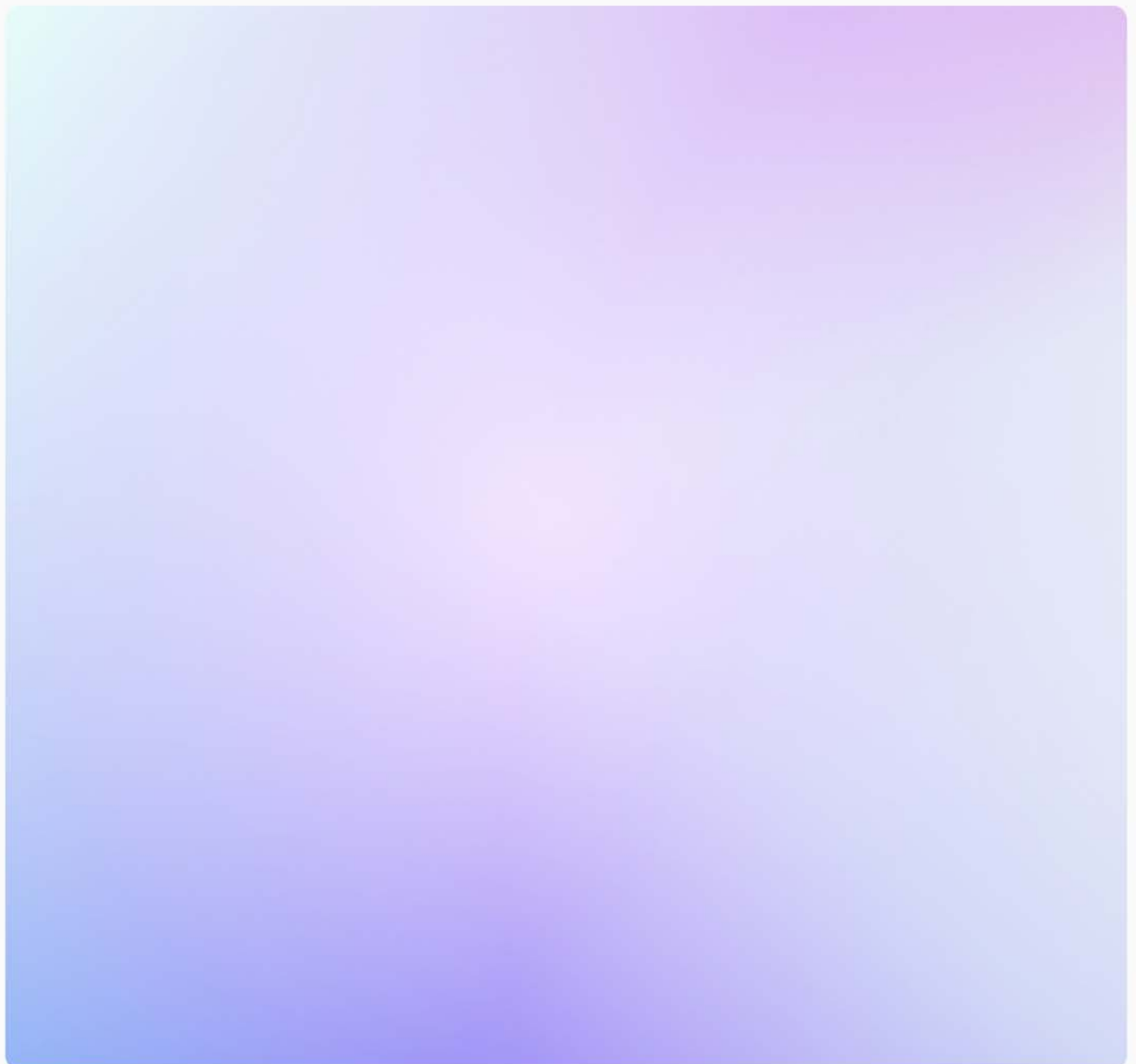


New South Wales AEC Members' Forum

Summary report of the workshop held 16 February 2024



About Understanding Animal Research Oceania

www.uaroceania.org

Understanding Animal Research Oceania (UAR Oceania) is an Australian non-profit organisation that explains why animals are used in medical and scientific research. We support greater understanding of how and why animals are used in medical, veterinary, scientific and environmental research in the Oceania region.

UAR Oceania works to help everyone understand how society benefits from the humane use of animals in research, and works with the scientific sector to ensure that when research uses animals, it meets the high standards of ethical conduct expected by the international research community and the public. We support the life-sciences community across Oceania to be open, courageous and credible in the way it approaches and discusses research, drawing together research organisations, industry associations, professional bodies, charities and others.

For further information or to join UAR Oceania please contact ajlear@uaroceania.org

About the AEC Members' Forum

UAR Oceania, in collaboration with The University of Sydney, has created the AEC Members' Forum which provides a platform for AEC members from across a specified region to meet face to face and discuss key and current issues that they may encounter or need to take decisions on as part of their AEC role.

While all AEC members undergo training for their position, science does not stand still, and neither do the related policy issues. The Forum provides space for AEC members to meet their counterparts from other committees and institutions, deliberate on topics that are emerging, changing or which can be challenging, and to discuss the roles of the AECs. The sessions are participatory and focused on knowledge building and sharing among AEC members.

The aim of the Forum is to strengthen the understanding and networks of AEC members and to support their deliberations on their committees. It does not replace AEC member training required by regulatory authorities, including that provided through ANZCCART's ComPass programme.

If you are interested in holding an AEC Members' Forum in your region, please contact policy@uaroceania.org.

Disclaimer

Opinions expressed in this report do not necessarily represent the views of all participants at the event, Understanding Animal Research Oceania, The University of Sydney or any other AEC Members' Forum partners.

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All web references were accessed in February 2024.

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Executive Summary

This report presents the discussions and findings from a forum focusing on various aspects of animal ethics and research methodologies. The discussions are categorised into four areas: Openness and Animal Ethics Committees, Reviewing Statistics for Animal Ethics Committee Members, Humane Endpoints, and the 3Rs (Reduction, Replacement, Refinement).

Discussion 1, on Openness and Animal Ethics Committees, led by Bella Lear, highlighted the importance of openness in life-sciences research to combat misconceptions and misinformation. The Concordat on Openness on Animal Research in the UK was presented as a pioneering agreement, now adopted in many countries, including in Australia and New Zealand. Open communication was emphasized as crucial for building trust, reducing exposure risks, and supporting ethical responsibilities towards animals and staff. Challenges such as potential negative attention and the need for more resources were acknowledged, but the overall benefits of openness, including staff wellbeing and animal welfare, were deemed to outweigh these challenges.

In Discussion 2: Reviewing Statistics for Animal Ethics Committee Members, Associate Professor Kieron Rooney discussed the importance of justifying the number of animals used in research projects, in line with the NHMRC Code's recommendations. The session focused on understanding the parameters for power calculations in ethics applications and exploring alternatives to power calculations. The need for a checklist to help AEC members evaluate the validity of sample size justifications was highlighted, promoting the minimum use of animals without compromising the validity of results.

