



Department of Primary Industries

ANIMAL RESEARCH REVIEW PANEL

3R's Webinar : 6 June 2023 : Additional Q&A document

The introduction to The [Australian Code for the Care and Use of Animals for Scientific Purposes](#) (the Australian Code) promotes the ethical, humane and responsible care and use of animals for scientific purposes. A governing principle of the Code is that 'respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes' (section 1.1). One way this is achieved is through applying the 3R's:

- the *Replacement* of animals with other methods
- the *Reduction* in the number of animals used
- the *Refinement* of techniques used to minimise the adverse impact on animals

During the webinar, we heard from three presenters who explored:

- alternatives to the use of animals such as machine learning,
- ways to reduce the numbers of animals used through the use of animal models and development of micro-skills (such as dexterity and spatial awareness), and
- methods for alleviating pain and distress in animals through improved handling techniques, positive training and increased enrichment.

Below are follow up questions that the speakers did not have time to answer during the webinar

	How do you suggest we can strike a balance between ensuring welfare is catered for, but also to the extent that it doesn't impact the disease progression that your research might be looking into? (i.e what if new enrichment provided turns out to be a confounding factor that improves disease, but you want to see the opposite effect so that you can test the efficacy of a particular treatment?) Researchers are often wary of changing too many factors that, although beneficial for the animal, may lead to more confounding factors that might affect their research negatively.
Assoc Professor Brett Lidbury	My advice is to read up on the "Refinement" "R" of the 3Rs that underpin our Code of Practice. Over the previous 10-15 years this has been an active area of research, with some nice recommendations on environments, enrichment (etc) for research animals (particularly rodents). In short, don't try these approaches randomly, because as you suggest, many well-intended changes will cause stress, and thereafter negatively impact your research results. As a former biomedical animal model focussed researcher, I now understand that many of my practices at that time were stressful for the animals, and hence very likely had a negative impact on my eventual research results (e.g., enhanced cortisol secretion – immune suppression). If animal experiments must be conducted, the highest standards, including for the cage and animal house environments, are essential to high quality research results, and less waste.

Dr Shari Cohen	Great and very common question! My advice is similar to Brett's advice. Good animal welfare is foundational for good science. We have a translatability and reproducibility crisis across all scientific disciplines with up to 80% of research non-reproducible in some cases. There is also an increasing number of scientific studies demonstrating poor animal welfare increases variability, decreases reliability, skews data, increases the potential for premature dismissal of potentially valid science and the premature inclusion of flawed science. Similar to other types of research, animal welfare science evolves over time. What we did 10 years+ ago may not always be the best available method. Research that does not conform to good animal welfare science is also subject to greater challenge, is less likely to be comparable with other recent studies/publications, can be less reproducible and may be at a higher risk of accepting results that are not significant/accurate and vice versa. The motto "Excellence in welfare = Excellent in research" is being increasingly validated with the literature only expanding over time and disciplines. Please find a collection of links to papers from 1997 through to 2022 as well as special editions with papers that might be helpful demonstrating improvements in welfare improve research outcomes (however please note this is not a comprehensive list): https://www.science.org/doi/10.1126/science.abq7198, https://pubmed.ncbi.nlm.nih.gov/9175008/, https://www.mdpi.com/journal/animals/special_issues/Improving_Research_Animal_Welfare_and_Quality_of_Science, https://link.springer.com/chapter/10.1007/164_2019_292, https://journals.sagepub.com/doi/10.1177/026119291804600501 https://www.theglobeandmail.com/canada/article-animal-stress-may-put-medical-research-results-in-question-canadian/, https://bmcbiol.biomedcentral.com/articles/10.1186/s12915-021-01184-0, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2852022/, https://theconversation.com/laboratory-mice-are-usually-distressed-and-overweight-calling-into-question-research-findings-175812, https://elifesciences.org/articles/27438, https://phys.org/news/2020-08-good-animal-welfare-lab-mice.html, https://pubmed.ncbi.nlm.nih.gov/32842924/, https://pubmed.ncbi.nlm.nih.gov/34983085/, https://www.frontiersin.org/articles/10.3389/fnbeh.2021.763428/full, https://www.science.org/content/article/are-happy-lab-animals-better-science Please also be aware it is a very important requirement for all NHRMC funded projects and overall regulatory requirement for any research approved under an AEC in Australia to meet <u>best practices</u> for animal care and use: https://www.nhmrc.gov.au/about-us/publications/best-practice-methodology-use-animals-scientific-purposes and https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes (section 1.9 governing principles and throughout the document requires best practices; also requires minimising of animal distress in sections 1.10-1.17
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	and repeated throughout the document). Therefore, it may be considered a breach of these regulatory Codes if these key principles and requirements are not met. Additionally, the social license of continued research with animals depends on maintaining the best possible welfare and conditions for the animals and applying the 3Rs (developed by animal research scientists Russell and Burch (1959)). More and more scientific and non-scientific communities are challenging the robustness of research performed with animals. Having a strong foundation in animal welfare and the 3Rs can help counteract future challenges to experimental studies. My own personal experiences working in animal facilities and with researchers is that well thought-out improvements in welfare and the 3Rs routinely result in significant increases in scientific validity/significance, potential ability to reduce animal numbers/workload per experiment, offer equivalent if not better research results and overall save on valuable research-related resources. By implementing animal welfare improvements, researchers are better able to focus on their research rather than on potential confounding effects from poor welfare. We can also minimise rates of attrition and maximise overall success rates of their studies. On a practical level, I would always recommend to try and work closely with your animal welfare/facility teams and where appropriate seek the advice of experts in the field of lab animal & welfare science. We have a whole ANZ and world-wide community available to support better research with animals. I would also recommend a thorough review to properly understand the research aims, husbandry and experimental protocols/design. If there are concerns about potential negative research outcomes, a robustly designed pilot study and/or a few parallel studies can be useful. When these are done correctly, the effects are usually very positive and can result in more scientific publications, more funding opportunities and greater recognition for researchers/research teams. While it might not always be initially easy to make changes, or seem too hard at first, the impacts in better implementation of animal welfare and the 3Rs is overall typically positive and long-lasting. With a little creativity, the right knowledge and good support both research and animal welfare outcomes can be improved.
	It seems that machine learning is dependent on the provision of some raw / primary data. One of the examples in the talk was ALT liver enzymes. This makes me wonder that in the world of total replacement within silico work, there must still be some reliance on the provision of data from animals. Or is it only from human projects one would rely? Is there an assumption within machine learning that all the raw data we need from animals has been collected and as such no more animal use is necessary, or, is it that machine learning can take us some way forward but there will still be a need for some animal experiments to provide data within which to refine the model??
Assoc Professor Brett Lidbury	I envisage a future, via innovations in machine learning and other NAMs / alternatives, where animal models of human disease will not be required. As stated during my talk though, fundamental experiments on mammalian systems can still be expected on appropriate model animal species.

