



## REPRODUCIBILITY – IS IT A FOURTH R?

### CCAC's Views on Reproducibility, Considerations of Translatability of Animal-Based Science, Maximizing Data Obtained from Animal Experiments, and Reducing Animal Use

**DATE OF PUBLICATION:** March 2019

#### OVERVIEW – PROBLEM

The Three Rs (replacement, reduction, and refinement) are universally recognized as providing the overarching ethical principles for animal-based science (Russell and Burch, 1959). The Canadian Council on Animal Care (CCAC) bases its standards and its assessment and certification of institutions on these principles (CCAC, 1989), which underpin the values of the Canadian public, requiring sound justification for the involvement of animals in science; sound justification for the numbers of animals involved; and minimization of any potential pain or distress.

As in other countries, full implementation of the Three Rs in Canadian science requires the collaboration of many stakeholders involved in the research enterprise. While there are elements relating to the Three Rs that can be directly influenced by the CCAC, other elements fall within the domains of research funders, institutions, and research scientists themselves and should be viewed as a shared responsibility.

Concern about the reproducibility of scientific research has been increasing in recent years, with failures to replicate findings detailed in scientific journals (e.g., Begley and Ellis, 2012; Prinz et al., 2011), and discussed in the mainstream media (e.g., *The Economist*, 2013).

Lack of reproducibility not only impacts scientific progress and translation of animal-based studies to treatments for humans and animals, it threatens the reputation of biomedical science, thus destroying public trust in animal-based research, and is wasteful of both research dollars and animal lives.

Where animals continue to be used in research, there is a moral responsibility to ensure that the work is carried out to internationally acceptable standards, so that data obtained from each animal can contribute meaningfully to the stated aims of the research project. This requires an extraordinary attention to detail throughout the entire cycle of study planning, funding, ethical review, execution, analysis, reporting and publication. Independent analyses in a number of research areas have highlighted failings in each of these steps which may be contributing to the irreproducibility of animal-based studies.

Reproducibility as defined by Goodman et al. (2016) relates not solely to the inability to reproduce research results, but also to methods and inferences (including generalizability and

robustness). The elements discussed below are key aspects which can impact reproducibility in the Canadian animal research community, with illustrations of current attempts and possibilities for remedial action.

## STUDY PLANNING

Study design has been singled out as a key culprit in poor reproducibility, as animal-based researchers do not necessarily use the most efficient designs (Kilkenny et al., 2009). In 2012, the CCAC and other international colleagues published an article encouraging periodic synthesis of evidence as part of sound planning for animal research (Leenars et al., 2012). Ironically, systematic reviews (one way to synthesize available evidence) has highlighted poor study planning in many disciplines, underscoring the need to encourage a sound understanding of experimental design (e.g., Egan et al., 2016).

The need for better planning is beginning to be acknowledged. The PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines, recently published by NORECOPA (Norway's National Consensus Platform for the Advancement of the Three Rs) is one attempt to encourage full and thoughtful planning of any animal-based study, to ensure that the best use is made of any animal involved in research (Smith et al., 2018). In the EU, learning outcomes for researcher training have been published, which include the design of procedures and projects (National Competent Authorities for the Implementation of Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes, 2014).

BioCanRx recently organized a [workshop on Preclinical Experimental Design and Reporting](#), (October 2018). The aim of the workshop was to improve BioCanRx scientists' knowledge of research design, analysis, and reporting of key methodological details. These types of workshops are welcome, given the apparent misunderstandings of experimental design (Egan et al, 2016); however, more time should be accorded to training researchers in the importance of sound experimental design (Fry, 2014). As one aspect of improving experimental design, recent articles highlight training initiatives on the part of the US National Institutes of Health and the Canadian Institutes of Health Research that encourage the understanding of how to address sex as a biological variable (Tannenbaum et al., 2016; Clayton, 2018). Encouraging the use of more efficient designs, such as the split-plot design, can reduce the numbers of animals required for a study while maintaining (or enhancing) the power of the study. Walker et al. (2016) maintain that implemented globally, such efficient designs could, conservatively, reduce mouse use by a minimum of 10%, or three million mice annually, with no loss in data quality.

## FUNDING

Funding agencies have a role to play in ensuring that research programs are well conceived. In the UK, the Biotechnology and Biological Sciences Research Council, the Medical Research Council, and the Wellcome Trust, in response to the Bateson Review Recommendations (Bateson et al., 2011), require "...that for each proposal that involves the use of animals, reviewers and funding panels...assess whether animals are needed, whether the potential benefit justifies the adverse effects on the animals, whether the numbers are appropriate, and whether the species is the most appropriate". In the US, the National Institutes of Health has developed guidelines and training materials in an attempt to improve the rigour and reproducibility of research that they fund (NIH, 2018). In Canada, the major research funders have developed the

*Tri-Agency Framework: Responsible Conduct of Research* (2016), which touches on aspects that contribute to improving reproducibility. The recent North American Gender Summit pointed to the need to support training and education for peer reviewers (Holmes et al., 2018). The Canadian Institutes of Health Research College of Reviewers provides reviewers with the knowledge and resources to conduct consistent, high-quality reviews, and affords the opportunity for consideration of matters of direct relevance to improving reproducibility.

