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## Contact Us:

Web:

[www.adelaide.edu.au/ANZCCART](http://www.adelaide.edu.au/ANZCCART)

Email:

[ANZCCART@adelaide.edu.au](mailto:ANZCCART@adelaide.edu.au)

# Ask a simple question

*J. R. Webster and M. A. Sutherland, AgResearch Ltd, New Zealand*

A common question posed to animal welfare researchers is "is the welfare of animals (in a specific situation) OK?" Whilst this might appear to be a simple question, in fact, it is far from simple. The example described below has taken three years to reach the stage where we can begin to have an answer. This is due in part to the complexities of what actually defines animal welfare and what "OK" means in that context. It has also been complicated by the fact that there was only an annual window of opportunity for this specific research question to be addressed. In addition to these factors, it is necessary to understand the scope of the question with regards to animal welfare and allow this understanding to guide us in how to best answer it.

Animals do not inherently have 'welfare' unless a human is judging them. That is, welfare is about how we see and assess an animal's state against our expectations and understanding of how its life should be. Given that, welfare is determined by the actual state of the animal and

cannot be determined directly from the quality of the environment or resources provided. The assessment of welfare must therefore be carried out in the best way that we can, to determine how life is for an animal. However, the question remains, what aspects of life are important in determining the quality of that life? As welfare is ultimately judged against our expectations, we tend to focus on three areas; biological functioning (an animal's physical and health state), affective state (an animal's feelings) and naturalness (an animal's natural behaviours) which correspond to the main societal orientations of importance to animal welfare. We therefore need to objectively assess the animal's status in these three dimensions in order to answer the second half of the question, "is the animal's welfare OK?" This means assessing whether the amalgamation of welfare across the three dimensions is acceptable with respect to the current societal expectations for animals in that situation. While science alone cannot make this judgment, it can assist by providing comparisons and interpretations of the measurements made, in terms that can be used for this purpose.

A recent example of a question along the lines described above concerned the substrate upon which dairy calves are reared in New Zealand. Calf-rearing is a process that happens in the dairy industry when a calf is removed from its mother shortly after birth and taken to a facility where it is fed colostrum and then milk and supplements until an age when it can be weaned. To facilitate feeding and to provide adequate shelter and cleanliness during this period, the rearing area is usually indoors or in a sheltered area. The underfoot surface must therefore be comfortable enough for the calf to live on and remain hygienic for this period. Traditionally, wood-based materials such as sawdust or woodchips have been used successfully for this purpose; however recent demand for these materials for other uses has meant it has become increasingly difficult and/or expensive for farmers to obtain sufficient quantities of these products.

Several years ago, farmers started looking for alternatives to the traditional sawdust or woodchips and some started to use river stones because they were easy to access, economical and they had perceived health benefits associated with improved calf cleanliness. This practice came to the attention of the Ministry for Primary Industries and the dairy industry and we were subsequently approached to evaluate the welfare of calves reared on river stones.

The first stage of answering this question was to carry out an initial study on a commercial farm where river stones were being used. Due to the late-spring timing, a farm in Southland that was still rearing calves was selected. A basic experimental design was used to compare calves reared on river stones with those reared on sawdust, which was perceived to be best practice. As rearing normally lasts from one day to six weeks of age, comparative measures were timed to occur during week one and week five. The measurements included behaviour (using 24hr video recording), body weight, cleanliness (using a score), health (by veterinary check for signs of disease and injuries) and skin temperature (using adhesive temperature loggers).

The result obtained indicated that after one week, the calves reared on river stones played less (less time running and fewer head shakes, jumps, kicks and leaps) than calves reared on sawdust. After five weeks of rearing, the calves kept on river stones spent less time lying and performed fewer head shakes and kicks than calves reared on sawdust. Calves reared on river stones also had a cooler skin temperature (1.2° C) at both time points compared to calves reared on sawdust. There were no differences between the calves reared on either substrate when parameters such as body weight and cleanliness were assessed

and there were no clinical signs of disease, lameness, leg lesions, injury or abnormalities detected in either group.

Thinking back to the three important indicators of welfare, it could be concluded that biological functioning was similar for calves raised on either surface (with the exception of the small difference in skin temperature). However, the reduced lying and play behaviour exhibited by calves raised on river stones indicated that the naturalness and affective state of these calves might be impacted. It is possible that calves find river stones more uncomfortable and/or more difficult to move about on and the reduced insulating properties could add to the reduced comfort under the conditions studied.

Given this potential for decreased welfare, it was decided that more evidence was needed in a research setting and a second study was carried out. A number of additional issues associated with the rearing of calves on river stones had been raised subsequently, which may have affected the results of the first study, including the space allowance of calves used and the nature of the stones. These questions were supported by published evidence indicating that space allowance can indeed affect calf behaviour.

In the first study, the space allowance was based on standard practice for that farm, which was 1.8 m<sup>2</sup> per calf. Although there are currently no minimum space allowances for rearing calves in New Zealand, the industry standard is approximately 1.0 m<sup>2</sup>/calf and industry bodies have recommended 1.5 m<sup>2</sup>/calf. In the subsequent study we therefore compared 1.0 m<sup>2</sup>/calf, 1.5 m<sup>2</sup>/calf, and a larger space allowance of 2.0 m<sup>2</sup>/calf. There was also the matter of the stones themselves. In Southland the stones used in the first study were river stones, approximately 3-12 cm in length with a smooth surface. In the North Island these stones were not readily available and quarried stones were used in the second study instead, which were a similar size but had rougher edges.

