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Protecting Australian native mammals in research and teaching

Alexis Cooper and Mary Bate, NHMRC

From pygmy possums to wombats, ensuring that our native mammals are treated with dignity and afforded a high standard of care in research and teaching is the central concern of a new guide released this month.

The National Health and Medical Research Council's revised *Guide to the care and use of Australian native mammals in research and teaching* will help to support researchers, carers of Australian native mammals, members of animal ethics committees, and state and territory regulators in this task.

Given that the biology, diet, habitat and reproductive mode of native mammals varies enormously between species, it is essential that researchers and carers have access to up-to-date information regarding the specific and unique needs of individual species.

The revised Guide is incredibly comprehensive and provides guidance on current best practices for the care and use of over 120 Australian native mammal species. The advice was sourced from experts of each of the species, and covers areas including:

- capture, handling, marking for identification, transport;
- anaesthesia, analgesia and sedation;
- health issues, disease control and zoonoses;
- conservation status, distribution and natural diet;
- captive husbandry;
- diet for captive animals;
- routine sample/data collection;
- humane killing;
- specific breeding requirements.

The Guide also emphasises the need for those who have not previously or recently worked with a species of Australian native mammal to seek expert advice from knowledgeable sources before obtaining animals or commencing research, and provides a contact list for expert contacts is included.

The Guide was released in October and complements the *Australian code for the care and use of animals for scientific purposes, 8th edition* (2013). The Guide replaces two earlier publications *A Guide to the use of Australian Native Mammals in Biomedical Research - Sections 1-3* and *A Guide to the use of Australian Native Mammals in Biomedical Research - Section 4: Care of individual species.*

It seeks to ensure that any use of Australian native mammals for scientific purposes is ethical, humane and responsible and maintains NHMRC's commitment to the three key tenets of animal research – to use non-animal methods where possible; to reduce the number of animals used; and to refine studies to minimise harm.

The development of the Guide was overseen by NHMRC's Animal Welfare Committee, with guidance from an NHMRC working committee comprised of experts in the care and use of Australian native mammals and animal welfare. The process has included public consultation on a draft version of the Guide with input from a broad range of interested people.

The contribution, expertise and commitment of those who helped develop the guideline was invaluable. NHMRC acknowledges the Chair of the NHMRC working committee, Dr Kathrine Handasyde, as well as the members of the committee itself.

The Guide and details about the working committee are available on the NHMRC website: http://www.nhmrc.gov.au/guidelines/publications/ea29

Testing drugs in animal models

Geoff Dandie, CEO, ANZCCART

There seems to be a growing question mark over the validity of animal models of disease and the applicability of experimental data derived from animal experiments to clinical medicine. So perhaps it might be worth taking a step back to consider not only the validity of animal models, but also the processes of drug discovery and safety testing that precede the licensing of any new medicine.

To do this objectively, we need to discard any naive preconceptions about scientists and drug companies injecting a few animals and then rushing drugs onto the market, because while this might be how the process is portrayed by some sectors in the media, nothing could be further from the truth. Modern methodology for drug development begins *in silico* with computer modelling of chemical structures associated with both biological signalling pathways (i.e. signal receptors on our cells) and potential agonists or antagonists (i.e. potential drugs that might turn on those cell receptors or turn them off) to treat various conditions. Candidate molecules that pass this preliminary stage are then generally subject to *in vitro* testing, which often begins with what are termed 'high throughput screening assays'. These assays will generally involve growing target cells (usually, but not always, human in origin) for the action of the drugs being developed in 96 – or increasingly 384 – well plates so that a large number of potential molecules, compounds, or fractions can be screened for activity as quickly and as economically as possible. Samples that pass this test will then be re-tested *in vitro* to confirm activity and determine the effective dose ranges and get some idea of response kinetics, etc. If those results are also positive, these samples may then be tested in animal models. At this stage, standard safety testing protocols still require any potential new drug to be tested in at least two different species of animal before human testing and while this is controversial on the one hand, there is also still community support for the idea of animal safety testing preceding any administration to human subjects.

