

Inside this issue:

Moves to Tighten Research Statistical Parameters and Their Potential Ramifications for Animal Use

ANZCCART AEC Members of the Year Announced 4

In Search of a Category C (Animal Welfare) Member for Your AEC? 4

2017 ANZCCART Conference in Review 5

Recent Articles of Interest 5

- Tickling Improves Laboratory Rat Welfare
- Sea Snakes Lose Their Stripes to Deal With Pollution
- Fish Reveal Clues to Healing Spinal Cord Injuries
- New Animal Models for Hepatitis C Could Pave the Way for a Vaccine

Moves to Tighten Research Statistical Parameters and Their Potential Ramifications for Animal Use

Geoff Dandie, CEO, ANZCCART

According to a recent article in the highly respected journal, *Nature* (*Nature News*, 26 July 2017), there are moves afoot to shake up the levels of statistical significance (*P value*) required for scientific research and the publication of results obtained. The much-maligned *P value* is commonly used by scientists to demonstrate whether or not their experimental results are statistically valid and the current standard practice adopted for the publication of results is generally to use a threshold *P value* of less than 0.05 to demonstrate statistical significance. The move to increase the stringency for demonstrating significance has apparently come from a group of over 70 prominent researchers, who believe that we are currently in the throes of a reproducibility crisis and weak statistical standards are one cause of the problem. The solution being proposed is to change the current, commonly used threshold of statistical significance *P* being less than 0.05 on the grounds that this threshold limit has caused too many

false positives in the literature. In essence, having a *P value* of less than 0.05 means that there is less than a 5% chance that the Null hypothesis is true, or if you prefer, that there is less than a 5% chance that the results obtained are purely due to chance. The proposal being put forward by this group of international luminaries is that a *P value* less than 0.005 should be adopted as the new standard. This would mean that there would need to be less than 0.5% chance of the result being in error due to random events.

It is reasonable to assume that everyone engaged in research would strive to ensure their results are correct and applicable to the goals of their study, and so this proposal looks to be a reasonable and logical step towards increasing the stringency of research standards and the resulting publications. It also seems to be attracting broad support as demonstrated by the results of a survey of *Nature's*

readership which showed approximately two thirds of respondents supported the lowering of *P value* thresholds. While ANZCCART remains an advocate for the highest standards being applied to research, we might also suggest that moves such as these need to be considered very carefully and from various perspectives before they are universally applied. It might also be suggested that while statistical analysis is a vital aspect of experimental design, it is certainly not the only factor with potential to impact on the reproducibility of any experimental results.

Considering the effects of reducing the *P value* thresholds from a purely statistical perspective, it is possible that increasing the sample size may also increase the odds of a false negative, which means that the experimental results would indicate that effects do not exist when in fact they do. Not surprisingly, the statistical experts promoting this change have recognised this risk and the significant additional costs that would come with the required increase in sample size and these factors have resulted in their suggestion that it might be best to limit the increase in sample size to 70% of the differences that would normally result from a change in stringency limits to $P < 0.005$, with a view to reducing the risks of such potential complications.

Certainly when it comes to animal-based experiments, one of the biggest and, from ANZCCART's perspective, one of the most important impacts of this proposal, would be the effect it will have on the number of animals required for each experiment. While a ten-fold change in the level of statistical significance may not necessarily result in a ten-fold increase in the number of animals required, it is safe to assume that the increase in animals required will be significant and more often than not, substantial. This would still be true, even if sample size increases are limited to an additional 70%, so the number of animals normally required would still need to increase substantially.

Equally, when consideration is given to more abstract concepts, such as biological significance, it might be fair to ask whether these measures will actually translate to improved reproducibility of data? To illustrate the important difference between biological and statistical significance, experiments designed to test the efficacy of a new anti-tumour agent might result in a reproducible reduction in tumour growth of between 2 and 5% over the course of each experiment, even though the tumour survives and would eventually kill the animal if allowed to do so — a difference that can be shown to be statistically significant using either threshold value if the result is sufficiently

reproducible. However, the potentially important consideration of biological significance would require showing a different outcome, ideally a cure. This difference is important because a statistically significant difference in tumour growth rate is not the same as eliminating the tumour altogether.