There are additional elements that have been discussed as possible conditions of funding. For example, at strategic times, investigators could be encouraged to undertake a systematic review, to ensure that the model used is capable of answering the research question (De Vries et al., 2014). As is the case for clinical studies, where preclinical animal studies have reached a confirmatory stage, investigators could be required to pre-register their protocols. This, along with a requirement to publish all results, is aimed at reducing the duplication of studies and preventing publication bias (e.g., Vogt et al., 2016).

## **ETHICAL REVIEW**

There has been considerable debate both in the US and Canada concerning the extent to which animal care committees (or institutional animal care and use committees in the US) should involve themselves in the consideration of scientific issues outside of those directly impacting animal welfare and humane use.

Salman et al. (2014) in a *Lancet* series on research waste indicated that ethical committees were at fault for reducing the number of animals, leading to underpowered studies. Avey et al. (unpublished data) subsequently carried out a survey of Canadian animal care committees, and determined that Canadian animal care committees do not willingly drive down the number of animals; however, to what extent animal care committees impact animal numbers needs to be better substantiated. Regardless of the reason(s), it is evident that both researchers and animal care committees often underestimate the number of animals needed for a sufficiently powered study (e.g., Button et al., 2013; Jennion and Møller, 2003), thus risking the validity of the data.

In addition to concerns about numbers of animals, there are additional concerns that animal care committees do not have the capacity or competency to be able to adequately analyze study protocols in sufficient detail to ensure the reproducibility of studies they review and approve. Nevertheless, Everitt and Berridge (2017) maintain that animal care committees have a key role to play within the institution to foster a culture of openness and trust that seeks to optimize the involvement of animals in high-quality reproducible studies that are then able to contribute to translational success. For example, animal care committees are in a position to influence reproducibility through attention to scientific details at the planning stages of a research project (Smith et al., 2018) and are able to broker interdisciplinary discussions at the institutional level that contribute to a culture of high-quality science (Everitt and Berridge, 2017).

## **EXECUTION**

Several studies have shown that investigators do not fully understand the importance of ensuring that bias is not introduced into their work (e.g., Abdel-Sattar et al., 2015). In 2009, Kilkeny et al. found that only 13% and 14% of animal-based studies reported use of randomization or blinding respectively. With the implementation of reporting guidelines such as ARRIVE (Animal Research Reporting of In Vivo Experiments; Kilkeny et al., 2010), this should have improved.

However, subsequent reviews have found only limited improvement (Baker et al., 2014), leading the UK-based National Centre for the 3Rs (NC3Rs) to consider how best to [revise the guidelines](#) to encourage full implementation. Garner et al. (2017) among others, have also addressed the problems of external and internal validity of animal studies, which should be considered during the planning stages, so that spurious data is not obtained due to the manner in which experiments are conducted (e.g., not habituating animals to handling), or because of details that are ignored (e.g., impact of bedding type, or sources of non-systematic variation).

## ANALYSIS

Numerous studies have now been published highlighting inappropriate use of statistical methods (e.g., Baker et al., 2014; Ioannidis, 2005), suggesting widespread statistical naivety among researchers and reviewers. Weissgerber et al. (2016), listed common errors as including the use of incorrect or suboptimal tests, summarizing data that were analyzed by nonparametric techniques as mean and standard deviation or standard error, reporting p-values that are inconsistent with the test statistic, p-hacking, and analyzing non-independent data as though they are independent. In addition, they point to additional problems arising from inadequate reporting of statistical methods, including failure to provide a power calculation, not reporting which statistical test was used, or not providing adequate detail about the test (e.g., paired versus unpaired t-test), and not addressing whether the assumptions of the statistical tests were examined (Weissgerber et al., 2016). Interviews with biomedical researchers, policy-makers, funders, and regulators led Fitzpatrick et al. (2018) to assert that researchers with well-established collaborations with statisticians were more confident about their data analysis. In short, there is overwhelming evidence that improved statistics education is needed for research scientists.