Assuming the animal testing yields positive results, permission may be sought for the drug to enter phase 1 clinical trials, where a small number of volunteers might be treated with the drug. If that goes well, it may progress to phase 2 and possibly even phase 3 trials where increasing numbers of volunteer subjects and / or patients might be given the drug. Beyond this stage, the drug woud potentially become the subject of a large-scale clinical trial which would normally involve thousands of patients in various parts of the world being part of a blinded and carefully controlled study where the candidate drug would be tested in patients and compared with the best treatment currently available. Assuming it passes those trials, proving safe, successful in treating disease and at least as good if not better than current treatments, it could be submitted for licensing.

Regrettably, history shows that even after going through all the levels of testing for safety and efficacy, some new drugs end up causing serious side-effects and need to be withdrawn from the market.

So this begs the question, does the failure of any new drug or treatment demonstrate the inadequacies

of animal experimentation and how the use of animal models is inappropriate for such testing? In all honesty, it probably is reasonable to suggest that such failures do show that the use of animals is not a perfect system for testing human medicines. However, such failures must equally be seen to highlight the current inadequacies of computer modelling, for in vitro testing systems and even for testing in human volunteers and human patients as well. So having used medical failures to demonstrate the inadequacies of all currently available testing methods, it might be more productive to turn this around and focus on what we have learned from such failures and aim to improve current safety and efficacy testing procedures.

Possibly the first and clearest lesson from any failure is that the system we have in place is imperfect and potentially fallible. While most people would be happy to concede that model systems such as data mining programmes and computer simulations will provide some valuable guidance, they cannot give definitive answers. On the other hand, how many would have considered that humans subjects were also so imperfect and would fail to predict the problems that necessitated withdrawing those treatments from the market? Equally, most people would concede that cell culture systems or even animal models are only going to have limited predictive value, but again the popular concept seems to be that testing in humans should be more reliable and provide definitive information. Yet clearly that is not always the case. What we can say is that even in the more extreme cases where a so-called 'failed drug' has needed to be withdrawn from the market because of the potential for it to cause death, that drug has obviously performed very successfully during clinical trials and presumably in some patients after those trials had been completed. So what we are really highlighting is the genetic diversity among the people who participated in the trials and the patients who need help.

Looking at this from a pharmaceutical perspective, diversity among the population should be no surprise as clinical medicine has always involved an element of trial and error to see which of the myriad of drugs available to treat some conditions will work best for each individual patient. The fact that there are multiple therapies available for some common conditions is only part of it, as the required dose to treat a condition might also vary significantly between patients. So part of the skill of the medical practitioner is to assess (based on their knowledge and experience) which particular therapeutic agent might work best and at what dose for each patient they treat. This means essentially that every doctor will try to tailor each patient's treatment to that patient's individual needs and situation. While some of this variation will be due to environmental factors, the individual genetic makeup of each patient will also be a key factor in determining how they respond to treatment and so the potential for genetically matching each patient to the best therapy for treating their disease at a genetic level, which is now starting to emerge, may prove to be the key.

Success at the level of genotyping patients and being able to tailor therapies to each patient's health needs would, however, mean more than that. Suddenly we have the prospect of considering the use of therapies that have previously been discarded on the basis of perceived risk in some patients by virtue of the fact that it might be possible to predict who would respond well and who might experience an adverse reaction to each potential treatment. It would also potentially mean being able to go back and review the data obtained from so-called 'failed trials' with a view to reassessing the risks and efficacy of those agents on a patient-by-patient basis.

In the meantime, we still need to remember that animal models are just that – they are models that can be used to predict outcomes, just the same as computer models can predict an outcome. But, like computer models, animal models are not going to provide a perfect answer every time. What we do need to focus on more carefully perhaps, is ensuring that where animal models have to be used, we make sure that we are using the best and most appropriate species and the best model in each case and this perhaps highlights the compounding variable of all – the fact that humans are not infallible either.

