rats or mice in their home cage with automated analysis of their behaviour and activity.

Challenge Contractor:
Professor Douglas Armstrong

Organisation:
Actual Analytics

Start date:
2012 / 2013

Duration:
3 years + 3 years

Amount:
£0.5M + £0.5M

Sponsors:
Rodent Big Brother – AstraZeneca
Rodent Little Brother – MRC Harwell

The Challenge

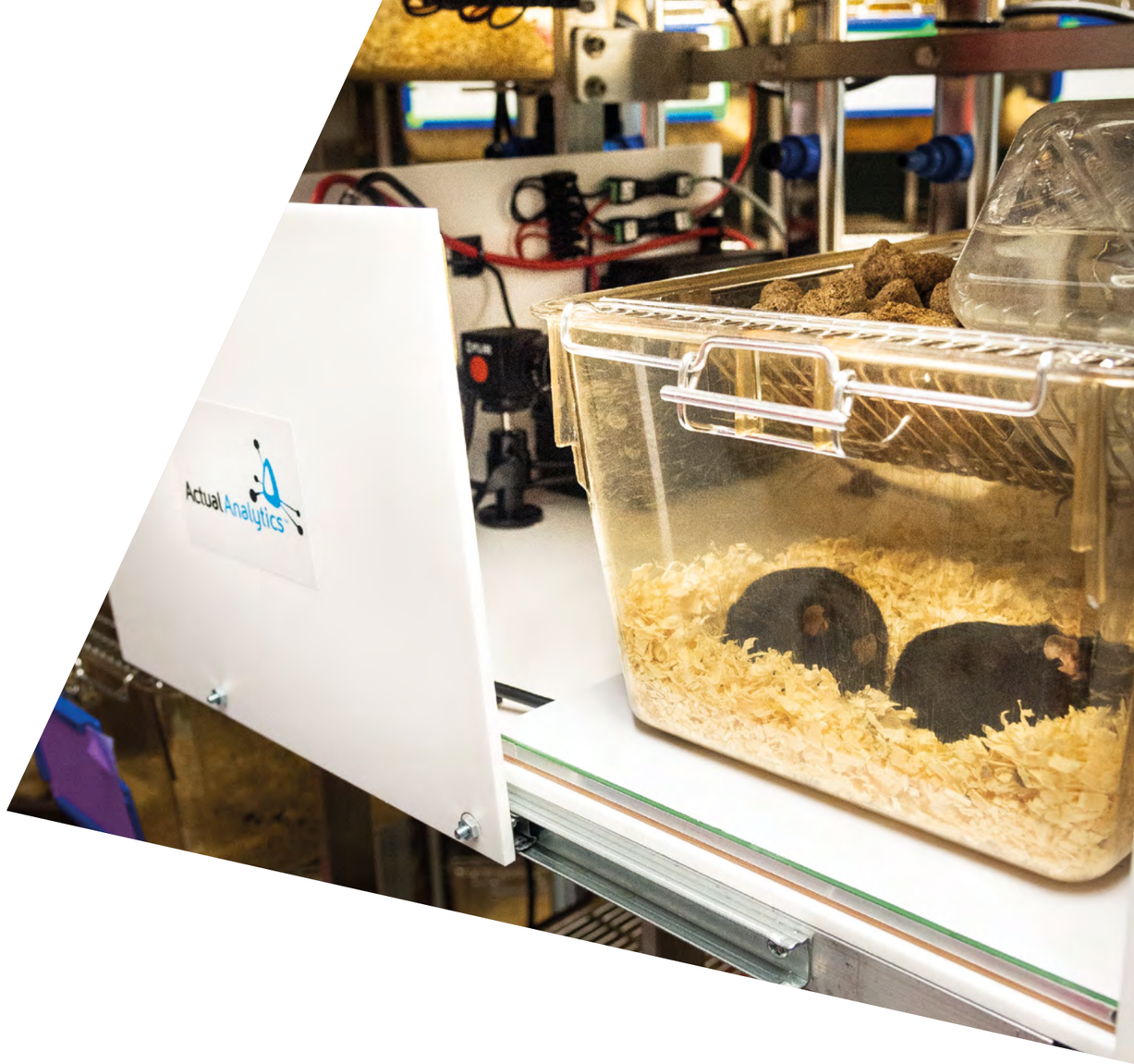
Measurement of the activity and behaviour of individual rats provides critical information in studies from basic research through to drug development. For example, rats are used in safety pharmacology studies to assess potential drug toxicities on the central nervous system (CNS). Locomotor activity is measured in rats placed singly in an unfamiliar test arena, whereas behaviour is assessed manually in the home cage environment or in a testing arena (e.g. the open field test) to detect signs such as lethargy, seizures and sleep disturbances which may be relevant to the clinic. Single housing and unfamiliar environments can affect animal welfare. Observing behaviour is labour intensive and often conducted during the daytime when rats are naturally less active. For some studies body temperature is measured manually or by using surgically implanted telemetry transducers. Depending on the company and the drug being tested, up to 100 rats may be used per drug in such studies.

Addressing the welfare concerns associated with monitoring individual animals, AstraZeneca posed the Rodent Big Brother Challenge to develop an automated non-surgical system, to measure individual activity, behaviour and body temperature of group-housed rats over a minimum of a 24-hour period in their home cage.

The product

The Rodent Big Brother Challenge was awarded to Professor Douglas Armstrong from the Edinburgh-based SME Actual Analytics. Working with AstraZeneca, the team created the Actual Home Cage Analyser (HCA) system, which provides unobtrusive, continuous monitoring of group-housed rats for the full study duration, avoiding the need for single housing and allowing testing in the familiar home cage environment. The HCA system can be incorporated into standard vivarium racks with the position, activity and temperature of individual rats measured by tagging the animals with subcutaneous RFID chips that are tracked using a baseplate containing a series of RFID antennae.

Behavioural data is obtained using high grade video recording and infrared LED lighting placed above the cage. The video analysis software has been trained to automatically detect specific behaviours associated with neuro-behavioural or CNS issues monitored during safety pharmacology studies, including rearing and sleep disturbance. The technology evaluation and verification work were published in *PLoS ONE* in 2017.



3Rs and scientific benefits

Actual Analytics and AstraZeneca validated the HCA system using sedative and stimulatory drugs in dosing protocols designed to compare data between light and dark phases. AstraZeneca demonstrated that the HCA system can detect changes in ambulatory and vertical activity, and subcutaneous temperature, in group-housed rats, consistent with published data. Importantly, the system detected drug effects missed by traditional methods which could have an impact in the clinic. This study was published in the *Journal of Pharmacological and Toxicological Methods* in 2018.

To build further confidence in the HCA system for safety pharmacology studies, the NC3Rs has convened an industry consortium which involves the contract research organisation, Charles River, contributing their time and resources to test three compounds provided by three major pharmaceutical companies.

Wider impacts

The Actual HCA system was adapted for use with mice to address the Rodent Little Brother Challenge posed by MRC Harwell in 2012. Validation of the system was performed by MRC Harwell with three commonly used mouse strains. This revealed novel insights into mouse social interactions and behaviour which have major implications for the choice of strain used and the interpretation of scientific findings. For example, in studies on circadian biology, bouts of activity were identified consistently in mice during the light phase, whereas traditional approaches, which monitor activity using running wheels, show negligible activity during this phase. There were also strain specific alterations in the levels of activity over a 24-hour period that had not been previously detected. MRC Harwell now routinely use the HCA system for the identification of early deficits in mouse models of neurodegenerative diseases helping to better characterise the models and identify humane endpoints to limit animal suffering. To help annotate the extensive behavioural data sets obtained from the HCA system, working with Zooniverse, the NC3Rs and MRC Harwell have launched a citizen science project – a first for the 3Rs.

Through the Rodent Big and Little Brother Challenges, Actual Analytics has developed a global client base of academics and companies. They have gone on to secure CRACK IT Solutions seed funding with collaborators at Queen Mary University London to apply the HCA system to monitor the welfare of rats used in research on spinal cord and traumatic brain injury.