It must be remembered that the use of animal models in medical research for example, already comes with limitations. Simply the fact that you are using an animal model of the disease or condition for which a treatment is being sought, is only the first and perhaps most obvious one to consider. Even the best and most reproducible animal models of human disease may fall short of expectations, simply because they do not adequately allow for the very high degree of variability that exists between individual humans and the way they respond to both the disease and the potential treatment. It may also be true to say that even though the animals in a study exhibit the same symptoms or clinical effects as the patients for whom a cure is being sought, it does not always follow that the cause / aetiology of disease is the same as the human condition. Accordingly, it is reasonable to question whether responses to the potential treatment in the animal model, will mimic the responses in human patients. None of these questions are new, but bearing them in mind, it might be reasonable to question whether or not increasing statistical stringency will solve many of the problems associated with the translation of research findings to clinical practice.

Certainly, when it comes to animal-based experiments in particular, ensuring the optimal design of experiments remains a major consideration and one that might still prove to be far more important than increasing the stringency of statistical significance. Simple measures such as ensuring appropriate randomisation of animals between test and control groups, adequate blinding to ensure those measuring the experimental outcomes are not unwittingly biased in the way they read results, etc., are all recognised as being extremely important and provide effective ways to improve the reproducibility of experimental results.

This is where the simple concepts of good science, as well as excellence and accuracy in reporting the results of experiments is so important and this is the aim of the ARRIVE guidelines (Animals in Research: Reporting In Vivo Experiments), which were developed by Carol Kilkenny, William Browne, Innes Cuthill, Michael Emerson and Douglas Altman. These guidelines were first published in the journal, *PLoS Biology* (<http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1000412>) and were derived with the support of the National Centre for

the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), so they also feature on their web site (<https://www.nc3rs.org.uk/arrive-guidelines>). The key aim of the ARRIVE guidelines is to improve the reproducibility of science that involves the use of animal models, but in truth, they can and should be equally applied to all manner of experimental work and subsequent publication of results. Accordingly, they not only include mention of important experimental design features but also focus heavily on the need to include appropriate details in publications. Details such as the housing / husbandry conditions or any unexpected adverse events that may have impacted on the study, as well as the full details of any surgical procedures, anaesthetic administration, analgesia provided, drug formulations and the site, route and dose for everything administered, etc. all need to be described in order for the experiments to be reproducible.

Looking objectively at both proposed strategies for improving the reproducibility of animal-based studies, there are clear differences between them that may help decide which would be best to adopt. One strategy vastly increases costs associated with the research and greatly increases the number of animals that will need to be used for all such work. The other, requires greater care in planning experiments and attention to details as well as a far higher standard of reporting the details of methodology and outcomes of all animal-based research. When considered from a perspective of animal welfare, the choice between these two models seems a relatively simple one. Equally, a comparison of the two options in light of the 3Rs of Replacement, Reduction and Refinement, or possibly even from an economic point of view, would indicate that there really should be only one viable choice.

Assuming that the implementation of the ARRIVE guidelines is a better option than changing the statistical parameters associated with data analysis, scientists, journal editors and potentially even Animal Ethics Committee (AEC) members, all need to consider how this can be implemented in a timely and broadly based (if not universal) fashion.

Perhaps the first and simplest step would be to raise awareness of the ARRIVE guidelines. This would mean ensuring that they are featured in all ethical and / or animal welfare training given to both new and established researchers. It would also require AEC members to become familiar with them and use them to guide their deliberations, particularly when it comes to forming an opinion of the quality of the science they are considering so they can measure it against

the potential cost to the animals. This approach does potentially carry additional benefits as it effectively provides all members of an AEC (science-based or not) with some clear standards against which they can measure the approach described in any application.

The second stage of implementation relies on both the authors of all relevant scientific papers and the editors of the journals in which the work will presumably be published. The biggest potential problem here is enforcement as there is no single authority capable of taking on such a role. This is an important consideration as adherence to the guidelines does require diligence and already we are seeing this as a complicating factor. Even though a number of journals have endorsed and agreed to abide by the ARRIVE guidelines, it has already become apparent that this is not a guarantee that all papers published in that journal meet the standards of reporting described in those guidelines. While it must be acknowledged that ensuring compliance with the ARRIVE guidelines will place additional pressures on busy reviewers and editors, it is something that ANZCCART would suggest is worth the effort – both from the perspective of the stated aim of making research more reproducible and also in terms of enhancing the reputation of the journals themselves.