Can we further refine humane killing with CO_2?

Geoff Dandie, CEO, ANZCCART

After the first international consensus meeting on CO_2 euthanasia the principle of slow fill CO_2 (adding CO_2 at the rate of ten percent of chamber volume per minute) was seen as a more welfare-friendly way of using a long-standing, cheap and effective method for killing rodents and so it gained widespread acceptance. A key feature of this method that helped it become accepted as 'current best practice' for the humane killing of rodents in laboratories across the world was the fact that the animals lost consciousness before the most profound effects of CO_2 narcosis became apparent. Yet there has always been (and still is) an underlying

suspicion that this method can still be improved to make it more humane.

One of the key factors pushing this desire to further improve the technique is the knowledge that rodents are far more sensitive to CO_2 levels in the atmosphere than many other species, including humans, and they do find levels of CO_2 well short of the 40% concentration required to anaesthetise rats to be unpleasant. In fact, when this has been measured in the laboratory, all evidence indicates that rats find concentrations of CO_2 of around 12% or greater to be strongly aversive, sufficiently so to cause them to abandon their quest for sweet cereal treats and seek a way out of that situation, leaving the chamber if at all possible.

The fact that in most cases naïve rats in particular seem to find an atmosphere of increasing CO_2 concentration to be more aversive than one containing a volatile anaesthetic gas such as Isoflurane is potentially very instructive and is progressively leading welfare agencies to recommend moving away from the use of slow fill CO_2 alone, in favour of using an anaesthetic gas to initially render the animals unconscious before increasing the CO_2 concentration to lethal levels.

At this stage, there are still some questions that need to be addressed regarding the best anaesthetic agent to use in a CO_2 chamber as there is some evidence indicating that prior experience with agents such as Isoflurane can have a profound effect on the response of rats to subsequent exposure. Whether the response of animals to repeat exposure to anaesthetic agents of this kind is potentially more or less distressing than their response to CO_2 alone remains a question and this is one reason why ANZCCART does not yet feel ready to make any definitive recommendations. The evidence is increasingly clear that the use of some form of anaesthetic gas as the first stage of humane killing with CO_2 does represent a significant improvement to the welfare of laboratory rodents. Accordingly, it might be prudent to consider this to be the start of a conversation rather than the conclusion and we would welcome comment and advice from those of you who have experience with the inclusion of volatile anaesthetic agents in your humane killing with CO_2 protocol. We will also continue to work with those most actively researching this area with a view to presenting more on this subject in subsequent issues of ANZCCART News.

Science, society and the ethics of animal use

Pete Hodgson

Science does not occur in a vacuum or on any splendidly isolated pedestal. Instead it occurs in a society. That society nurtures it, supports it, funds it, permits it, forbids it and regulates it. So all science must take place within parameters that are usually taken for granted. For example, science values honesty and punishes dishonesty more than most areas of human endeavor. Science is usually collegial by nature, more so than most occupations. Science is expected to be obscure or esoteric but there is societal interest in learning what it is all about.

Some parameters are unusually permissive. Scientists are not only allowed to fail, it is somewhat expected. Society values risky science more than safe science. Such a cavalier approach to failure is obviously not accorded in fields such as medicine or aviation. Scientists are permitted to offer a surprising or very different public perspective, whereas we other mortals should not step beyond bounded rationality lest we be thought of as weird.

Parameters change with time often as new technologies emerge. Currently our approach to nanotechnology, for example, is still being framed up. Caution seems in the ascendancy. Equally, parameters also change from place to place. Embryonic stem cell research for example, is a much more political issue in the US than in Australasia, while the converse applies to genetic modification where the US has a more liberal attitude than the population in our region. In Japan there is either support for or indifference toward scientific whaling and yet elsewhere, the idea is widely ridiculed. So, a first and obvious conclusion is that science operates in a social context which is important or even powerful, which varies, but which persists.