Sponsor in-kind contributions

AstraZeneca provided a range of in-kind contributions. This included in-house testing of prototype systems in real-world experimental settings and scientific validation studies to build confidence in the system. MRC Harwell provided space and caging equivalent to 100 cage-spaces a week (approximately £1k per week in caging revenue).

AstraZeneca and MRC Harwell also provided 0.5 and 0.35 of a full-time equivalent (scientific staff) respectively per year over the project period.

NephroTube: kidney toxicity screening *in vitro*

We have funded the creation of NephroScreen, an organ-on-a-chip platform that models human renal tubular injury and has the potential to replace *in vivo* nephrotoxicity studies in drug development.

Challenge Contractor:
Dr Martijn Wilmer

Organisation:
Radboud University Medical Centre, The Netherlands

Start date:
2013

Duration:
3 years

Amount:
£1M

Sponsors:
GSK | Pfizer | Roche

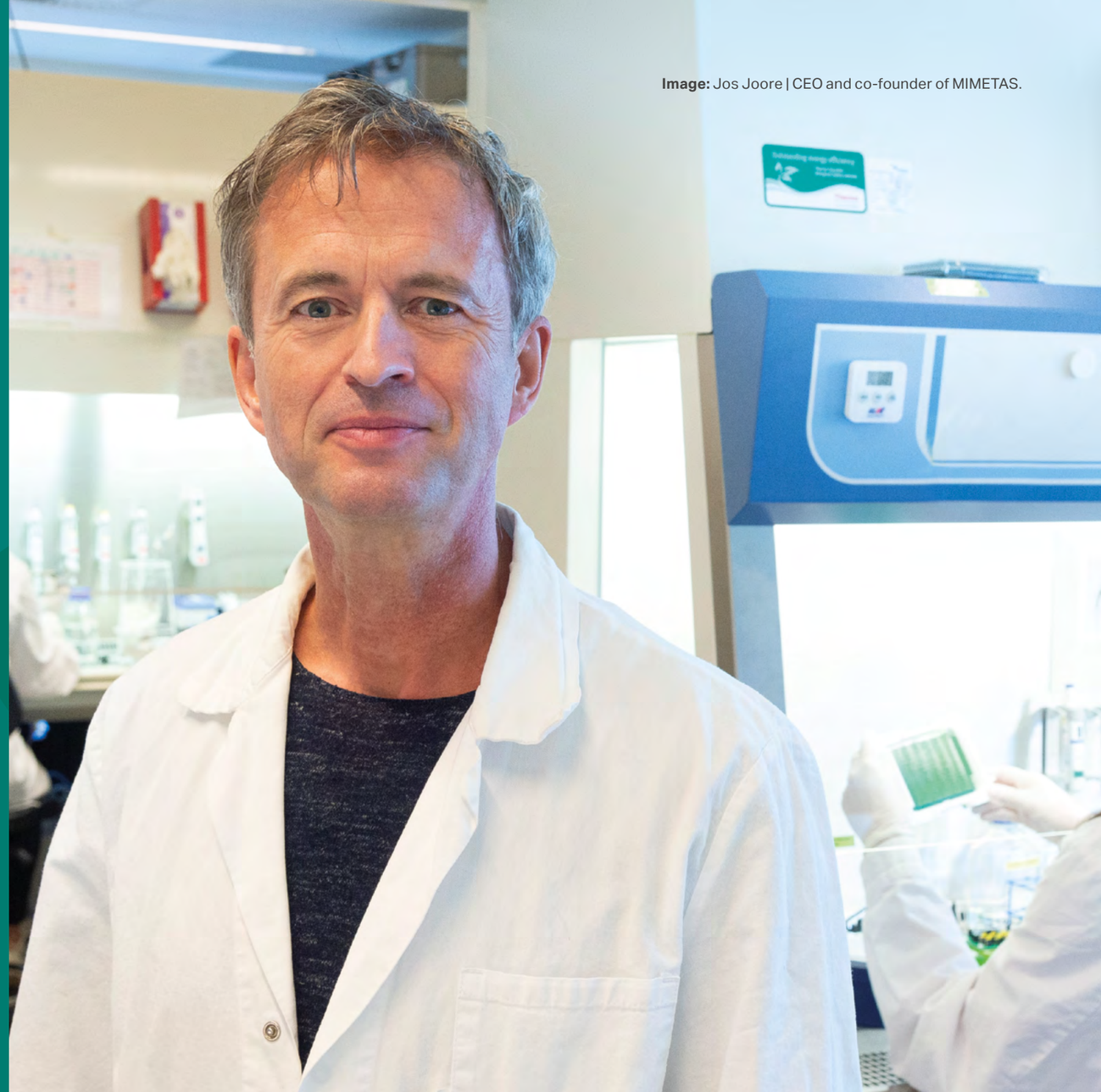


Image: Jos Joore | CEO and co-founder of MIMETAS.

The Challenge

Preclinically, drug candidates are assessed for their nephrotoxic potential using cell assays and subsequently as part of chronic toxicity tests carried out for regulatory purposes, often in two species (a rodent and a non-rodent). Nephrotoxicity accounts for 2% of drug failures in the preclinical stages of development and 19% of all failures in Phase III clinical studies.

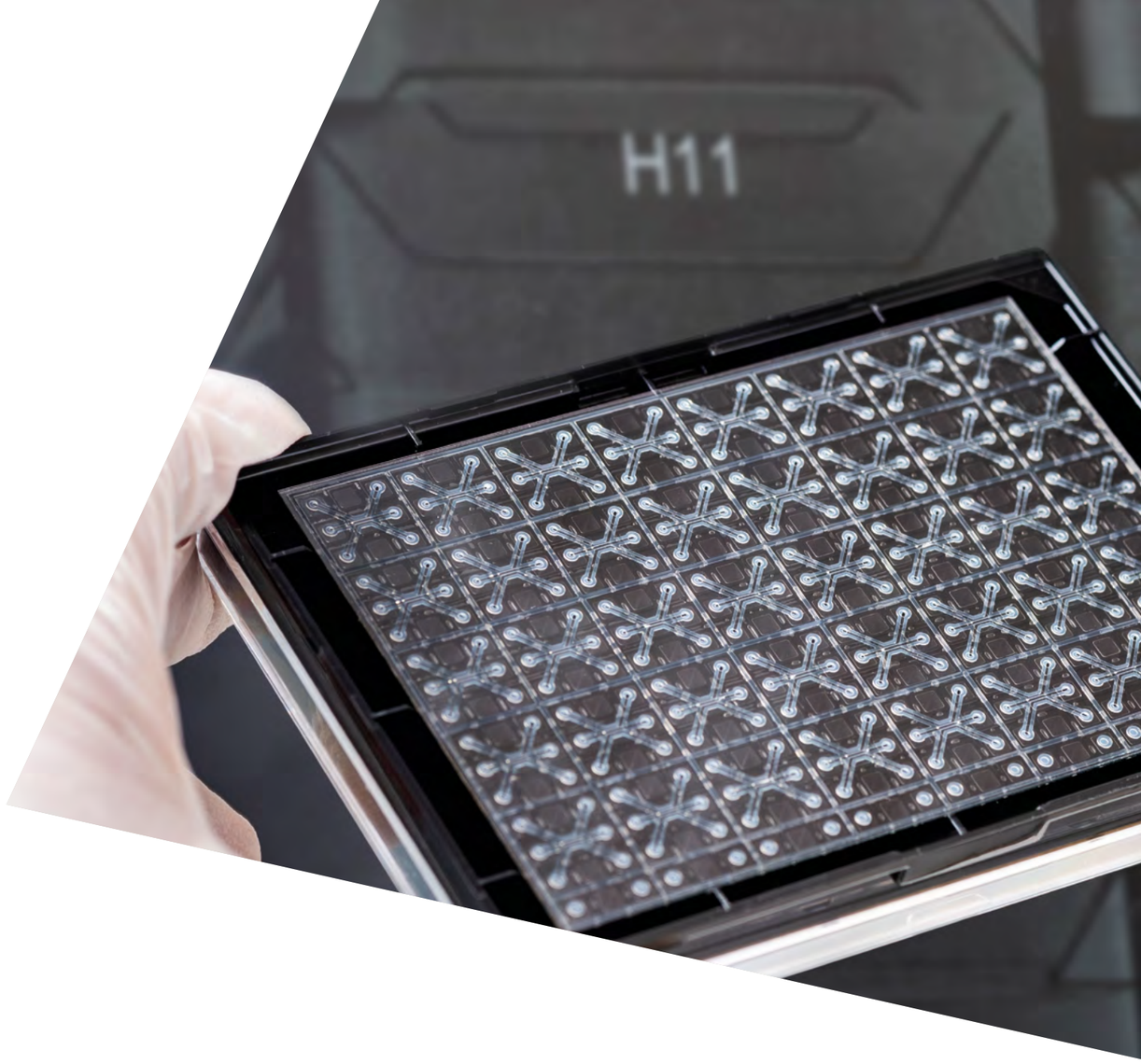
The translational gap between the preclinical toxicology models and their predictive value to the clinic means that compounds are tested in animals rather than being screened out early in development, or that standalone *in vivo* studies are required to investigate the relevance of a nephrotoxic signal.