Dr. Malcolm France led Discussion 3 on humane endpoints in animal research, emphasising the shared responsibility in ensuring minimal welfare impact while achieving scientific goals. Common humane endpoints and their practical application were examined, alongside the challenges in accurately assessing pain and distress using tools like the mouse grimace scale. The complexity of applying humane endpoints that are both practical and fit for purpose was underscored.

Discussion 4: The 3Rs was led by Professor Kay Double, and included presentations on the principles of reduction, replacement, and refinement in animal research. Challenges in fully implementing the 3Rs, the potential for new funding, and training initiatives in New South Wales, and the potential for a 3Rs Centre were discussed. Four presentations introduced the Forum to current 3Rs research taking place in New South Wales institutions: Micropipette-guided drug administration, the use of *Galleria Mellonella* as an animal model replacement, the UNSW 3Rs Grant Scheme as a unique funding opportunity to support 3Rs projects, and a study to support the reduction of rats used in psychology experiments.

Welcome

The meeting was opened by Dr Susan Maastricht, Director of Ethics and Integrity at The University of Sydney, and Director at Understanding Animal Research Oceania.

She welcomed speakers and participants to Eora Country of the Gadigal people, on whose lands the meeting took place. She continued to welcome them to the first AEC Forum, developed as a collaboration between The University of Sydney and UAR Oceania, to provide a place where AEC members could meet, discuss and learn more about their duties in supporting the ethical and correct treatment of animals in scientific research.

AEC Forum aimed to generate discussion and debate around key topics which create sticking points or uncertainty for AEC members, raising awareness of these topics and suggesting new ways to approach them.

Discussion 1

Openness & Animal Ethics Committees

Bella Lear's presentation introduced openness as a way to protect research from misconceptions, misinformation and disinformation. Showing how the Concordat on Openness on Animal Research, the first openness agreement to be launched, changed the way that life-sciences research is communicated in the UK, she went on to discuss the adoption of this approach in Europe and beyond, with ANZCCART launching Openness Agreements in both New Zealand and Australia. The University of Sydney is a current signatory to the Australian Openness Agreement, while most other research institutes and universities represented at the Forum are considering signing up.

Bella outlined three principal ways in which open communication supports research: it builds trust by sharing ideas and giving research context; it reduces risk of exposure by eliminating secrecy; and research organisations' have a duty to their animals and staff to openly and clearly support the work they do for their institution. Discussion among participants confirmed that the Australian public considers the life-sciences sector to be secretive (ANZCCART survey, 2022).

Openness is particularly relevant to AECs because it shines a light on research practices and invites scrutiny, allowing new and different new voices to be heard. AECs work with continually evolving issues, and to change with them committee members must listen to and be mindful of social concerns.

Challenges that come with openness were acknowledged and three were discussed: that openness can invite negative attention and criticism; that resources to do more communications work are needed; and that many organisations do not know how to communicate using this new approach. Bella emphasised that the benefits of openness outweigh these challenges, including reported impacts of openness on staff wellbeing and inclusion, and on animal welfare. The Forum heard that support is available to help organisations that are new to this work, and that signatories to the Openness agreement and members of UAR Oceania, such as University of Sydney members, qualify for openness-support on request.

In an interactive session, participants discussed the state of play of openness in their different organisations. Most participants felt their AEC's organisations' were moving towards greater openness at a pace which is about right, while four out of the 25 participants who commented felt their organisations were not progressing quickly enough.

The majority of respondents in the interactive session felt that initial progress towards openness was best achieved through internal communication or website content. Participants views of the challenges openness created for their organisations were themed and are shown in **Figure 2**. Key challenges were identified around coordination and resources, but components of communications such as messaging as well as seeking clarity on the necessary culture change that supports an open approach were also seen as important.