The use of animals in society is similarly bound by parameters and they too are changing. Animal use is supported and controlled by society, and codified in law. However, law doesn't tell us all we need to know about attitudes to animals. It does not, for example, include the influence of the predominant religion our part of the world. The Bible tells us that man enjoys dominion over animals – the fowl of the air and the fish of the sea.

Like other cultures we own animals - after all we buy and sell them. We kill them too. Within rules to be sure, but we generally don't hesitate to take an animal's life if we want to. All of this we know well, but things

can still get tricky in a number of ways that are worth exploring. For starters whilst vast numbers of animals are killed in Australasia, not many of us associate with killing animals. Farmers, vets and slaughter men do, but not the rest of society. Most of us avert our eyes. However, if we broaden the discussion to include fish, then many more of us are involved in killing. Enter rat bait and the numbers of humans who kill mammals rises steeply, even if they are only little mammals, even though we kill them sort of indirectly and even if they conveniently take themselves away to die. This means that almost all of us are involved, one way or another, in killing animals.

Yet our attitude to animals is not always utilitarian. We love animals too. Our attitudes are strongly influenced by the species we are talking about. We just happen to like some species more than others and we freely discriminate accordingly. So speciesism is alive and well folks, even if it doesn't make it through my spell checker. When these special species are being discussed we stop using words like kill and instead inject words like euthanase - for a cat - or perhaps murder if it is a mountain gorilla.

These special species fall in to three groups. The first are the companion animals. They have been with us for millennia in a successful system of commensalism. I don't need to dwell on these relationships; we all understand them. Most of us have one or more of them on the go right now. The second group is a bit more ethereal - it is whales and dolphins and perhaps pandas or elephants. These are animals to which we seem strongly attracted even though we have little to do with them. They make awkward pets. Yet we feel a need to get to understand them and certainly to protect them. Maybe they partly meet our need for myth and mystery. The third group of animals which we love is also strongly supported by a rational analysis. It is the four species of great ape: the gorilla; the chimpanzee; the bonobo: and the orangutan. We love these animals because we see ourselves in them. Empathy seems to be strongly at play. A human rights argument is never far away.

The important feature of this analysis of love is that the list of species involved is undergoing quite fast change over time. During my childhood, piano keys were topped with bits of elephant tusk and whales were harvested – murdered - in Cook Strait. Conservation has been a big driver of change, but so has the changing relationship that we have forged in our minds. Most dolphin species, for example, have been under no population threat, yet our relationships have blossomed to an entire eco-tourism niche nonetheless.

It is time to draw a second conclusion. The first was that science is done, not in a vacuum, but in a social context. The second is that our relationships to some species change quite quickly - over decades not centuries. A third conclusion can also be drawn because it is self-evident. All animal welfare issues, irrespective of the species in question, are moving in only one direction - towards greater animal welfare protection. So there are shifts in how society values certain chosen species, leading to them having enhanced protection or status. However, there is also a generic shift in animal welfare across all species and that shift is always and everywhere, towards greater protection.

So where does that leave the future for animal use in science (including teaching and testing)? One analysis is pretty bleak. After all, science occurs only with society's permission and society is getting fussier about animal welfare, so it stands to reason that it is only a matter of time before permission is withheld, in part or in whole. A contrary analysis is that the use of animals in science has become much better regulated. The nature and quantity of the experiments is openly disclosed, there are a variety of techniques to reduce, refine or replace, and literature that supports more progress in that direction.

My own view is that animals will be used in science foreseeably. However, I have identified three caveats and all three matter. Two out of three will not do. The first caveat is to avoid the big error. The reason is that it will result in a permanent societal reaction. In Australasia, a shared example of a big error is live sheep exports. Our respective societies have reacted to that trade, in New Zealand's case by logically withdrawing society's permission altogether. A research example might be the unfortunate cervical cancer experiment in humans in New Zealand in the eighties or the dodgy supplementary medicine events in Australia more recently. The first resulted in a commendably strong doctrine of informed consent in New Zealand and the second resulted in rather strong regulation in Australia.