To minimise the use of animals and address the difficulties of predicting nephrotoxicity, GSK, Pfizer and Roche posed the NephroTube Challenge to develop a complex, human relevant, microfluidic platform capable of accurately identifying nephrotoxic effects.

The product

The NephroTube Challenge was awarded to a team led by Dr Martijn Wilmer at Radboud University Medical Centre in the Netherlands, that included expertise in drug transporters, advanced microfluidics and *in vitro* toxicology.

The team developed NephroScreen, a microfluidic human kidney proximal tubule-on-a-chip platform that can be used as an early *in vitro* screen for drug-induced nephrotoxicity. Consisting of 40 kidney proximal tubules per plate, the platform does not require complex pumps, making it suitable for high throughput and routine drug screening.



3Rs and scientific benefits

The team demonstrated that polarised 3D proximal tubules can be formed from epithelial cell lines cultured in a microtiter plate-based microfluidic chip (termed OrganoPlate®) under fluid flow, with cilia present on the apical side of the tubules. The tubules can be accessed from the apical and basal sides, enabling transporter mediated cellular efflux and influx to be measured. Importantly, the tubules from one of the cell lines used, have barrier integrity with the formation of tight junctions – the disruption of these is a key measure of nephrotoxicity.

NephroScreen was independently characterised at three different laboratories using two proximal tubule human cell lines and validated with 12 nephrotoxic compounds provided by the Sponsors. This demonstrated that the platform can accurately detect toxicities when compared with preclinical and/or clinical data, and that multiple endpoints such as drug transporter function, barrier integrity, cell viability and genetic variations – essential endpoints for assessing drug-induced toxicity – can be measured. Using NephroScreen, the team identified novel indicators of kidney injury, such as upregulated miRNAs, which may be better markers of cell viability than the currently used proliferation assays.

The development and preliminary characterisation of NephroScreen has been described in five publications to date. Workshops have been held to facilitate transfer of the technology to the Sponsors, with supporting Standard Operating Procedures for 20 nephrotoxicity endpoints published online. The utility of NephroScreen is also being evaluated by six other pharmaceutical companies. It is early days to assess the 3Rs impacts but as confidence and experience builds in the use of microphysiological systems, such as NephroScreen, there will be an impact on animal use – in the short-term to screen out compounds prior to *in vivo* tests and ultimately in the long-term potentially as part of a suite of *in vitro* approaches to replace chronic toxicity studies.

Wider impacts

All components of NephroScreen are now available commercially. Martijn has recently co-founded Cell4pharma, a spin-out company from Radboud University, to provide human renal cell lines. For end-users wanting to purchase an off-the-shelf product, MIMETAS, a Dutch SME, is investigating the potential to turn NephroScreen into a single commercial offering to further increase the uptake and reproducibility of the model.

The OrganoPlate® was further adapted for neurotoxicity studies in the Neuratect Challenge posed by Abbvie, GSK and Sanofi in 2014. A team led by MIMETAS was awarded £1M to generate a human stem cell-based model for the assessment of neurotoxicity and seizure liability in safety pharmacology studies. Behavioural observations in animals, usually rats, are used to predict whether a drug may cause seizures in the clinic.

In vitro modelling of seizurogenic activity offers the potential to screen large numbers of compounds in early development and mitigate downstream seizure concerns without using animals. The team developed a microfluidic cell culture platform, termed NeuroScreen 3D, using the OrganoPlate® system coupled with a co-culture of human-induced pluripotent stem cell-derived neurons and astrocytes from Cellular Dynamics International. The cultures form neuronal networks and demonstrate burst activity which can be measured by calcium imaging. Measurements of burst activity showed that NeuroScreen 3D can discriminate between compounds with known seizurogenic activity selected by the Sponsors. The platform provides the ability to multiplex calcium imaging assays with neurite outgrowth and cell viability measurements and integrates single neuron activity detection for highly granular data analysis. The Sponsors are at various stages of evaluating the technology in-house. The components of NeuroScreen are available commercially.

Sponsor in-kind contributions

The Sponsors provided a blinded compound set for nephrotoxicity testing across multiple laboratory sites and related preclinical and clinical data for validation purposes.

Virtual Infectious Disease Research: The Leishmania Virtual Laboratory

We have funded the creation of an *in silico* model of the host response to chronic infection by *Leishmania* parasites, to reduce the number of animals used in the development of drug treatments and vaccines.

Challenge Contractor:
Professor Paul Kaye

Organisation:
University of York

Start date:
2013

Duration:
3 years

Amount:
£1M

Sponsor:
NC3Rs

The Challenge

Large numbers of animals, typically rodents, are used in efficacy studies for new antibiotics or vaccines each year. The animals are infected with the pathogen of interest after vaccination or treated with the experimental drug. Untreated controls are always used. The resulting disease in control animals and those in which the vaccine or drug are ineffective can cause significant suffering. The use of *in silico* approaches to study disease biology and predict efficacy has the potential to replace some animal use.

Addressing the animal welfare concerns associated with predicting drug efficacy, the NC3Rs posed the Virtual Infectious Disease Research (VIDR) Challenge to develop a model of infection and the host response to pathogen insult for use in basic research and target development.

The product

The VIDR Challenge was awarded to a team led by Professor Paul Kaye, an expert in the immunology of the tropical disease leishmaniasis, and his collaborators which included Simomics, a software SME that develops tools for pharmaceutical and life sciences industries. The team developed the LeishSim Virtual Laboratory, a computational model of a mouse spleen chronically infected with the parasites that cause visceral leishmaniasis. The model is parametrised to allow the interrogation of various genetic, immune and pharmacokinetic factors over the time-course of the infection and the impact of potential treatment interventions.

3Rs and scientific benefits

Around 40,000 animals are used globally each year for leishmaniasis drug and vaccine research and development. The LeishSim Virtual Laboratory provides the opportunity to minimise animal use by testing hypotheses *in silico* and prioritising those experiments where an *in vivo* approach is essential. For example, Paul and colleagues have used LeishSim to investigate monotherapy treatments. 1,248 simulations were run over four days to analyse potential knockout phenotypes across approximately 95 independent variables (genes) with each simulation providing data for as many timepoints as required. The comparable study in mice would have taken at least three years to run the assays and required more than 3,500 animals. Using LeishSim, the team were able to identify the best genes to focus on, using 62 mice for the *in vivo* validation of the best "hits" from the model output.

LeishSim has been validated for use with both the major parasite species that cause visceral leishmaniasis (*L. donovani* and *L. infantum*). Generating the necessary parameterisation data has delivered new understanding of leishmaniasis immunology, including tissue-specific alterations in the transcriptome; new understanding of immune changes during and after treatments; and novel data on drug pharmacokinetics that will inform the formulation of topically applied treatments. The model also has the potential to be applied to other forms of leishmaniasis such as post kala-azar dermal leishmaniasis where there is no animal model available.