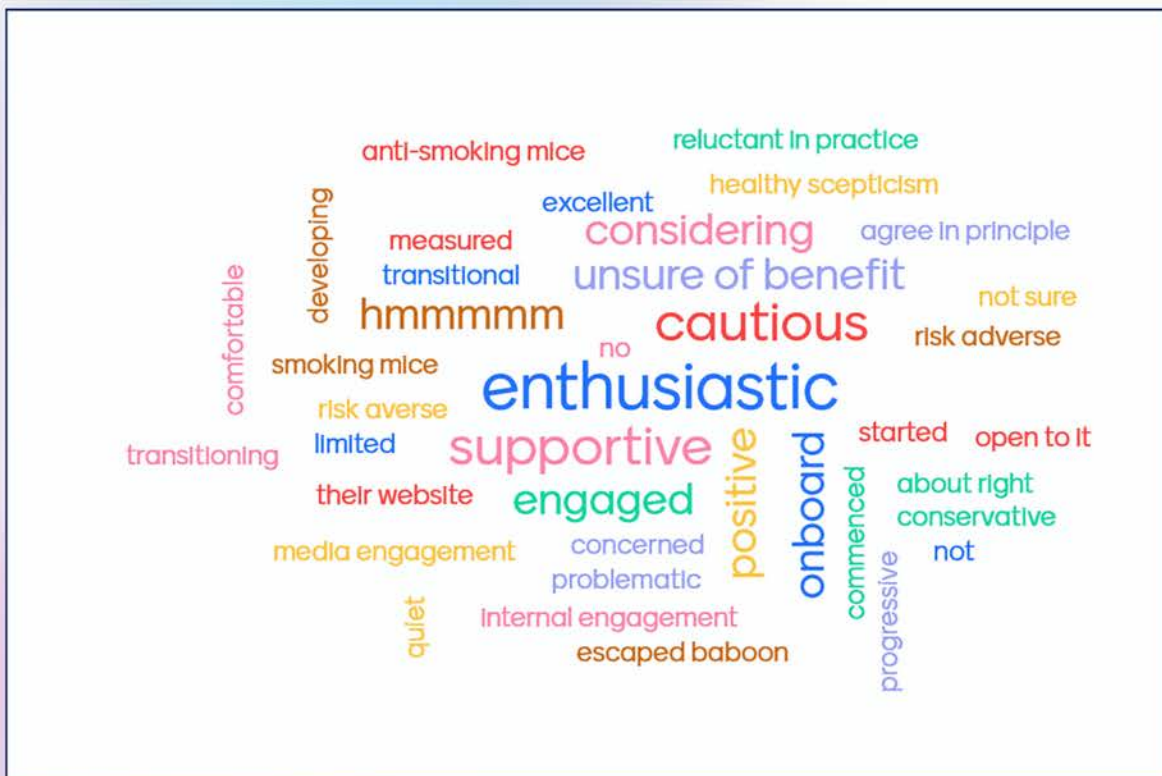


Figure 1. A word to describe your institution's approach to openness

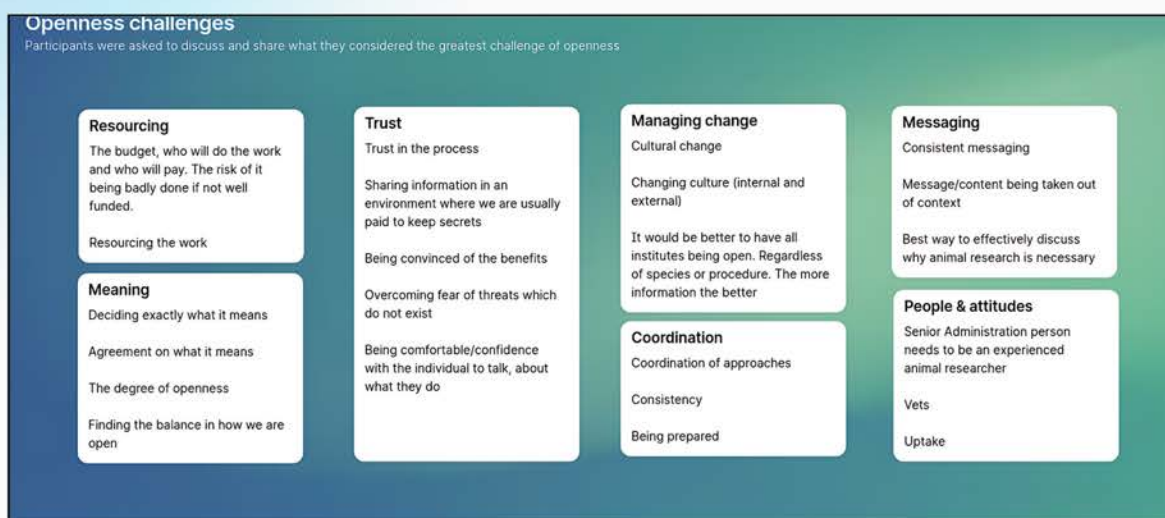


Figure 2. Themed openness challenges

Discussion 2

Reviewing statistics for Animal Ethics Committee Members

This session was led by Associate Professor Kieron Rooney, from The University of Sydney, who introduced the topic by citing the NHMRC Code's recommendations on statistics in project licence applications.

The Code 1.5 (iii)

Evidence to support a case to use animals must demonstrate that the project involves the minimum number of animals required to obtain valid data.

The Code 1.21

The number of animals used in a project must be the minimum necessary to achieve the proposed aim(s) and to satisfy good statistical design. The use of too few animals may invalidate the experimental result and result in wastage of animals.

In this session, participants were supported to:

- Identify the key parameters of a power calculation that should always be stated in an ethics application that uses power calculations to justify animal numbers
- Discuss alternatives to power calculations and their general acceptability
- Employ a checklist developed to help AEC members identify the most valid approach to justifying the number of animals requested in the context of the study design and validity of the study

Dr Rooney showed the session participants that the determination of the number of animals needed to validate a study (the sample size) is intrinsically linked to the study design, and that often a power calculation is not the most appropriate method of determining how many animals should be used.

A power calculation is specifically required for determining the sample size needed for a statistically significant effect in hypothesis testing. In fact, the most appropriate method for determining the number of animals needed to validate a study is intrinsically linked to the study design. Many studies presented to AECs are exploratory rather than hypothesis testing, and so do not require a power calculation to determine the appropriate sample size.

When a power calculation is used, participants were shown that even when well-articulated in the licence application, the power calculation is only as accurate as the assumptions behind the inputs. Further, many project applications fail to specify the primary outcome of their investigation, even though the power calculation is only relevant to a particular (primary) outcome.

The following preliminary checklist for sample size was shared with workshop participants. A final version is in development and will be made available to NSW AEC members soon.

Sample size check list for AEC members

Has the study identified itself as a hypothesis testing study?

1. Has a primary outcome upon which the significance, design and implementation of the study been justified?
2. Has an estimated sample size been requested using a power calculation?
3. Have each of the six critical parameters been articulated?
4. Has the mean and standard deviation and/or effect size and assumption of direction been based on animal data from a similar age, sex, and strain?
5. If not, has a reasonable attempt been made to justify similarity between the data used and the animal requested?

For studies that do not use power calculations, and utilise literature (either single papers or reviews):

1. If presenting a review, was it conducted in a systematic approach?
2. If not, have steps regarding how the investigator ensured their justification is based on the totality of evidence available and not just a selection of supporting studies been provided?
3. Does the literature provided investigate the same primary outcome as that proposed?
4. If not, has an adequate attempt to justify why the literature used is relevant been made?
5. Does the literature provided investigate the same primary outcome as that proposed, in a similar animal model to that requested?
6. If not, has an adequate attempt to justify why the literature used is relevant been made?

Figure 3. Sample size check list for AEC members

Discussion 3

Humane Endpoints: When is enough enough?

This discussion was led by Dr Malcolm France, who discussed humane endpoints, how they are defined and their scrutiny by AECs.

A humane endpoint of a study was defined as “a pre-defined point at which an animal’s involvement in a study is concluded in order to minimise the welfare impact while still achieving the scientific goals”. Ensuring that appropriate humane endpoints are adhered to is a shared responsibility of the researcher, the animal ethics committee, animal welfare officer / vet and the animal facility team.

Malcolm considered some common humane endpoints in detail, encouraging participants to consider the “3Ps”, that is whether each endpoint is **p**rospective, **p**ractical and fit for **p**urpose.


The assessments of pain and distress in animals, can easily be misinterpreted. The mouse grimace scale is now frequently used as a tool for assessing humane endpoints, and appears intuitive and straightforward, but are assessors as able to accurately determine indicators of pain as they believe?

Using demonstration images of mouse “pain faces”, used for training, Forum participants were invited to try their own assessment using the mouse grimace scale. In this test, more than 85% of participants assessed a mouse in severe pain as experiencing no pain at all in two of the five images presented, demonstrating that in most cases the mouse grimace scale is only fit for purpose when accompanied by a suitable standard operating procedure (SOP) and appropriate training.

Participants were invited to consider how other humane endpoints are used in studies or research areas:

- >15% body weight loss
- Decrease in food or water intake
- Heart or respiratory rate
- Lab-based fish research (what humane endpoints apply?)
- Activity or behavioural signs
- Neurological signs

The discussions showed the complexity of applying humane endpoints that are practical and fit for purpose. While the commonly used decrease in body weight is often reliable, it is not fit for purpose in all studies, and in more complex protocols a full discussion with the AEC and animal care team may be needed to ensure that humane endpoints are truly fit for purpose.


















**NC
3R^s**

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

The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice.