The best protectant against a big error is transparency. The more sunlight, the less likelihood of a big mess. The second caveat is to do with humility, or a lack of arrogance. We all get this stuff because in Australasia we are acculturated accordingly. A scientist who demands the right to do dodgy things to an animal because he or she is brilliant is likely to be pilloried publicly and then denied. We all get that, so it needs no further consideration.

The third caveat is the need to earn permission, continuously and that means continuous improvement. In my view, this is the bit that matters most of all. Standing still will not work. After all, society isn't standing still. Animal protection is continually

advancing, just as we want it to. That means that our work must continually advance too. That is what the 3Rs are about and that is why ANZCCART continues to focus on the 3Rs.

So if you buy my analysis then you will be wanting to progress continuous improvement. Our system is a good one, not a troubled one. It is functional and not broken. It is easily defensible, ethical, somewhat dynamic, somewhat transparent, periodically reviewed, and so on. We can be pleased, as long as we do not reach the conclusion that we have done enough. By definition, continuous improvement says that the destination is never reached.

Recent Articles of Interest

Penn surgeons make cancer glow green

A surgeon and a team of scientists at the University of Pennsylvania School of Veterinary Medicine have demonstrated that an injectable dye can make cancerous tumours glow. The dye called indocyanine green, or ICG, was approved for use in people by the Food and Drug Administration in 1958, and glows a fluorescent bright green under near-infrared light (NIR). When the dye is injected it masses in the tumour tissues as they have “leaky” blood vessels whereas blood vessels in normal tissue do not.

After using ICG on mice with lung cancer, the researchers discovered they could distinguish tumours from normal lung tissue after 15 days. The technique was next tried on dogs that had developed cancer naturally. During each operation and after the tumours were removed, the cancerous tissue glowed green under the NIR whereas the normal tissue did not.

As the technique had worked in a spontaneous large animal model, approval was then given to begin a pilot study in humans with cancer in their lungs or chest. As in the canine surgeries, the tumours glowed, giving them confidence that the technique was successful.

Scientists continue to refine the technique looking for different dyes which are even more specific to tumour cells.

http://www.upenn.edu/pennnews/current/2014-09-11/research/penn-surgeons-make-cancer-glow-green

Eye drops treat blindness in glaucoma model

The World Health Organisation has listed glaucoma as the second leading cause of blindness behind cataracts. In glaucoma, a build-up of fluid in the eye creates pressure which can result in damage to the optic nerve and loss of sight. The eye makes new fluid continuously and it is essential to have a proper drainage system so the fluid can escape.

Using laboratory mice, Northwestern Medicine scientists have identified a chemical pathway in the body which is essential for the development of a proper fluid drainage system. Mice that were genetically modified to block that pathway quickly showed signs of glaucoma.

With the development of the new animal model of glaucoma scientists are looking at developing an eye drop that will activate the chemical pathway to improve drainage, helping to reduce pressure and prevent blindness.

http://www.alnmag.com/news/2014/09/eye-drops-treat-blindness-glaucoma-model?et_cid=4148307&et_rid=497549351&type=cta
and a similar article
http://www.voanews.com/content/study-blockage-on-eye-chemical-pathway-leads-to-glaucoma/2445542.html

Animal rights group ends 15-year campaign against experiments at Huntingdon

Legislation and a police operation across Europe has forced the Stop Huntingdon Animal Cruelty (SHAC) organisation to end its 15-year-old campaign against the Huntingdon Life Sciences (HLS) testing centre in the UK.

During their campaign, SHAC tried to force more than 40 companies to sever their links with the HLS. It has been reported that SHAC has carried out a merciless operation: making false allegations of child abuse; sending hoax bombs; torching employee's cars; and even attacking the Managing Director of HLS with a pickaxe handle.

With the establishment of new laws and legal actions continuing against some of the protestors SHAC announced on their website "with the onslaught of government repression against animal rights

activists...it's time to reassess our methods, obstacles and opponents weaknesses and to change our tactics".

HLS has welcomed the end of the merciless campaign and praised the law enforcement agencies in taking action to control animal rights extremism.

