anzccart
HUMANE SCIENCE

NEWS

Volume 30, Number 3, 2017

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ANZCCART Formally Endorses ARRIVE and PREPARE Guidelines

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In line with ANZCCART's long-standing policy of promoting the highest professional standards when it comes to research that involves the use of animals, the ANZCCART Board formally endorsed at its Meeting on 11 December 2017, both the ARRIVE Guidelines (as published on the NC3Rs website in the UK at: https://www.nc3rs.org.uk/arrive-guidelines) and the PREPARE Guidelines that have recently been published on the Norecopa website (https://norecopa.no/PREPARE).

The decision to formally endorse these two Guidelines documents reflects the very sensible and logical steps they promote when it comes to the planning, execution and reporting of scientific experiments, particularly those that involve the use of animals. This is to some degree, a response to on-going concerns being expressed in both the mainstream media and the scientific literature about the lack of reproducibility of some published studies that have relied on data generated by use of animal models.

ARRIVE Guidelines:
The ARRIVE Guidelines were compiled by a group of British scientists from the NC3Rs and a variety of well-regarded research institutions around the UK and were first published in the journal, *PLoS Biology* in June 2010. The authors, Carol Kilkenny, William J Browne, Innes C Cuthill, Michael Emerson and Douglas G Altman originally published these guidelines under the title: Animal Research: Reporting *In Vivo* Experiments, which not only serves to name the guidelines, but also fulfils the first requirement of the guidelines themselves by providing an accurate and concise description of the contents of the article.

The guidelines outline best practice steps under a series of headings and sub-headings that parallel the standard headings publishing scientists would all be very familiar with and use in all publications.

The sub-headings under each major heading are indicative of the standard key points that should be a part of every scientific publication. They essentially fulfil the criteria undergraduate students are taught to include in a written report. Using such guidelines ensures sufficient details are provided to allow other scientists to accurately reproduce the study.

Not surprisingly, ANZCCART is not the first organization to formally recognise these guidelines and certainly will not be the last. Already a number of leading international research institutions and many key international scientific journals have formally adopted the ARRIVE Guidelines and ANZCCART would encourage others to follow suit. Equally or perhaps even more importantly, we encourage research scientists to use the ARRIVE Guidelines to guide them in the planning, execution and reporting of all research work, particularly when it involves the use of animals.

When it comes to seeking approval from the presiding Animal Ethics Committee (AEC) to use animals as a part of any research project, we would encourage both researchers and members of the AEC to consider and be guided by the ARRIVE Guidelines as part of the process and pay particular attention to the Methods section. Other sections are also important to consider, but either include areas that are generally very well addressed already by the great majority of applicants, or focus on areas that are more relevant to post experimental reporting of data and so of lesser importance to the AEC approval process itself.

The ARRIVE Guidelines are reproduced below:

	ITEM	RECOMMENDATION
Title	1	Provide as accurate and concise a description of the content of the article as possible
Abstract	2	Provide an accurate summary of the background research objectives including details of the species or strain of animal used, key methods, principal findings and conclusions of the study
INTRODUCTION		
Background	3	a. include sufficient scientific background including relevant references to previous works to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and where appropriate, the study's relevance to human biology
Objectives	4	Clearly describe the primary and any secondary objectives of the study or specific hypotheses being tested
METHODS		
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal Scientific Procedures) Act 1986) and national or institutional guidelines for the care and use of animals that cover the research
Study design	6	For each experiment give brief details of the study deign including a. The number of experimental and control groups b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when)

	ITEM	RECOMMENDATION
		c. The experimental unit (e.g. a single animal, group or cage of animals) A time-line diagram or flow chart can be used to illustrate how complex study designs were carried out.
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier (s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naive, previous procedures, etc.
Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc. for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.
Sample Size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).

	ITEM	RECOMMENDATION
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.
RESULTS		
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naive) prior to treatment or testing (this information can often be tabulated).
Numbers Analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%) b. If any animals or data were not included in the analysis, explain why.
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.
DISCUSSION		
Interpretation / scientific implications	18	a. Interpret the results, taking into account the study objects and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.
Generalisability / translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.

PREPARE Guidelines:

More recently, we have seen the publication of the PREPARE Guidelines. While the ARRIVE Guidelines are an excellent guide when planning research using animals, they are designed more to cover the important issues that need to be considered when reporting the findings of research projects (as the name implies), the PREPARE Guidelines focus on the key issues that need to be considered when planning research using animals. These have been published by Norecopa and have been designed to operate in concert with the ARRIVE Guidelines. So much so, that they are published on the Norecopa website under the heading "PREPARE before you ARRIVE". However, as indicated above, the PREPARE Guidelines focus on areas that are not specifically emphasised in the ARRIVE Guidelines and they concentrate on the earlier stages of planning animal-based experiments and the process of applying for permission to use animals. It is therefore interesting to note the very close parallels between the points raised in the PREPARE Guidelines and the requirements outlined to be part of the application process in the Australian Code for the Care and Use of Animals for Scientific Purposes (2013). South Australian readers may even recognise the near perfect resemblance to the questions on the South Australian common AEC application form.