The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment. The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
<p>Orbital tightening</p> <ul style="list-style-type: none"> • Closing of the eyelid (narrowing of orbital area) • A wrinkle may be visible around the eye 			
<p>Nose bulge</p> <ul style="list-style-type: none"> • Bulging on the bridge of the nose • Vertical wrinkles on the side of the nose 			
<p>Cheek bulge</p> <ul style="list-style-type: none"> • Bulging of the cheeks 			
<p>Ear position</p> <ul style="list-style-type: none"> • Ears rotate outwards and/or backwards, away from the face • Ears may fold to form a 'pointed' shape • Space between the ears increases 			
<p>Whisker change</p> <ul style="list-style-type: none"> • Whiskers are either pulled back against the cheek, or pulled forward to 'stand on end' • Whiskers may clump together • Whiskers lose their natural 'downward' curve 			

Read the original paper:
Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingnao J, Klassen-Ross T, LaCroix-Fralish ML, Matsunoya L, Sorge RE, Sotoocanal SG, Tibaka JM, Wong D, van den Maagdenberg AMJM, Ferrari MD, Craig KD, Mogil JS. 2010. Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* 7(9): 447-449. doi:10.1038/nmeth.1455

For guidance on using the Mouse Grimace Scale, research papers that underpin this technique, and for grimace scales in other species, visit: www.nc3rs.org.uk/grimacescales. To request copies of this poster, please email: enquiries@nc3rs.org.uk. The NC3Rs provides a range of 3Rs resources at: www.nc3rs.org.uk/resources. Images kindly provided by Dr Jeffrey Mogil, McGill University.

Figure 4. The mouse grimace scale: frequently used to determine humane endpoints

Discussion 4

An overview and four short presentations on the 3Rs

The 3Rs in New South Wales: what will it take to make advances? Professor Kay Double, The University of Sydney

Professor Kay Double introduced the 3Rs of reduction, replacement and refinement (Russell, W.M.S. and Birch, R.L.,1959) as internationally accepted principles for humane animal research, embedded in both the Australian Code for Care and Use of Animals for Scientific Purposes (2013) and Best Practice Methodology in the Use of Animals for Scientific Purposes (2017).

But while the 3Rs are recognised by the scientific community, their concepts are incompletely understood, and their implementation is limited. This is shown by a lack of public 3Rs strategies held by Australian universities, and the low number of Australian universities (37%) that reference the 3Rs.

Full implementation of the 3Rs in Australia is impacted by a lack of:

- 3Rs research
- funding
- data sharing
- training and mentoring
- cultural expectations

The first national centre for the 3Rs (NC3Rs) was founded in the UK in 2004, and since then 3Rs centres have been founded across Europe and around the world, including North America and further afield. In Australia ANZCCART is the only current dedicated 3Rs organisation (Norecopa, 2024).

A group led by The University of Sydney and University of New South Wales, supported by a research consortium of NSW universities and research institutes, together with the support of research sector stakeholders and the International 3Rs Centres is currently seeking support to establish a 3Rs centre in New South Wales. Such a centre would bring significant benefits and opportunities for progressing the sustainable and ethical use of animals in research, such as:

- New technology
- Education and training
- International reputation
- Community confidence in research outcomes and animal use



Figure 5. Proposal for a 3Rs Centre in New South Wales

The micropipette-guided drug administration (MDA) procedure: a safer alternative to oral gavage in rodents

Dr Benjamin Rowlands, The University of Sydney

At present, oral gavage which administers a compound directly into the stomach of a rodent is the most widely used method for precise oral dosing in experimental studies using mice, and is used in 89% of cases (Chamorro et al., 2023). But it is a technically complex method that requires considerable training, and which must be performed by competent handlers to prevent distress and injury to the animals. This short talk presented micropipette-guided drug administration (MDA) as a less invasive alternative to oral gavage. With MDA the mouse voluntarily ingests the compound from the end of a micropipette, so that an accurate dose of a compound such as a drug can be reliably delivered.

In a pilot study carried out in 2001, rodents were trained over a two-day period to drink a sweetened solution from a micropipette. On the first and second day of training, the mouse was placed on the metal grid of a food hopper and restrained solely by the base of the tail. The mouse was exposed to the solution by offering the pipette tip (P100-200) to the mouse until it began to drink voluntarily. Once treatment with drug solution began the rodents no longer required any restraint, but drank the solution voluntarily. The team aimed to ensure the animal had a positive experience, which was important for ensuring that they latched onto the micropipette and drank all the solution.

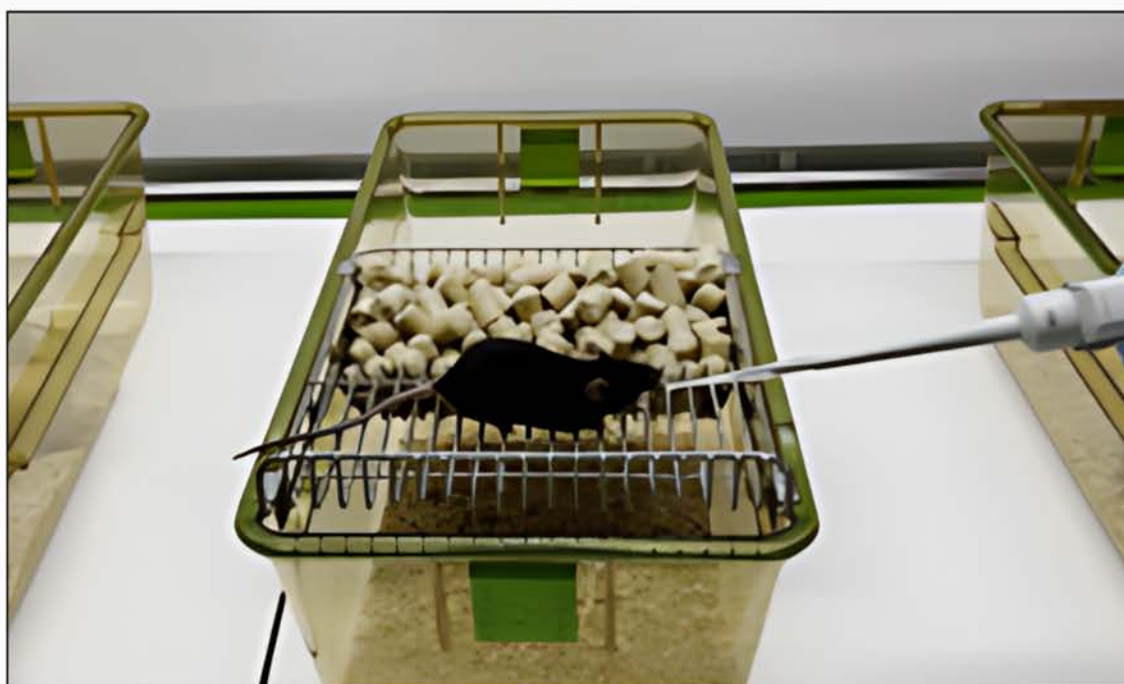


Figure 6: A trained mouse drinking from a micropipette



Figure 7: Image by Dr. Benjamin Rowlands, The University of Sydney

MDA is a non-invasive, voluntary, cost-effective, low-impact and flexible alternative to oral gavage. The mice show no increase in the stress hormone corticosterone. As this technique is not dependent on pH, and chemical stability, it can apply to a broader range of compounds than oral gavage and it is easy to master. This means it can be quickly and safely adopted by new staff and students without the risk of oesophageal, tracheal, or stomach perforations. However, because this method relies on the mouse voluntarily drinking the solution, compliance is dependent on the palatability of the drug being given.