Wider impacts

The tissue transcriptomic data and histopathology on the early and late response of mice to *L. donovani* infection in the presence or absence of drug treatment, and associated metadata, generated through the VIDR Challenge has been published in *Wellcome Open Research* and whole slide images are available on the global pathology network, LeishPathNet. The samples used to generate the data are banked at the University of York, enabling other researchers to request additional tests on the existing samples rather than conduct new animal studies.

The development of LeishSim has resulted in the growth of Simomics from two to eight staff. The underpinning software can be adapted and customised for other areas relevant to the 3Rs including toxicological risk assessments. For example, Simomics has secured a further £0.2M through CRACK IT for Challenges which focus on *in silico* modelling of reproductive and developmental toxicity (the DARTpaths Challenge) and acute toxicity for the classification and labelling of chemicals (the Maximise Challenge). They also led a consortium awarded £0.45M from Innovate UK (in a competition co-funded by the NC3Rs) to provide a Virtual Fish Ecotoxicology Laboratory for environmental toxicity testing of new and legacy drugs that were tested prior to current environmental regulations coming into force. The Virtual Fish software, developed in partnership with AstraZeneca and scientists at the University of York, integrates mathematical models for toxicity, exposure, uptake and metabolism. It forms the basis of a €10M Innovative Medicines Initiative partnership of nine pharmaceutical companies, academics and European regulators on the intelligent prediction of environmental risks posed by human medicinal products. Over the next decade the partnership is expected to save one million fish, and industry over £500M in unnecessary testing, without compromising environmental protection.

Paul and Simomics are also part of a consortium that has received $148k from the Foundation for Sarcoidosis Research to adapt the model for sarcoidosis, an inflammatory disease which, like leishmaniasis, is characterised by the formation of granulomas.

Sponsor in-kind contributions

This Challenge was sponsored by the NC3Rs. There were no in-kind contributions.

EASE: Eliminating surgical embryo transfer in mice

We have funded the development of a microfluidic device that improves the developmental competence of *in vitro* derived mouse embryos to allow the use of non-surgical embryo transfer (NSET) in the generation of transgenic mice.

Challenge Contractor:
Dr Virginia Pensabene

Organisation:
University of Leeds

Start date:
2016

Duration:
1 year

Amount:
£100k

Sponsor:
MRC Harwell

The Challenge

In 2018, 1.49M procedures were carried out in Great Britain for the creation and breeding of genetically altered mice. Generation of these mouse models involves the transfer of genetically altered embryos into pseudo-pregnant mice. Most transfers are done surgically via a laparotomy which can cause the mice pain. NSET avoids the need for surgery but is only optimised for the transfer of late stage pre-implantation embryos (i.e. blastocysts). This makes it unsuitable for earlier embryonic stages generated through *in vitro* fertilisation (IVF) and pronuclear injection methods, both of which are necessary to create transgenic archives or to manipulate the embryo using CRISPR/Cas9 gene editing.

Addressing the animal welfare concerns associated with surgical embryo transfer, MRC Harwell posed the EASE Challenge to maximise the use of NSET by developing a reliable system for culturing *in vitro* manipulated embryos through to the blastocyst stage.

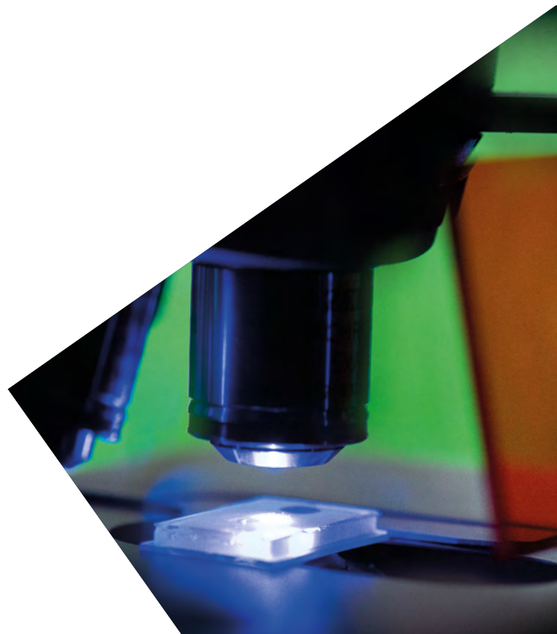
The product

The EASE Challenge was awarded to a team, with expertise in microfluidic technology and reproductive biology, led by Dr Virginia Pensabene. The team developed a microfluidic device that reliably permits the culture of embryos to the blastocyst stage – the technology is cheap, transparent and compatible with standard laboratory equipment. Up to 15 embryos can be cultured in the same device although there is the potential to increase this number.

3Rs and scientific benefits

In this year-long project, the Leeds team and their colleagues at MRC Harwell undertook studies to evaluate the success rate of culturing IVF embryos in the device and to test their subsequent viability (i.e. number of pups born/embryos implanted) when transferred surgically. This demonstrated that there was a high success rate for morula/blastocyst formation from fresh and cryopreserved two-cell IVF embryos and that birth rates of more than 50% could be achieved – consistent with that achieved with IVF blastocysts formed using traditional micro-drop culture.

The number of pups born using morula/blastocysts cultured in the microfluidic device and implanted by NSET was variable. Although pilot experiments showed that birth rates better than those with conventional culture and surgical transfer could be achieved, there is more work to be done to optimise the NSET as part of the collaboration. Ultimately this could replace 90% of the 2,500 surgical embryo transfers performed at MRC Harwell each year. More broadly, given the predicted growth in the use of gene editing approaches, tens of thousands of surgeries could be avoided worldwide by the ability to enhance the formation of blastocysts *in vitro*.



Wider impacts

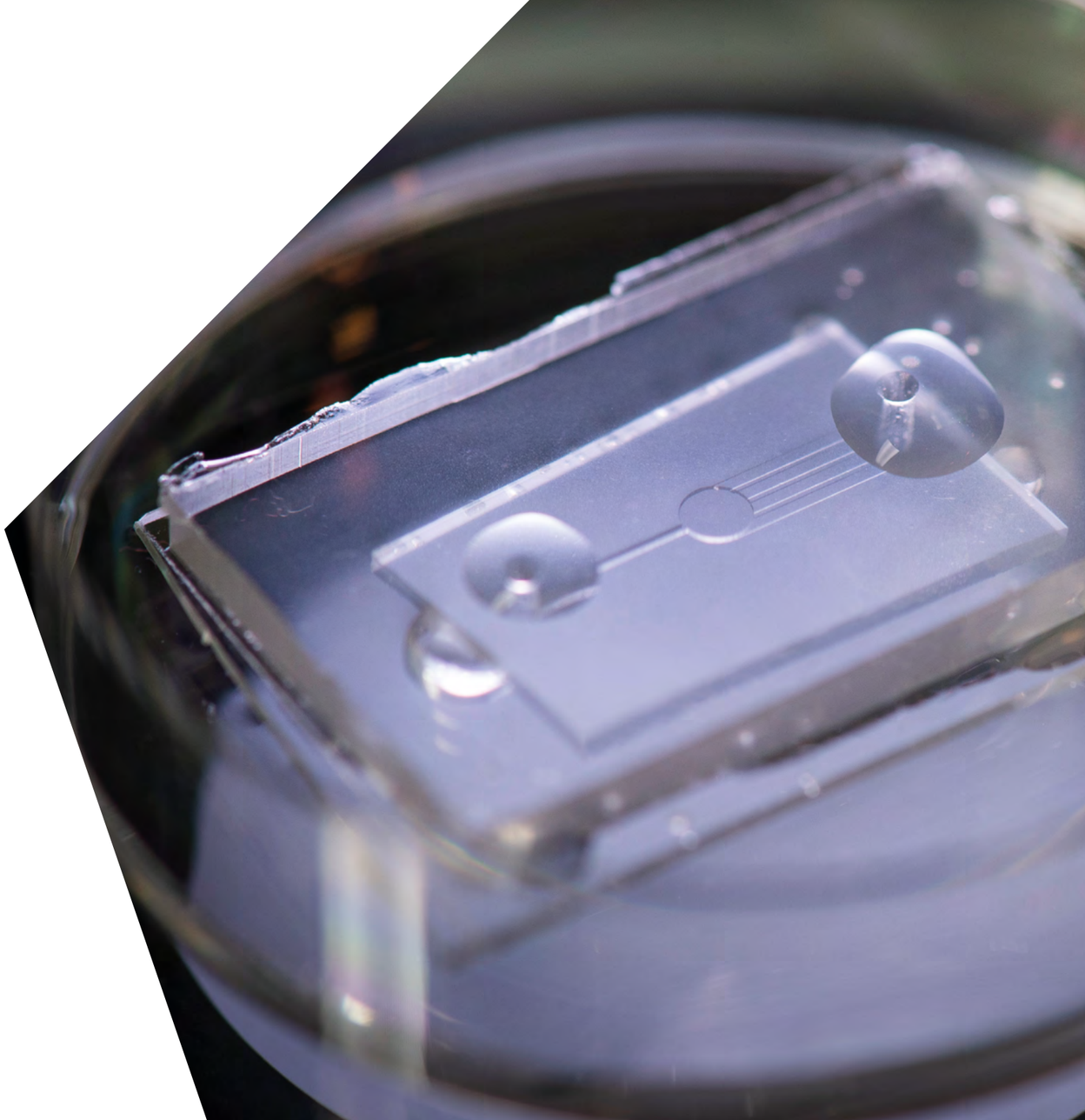
The microfluidic device reduces the exposure of embryos to *in vitro* stressors and improves their viability through to the blastocyst stage by manipulating the volume and composition of the medium, pH and oxygen tension. A functional prototype has been developed and two patent applications have been submitted in the UK to protect the technology. Virginia is in the process of setting up a spin-out company to commercialise the technology.