On balance therefore, we would like to suggest that the idea of increasing the required level of statistical stringency required for publication in the international literature is an interesting proposal that makes a lot of sense from a mathematical point of view. However, from a practical perspective as well as an animal welfare and 3Rs point of view, a better solution would be the endorsement and adoption of the ARRIVE guidelines.

It is perhaps telling, that during the preparation of this edition of ANZCCART News, the Journal *Science* published an article that also challenges scientists and publishers alike to pay more attention to the ARRIVE Guidelines. The article title “*Sloppy reporting on animal studies proves hard to change*” and even the subtitle “*Scientists appear to ignore guidelines adopted 7 years ago*” seem to suggest that complete and accurate reporting of experimental conditions and co-variables is a critical aspect when it comes to improving the reproducibility of animal based experiments and their relevance to the research goals that led to that work: (*Science* **357** (6358) pp 1337 – 1338) at: <http://science.sciencemag.org/content/357/6358/1337/tab-pdf>).

So let's see what can be done to improve compliance with the ARRIVE guidelines.

ANZCCART AEC Members of the Year Announced

At this year's conference in Queenstown, we were delighted to be able to announce the two recipients of the ANZCCART AEC Member of the Year Award for 2017. As previously announced, there were two awards made this year as the ANZCCART Board decided to make separate presentations to an Australian AEC member and one from New Zealand as well, with each recipient being decided from the pool of nominees by the local ANZCCART Board / Committee.

The Australian recipient of the ANZCCART AEC Member of the Year Award was Mr Robert Beattie from Queensland. Robert was nominated for his work with both the Queensland Institute of Medical Research (QIMR) and the CHM Alliance AECs for over seven years and almost six years respectively.

Mr Beattie's initial introduction to animal welfare and ethics came via his daughter's PhD studies at QIMR. He became aware of the valuable research conducted at QIMR and the "how and why" role an animal ethics committee plays in research. When a vacancy came up on the Queensland Institute of Medical Research Berghofer Medical Research Institute (QIMRBMRI) AEC some years later, Bob saw this as an opportunity to repay the Institute for its support towards his daughter's career development and the contribution the Institute makes to society in general. Bob was subsequently appointed to the CHM Alliance Pty Ltd Animal Ethics Committee in 2011 as a Category D (Lay) member.

In 2011 Bob was invited to join and contribute to the triennial review of the University of Queensland Animal Ethics Committee and then two years later he was asked to chair the QIMRBMRI AEC and so became one of a small group of non-scientific/external members to hold this position. His role as Chair has seen Bob working closely with the QIMR AEC executive, demonstrating the high regard in which he is held by that organisation. Bob has attended recent ANZCCART conferences and provides updates on current issues and topics of discussion to committee members. These updates provide valuable learnings for their committee. Bob also provides feedback and advice where a committee can make improvements, thus providing invaluable support to the Chair and administration officer.

Having been a Chair, Deputy Chair and Category D member of two very different AECs places Robert in a unique position. Being involved in both high level

medical research in a state capital city and farm industry research in a rural location, Bob's extensive experience and his commitment to community service is having a significant impact and helping to ensure successful outcomes in two very different but important fields of research. The Board was therefore very pleased to be able to recognise Bob's service with this award.

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The recipient of the inaugural ANZCCART AEC Member of the Year Award granted by the New Zealand Committee was Miralie Thomas Vincent. Miralie was recognised for her excellent service as the Secretary of the Massey University AEC since 1994.

When Miralie took on this role, it was a voluntary committee set up by Massey University (prior to the passing of the Animal Welfare Act) and she has supported the work of the committee with a level of commitment and expertise that can only be described as exceptional. She has also helped to oversee the adoption of a formal code of welfare and many such developments in practice under the leadership of five Chairs.

Miralie's knowledge of Massey University's Code of Conduct as well as their working practices and the precedents set by previous decisions has been described as encyclopaedic and her constant attention to detail in dealing with all the applications submitted each year is invaluable.