Around 500 animals have now been treated using this method to replacement gavage, across five research projects at The University of Sydney.

Galleria Mellonella: a safe, cost-effective and ethical animal model alternative

Associate Professor Amy Cain, Macquarie University

Associate Professor Amy Cain introduced the Forum to the work of the Galleria Research Facility at Macquarie University. While relatively unknown in Australia, the larvae of *Galleria mellonella*, the greater wax moth is an established invertebrate species replacement model, used in the study of drug toxicity and efficacy, virulence of bacterial strains and gene function with mutants in the UK and Europe. The Macquarie Galleria Research Facility is Australia's first fully functional, high throughput facility for *G. mellonella*, where drug toxicity and antimicrobial efficacy can be tested using a plentiful, non-sentient insect model in 3-10 days, depending on the assay used.

Amy explained that *G. mellonella*, first used as a toxicity testing model in the 1950s, and an infection model in the 1970s, saw greater uptake among researchers when research-grade larvae became commercially available in the UK in 2015. Sequencing of the *Galleria* genome in 2019 has now accelerated its popularity as a robust and reliable research model.



Figure 8. *Galleria mellonella* caterpillars

The large caterpillars (around 200 mg) can be easily injected without using microscopes. Their simplicity and ease of use means this model requires little resources, and can provide an effective bridge from *in vitro* to *in vivo* research without the ethical or administrative burden of moving directly into vertebrate animal models. As a tropical species, the caterpillars can be used at 37°C to mimic human body temperature. Although they have no adaptive immune system, their innate immune system is relatively advanced, and because their genome has been fully sequenced they can be used with genomics methods, as well as in drug toxicity and efficacy studies.



Figure 9. The melanisation response of *G. Mellonella*

At Macquarie, the caterpillars are reared at around 30°C and 60% humidity using flexible light-dark cycles, taking about six weeks to grow to injection stage (200 mg). The larvae can be injected easily into each of their prolegs with up to 10 µl of solution, and a frequently used protocol is to inject a pathogen into one side and a drug into the other.

A health check scoring system is used to determine their response after 5 or 10 days depending on the assay. A key benefit of the Galleria model is that when they start to mount an immune response a melanisation effect turns them from white to black (**Figure 8**), and this immune response is a simple marker which removes any ambiguity over whether they were impacted by a test-pathogen. For bacterial or pathogenicity studies bacterial load counts and efficacy counts can be made, while antibiotics can be studied by analysis of the hemolymph, which is analogous to blood in the caterpillars. Other studies concern whether co-infection of pathogens increases their virulence. Using Galleria it is possible to show that some pathogens affect the Galleria more in combination, and these can be confirmed against *in vitro* results.

Amy concluded with brief examples of Galleria use in current and future studies, including partnerships with the University of Queensland to screen compounds in their open source drug library and identify those with anti-microbial properties, and with Brunel University, London to test treatments for small burn wounds, and screening studies that can help to identify key questions and parameters for more detailed follow-up studies in mice.

The University of New South Wales' 3Rs Grant Scheme, an initiative of UNSW Research Infrastructure

Karen Brennan, University of New South Wales

Karen Brennan, Director of Animal Services at University of New South Wales (UNSW), gave a presentation on the University's 3Rs grant scheme, which offers specific funding to support 3Rs initiatives at the University, or by the University's partner institutions.

Launched in 2019 and funded by the Pro Vice Chancellor of Research Infrastructure's Office, UNSW's 3Rs grant scheme is unique both nationally and internationally. The fund allocates \$250,000 annually to specifically support proposals for 3Rs projects, which are evaluated on their scientific merit, potential to advance the 3Rs, expected impact and feasibility. Over the lifespan of the scheme, \$1.16 million has now been awarded for projects whose primary focus is developing the 3Rs in Australia.

While most other 3Rs awards available both in Australia and worldwide recognise completed work, this scheme supports the development of new methodologies and innovations by funding future projects. Grants of up to \$100,000 for 3Rs-focused projects lasting between 12 to 18 months are available to UNSW researchers, researchers in affiliated institutes and their external collaborators.

The scheme funded three projects in its first round (2019), and two in its second round (2020). It continued to run through COVID, but in 2020 and 2022 a number of projects focusing on the development of organoids as alternatives to animals were submitted. In response, some of the 3Rs funding was diverted to building and seeding a core facility for 3D cell culture, which is now complete and providing a service for 3Rs projects focused on organoid development across the university (examples shown in **Figure 10** and **Figure 11**).

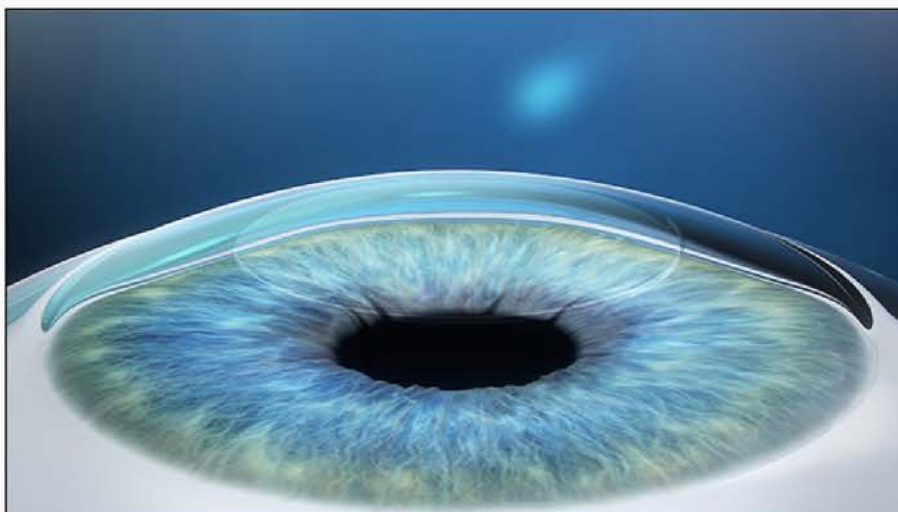


Figure 10.

Lenticule samples from normal human corneas are a by-product of the refractive surgery called SMILE and can be used as a matrix in a bio-engineered 3-D cornea model to replace the use of animals to study pathophysiological mechanisms in a dish. Image: Prof. Nick Di Girolamo, UNSW.