The device has many applications in developmental biology and to help exploit this Virginia has received £50k from the EPSRC Impact Acceleration Account to make the device compatible with time lapse microscopy. The Leeds team has already used the device in studies to understand the role of endometrial cells in embryo development, and to test the embryo toxicity of polydimethylsiloxane, a silicone used in a variety of products including medicines and cosmetics.

There are also potential non-research applications for the device. This includes clinical and agricultural applications where techniques for IVF and *in vitro* production of embryos are sub-optimal. IVF is an invasive and expensive treatment. There are around 60,000 fertility treatments in the UK each year, with birth rates ranging from 2% to 30% (per embryo transferred) depending on the woman's age. In agriculture, IVF is increasingly used in cattle breeding to improve genetic selection. There is a significant market opportunity and Virginia has been awarded £80k from Grow Med Tech (a consortium of six universities across the Leeds and Sheffield areas) to modify the device so that it is suitable for human embryo culture, and £100k from the MRC's Confidence in Concept scheme to scale-up the system for bovine embryo culture.

Sponsor in-kind contributions

MRC Harwell provided a range of in-kind contributions. This included fresh and frozen one and two-cell embryos for validation and optimisation work, and mice as embryo recipients. MRC Harwell also provided 0.2 of a full-time equivalent (technical staff).



PREDART: non-mammalian testing for developmental and reproductive toxicology

We have funded the creation of phenotypic screens using two model organisms to assess chemicals for developmental and reproductive toxicity (DART).

Solution provider:
Professor Raymond Pieters

Organisation:
University of Applied Sciences Utrecht, The Netherlands

Start date:
2012

Duration:
3 years

Amount:
£0.75M

Sponsors:
Shell | Syngenta

The Challenge

Chemicals and pharmaceuticals are tested for potential reproductive and/or developmental toxicity, usually in two species as specified in OECD and ICH test guidelines for chemicals and pharmaceuticals respectively. The studies use large numbers of rats and rabbits with the standard multi-generation reproductive and developmental toxicity study involving around 2,500 animals per substance. The tests are also resource intensive and expensive because of their long duration and the associated animal housing and husbandry costs.

In academic research, many of the biological phenomena associated with development and reproduction are studied in model organisms such as the nematode *Caenorhabditis elegans* and zebrafish embryos. Nematodes have highly reproducible developmental timings and in zebrafish most of the organs are formed within three days post-fertilisation. These models have 3Rs potential since they are not covered by regulations on the use of animals in scientific procedures[7], however, despite the evolutionary conservation of the molecular pathways involved in development and reproduction, the use of *C. elegans* and zebrafish embryos by industry for DART studies had not been fully explored. Shell and Syngenta posed the PREDART Challenge to develop the use of these model organisms for the early and rapid prediction of DART.

The product

The PREDART Challenge was awarded to a consortium led by Professor Raymond Pieters at the Institute of Risk Assessment at the University of Applied Sciences Utrecht, that included collaborators from four Dutch institutions and the University of Oxford. The consortium developed a phenotypic screen for DART hazard assessment using *C. elegans* and zebrafish embryos (up to five days post-fertilisation).

3Rs and scientific benefits

The phenotypic screen employs assays for various developmental and reproductive effects, including for the nervous, intestinal and reproductive systems. The consortium tested the assays using a range of concentrations of well-characterised DART chemicals identified by Shell and Syngenta. This demonstrated that 28/32 (87%) and 26/35 (74%) of the chemicals tested were correctly identified as having a DART-phenotype by the nematode and fish embryos respectively. Those that were missed by one of the two models were picked up as DART chemicals by the other, such that all chemicals were scored correctly by combinatorial testing using nematodes and zebrafish. Testing in these two model organisms is cheap and fast, taking one week to complete compared to mammalian studies conducted to comply with OECD test guidelines which can take up to 30 weeks.

Achieving full 3Rs impact requires regulatory acceptance and considerably more development and validation work, likely to take many years. But the study shows that the models could be utilised as part of a weight-of-evidence approach to avoid testing in rodents and rabbits for DART purposes and this has generated further interest and investment. A spin-out company, Vivaltes, has been formed as a result of the Challenge to provide DART screening using *C. elegans* (with the sub-contracting of testing in zebrafish embryos). It has conducted assignments from Shell including testing water samples, expanding the application of the screen to ecotoxicology as well as human health.

Vivaltes has received two Dutch development grants totalling €80k to support the commercialisation of the screens and to further validate the *C. elegans* test system against a broader range of chemicals to build confidence in its utility for mechanism of action studies. Vivaltes and the University of Applied Sciences Utrecht are also part of a consortium with Shell working on the €4.28M 3R TOXFLOW project, applying DART testing using *C. elegans* and zebrafish embryos to complex petrochemical mixtures to address the REACH regulation requirements.

[7] Zebrafish are protected, by EU legislation on the use of animals in scientific procedures, five days after fertilisation.

Wider impacts

During the Challenge, the consortium incorporated high content assays into the nematode and fish embryo phenotypic screens as well as conducting some analyses in the social amoeba, *Dictyostelium discoideum*, a model commonly used to study cell signalling pathways. For eight DART chemicals, RNAseq analyses were carried out on the three model organisms. Although different phenotypic outcomes were observed, toxicogenomic profiles were common across the test species identifying potential molecular mechanisms with human relevance. This type of mechanistic information supports the development of Adverse Outcome Pathways (AOPs) relevant to DART. A team led by Raymond was subsequently awarded £1M in the DARTpaths CRACK IT Challenge sponsored by Shell and Syngenta to integrate information on genes involved in DART in model organisms and humans into functional AOPs that can be used to predict DART and select the best non-mammalian model for testing.

Members of the consortium from the University of Oxford have been awarded an NC3Rs grant of £0.31M to develop fluorescent reporter constructs for different developmental events in *C. elegans* and *D. discoideum*, further expanding their utility for mechanism of action data for DART. Through CRACK IT Solutions, the Oxford team have partnered with Syngenta to conduct validation work on the reporter constructs.