The PREPARE Guidelines provide researchers with an excellent insight to the deliberations of your AEC. Each point raised in these guidelines represents an area of concern to your AEC and so it is true to suggest that addressing each of those points (where relevant to your work) will improve both your application to the AEC and your chances of gaining approval.

As indicated, the fifteen topic headings that make up the PREPARE Guidelines will cover most of the key issues that your AEC want to see addressed.

1. Literature Searches: These not only help researchers to ensure they are using the best and most appropriate models and methods, but also provide an important framework that will help to form a clear and logical hypothesis (or hypotheses) to test. The importance of asking significant and very clear questions when designing a project cannot be over emphasised as this will provide the focus that is a part of every good application.

2. Legal Issues: This broadly covers everything from ensuring that the relevant permits (eg wildlife, biosafety, radiation, transport, etc) are all either in place or ready to go once AEC approval has been obtained. It is also important to ensure that the work will comply with any local or Institutional procedures or requirements that may be relevant.

3. Ethical Issues: The Code requires that researchers satisfy themselves that the work they propose is ethically justified and the AEC must also ensure that they too are comfortable that this is the case. Therefore, it is important to ensure the work is ethically justified and also consider the harm versus benefit equation to guide your project plans for reducing the impact on your animals. Consider and ensure you address all of the 3Rs (Reduction, Refinement & Replacement) and define easily measureable, unequivocal and humane endpoints.

4. Experimental Design and Statistical Analysis: Consider setting up pilot studies and / or check prior published work to determine appropriate details for calculation of statistical power and significance levels so that optimal animal numbers may be determined. Plan methods of randomisation and ways to prevent observer bias through blinding etc. Set parameters to determine criteria for inclusion or exclusion.

5. Objectives and timescale, funding and division of labour: This section highlights the importance of liaising with Animal Care Staff to plan work and determine all details from the costs associated with the work right through to defining who will be performing all tasks (including defining roles in animal monitoring).

6. Facility Evaluation: Physical inspection of all relevant facilities to determine that all equipment, drugs, etc are available and ensure availability of staff as required.

7. Education and Training: Assess current competence of all staff and arrange additional training or refresher training as required.

8. Health risks, waste disposal and decontamination: Discuss with Facilities management any requirements for security, containment, decontamination etc., as well as any specific arrangements that may be required for the disposal of all items associated with the project.

9. Test substances: Provide as much information about test substances and the feasibility of administering them etc. Remember that the AEC is bound by confidentiality, so there should be a full and frank discussion of novel compounds being used.

10. Experimental Animals: Determine the best animal model to use, including species, strain, etc. Decide what characteristics are of importance and if age or gender is important. If not, both genders should be used to help ensure broadest applicability of findings.

11. Quarantine and Health Monitoring: Discuss the health status of animals and any needs for transport, quarantine, isolation, acclimatization and health monitoring, including any potential consequences for staff.

12. Housing and husbandry: In consultation with expert facility staff, determine and attend to any specific instincts and needs of the animals and what forms of environmental enrichment are appropriate. Consider acclimatization, optimal housing conditions and any specific requirements such as isolation, food deprivation, etc.

13. Experimental procedures: Determine how procedures might be refined, including those associated with capture, handling, immobilization, marking and release or rehoming if feasible. Consideration should also be given to refining procedures for substance administration, sampling, sedation and anaesthesia, surgery and any / all other techniques.

14. Humane killing, release, re-use or re-homing: Consult relevant legislation & guidelines during planning stage. Define primary and emergency methods for humane killing and assess the competence of all those who may be called on to perform such tasks.

15. Necropsy: Consult with Animal Welfare Officer (AWO) and facilities staff to construct a plan for necropsy, including location, staff involved and identification of all animals and samples.

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The paper describing the Guidelines can be downloaded free of charge at:

http://journals.sagepub.com/doi/full/10.1177/0023677217724823

NHMRC publishes New Guidelines

The National Health and Medical Research Council (NHMRC) is also working hard to help improve and ensure the reproducibility of biomedical research in Australia and so their CEO, Professor Anne Kelso recently announced the release of a new guidelines document: The Best Practice methodology in the use of animals for scientific purposes (the Guidance). The Guidance provides advice on best practice for the conduct of animal-based studies to ensure the continued ethical and humane use of animals for scientific purposes.

The ethical use of animals for scientific purposes and the value of the outcomes of their use are dependent on the studies being rigorous, transparent and reproducible. This in turn is dependent on the quality of the methodologies used and the reporting of key information on how animal studies are designed, conducted and analysed.