The fact that Miralie was effectively nominated by every member of the Massey University AEC, demonstrates the extraordinarily high regard in which she is held and they expressed a strong belief that their AEC would not be able to operate to its current very high standard without her oversight and input. Accordingly, the New Zealand ANZCCART Committee was delighted to be able to recognise Miralie's hard work over the past twenty years with this award.

In Search of a Category C (Animal Welfare) Member For Your AEC?

Julia Nicholls AOM

As the current President of the Welfare Special Interest Group of the Australian Veterinary Association (AVAWA) I wanted to remind readers of *ANZCCART News* that vets with the appropriate

background can be appointed as category C members of an AEC. Recognising that this is a role which can be notoriously difficult to fill, our members are available to serve in this capacity if required. We can serve as category A (Veterinarian) members as well of course.

Any AEC interested in recruiting one of our members should contact our Executive Officer, Laurie Dowling at the Queensland Division, Australian Veterinary Conservation Biology, Australian Veterinary Animal Welfare and Ethics.

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2017 ANZCCART Conference in Review

This year's conference was organised by the New Zealand Committee of ANZCCART and held in Queenstown New Zealand as part of the annual Queenstown Research Week. The theme for this year's conference was "Maintaining a Social Licence in a Changing World" and focused on both the importance of public acceptance of the scientific use of animals as well as what the public expects of science in return for maintaining that approval.

The programme was based largely around a selection of invited speakers who each tackled a particular aspect of the theme in a way that challenged members of the audience to think about how and why their area of interest applied to the everyday work of their Animal Ethics Committee (AEC) and their own personal attitudes and beliefs. It also offered delegates some excellent opportunities to network and discuss issues of mutual interest in a less formal atmosphere with a lake cruise and dinner offered on the first night and a gala dinner on the final night of the conference.

Keynote invited speakers came from as far away as the Center for Alternatives to Animal Testing in the USA, Kings College London, UK and Winchester University, UK along with an impressive array of speakers from around Australia and New Zealand. Topics covered by the keynote speakers included the importance of communication, the potential value of a Concordat of openness, the results of a systematic

review of animal-based research and some new 3Rs techniques. Local speakers covered the usual array of relevant topics that included some alternatives to the use of animals, a critical examination of humane killing techniques, trends in media coverage, ways to improve the efficiency of trapping techniques and even some of the moral and ethical issues associated with the possibility of bringing back extinct species. There was also some in-depth discussion of the style, quality and consistency of training offered to researchers and teachers who wish to use animals in their work along with the AEC members who consider such applications.

Suffice to say, delegates were all very impressed by the range, depth and quality of all speakers at the conference and the discussion during breaks and between sessions certainly reflected the ability of all speakers to stimulate thoughts and further debate of the topics they had covered – discussions that in a number of cases appeared to extend well beyond the timeframe of the conference itself and that surely must be a strong indicator of a very successful meeting. Thanks and congratulations must therefore go to the New Zealand Committee and Dr Marc Rands (ANZCCART Executive Officer in New Zealand) for organising such a successful event.

The 2018 conference will be held in Canberra from the 24th to the 26th July. We are currently seeking volunteers to assist on the local organising committee. Anyone able to volunteer a small amount of time to help out is asked to contact ANZCCART via email at your earliest convenience.

Recent Articles of Interest

Tickling Improves Laboratory Rat Welfare

Many laboratory animals are stressed by contact with animal care staff which can change their organ and biochemical functions and have a major effect on the consistency and validity of research results. One way to help alleviate some of that stress in rats might be to tickle them, according to a systematic review published in the April 2017 edition of PLOS.

Studies have found that tickling rats before an injection can reduce stress levels and a review article in *Physiology & Behavior* by Dr Jaak Panksepp, a proponent of tickling and author credited with the design of one popular method for tickling rats, has also suggested that rats being tickled produce ultrasonic sounds (detectable at around the 50KHz using ultrasonic sound equipment or a bat detector), which might imitate laughter.