Sponsor in-kind contributions

Shell and Syngenta provided a range of in-kind contributions. This included DART compounds for testing, developmental toxicity data from rats and zebrafish for validation purposes and toxicogenomic data. Each company provided 0.36 of a full-time equivalent (scientific staff) over the three-year period.

CRACK IT Solutions Case Studies

Reducing animal numbers in tasks of memory

We have funded a partnership between Dr Alex Easton and GSK to develop and validate a new apparatus for industry use which reduces the use of mice in spontaneous recognition tasks.

Solution provider:
Dr Alex Easton

Organisation:
Durham University

Start date:
2013

Duration:
12 months

Amount:
£30k

Project partner:
GSK

Alex and his team, with NC3Rs funding, had previously developed a continuous trials apparatus for studying spontaneous recognition tasks in rats. The tests are used to assess memory, but the reliability of the data collected can be confounded by environmental factors such as handling which affect the animal's behaviour.

With the continuous trials apparatus, Alex had shown that multiple trials can be run by a single rat in a single session with no handling between trials. This minimises the inter-animal and inter-trial variability and allows the number of rats used per experiment to be reduced. However, with an increasing number of genetically altered mouse models of cognitive decline being produced, the apparatus required adaptation to allow for wider uptake.

Working with scientists at GSK, Alex and his team validated the apparatus for use with mice. This showed that the number of mice used could be reduced by 50%, to eight per experiment. Importantly, by minimising the behavioural noise, the team identified new insights into age-related memory decline in the TASTPM mouse model of early onset Alzheimer's disease. Alex has used the data generated in the collaboration to secure £74k from Innovate UK to work with Campden Instruments, to develop a semi-automated version of the apparatus, that includes a camera to monitor behaviour in the testing chamber and an app for behavioural scoring. In 2019 Alex and Campden Instruments received further CRACK IT Solutions funding to assess the prototype apparatus in a range of mouse and rat recognition studies as part of a collaboration with scientists from four UK universities.

In-kind contributions

Durham University provided matched funding to support a PhD studentship. Access to mouse models of Alzheimer's disease were provided by GSK.

Evaluating a microfluidic assay for botulinum neurotoxin testing

We have funded a collaboration between Dr Greg Stevens and eight partners including academics, small companies and government organisations to validate an *in vitro* test for detecting botulinum neurotoxin.

Solution provider:
Dr Greg Stevens

Organisation:
Albert Ludwig University of Freiburg, Germany

Start date:
2016

Duration:
12 months

Amount:
£30k

Project partner:
toxogen, Germany

The Botulinum neurotoxin LabDisk-Test (BLD-Test) was developed by Greg at Albert Ludwig University of Freiburg. It is a microfluidic assay for detecting botulinum neurotoxin (BoNT), a potent toxin, produced by the bacterium *Clostridium botulinum*, which inhibits neurotransmitter release resulting in potentially fatal paralysis. The BLD-Test detects bioluminescent or fluorescent reporter molecules that are cleaved from naturally occurring substrates of BoNT by the enzymatic activity of the toxin, with the intensity of the reporter signal reflecting the amount of BoNT present in the test sample.

The BLD-Test is automated and can detect the six major botulism-causing BoNT serotypes including in human sera, animal, foodstuff and environmental samples. It has the potential to replace the mouse bioassay currently used, which can cause significant suffering. However, the BLD-Test requires validation to a standard where end-users and regulators can have confidence in the assay's ability to reliably and reproducibly detect, distinguish and quantify BoNT contaminants in complex biological samples.

Working with eight partners, including four from Germany – toxogen, QIAGEN Lake Constance, the research institute Hahn-Schickard and public health Robert Koch Institute – as well as the UK National Institute for Biological Standards and Control, Greg characterised and validated the performance of the BLD-Test. In an international ring trial of the BLD-Test, five participating laboratories could identify BoNT-containing samples (without serotyping) in different concentrations in serum, bean juice and carrot juice in only three hours compared to up to 24 hours for the mouse bioassay.

Around 600,000 mice are used annually for screening and serotyping BoNT specimens, and for assessing the safety of botulinum products for therapeutic purposes. Applying the BLD-Test could substantially reduce the number of mice used for diagnostics. Based on the findings of the ring trial Greg, Hahn-Schickard and toxogen continue to collaborate to further improve the sensitivity, specificity and stability of the BLD-Test, with the aim of marketing it as an alternative to the mouse bioassay. The BLD-Test is protected by European and US patents.

In-kind contributions

A range of in-kind contributions were provided. This included: the production of assay components and tests in the ring trial from toxogen; Hahn-Schickard enabled the microfluidic implementation of the assay; QIAGEN Lake Constance provided processing devices; tests in the ring trial were carried out by five partners, including Robert Koch Institute and National Institute for Biological Standards and Control.

Advancing the application of *Galleria mellonella* in industry

We have funded two collaborations to integrate an insect model marketed by BioSystems Technology into the development pipeline of Demuris, an antibiotic drug discovery company, and to evaluate the potential to replace the use of rats in the acute oral toxicity testing of chemicals at Covance CRS, a global contract research organisation.

Solution provider:
Dr Olivia Champion

Organisation:
BioSystems Technology

Start date:
2016

Duration:
6 months + 6 months

Amount:
£12k + £12k

Project partners:
Demuris + Covance CRS

BioSystems Technology, a spin-out company founded by Dr Olivia Champion from the University of Exeter, was formed to provide research grade larvae of the wax moth *Galleria mellonella* (referred to as TruLarv). Wax moth larvae have been used extensively to study host/pathogen interactions, replacing the use of vertebrate models for some experiments. However, with pet-food grade larvae the main source, concerns about quality assurance and reproducibility have limited wider uptake of this model organism. TruLarv are weight and age defined, surface decontaminated and bred without the use of antibiotics or other drugs, making the model more amenable to industry users.

Working with Demuris, Olivia and her team demonstrated that TruLarv can be used to assess the toxicity and efficacy of naturally occurring antibiotics against Gram-negative and Gram-positive human pathogens. Based on this, Demuris is now purchasing the larvae from BioSystems Technology to evaluate their antibiotic compounds against a range of bacterial pathogens, saving around 200 animals a year.

Collaborating with Covance CRS, the team investigated whether TruLarv can minimise the use of rats in the acute oral toxicity testing of chemicals. They tested 19 chemicals provided by Covance CRS and compared the findings with historic data from rodent acute oral LD50 studies and two *in vitro* cytotoxicity assays. For chemicals classified as non-toxic, the level of concordance with the rodent data was 100% for TruLarv and 44% for the cytotoxicity assays. The findings were published in *Chemosphere* in 2018.

The high rate of false positives with the cytotoxicity assays suggests that fewer animal studies could be conducted by using TruLarv to generate data contributing to the evidence base for waiving testing for some chemicals. Covance CRS estimates they could replace the use of around 450 rats a year with TruLarv and Olivia continues to work with Covance CRS to realise this potential.

BioSystems Technology now export TruLarv to customers in 11 countries. In 2017, they received £100k from Innovate UK for the development of genetic tools to engineer the larvae.

In-kind contributions

Demuris provided crude actinomycete extracts for validation studies. Covance CRS supplied compounds for testing in TruLarv.

An *in ovo* model for safety and efficacy testing

We have connected the biotechnology company Inovotion with AstraZeneca, to test the utility of a chick egg embryo model to potentially replace some mouse xenograft studies in drug development.

Solution provider:
Dr Jean Viallet

Organisation:
Inovotion

Start date:
2016

Duration:
6 months

Amount:
£30k

Project partner:
AstraZeneca

In the *in ovo* model, human tumour cells are grafted on the chorioallantoid membrane (CAM) of embryonated chicken eggs. The CAM is a highly vascularised extra-embryonic membrane that is readily visualised and experimentally manipulated. The model is well established in academic research for studying tumour growth, invasion and metastasis but its use in industry has been relatively under-explored. Inovotion had previously tested 12 different human tumour cell lines and a small number of commercial drugs in the *in ovo* assay but needed a pharmaceutical partner to build confidence.