The last funding round saw two years of funding combined. Twenty-eight proposals were submitted around around half of which involved 3D cell culture techniques such as organoids or the development of immortalised cell lines to replace a specific animal model. Other proposals used bioengineering, or imaging tools to gain more information from animal models or replace them completely, and two very interesting proposals aimed to reduce the number of animals used for the production of reagents for research by replacing components of those reagents with plant-derived materials, a particularly interesting approach in light of the shift towards cell culture and use of organoids. Of these proposals five projects were awarded, to a combined sum of \$495,000.

As Chair of the Award's Evaluation Panel, Karen told the Forum that they would also encourage future proposals focusing on the replacement of animals with non-sentient organisms or lower sentient organisms, and the use of tissue sharing through the development of biobanks. Another interesting approach could be to develop technical competencies, by supporting people to learn new refinement skills that have been developed elsewhere, and bring them back to use in their laboratory and disseminate amongst the local research community.

It is not common to find such a generous scheme to support the 3Rs from a non-government body anywhere in the world, and this scheme enables fantastic outcomes for developing infrastructure and supporting the 3Rs. With a comprehensive review process and clear guidance to encourage innovative proposals, the scheme sets a precedent for 3Rs support.

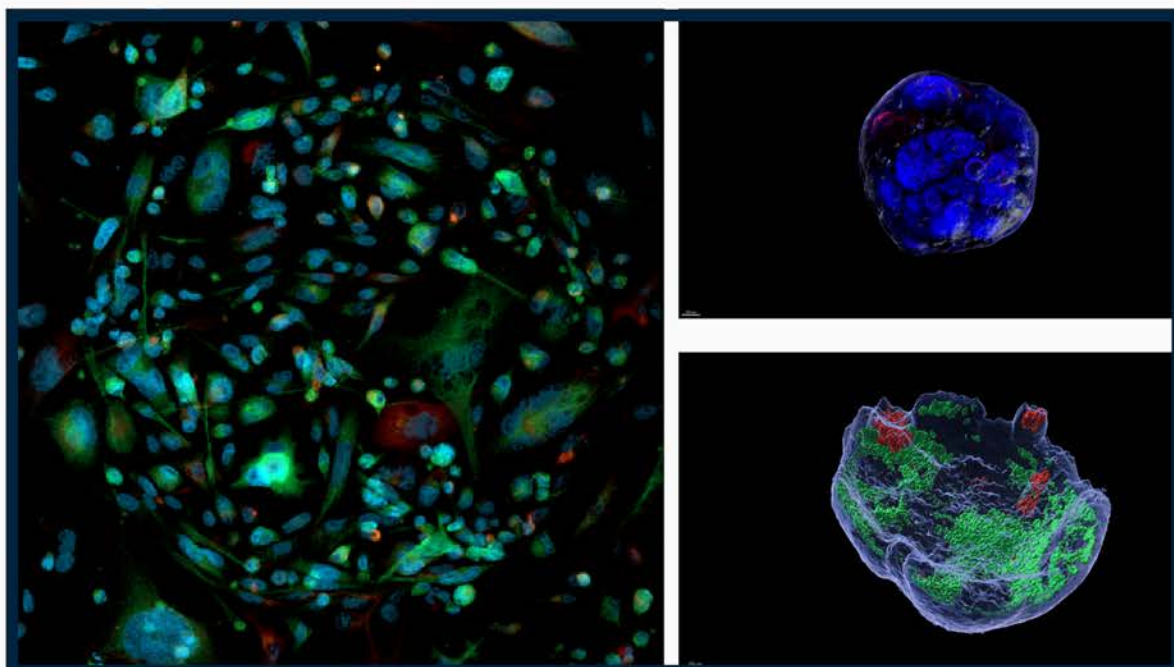


Figure 11. Cerebral organoids imaged at 5x, and 20x depicting self-organisation of the different cell types present. Blue (DAPI), Green (Beta-Tubulin III) and Red (cytokeratin). Images were obtained using the Zeiss LSM 900 and rendered using the Imaris software. Images by Dr. Alison Ferguson, 3D Culture Facility, UNSW.

A study on the Reduction Principle: Can laboratory rats be reused?

Dr Justine Fam, University of New South Wales

Justine, a research fellow at the University of New South Wales, was unable to attend the Forum in person, and sent a presentation to be shown at the end of the lunch break. Her presentation played to a full room, preceding the afternoon sessions.

Because psychology studies rely on being able to identify the neurological basis of behaviours in animals, many 3Rs approaches, such as tissue culture, are unsuitable. Instead, this 3Rs project looked at the feasibility of reducing the numbers of rats needed for research. Thousands of rats are used for psychology studies each year, and many are naive animals that are used for a single study and culled at the end of the experiment.

Justine's study sought to challenge an entrenched norm: that experience of a prior experiment will contaminate an animal's behaviour, so that naive animals provide better data. Although reuse of rats in psychology is believed to be detrimental to the integrity of the data, other animals are reused in research. The aim of this project was to change researcher attitudes towards rodent reuse by providing experimental evidence that rats can be reused, with a potential outcome of saving hundreds of rats every year.

The first study looked at whether a rat's participation in a fear-conditioning experiment would impact outcomes if it later took part in a reward conditioning experiment. Unsurprisingly, rats showed less reward-response to a tone if they had been fear conditioned to that same tone, but if a different stimulus was used in reward conditioning the results were similar to those seen in naive rats, indicating that changing the stimulus used for conditioning across experiments enables rats to be reused.

The next pair of experiments examined the impact of fear conditioning on naturalistic exploration and vice versa. In these experiments some rats received fear conditioning and fear testing as before, and these reused rats showed an equivalent proportion of time exploring objects to rats that were naive, showing that rats that have been fear conditioned can be reused for tests of naturalistic exploration and vice versa.

Justine's study showed clearly that rats can be reused in behavioural testing. She demonstrated that simply using a different stimulus across experiments permits the reuse of rats, and that if the behaviours concerned are largely unrelated then the reuse of rats is possible without compromising the quality of the data collected. She hopes to encourage a change in attitudes regarding the reuse of rats and to inspire researchers to think creatively about what they can do across experiments to enable rats to be reused.



Figure 12. Brown rats in their home cage. Image by [Understanding Animal Research](#)

Annex I

Agenda

9:30 - 10:00	Arrival and refreshments
10:00 - 10:30	Welcome and introductions
10:30 - 11:30	Discussion 1: Openness
11:30 - 11:40	Comfort break
11:40 - 12:40	Discussion 2: Statistics
12:40 - 12:45	Morning wrap-up
12:45 - 13:45	Lunch
13:45 - 14:45	Discussion 3: End-points
14:45 - 15:15	Break
15:15 - 16:15	Discussion 4: 3Rs Update
16:20 - 16:30	Closing comments

Annex II

Speakers

Dr Susan Maastricht

Director, Research Integrity & Ethics Administration, The University of Sydney
susan.maastricht@sydney.edu.au



Susan is a post-graduate qualified veterinarian who has worked in the vocational education, animal shelter, university and research sectors as a senior executive, manager and leader, with responsibility for the operation and management of complex scientific, educational and welfare facilities. She has extensive experience in human and animal ethics and welfare and has served on multiple advisory, ethics and management committees and Boards. She is past president of several industry associations. Holding qualifications in business and teaching, Susan has held executive or senior management positions responsible for educational, research and welfare outcomes for the past 10 years. Her work focuses on integrity, and empowering individuals and teams to be accountable in their own domain.