An activist from the newly reformed SHAC reported they are now approaching the Government to change the law to prevent animal cruelty.

http://www.independent.co.uk/news/uk/crime/animal-rights-group-ends-15year-campaign-against-experiments-at-huntingdon-9687843.html

Human speech gene makes mice smarter

Although all animal species communicate with each other, humans have a unique capability to produce and understand language. FOXP2, believed to be one of the genes that enable speech, was discovered in the 1990s in a family with severe speech difficulties caused by a mutation of this gene.

Researchers from the US and several European Universities engineered mice with the human version of the gene and used them in various maze tests. The results suggested that they were better at learning to transfer behaviour from conscious to automatic control. FOXP2 may help in the transfer of behaviour in the development of human speech as the ability to speak is thought to be a conscious action and mouth movements to be automatic.

These experiments have enabled researchers to have a better understanding of speech and have led to further investigation into other kinds of learning processes affected by the gene.

http://www.alnmag.com/news/2014/09/human-speech-gene-makes-mice-smarter?et_cid=4156804&et_rid=497549351&type=cta
and a similar article
http://www.newscientist.com/article/dn26216-human-language-gene-makes-mice-smarter.html#.VBhFJRbCrRA

Work still needed to reduce animals in research

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has recently celebrated 10 years in operation. When the Centre was first established, the scientific community were doubtful. However, since that time the organisation has funded numerous research projects and has been influential in improving animal welfare in research.

However, there are still many scientists who are not committed to the 3Rs in their research, says Vicky Robinson, Chief Executive, and they are concerned that changing laboratory conditions to improve animal welfare could have negative impacts on their research outcomes.

The recently released strategy for the NC3Rs for the next 10 years includes: working on the standardisation of improvements to laboratory conditions to benefit both research outcomes and the welfare of animals; ensuring that all scientists are committed to the 3Rs; and expanding these strategies to other countries.

http://blogs.nature.com/news/2014/10/work-still-needed-to-reduce-animals-in-research.html

Remembering the sacrifices that mice have made to science

In 2013, British universities were responsible for the death of 1.3 million animals used in research with nearly 1 million of those being mice. Mice are widely used as "it's easier for scientists and cheaper if everyone is using the same animal" reported Daniel Engber in a series of articles for *Slate* magazine.

With that in mind, researchers at the US Davis Center for Neuroscience and the Department of Psychology have conducted a study on mice to see if memories can be removed from their brains. By using genetically modified mice and light through a fiber-optic cable, researchers were able to see which cells were used in the learning and memory recollection process. After further testing they discovered they were able to erase bad experiences using pulsing lights through probes in the mice's brains.

The technology needs to be researched further before it is compatible with humans. However, if successful it may be able to help people suffering with post-traumatic stress cope better.

http://www.dailytitan.com/2014/10/of-mice-not-men-remembering-the-forgotten-sacrifices-that-mice-have-made-to-science/

Lab animal protection overdue

In a recent letter to the Journal *Science*, Aysha Akhtar has questioned the system of accreditation for laboratories using animals employed by organisations such as the Association for Assessment and Accreditation of Laboratory Animals (AAALAC).

The main point raised in this letter is that the 2011 review of chimpanzee use in biomedical research in the USA conducted by the Institute of Medicine, concluded that much of the work did not have to be undertaken in chimpanzees. Bearing in mind that many of the laboratories involved had current accreditation from AAALAC, the author has surmised that the process of accreditation by such organisations is substantially flawed. Whether or not this is true is a matter of opinion, but the observations covered by this letter do potentially raise questions about the review process and what factors are considered as a part of such accreditation visits.

The fundamental issue that needs to be considered is that any form of national or international accreditation is (at least in part) aimed at bolstering public confidence in the work that is undertaken within the accredited facility. It might therefore be important to ensure that accreditation assessments are done in a way that is in line with public expectations as well as the usual professional standards that are used to guide such examinations.

http://www.sciencemag.org/content/345/6203/1461.2.full

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