The Guidance highlights common flaws in methodologies used in animal-based studies and issues that affect their reproducibility. It outlines best practice for the conduct of high quality animal-based studies, and provides practical strategies for implementation of best practice methodology during the planning, conduct and reporting of animal-based studies. The Guidance is intended for use by institutions, investigators, members of institutional animal ethics committees and animal carers.

The development of the Guidance was overseen by NHMRC's Animal Welfare Committee comprising members with expertise in veterinary science and animal welfare, as well as scientists and community representatives. The development of the Guidance included consultation with the sector to ensure the practicality of the proposed strategies.

The Guidance supports the implementation of the Australian code for the care and use of animals for scientific purposes, which is adopted in all relevant state and territory legislation.

A copy of the Guidance is available to download from the NHMRC website at:

https://www.nhmrc.gov.au/_files_nhmrc/file/health_ethics/animal/ea20_best_practice_methodology_in_the_use_of_animals_for_scientific_purposes_2017.pdf

Articles of Interest

Zebrafish larvae could help to personalize cancer treatments

Often cancer treatment is experimental and can depend on what works best for the patient, so the use of murine Avatars to determine the best therapeutic regime for use in an individual patient has been trialed. Unfortunately, this is an expensive and time-consuming process that can often take longer to achieve than is available. A group in Lisbon is working with the implantation of human tumour cells in zebrafish larvae.

Zebrafish are cheaper and faster to raise, but there is a greater evolutionary distance between zebrafish and humans than the rodent models, however, when tested, the human tumour cells still grew and showed signs of rapid cell division, formation of blood vessels and the ability to spread. Most importantly, they also showed a differential response to chemotherapy drug combinations and in four of five test cases, accurately predicted the patient response to therapy after surgery.

Read more on this study at:
http://science.sciencemag.org/content/357/6353/745.full

PETA targets early-career wildlife researcher. Critics say animal rights organization is trying to intimidate vulnerable scientists

In 2014, Christine Lattin of Yale University established the use of medical imaging techniques to study how stress affects hormones and neurotransmitters in living birds. The birds she worked with were euthanized, however, she hopes the imaging technology will eventually allow her to release them back into the wild.

On their website earlier this year, People for the Ethical Treatment of Animals (PETA) accused Yale of tormenting birds and Lattin of abusing house sparrows. Within 3 months PETA had organised three protests against the researcher and Lattin was receiving around 50 abusive emails a day. Kathy Guillermo from PETA is running this battle and believes the research is cruel and not relevant to conservation or other species. She also claims they are pursuing Yale and not the researcher.

The University stated that the research activities were approved by Yale and there were no signs of inappropriate care.

PETA is being accused of trying to destroy Lattin's career as she is younger, more vulnerable and not as well-known as other scientists they have previously targeted. Her research is also less invasive than the previous studies PETA have targeted. PETA denies this accusation.

Christine Lattin is not giving up and PETA refuses to back off until Yale stops their animal research. The researcher has become more transparent on her website with clearer details on her studies and adding FAQs. Lattin also believes her goals are similar to PETAs as she's "trying to reduce the number of animals used in research, protect endangered species, and help animals in general". Read the full article here:
http://science.sciencemag.org/content/sci/357/6356/1087.full.pdf

Revamp animal research rules, report urges research organizations call for single agency to be in charge, but critics raise concerns

Moves are afoot within the USA to restructure the way government maintains oversight of research animal use and welfare. For years, scientists and universities have complained about the patchwork of U.S. regulations governing the welfare of animals used in research. Studies involving rabbits and larger mammals, for example, are overseen chiefly by the U.S. Department of Agriculture (USDA) in Washington, D.C. Federally funded studies of rats, mice, and birds are subject to different rules and a different overseer, the National Institutes of Health (NIH) in Bethesda, Maryland. Many privately funded animal studies, meanwhile, get relatively little federal oversight.

To the outsider, the changes suggest that more representative animal use statistics may be published in the future. Currently, the published animal use statistics that are cited internationally, come from the USDA and do not include the millions of rats and mice used each year, let alone the numbers of fish, birds, reptiles, amphibians, etc., so the prospect of seeing more accurate animal use numbers is a potential benefit. However, as a number of US animal welfare organizations are quick to point out, the current differences in approval, monitoring, renewal and reporting practices will need to be considered and are more likely to be rationalised in line with the least stringent practices currently in place, in order to minimise the administrative burden on researchers and this may potentially have some negative effects on welfare standards.

Clearly, if such a rationalisation of systems is to be implemented, it will require a lot of work and, potentially, significant compromise from all quarters. It will be interesting to see how this all plays out over the next 12 months, which is the timeframe proposed by the US government.