Working with AstraZeneca scientists, Inovotion conducted proof-of-concept studies to assess the efficacy and toxicity of therapeutic antibody-drug conjugates (ADCs) using the CAM model. Using a cytotoxic pyrrolobenzodiazepine (PBD) warhead bound to a HER2-targeting antibody, a comparison with clinical data showed that very low doses of the ADC could significantly reduce tumour weight in the CAM model, and that total tumour regression was possible at concentrations equivalent to clinically-relevant doses of trastuzumab, the current gold standard treatment.

In a further study, the efficacy of four ADCs using the same antibody, but with different cytotoxic PBDs, was assessed and ranked by comparing tumour weights and metastatic invasion. The ranking from the most to the least efficacious was the same in the CAM as that observed in the mouse model, demonstrating strong correlation.

There are around 60 companies involved in developing ADCs and the market is set to grow at 19% a year over the next four years. With further work, the CAM model has the potential to replace some mouse xenograft studies.

Inovotion and AstraZeneca continue to collaborate on assessing whether the CAM model can reliably predict the toxicity and pharmacokinetic measurements of ADCs.

In-kind contributions

AstraZeneca provided a range of in-kind contributions. This included cell lines, ADCs, conjugate molecules and control compounds; processing and statistical analysis of new data; and historic *in vivo* data for validation studies.

Annexes

Annex 1:

Publications list

Rodriguez-Villegas

Jiang Z *et al.* (2017). TaiNi: Maximizing research output whilst improving animals' welfare in neurophysiology experiments. *Scientific Reports* 7:8086. doi: 10.1038/s41598-017-08078-8

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Leishmania Virtual Laboratory
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Annex 2:

CRACK IT Challenges overview, including Sponsors

2011

Nephrotoxicity
No awards made

Sponsors: AstraZeneca | Roche
Amount awarded: –
Aim/outcome: *In vitro* model of kidney toxicity.

Cognition
Complete

Sponsors: Eli Lilly
Amount awarded: £0.5M
Aim/outcome: **Product:** TaiNi, an ultra-lightweight wireless device for recording neural activity from the brains of mice.

Rodent Big Brother
Complete

Sponsors: AstraZeneca
Amount awarded: £0.5M
Aim/outcome: **Product:** The Actual Home Cage Analyser System for rats – continuous monitoring of group-housed rats in their home cage with automated analysis of behaviour and activity.

Cytokines
Complete

Sponsors: Envigo
Amount awarded: £0.5M
Aim/outcome: Human immune cell-based assay for predicting the safety of therapeutic monoclonal antibodies. Not taken for further development.

IVIVE
Complete

Sponsors: AstraZeneca | Syngenta | Unilever
Co-funders: Defra
Amount awarded: £1M
Aim/outcome: **Prototype:** A zonated hollow fibre bioreactor that closely replicates the architecture and physiology of the liver for toxicity testing.

BADIPS
Complete

Sponsors: Eli Lilly | Janssen
Amount awarded: £1M
Aim/outcome: **Product:** Human-induced pluripotent stem cell lines from multiple family members affected by bipolar and major depressive disorder for disease modelling and drug discovery.

2012

Rodent Little Brother
Complete

Sponsors: MRC Harwell
Amount awarded: £0.78M
Aim/outcome: **Product:** The Actual Home Cage Analyser system for mice – continuous monitoring of group-housed mice in their home cage with automated analysis of behaviour and activity.

ProBE IT
No awards made in Phase 2

Sponsors: GSK
Amount awarded: £0.2M
Aim/outcome: A non-invasive imaging modality to determine the biodistribution of macromolecules *in vivo*.

DRGNET
Complete

Sponsors: Grünenthal | Metrion Bioscience
Amount awarded: £0.95M
Aim/outcome: **Prototype:** Proof-of-concept demonstrated for the collection and distribution of human dorsal root ganglia material.

Annex 2:

CRACK IT Challenges overview, including Sponsors

2012 continued

PREDART
Complete

Sponsors: Shell | Syngenta
Amount awarded: £1M
Aim/outcome: **Service:** A medium throughput non-mammalian test system to pre-screen compounds for developmental and reproductive toxicity.

RETINAS
Complete

Sponsors: GSK
Amount awarded: £50k
Aim/outcome: **Prototype:** Device for refined intravitreal injections in rabbits.

2013

UnTangle
Complete

Sponsors: Alzheimer's Research UK | Eli Lilly | Janssen
Co-funders: Alzheimer's Research UK
Amount awarded: £1.2M
Aim/outcome: **Prototype:** Microfluidic device composed of human-induced pluriopotent stem cell-derived neurons carrying mutations in the tau gene for drug discovery.

InPulse
Complete

Sponsors: GSK
Amount awarded: £1.3M
Aim/outcome: **Products:** *In vitro* assays for assessing drug-induced effects on cardiac contractility including 2D monolayers and 3D engineered heart tissue, and software for measuring contractility.

Inhalation Translation
Complete

Sponsors: GSK | Envigo
Amount awarded: £1.3M
Aim/outcome: **Prototype:** An *in vitro* high throughput screen for identifying compounds which induce a foamy phenotype in alveolar macrophages.

NephroTube
Complete

Sponsors: GSK | Pfizer | Roche
Amount awarded: £1.4M
Aim/outcome: **Product:** NephroScreen – a proximal tubule-on-a-chip for nephrotoxicity studies.

Virtual Infectious Disease Research
Complete

Sponsors: NC3Rs
Amount awarded: £1.2M
Aim/outcome: **Product:** LeishSim Virtual Laboratory – a computational model of the host response to chronic infection by *Leishmania* parasites.

2014

Neuratect
Complete

Sponsors: Abbvie | GSK | Sanofi
Amount awarded: £1.5M
Aim/outcome: **Product:** Neuroscreen-3D – an *in vitro* organ-on-a-chip model for neurotoxicity and seizure liability.

Targeting off targets
No awards made in Phase 2

Sponsors: Dow AgroSciences | Unilever
Amount awarded: £0.28M
Aim/outcome: *In vitro* and *in silico* approaches to predict safety issues arising from chemicals interacting with off-target receptors.

Annex 2:

CRACK IT Challenges overview, including Sponsors

2014 continued

QSARs Mix
Complete

Sponsors	Shell
Amount awarded	£97k
Aim/outcome	**Product:** High accuracy QSARs for predicting the skin and eye irritation potential of chemicals.

2015

Metaboderm
In progress

Sponsors	Dstl | GSK | Unilever
Co-funders	Dstl
Amount awarded	£1.1M
Aim/outcome	A model to assess metabolism and xenobiotic availability in human skin.

InMutaGene
In progress

Sponsors	GSK | Novartis
Amount awarded	£0.98M
Aim/outcome	Human relevant *in vitro* and *in silico* assay(s) to improve the safety of gene therapy products.

2016

Osteo-chip
In progress

Sponsors	GSK
Co-funders	EPSRC | Versus Arthritis
Amount awarded	£1.3M
Aim/outcome	An *in vitro* model of the human osteoarthritic joint.

Retinal3D
In progress

Sponsors	Merck | Novartis | Roche
Amount awarded	£1.3M
Aim/outcome	A 3D model of the human retina.

EASE
Complete

Sponsors	MRC Harwell
Amount awarded	£96k
Aim/outcome	**Prototype:** Microfluidic device for culturing embryos.

Maximise
Complete

Sponsors	Dow AgroSciences | Syngenta
Co-funders	EPSRC
Amount awarded	£98k
Aim/outcome	**Prototype:** *In silico* model for classifying agrochemical mixtures for acute oral toxicity, and skin and eye irritation.

2017

DARTpaths
In progress

Sponsors	Shell | Syngenta
Amount awarded	£1.3M
Aim/outcome	Mapping developmental and reproductive toxicity genes and pathways for cross-species comparison of toxic compound effects.