	The issue of “whole organism” modelling is countered by the fact that rodents and primates (including us) diverged around 60 million years ago, so the question of extrapolation between species is relevant. You are correct to say that we have collected an enormous amount of animal data, which may be useful. In the world of animal alternatives in research, colleagues have suggested that a central animal data repository would be desirable (and I agree). However, in the meantime I would encourage you to interrogate the meta-data that can be compiled via the published literature (using systematic review (SR) methods. There has been talk of a Cochrane library animal studies database, but I have lost track of how this is going?). There are many papers describing SR, and the host of our discussion last week, A/Prof Kieron Rooney, is an expert in this field, so help and advice is close at hand. The only problem with the animal literature is that many of the published studies are excluded due to poor quality (see the ARRIVE guidelines for what aspects are required for an animal research protocol of appropriate quality). To answer the second theme of your question, my view is that animal data and further biomedical animal experiments will not be required, and yes, that we will focus entirely on human datasets. Why? For the reasons suggested above – the species barrier - it makes sense that we study the target organism. We must remember that animals are used in biomedical research as human proxies, and this function has been shown to be fallible and mostly not an accurate predictor of the human condition (although some have, but it seems a minority). And leading from this point, animal data will not be required to validate human results for the reasons explained. Accessing human data can be challenging but is more-and-more feasible nowadays. As I explained last week, I utilise pathology results and these have allowed me to investigate human physiology and pathology in silico. I must add that for a proper animal alternative, other human-focussed cell-based, OMICS, SR etc methods are required to support the ML modelling (colourful figure in my talk).
	As researchers are encouraged to refine protocols do you see this as something that occurs in parallel with an old method within a study (that is, double measuring an outcome with two different techniques), or is there a need for bespoke animal use studies in which old methods are compared against new methods. In this latter approach, how do you see this work being funded given the desire for category 1 funding to be outcome driven rather than methods driven??
Dr Shari Cohen	Excellent question and common concern/query. I have partly answered this question above so some of this might be repetitive if you have looked at the above answered questions first. If not, please do feel free to read the above question for more info/context around the fundamental importance of refinements. My own personal experiences working with facilities and researchers to improve welfare and the 3Rs has often resulted in significantly greater increases in the validity/significance of their research as well as the ability to reduce animal numbers/workload and maintain equivalent or even better research results. I have also worked with teams to implement animal welfare improvements to enable researchers to focus more on their research outcomes rather than on potential confounding effects from poor welfare, as well as minimise rates of attrition, and maximise overall success rates of their research. It might not always be initially easy to make changes, but typically the impacts are both positive and long-lasting. With a little creativity, the right knowledge and proper support, improvements in animal welfare and the 3Rs can offer tangible improvements to research.

	On a practical level, I would always recommend to try and work closely with your animal welfare and facility teams and where appropriate seek the advice of experts in the field of lab animal welfare science. A review of your prior experimental data and set-up for animals is key. I would recommend a robustly designed pilot study and/or considering initial parallel studies depending on the circumstances. At times a completely different study can be developed to test these methods and outcomes. Like any kind of experiment, it might take a bit of time and tweaking to get the best results. Where possible, additional funding can be sourced and often collaborations can be found to maximise resources and opportunities. My personal experience is that there are often untapped resources within teams, institutions, and the wider research community that can support improvement/refinements whilst simultaneously achieving Type 1/major funding aims. Again, with some creativity, a thorough review, good planning/staging of the research, and some perseverance a lot can be achieved. When done appropriately these refinements or even replacements can be more effective models that can result in more scientific publications, more funding opportunities, more collaboration and greater recognition for researchers/research teams.
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