Researchers have found that the effects of tickling can include increased positive vocalisations, improved handling and approach behaviours and decreased anxiety. There have also been reports of this kind of activity reducing levels of stress hormones. Read the full article at:

https://www.alnmag.com/article/2017/07/tickling-improves-laboratory-rat-welfare?et_cid=6033481&et_rid=454969632&type=cta&et_cid=6033481&et_rid=454969632&linkid=https%3a%2f%2fwww.alnmag.com%2farticle%2f2017%2f07%2ftickling-improves-laboratory-rat-welfare%3fet_cid%3d6033481%26et_rid%3d%26type%3dcta

Sea Snakes Lose Their Stripes to Deal With Pollution

An ecologist from the University of New Caledonia in Nouméa, studying turtle-headed sea snake populations in the Indo-Pacific, found that some snakes had lost their stripes and were jet black, presumably as a mechanism for coping with the pollution. While the majority of adaption seen resulted in darkening of skin colour, some others showed pale banding or mottled white markings. A marine biologist from the University of Sydney came across a study of Parisian pigeons which suggested that darker feathers stored more pollutants than lighter coloured feathers because the elements associated with the pollution bound preferentially to the pigments in darker feathers. The researchers collaborated to see if this was the case with sea snakes.

The colours of 1,400 turtle-headed sea snake specimens from both industrial and non-industrial sites in New Caledonia and Australia were compared and they found that the black variety was most common in polluted areas and like the feathers in the reported study, these skins were found to contain higher concentrations of pollutants. The researchers also found that the black snakes shed their skins more often and consider this could be a way of getting rid of pollutants. Unfortunately however, there are limits to how much this can protect a population. Read the full article at:

http://www.nature.com/news/sea-snakes-lose-their-stripes-to-deal-with-pollution-1.22441?WT.ec_id=NEWS-20170817&spMailingID=54724489&spUserID=MTc2Njc3MzgqMwS2&spJobID=1222851175&spReportId=MTlyMjg1MTE3NQSQ2

Fish Reveal Clues to Healing Spinal Cord Injuries

New research into how Zebrafish repair their damaged nerve cells gives hope to restoring connections between the brain and muscles for people with spinal cord injuries.

Damage to the spinal cord for humans and other mammals is permanent and results in paralysis however Zebrafish can regain full movement within four weeks of injury to their spinal cord. By researching these fish, scientists at the University of Edinburgh have identified wound-healing cells called fibroblasts that produce collagen 12 by activating specific biochemical signalling pathways. The collagen 12 molecules change the support structures in the area around the nerve fibres, allowing them to regrow across the area and restore the lost connections between the brain and muscles.

The researcher's next plan is to study other animals to see if activation of the same signalling pathways could help repair nerve connections damaged by spinal cord injuries. Read the full article at:

https://www.alnmag.com/news/2017/07/fish-reveal-clues-healing-spinal-cord-injuries?et_cid=6034917&et_rid=454969632&type=cta&et_cid=6034917&et_rid=454969632&linkid=https%3a%2f%2fwww.alnmag.com%2fnews%2f2017%2f07%2ffish-reveal-clues-healing-spinal-cord-injuries%3fet_cid%3d6034917%26et_rid%3d%26type%3dcta

New Animal Models for Hepatitis C Could Pave the Way for a Vaccine

Hepatitis C affects nearly 71 million people worldwide and as the disease often shows no obvious clinical signs more than 80% of cases go undiagnosed. So even though there is now an effective treatment available, the best solution would be an effective vaccine to prevent infection. Unfortunately, the development of such a vaccine has been stymied by the lack of a good animal model as Hepatitis C is a very specific virus that only infects humans and chimpanzees.

Professor Rice from Virology at The Rockefeller University, in collaboration with researchers from Columbia University has now developed a way to mimic the disease in rodents, by virtue of discovering a virus that is closely related to Hepatitis C, but able to infect rats and mice. Importantly, they have found this model recapitulates much of the human disease, which should help accelerate Hepatitis C vaccine research. Read the full article at:

https://www.alnmag.com/news/2017/07/new-animal-models-hepatitis-c-could-pave-way-vaccine?et_cid=6030043&et_rid=454969632&location=top&et_cid=6030043&et_rid=454969632&linkid=https%3a%2f%2fwww.alnmag.com%2fnews%2f2017%2f07%2fnew-animal-models-hepatitis-c-could-pave-way-vaccine%3fet_cid%3d6030043%26et_rid%3d%26type%3dlocation%3dtop

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The opinions expressed in *ANZCCART NEWS* are not necessarily those held by ANZCCART.

Contributions to *ANZCCART NEWS* are welcome and should be sent to the Australian Office of ANZCCART.

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