## REPORTING

The quality of reporting animal-based science has been cause for concern for at least 30 years. In the more recent past, concerns have grown because of the focus on reproducibility. If the necessary components of the methodology used and the full data obtained are not made available, it is impossible to reproduce the findings of a study. A number of reporting guidelines have been published: for example, the Gold Standard Publication List (Hooijmans et al., 2010), the ARRIVE guidelines (Kilkenny et al., 2010), the Institute for Laboratory Animal Research *Guidance on the Description of Animal Research in Scientific Publications* (ILAR, 2011), and the Landis checklist in *Nature* (Landis et al., 2012). While a recent study indicated that requiring a checklist at the time of article submission was associated with an increase in the quality of reporting preclinical biomedical research (Han et al., 2017), other analyses have indicated that not to be the case (Enserink, 2017). The International Council for Laboratory Animal Science has tried to improve reporting globally through a harmonization exercise, aimed at distilling the important concepts into “an easily translatable minimum standard, upon which a more structured framework could be built” (Osborne et al., 2018). Ensuring that the relevant elements have been considered during the preparation of an animal-based protocol, as advocated by Smith et al. (2018), should help ensure that the important elements to be reported are available when the results of a study are prepared for publication. In this, animal care committees can play an important role by ensuring, during the protocol review process, that the necessary elements listed in the PREPARE guidelines have been addressed (Smith et al., 2018).

## PUBLICATION

In the interview study of Fitzpatrick et al. (2018), the most commonly cited problem concerning reproducibility stemmed from pressure to publish in high-impact-factor journals, despite the fact that the quality of work published in these journals is no better than average (Macleod et al., 2015). Because publication record (i.e. quantity of publications and the perceived prestige of the journals) continues to be an important factor in annual performance reviews, academic tenure and promotion, and future research funding, there are few incentives for scientists to perform high-quality, rigorous work. In fact, the opposite is likely true: scientists are incentivized to avoid rigorous practices such as blinding and randomization (which reduce the chance of finding a ‘significant’, publishable result [Holman et al., 2015]) and by the current system which rewards publication per se rather than true discoveries (Smaldino and McElreath, 2016). As noted at the 2014 Institute for Laboratory Animal Research workshop, the race to publish can lead to shoddy science that is irreproducible and cannot contribute meaningfully to the research record, thereby wasting animal lives and potentially putting human lives at risk (ILAR, 2015). In this respect, initiatives such as those described in the Gender Summit report to rethink the ways to assess excellence in research, are likely to be valuable in the context of improving reproducibility (Holmes et al., 2018).

There are also problems with not publishing ‘negative’ results of animal-based studies: the data cannot be incorporated into meta-analyses or systematic reviews; as a result, these studies may be unwittingly duplicated. In general, these problems arise due to an unwillingness on the part of researchers to put time into the preparation of scientific papers reporting negative results, likely in lower-impact journals, and an unwillingness of journals (in particular, high-impact-factor journals) to publish papers that are not reporting novel findings. This publication bias has been estimated by following the publication of full papers based on abstracts submitted to the 2008 Society of Critical Care Medicine Conference (Joffe and Conradi, 2017), and by surveying animal-based researchers (ter Riet et al., 2012). It has also been estimated as “missing data” in various meta-analyses (e.g., McLeod et al., 2004).

## CONCLUSIONS

Much as Russell and Burch originally intended, implementation of the Three Rs should be a holistic endeavour that seeks to improve animal-based research practices (Russell and Burch, 1959). As outlined above, improving reproducibility is a part of this and research funders, “regulators”, journals, institutions, and individuals all have a part to play in ensuring high-quality, animal-based research.

Institutions bear the responsibility of ensuring that research carried out under its auspices is of high quality, and reflects well on the institution. In addition, individuals, institutions, and funders share an interest in making the most of scarce research dollars. These elements may not have traditionally been considered to fall within the purview of the CCAC yet have a direct impact on the animals involved in research studies, and on the ethics of animal use.

Understanding how best to achieve progress in addressing reproducibility issues will require collaboration with various groups, research funders, research institutions, and centres interested in changing research practice, including, for example, the Institute of Gender and Health. These interdisciplinary approaches will be invaluable in building more robust scientific knowledge and improving research practices (Holmes et al., 2018).

Freedman and colleagues (2015) estimated that over 50% of preclinical research cannot be replicated, placing the approximate annual cost of irreproducibility in the US alone at US\$28 billion. Lack of reproducibility is not only an issue for preclinical work, but plagues all areas of animal-based science (Gerlai, 2018); there is no reason to think that Canadian animal-based science is immune. A conservatively estimated 58 million animals are used directly each year in scientific research worldwide, suggesting that many millions of animal lives are wasted in low-quality work (Taylor et al., 2008). With the goal of ensuring that animal-based research is carried out to high standards of animal ethics and care, the CCAC will continue to work with stakeholders to implement practices aimed at improving the reproducibility of Canadian animal-based science.

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