A more complete account can be read at: http://science.sciencemag.org/content/sci/358/6362/434.full.pdf

Alzheimer's Disease might be a "whole body" problem

Alzheimer's Disease, the leading cause of dementia, has long been assumed to originate in the brain. But research from the University of British Columbia and Chinese scientists indicates that it could be triggered by breakdowns elsewhere in the body.

Findings of murine studies published recently in *Molecular Psychiatry*, offer hope that future drug therapies might be able to stop or slow the disease without acting directly on the brain, which is a complex, sensitive and often hard-to-reach target. Instead, such drugs could target the kidney or liver, ridding the blood of a toxic protein before it ever reaches the brain. To do this, scientists used a technique called parabiosis, that involves surgically attaching two animals together so they share the same blood supply for several months. UBC Psychiatry Professor Dr Weihong Song and Neurology Professor Yan-Jiang Wang at Third Military Medical University in Chongqing attached normal mice, which don't naturally develop Alzheimer's Disease, to mice modified to carry a mutant human gene that produces high levels of a protein called amyloid-beta. In people with Alzheimer's disease, that protein ultimately forms clumps, or "plaques," that smother brain cells.

Normal mice that had been joined to genetically modified partners for a year "contracted" Alzheimer's Disease. Song says the amyloid-beta travelled from the genetically-modified mice to the brains of their normal partners, where it accumulated and began to inflict damage. Not only did the normal mice develop plaques, but also a pathology similar to "tangles" – twisted protein strands that form inside brain cells, disrupting their function and eventually killing them from the inside-out. Other signs of Alzheimer's-like

damage included brain cell degeneration, inflammation and microbleeds. In addition, the ability to transmit electrical signals involved in learning and memory – a sign of a healthy brain – was impaired, even in mice that had been joined for just four months.

Besides the brain, amyloid-beta is produced in blood platelets, blood vessels and muscles, and its precursor protein is found in several other organs. But until these experiments, it was unclear if amyloid-beta from outside the brain could contribute to Alzheimer's Disease. This study, Song says, shows it can.

"The blood-brain barrier weakens as we age," says Song, a Canada Research Chair in Alzheimer's Disease and the Jack Brown and Family Professor. "That might allow more amyloid-beta to infiltrate the brain, supplementing what is produced by the brain itself and accelerating the deterioration." "Alzheimer's Disease is clearly a disease of the brain, but we need to pay attention to the whole body to understand where it comes from, and how to stop it," he says.

https://www.alnmag.com/news/2017/10/alzheimers-disease-might-be-whole-body-problem?et_cid=6156763&et_rid=497549351&type=cta&et_cid=6156763&et_rid=497549351&linkid=https%3a%2f%2fwww.alnmag.com%2fnews%2f2017%2f10%2falzheimers-disease-might-be-whole-body-problem%3fet_cid%3d6156763%26et_rid%3d%%subscriberid%%%26type%3dcta

An interesting result of this work has been a follow-up study / clinical trial, where dementia patients have been given blood transfusions from young donors.

The first controlled, but controversial and small, clinical trial of giving young blood to people with dementia has reported that the procedure appears safe. It has also hinted that it may even produce modest improvements in the daily lives of people who have Alzheimer's disease.

Researchers who conducted the trial and others caution that the results are based on just 18 people and therefore are only a first step in exploring this type of treatment.

The results suggest the procedure is safe and hint that it could even boost the ability of people with dementia to undertake everyday tasks, such as shopping or preparing a meal. The trial tested people aged between 54 and 86 with mild to moderate Alzheimer's Disease. The team gave the 18 subjects weekly infusions for four weeks. They received either a saline placebo or plasma — blood from which the red cells have been removed — from blood donors aged 18–30. During the study, the team monitored the patients to assess their cognitive skills, mood and general abilities to manage their lives independently.

Blood-transfusion trials are controversial because the active molecules in plasma that seem to lead to the purported effects are unknown. However, the study detected no serious adverse reactions. It saw no significant effect on cognition, but two different batteries of tests assessing daily living skills both showed significant improvement.

The human trial grew out of earlier 'parabiosis' experiments, in which the blood systems of two rodents were surgically joined together to see what happened when molecules circulating in one animal entered another animal.

More details can be found at: http://www.nature.com/news/infusions-of-young-blood-tested-in-patients-with-dementia-1.22930?WT.ec_id=NEWS-20171102&spMailingID=55275469&spUserID=MTc2Njc3MzgzMwS2&spJobID=1280337990&spReportId=MTI4MDMzNzk5MAS2

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is free of charge and is published by the Australian and New Zealand Council for the Care of Animals in Research and Teaching Limited.

It is a publication for researchers and teachers: members of Animal Ethics Committees, staff of organisations concerned with research, teaching and funding, and parliamentarians and members of the public with interests in the conduct of animal-based research and teaching and the welfare of animals used.

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

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