Bella Lear

Chief Executive, Understanding Animal Research Oceania
ajlear@uaroceania.org



Bella is a science communicator, and social researcher, who supports positive social change around scientific issues. As Head of Engagement at Understanding Animal Research, Bella created stakeholder and public engagement initiatives to change thinking about animals used in research. She was an instigator of Concordat on Openness on Animal Research in the UK, which she led for many years, as a way to drive open and constructive communication between the research community, policy makers and the public. Now leading Understanding Animal Research Oceania, Bella provides communications support to build better understanding and representation of animal-based research in Australia, New Zealand and the Oceania region.

Ass. Prof. Kieron Rooney

Associate Professor, Sydney School of Health Sciences, The University of Sydney

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Kieron co-leads the Bias in Research Node of the Charles Perkins Centre, is Deputy Chair of the NSW Animal Research Review Panel and has been a category B member of the University of Sydney Animal Ethics Committee since 2015. He is an ambassador for the Systematic Review Center for Laboratory animal Experimentation (SYRCLE) and was a member of the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs, UK) working group that revised the ARRIVE guidelines (2018-2020). Kieron is a registered Nutritionist and has utilised both small animal models (rodent) and large animal models (human) investigating the role of diet and physical activity on parameters of fuel storage and utilisation as they pertain to dysregulated metabolic states.

Dr Malcolm France

Independent consultant

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Malcolm France is a consultant veterinarian specialising in the care and management of animals used in research. He has served as an animal facility director, chair of two Animal Ethics Committees, reviewer for the international journal *Laboratory Animals*, and ad hoc consultant for the accreditation organisation AAALAC International. He has also served in honorary roles for several industry bodies, including as inaugural president of ANZLAA, and is currently project officer with ANZCCART where he manages the Australian Openness Agreement and other initiatives.

Prof. Kay Double

Professor of Neuroscience, Brain & Mind Centre, The University of Sydney

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Kay heads the Neurodegeneration Research Laboratory. Her work identifies cellular pathways responsible for brain cell death in degenerative disorders, including Parkinson's disease, motor neurone disease, and dementia disorders, and developing and testing treatments to slow or halt the disease process. Her group recently identified a new type of abnormal protein in Parkinson's disease which is thought to be associated with brain cell damage. She is also developing novel methods to image disease-associated changes in the brain and spinal cord to improve diagnosis and monitor treatment effects. She leads multidisciplinary, international research groups, and in addition to her research awards, Kay is an award-winning supervisor of Early Career Researchers and students.

Dr Benjamin Rowlands

Research Fellow, School of Chemistry, University of Sydney

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Dr Benjamin Rowlands earned his doctorate from the University of New South Wales in 2020, mentored by Professor Caroline Rae. Following this, he worked as a postdoctoral researcher at the University of Sydney under Professor Kay Double's guidance where he worked on an animal model of Parkinson's disease until the end of 2022. Currently, Ben is a Research Fellow at the University of Sydney's School of Chemistry under the supervision of Professor Michael Kassiou where he is using a preclinical rat model of a developmental motor coordination disorder.

Ass. Prof. Amy Cain

Associate Professor of Molecular Biology, Macquarie University, Sydney

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Amy K. Cain is an ARC Future Fellow and Associate Professor of Molecular Biology in the School of Natural Sciences and the ARC Centre of Excellence in Synthetic Biology at Macquarie University. She has worked in academic groups, hospital labs and in the pharmaceutical industry across Australia, Cambridge and Oxford in the UK, Malawi (sub-Saharan Africa), and Boston (USA). Her current research focuses on developing new-to-nature antibiotics and uncovering bacterial stress response systems in hospital pathogens using functional genomic techniques. She is the founder and Director of the Galleria Research Facility, an ethical and cost-effective *in vivo* model for testing pathogenicity of microbes, and toxicity and efficacy of new drugs.

Karen Brennan

Director of Animal Services, University of New South Wales

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Karen has 29 years of experience managing research animal facilities and transgenic services in Australia and Germany, focusing primarily on the creation and analysis of genetically modified mouse models of human disease. She is passionate about providing ethical animal resources and services to the scientific community and promoting responsible use of animal models through effective production strategies, the application of ethical principles (3Rs) and awareness of reproducibility issues in animal experimentation.

Dr Justine Fam

ARC DECRA Fellow, School of Psychology, University of New South Wales

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Justine is a postdoctoral researcher in the School of Psychology UNSW with over 12 years experience investigating neural mechanisms that support learning and memory using animal models. Her work pairs neuroscience techniques with detailed analyses of behaviour for a better understanding of these mechanisms. After gaining a PhD in Psychology in 2016, Justine was awarded an ARC DECRA Fellowship in 2020. She leads several projects, examining the effects of oxytocin on learning and memory, the ways in which unhealthy diets can change taste perception, and how we can reduce animal use in psychology experiments.

Annex III

Participating organisations

The University of Sydney

Understanding Animal Research Oceania

Australian National University

Taronga Zoo

The Garvan Insitute

University of New South Wales

Virbac (Australia) Pty

University of Technology Sydney

University of Newcastle

Children's Medical Research Institute

University of Wollongong

Ingham Institute

Macquarie University

Charles Sturt University

Heart Research Institute

ANZLAA

Dept. Climate Change, Energy, the Enviornment & Water

Annex IV

Participant feedback

Overall experience

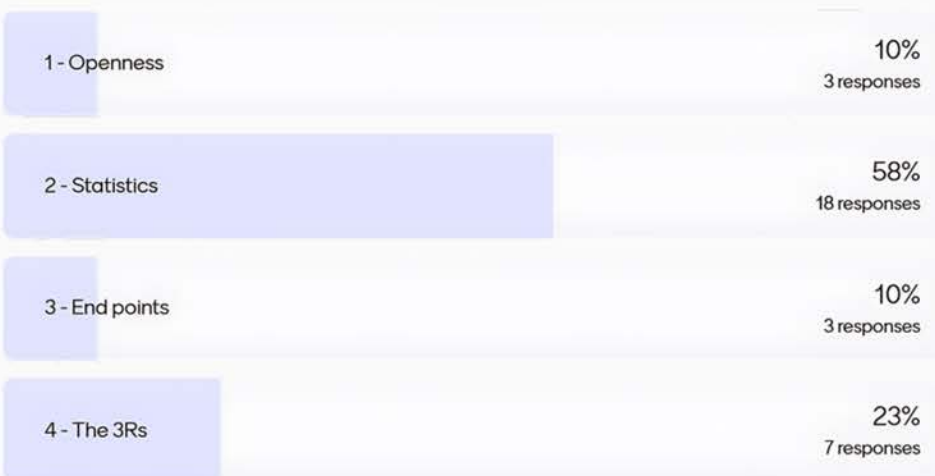
> Marks out of 10 for the programme today