DoCE
No awards made in Phase 2

Sponsors	Shell | Unilever
Amount awarded	£0.29M
Aim/outcome	Tools to better quantify and control the exposure of chemicals in *in vitro* assay systems.

Annex 2:

CRACK IT Challenges overview, including Sponsors

2017 continued

RespiraTox
Complete

Sponsors	Shell
Amount awarded	£100k
Aim/outcome	**Product:** A QSAR model to assess human respiratory irritancy potential of chemicals.

2018

ImmuLiver
In progress

Sponsors	Sanofi Pasteur EU
Amount awarded	£1.3M
Aim/outcome	An immunologically-competent *in vitro* model of the human liver for assessing Yellow Fever vaccine attenuation.

RaTS
In progress

Sponsors	Galvani Bioelectronics | GSK
Co-funders	EPSRC
Amount awarded	£1M
Aim/outcome	Handheld device for objective monitoring of rheumatoid arthritis progression in rodents.

Moshers
In progress

Sponsors	MRC Harwell
Amount awarded	£99k
Aim/outcome	A device to accurately measure individual food intake in group-housed mice.

2019

Transgene Track
Launched

Sponsors	GSK | Novartis
Budget	£1.3M
Aim/outcome	Quantification method for tracking AAV gene therapies and CAR T-cells *in vivo*.

CleanCut
Launched

Sponsors	Bayer | Novartis | Takeda
Budget	£1.3M
Aim/outcome	*In vitro* model for tumourigenicity studies for safety assessment of genome-edited haematopoietic stem cells.

Sharp and to the Point
Launched

Sponsors	AstraZeneca | GSK | The Royal Veterinary College The University of Sheffield
Budget	£100k
Aim/outcome	Improved needles for refined, high throughput and critical compound administration in mice.

Annex 3:

CRACK IT Advisory Panel membership and terms of reference

The Panel advises the NC3Rs on the CRACK IT programme, including:

- Assessing the Challenges proposed each year.
- Providing advice and feedback to Challenge contractors.
- Assessing applications to the Business Growth Scheme.
- Reviewing CRACK IT Solutions proposals.

Members are appointed for three years.

Member	Institution
Professor Jon Timmis (Chair)	University of Sunderland and NC3Rs Board member
Mr Stephen Browning	Innovate UK
Dr Kathryn Chapman	Milner Therapeutics Institute
Mrs Sue Dunkerton OBE	Knowledge Transfer Network
Mrs Margaret Parton OBE	Innovate UK
Dr Uday Phadke	Cartezia
Dr Martino Picardo	Discovery Park
Dr Malcolm Skingle CBE	GSK

Past members

Dr Diane Harbison	BioCity Scotland
Dr Ian Ragan	NC3Rs Board member

Annex 4:

CRACK IT Solutions overview, including Partners

2013

Dr David Hay FibromEd	Partners Amount awarded Aim	Kirkstall £30k To optimise the culture of stem cell-derived hepatocytes and assess their response to known hepatotoxins.
Professor Robin Williams Royal Holloway, University of London	Partners Amount awarded Aim	St George's University of London | GSK £30k To develop and validate *Dictyostelium* as a model for screening for emetic liability.
Dr Tamer Mohamed University of Manchester	Partners Amount awarded In-kind contributions Aim	Franhofer IME ScreeningPort £30k £32k To validate a cardiomyocyte model for use in cardiotoxicity studies.
Dr Alex Easton Durham University	Partners Amount awarded In-kind contributions Aim	GSK £30k £30k To develop the spontaneous recognition task apparatus for industrial use.

Annex 4:

CRACK IT Solutions overview, including Partners

2013 continued

Dr Dan Daly
Lein Applied Diagnostics

Partners	University of Oxford
Amount awarded	£30k
In-kind contributions	£50k
Aim	To combine confocal microscopy with an ultrasound enhanced drug delivery system for characterising drug distribution in a phantom tissue construct.

2014

Dr Edwina Wright
Ostara Biomedical

Partners	Cancer Research UK London Research Institute
Amount awarded	£29k
In-kind contributions	£10.5k
Aim	To evaluate a prototype vaginal pessary system for pseudopregnant mice.

2015

Dr Davide D'Amico
ZeClinics

Partners	Noldus | Pivot Park Screening Centre
Amount awarded	£30k
In-kind contributions	£21k
Aim	To validate a zebrafish embryo screen against compound sets with known preclinical and clinical toxicity data.

Professor Douglas Armstrong
Actual Analytics

Partners	Queen Mary University of London
Amount awarded	£30k
In-kind contributions	£10k
Aim	To expand the scope of the Home Cage Analyser system, for monitoring of rats with spinal cord and brain injuries.

Dr Gregory Stevens
Albert Ludwig University of Freiburg

Partners	toxogen | Hahn-Schickard | QIAGEN Lake Constance GmbH | Robert Koch Institute National Institute for Biological Standards and Control (NIBSC)
Amount awarded	£25k
In-kind contributions	£95k
Aim	To validate an automated assay for detecting botulinum neurotoxin.

Dr Philippe Cotrel
Avacta Life Sciences

Partners	MRC Harwell
Amount awarded	£30k
Aim	To develop affimers against inhibin as a tool for increasing the efficiency of genetically engineered mouse production.

Dr James Sidaway
Phenotox

Partners	Biogen
Amount awarded	£30k
In-kind contributions	£18k
Aim	To develop a mass spectrometry-based profiling method that can detect the binding of unmodified chemicals for efficacy and toxicity studies.

Annex 4:

CRACK IT Solutions overview, including Partners

2016

Dr Olivia Champion
BioSystems Technology

Partners: Demuris
Amount awarded: £12k
Aim: To validate use of the TruLarv model in antimicrobial drug discovery.

Dr Olivia Champion
BioSystems Technology

Partners: Covance CRS
Amount awarded: £12k
Aim: To compare acute oral toxicity testing in the TruLarv model with existing data from animal and cell culture methods.

Dr Jean Viallet
Inovotion

Partners: AstraZeneca
Amount awarded: £30k
In-kind contributions: £3k
Aim: To validate a chick egg chorioallantoid membrane assay for use in toxicity and efficacy studies of antibody-drug conjugates.

2017

Professor Zhihong Huang
University of Dundee

Partners: Keele University | Thea Pharmaceuticals
Amount awarded: £50k
Aim: To explore the use of optical coherence tomography for monitoring the structure and function of *in vitro* models of corneal wound healing.

Professor Adalberto Merighi
University of Turin

Partners: University of Chester
Amount awarded: £50k
In-kind contributions: £22k
Aim: To test spinal cord slice platforms for studying the effects of transplanting mesenchymal stem/stromal cells into the spinal cord.

Dr Jonathan Mullins
Moleculomics

Partners: Dow AgroSciences
Amount awarded: £50k
Aim: To validate an *in silico* structural/functional platform, against ten hepatotoxic compounds and ten non-toxic compounds.

2018

Dr Marloes Peeters
Manchester Metropolitan University

Partners: MIP Diagnostics
Amount awarded: £49k
In-kind contributions: £5k
Aim: To prepare and validate a biosensor using molecularly imprinted polymer nanoparticles instead of antibodies for the detection of cardiac biomarkers.

Mr Mike Dennis
Public Health England

Partners: The Veterinary Health Innovation Engine (vHIVE)
Amount awarded: £50k
In-kind contributions: £8k
Aim: To improve the usability and uptake of an animal welfare assessment tool.

Annex 4:

CRACK IT Solutions overview, including Partners

2018 continued

Professor Alison Woollard University of Oxford	Partners	Syngenta
	Amount awarded	£50k
	Aim	To demonstrate that the CombiDART non-mammalian model can provide DART alerts for new compounds.

2019

Dr Alex Easton Durham University	Partners	Campden Instruments | University of St Andrews University of Edinburgh | The Open University University of Bristol
	Amount awarded	£49k
	In-kind contributions	£45k
	Aim	To expand the application of the semi-automated continual trials apparatus for spontaneous object recognition tasks and assess the potential for running multiple apparatus simultaneously.

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