> Marks out of 10 for facilities, food and venue



Favourite discussion



Participants enjoyed

Malcolms talk	Meeting members from other institutions	Networking
Galleria	Networking opportunities at breaks	Variety of topics
Connecting with people	Variety of speaker topics	Interactive sessions all round
I enjoyed most sessions. Lots of breaks also good.	Talking to others	Catch-ups
The presenters and subjects were excellent	exposure to new ideas,	The 3Rs - fantastic stuff
All	Getting to know other people in this space	Calibre of presenters was excellent
Meeting others	Galleria	Variety of topics
Learning	Networking	Making new connections
Portuguese tarts!	Great day	Working in group discussions, networking, learning new things from presentations
Chance to meet/discuss with members of other AECs	Hearing people's perspectives on different topics	Involvement of audience
Meeting other people interested and engaged in this area and learning new things that will be helpful in my AEC work	Discussion around the need to justify the number of animals for a study	Meeting other members . Was surprised there were only 6 C and D members present ?
Excellent networking opportunity	Excellent networking opportunity	Hearing about the progress in the 3Rs
Learning new things	All the sessions!	Understanding other institutions experiences
Discussions	Chatting to other colleagues. Listen to others and had good open discussions. Learnt a lot from the talks	Statistics and 3Rs session
Discussions		End points discussion
challenging previous concepts	Seeing other perspectives	Getting it right is a challenge
End points	I really enjoyed meeting people within the industry. All the sessions gave lots of great ideas and food for thought and most of the sessions were interactive	More attendees
Looking forward to next one		

Participants learned

Valuable stats info	Galleria	End point information
Mice have different types	Power analyses!	Galleria
Information about alternative to power calculation	How to evaluate animal numbers	Power analysis is not relevant to every study
Statistics	We need a better way to define an endpoint	More stats are good for us
Good review of stats was given.	Humane endpoints assessment	NSW 3R centre establishment plans is exciting
A little bit more about statistics	Importance of statistics	Stats = the frenemy
Potential new research models, statistics for research	shake off of previous concepts	Factors to determine number of animals
Ethics committees work hard but need more support to help with understanding statistics and understanding the species	About 3rs centre	I hadn't really thought deeply about humane endpoints and would like to put more thought into this space. I also learnt about 3Rs initiatives which was exciting!
What to look for in a power calculation. Upcoming 3Rs	New technique replacing oral gavage. This would benefit so many animals and reduces their suffering when gavage is not applied properly	End points

Even better if...

We had more time for questions	Bourbon with lunch	we could have more of these sessions and if everyone came along!
Finished on time	Finished on time	More AEC members attended
Went for 2 days	More institutions participated	Less surveys
More networking	Drinks afterwards to increase mixing	Don't allow speakers to go overtime
Forewarning to revise openness agreement	More time, more case studies	Handouts before hand so can jot down notes
Cooler aircon	Can fit in more sessions	There was a category E person added to ethics committee to help with project approvals
The openness session was prefaced with some more outlining of the actual agreement and what it means, a lot of people were pretty confused and didn't know what they were discussing/ commenting on	Props	Forums are held more often
	there could be more sessions in future	We have more time to mingle with others. Perhaps more activities where we can move to different tables.
	More networking time/ opportunities to continue important discussions	
	This was great. More please.	

Final thoughts

Nil	This was great!!!!	Thank you for putting this on
Thanks Bella, this was really great	Good job to all the presenters!	Nada
thanks, really enjoyed the day	Thank you so much to Bella and all the presenters/organisers, it was an absolutely fantastic day!!	Would love more training opportunities like this again soon
If program details can be made available earlier for promotion	Well organised	Really enjoyed the day thanks, it was much more useful than I expected 😊
Great program	Caution re grouping re gender	It was a very helpful event- thanks for organising!
Thanks for a great day . Just the right amount of info . Interesting speakers . Great topics . More please	Thankyou. There are so many passionate people. It is ashame that in reality politics gets in the way of good progression moving forward on better research	Not all animal ethics considerations are about rodents. I think we would benefit from expansion.

Annex V

Selected references & resources

Openness

Concordat on Openness on Animal Research in the UK
<https://concordatopenness.org.uk/>

ANZCCART Australian Openness Agreement
<https://anzccart.adelaide.edu.au/openness-agreement>

ANZCCART 2nd report on the [New Zealand Openness Agreement](#)

Lear, A (2024) Changing Openness Agendas in Animal Research, In *Researching Animal Research*.
<https://doi.org/10.7765/9781526165770.00002>

Whittaker, DRA et al. (2022) Australian Community Attitudes towards the use of Animals in Research. <https://anzccart.adelaide.edu.au/ua/media/664/attitudes-animal-research-survey-report2.pdf>

Mouse grimace scale

NC3Rs Mouse Grimace Scale, hard copy resource: <https://www.nc3rs.org.uk/3rs-resources/grimace-scales/grimace-scale-mouse#paper>

Langford DJ et al. (2010). Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* 7(6): 447-449. [doi:10.1038/nmeth.1455](https://doi.org/10.1038/nmeth.1455)

3Rs

Russell, W.M.S. and Burch, R.L. (1959) *The principles of humane experimental technique*, London: Methuen & Co. Limited.

National Health and Medical Research Council (2013) *Australian code for the care and use of animals for scientific purposes*, 8th edition. Canberra: National Health and Medical Research Council.

National Health and Medical Research Council (NHMRC) 2017 (Updated July 2018), *Best practice methodology in the use of animals for scientific purposes*.

UNSW 3Rs Grant Scheme <https://research.unsw.edu.au/unsw-3rs-grant-scheme>

Norcopa website with details of 3Rs centres and events worldwide <https://norecopa.no/>

NC3Rs is the UK 3Rs centre. Their website contains many valuable resources for driving the 3Rs www.nc3rs.org.uk

NA3RsC is the North American 3Rs Collaborative, with a wide range of free resources.

The Macquarie Galleria Research Facility <https://www.mq.edu.au/faculty-of-science-and-engineering/departments-and-schools/applied-biosciences/our-facilities/galleria-facility>

Dinh, H et al. (2021) Microbiology's next top model: Galleria in the molecular age, *Pathogens and Disease*, 79 (2), <https://doi.org/10.1093/femspd/ftab006>

Chamorro, M. B., Swaminathan, G., Mundt, E., et al. (2023). Towards more translatable research: Exploring alternatives to gavage as the oral administration route of vaccines in rodents for improved animal welfare and human relevance. *Lab Animal*, 52, 195–197 DOI: [10.1038/s41684-023-01232-y](https://doi.org/10.1038/s41684-023-01232-y)



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