The second study was designed to compare calves reared on sawdust versus stones at the three space allowances described above. An additional sampling time point was added so that measures of the calves were taken at two, four and six weeks of rearing. Behaviour, skin temperature, body weight and cleanliness were recorded, as they were during the first study, but additional measures of hygiene were taken including swabs for *E. coli*. Blood samples were also collected for measurement of cortisol (to assess stress), creatine kinase (to assess muscle damage), and immunoglobulin-G, serum amyloid-A and total protein concentrations (to assess immune function). As the first study had indicated that naturalness and

affective state might have been affected, the second study set out to specifically determine if play behaviour was affected by rearing on stones. Play is a natural part of a young animal's behaviour and in addition is thought to indicate that the animal is in a positive affective state, as its occurrence is reduced in calves under negative circumstances such as pain, hunger and low space. To make the measurement of play easier, it was recorded in a situation in which play is stimulated, which was done by moving the calves into a separate arena that had a novel surface to all calves (bark chips). It was predicted that rearing calves on river stones and at smaller space allowances would increase their performance of play in the arena test, as a response to suppression of this behaviour by the home environment.

The findings were consistent with the first study with calves on stones spending less time lying and walking and more time standing after four and six weeks of being reared compared with calves on sawdust. When released into the arena, calves reared on stones played more than calves reared on sawdust. Calves reared on stones had lower skin surface temperatures than calves reared on sawdust once again. No differences were found between the rearing substrates in terms of body weight, cleanliness, hygiene stress and immune function, with the exception of fewer *E. coli* being recovered from the shoulder of calves reared on stones. The only effects of space allowance were on behaviour, with less time lying and more time standing and walking at 2.0 m<sup>2</sup>/calf than at 1.0 and 1.5 m<sup>2</sup>/calf and increased play behaviour of calves reared in the smaller space allowance when they went into the arena (supporting the inverse association between home pen play behaviour and play in the arena). There were some signs of rubbing and hair loss on the legs of the calves on stones at six weeks and we therefore kept the calves on stones and sawdust for an extra two weeks to investigate if this would worsen but it did not. This hair loss was not seen on the calves in the Southland study and may have been due to rubbing of the hair against the rougher surface of the stones used in the second study, when calves knelt to lie down.

The second study therefore provided further evidence that while biological functioning of calves reared on stones is not different to those reared on sawdust, the affective state and natural behaviour of calves on stones may well be impacted. In addition, it appears that not all types of stones are equal and there needs to be caution exercised with regard to the choice of stones. Furthermore, a higher space allowance may help compensate for some of the behavioural restriction that occurs on stones.

Overall, a picture was building up suggesting that while there was not enough evidence to recommend to the industry that the practice of rearing calves on stones

should be stopped, the differences between stones and sawdust were sufficient to conclude that rearing on stones was not best practice. Another important aspect of the question of acceptability of the practice still needed to be examined, however, and this was the calves' perception of living on a stone surface. During presentation and discussion of results with stakeholders it was highlighted that intuitively, the public perception of living on stones is that it would be uncomfortable and affective state would be impacted, but would the calves feel the same way? Additionally, from an industry perspective the difficulties of accessing and using sawdust were still present so if stones were not a best practice alternative, what other materials might be?

This year we therefore undertook two further experiments. The goal of the first experiment was to determine the preference of calves for different rearing substrates. Preference tests have been widely used in animal welfare research as testing an animal's preference is one of the most direct and simplest ways of asking an animal how it feels about its environment, and gaining an insight into which option the animal prefers. For this study, we compared sawdust and stones, but also added in two other alternatives, sand (used successfully for lactating cow bedding in free stalls) and rubber chip (a product made from used tyres with the metal removed). Preferences were evaluated in two ways. Initially, we wanted to assess calves preference for a given substrate when given free access to all four substrates simultaneously. Secondly, we wanted to rank calf preference between the four substrates and this was achieved by comparing two substrates at a time (pair-wise). Preference was determined by the proportion of time calves spent lying on each substrate. The purpose of the second experiment was to investigate the welfare implications of rearing calves on four different substrates, three novel options in comparison to sawdust. The substrates tested were sawdust, pea metal (small stones < 1 cm), sand and rubber chips. As in previous studies, comprehensive measurements of the health and welfare of calves on each surface type were made.

Preliminary results of these studies are now available. The preference study indicated that calves had the least preference to lie on stones, strongly favouring every other surface that was compared with stones in pairs. When all surfaces were compared together, calves showed a strong preference for lying on sawdust, reinforcing this substrate as a 'gold' standard for lying surfaces. The second experiment revealed very few differences between the substrates in terms of health and welfare, suggesting that the alternatives to sawdust all have potential as rearing substrates on this basis.

In conclusion, the process of answering what might

seem at first a very simple question was a complex and time-consuming one involving large multidisciplinary scientific teams across multiple organisations and many locations. In this instance the studies revealed that while biological function appears to not be impaired on stone surfaces, naturalness and affective state are likely impacted. The approach of including the main societal orientations towards animal welfare in coming up with an assessment of welfare that is both robust and acceptable is, however, a valuable one that ultimately will deliver the most benefit to the many stakeholders concerned.

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### **Editorial Comment:**

#### **Animal Welfare Cuts Across the Board**

*Geoff Dandie, CEO, ANZCCART*

During the past few years, we have seen governments around Australia face budget problems and a real need to cut back on expenditure. Regrettably, both federal and state government departments responsible for overseeing animal welfare legislation have not been immune from these cuts and neither have animal welfare programmes they run. This reality recently came to a focus at the end of February, when the federal government shut down the Animal Welfare Unit within the Department of Agriculture, Fisheries and Forestry and this marked the end of their support for the Australian Animal Welfare Strategy (AAWS). When this closure was announced, the Minister rather optimistically expressed a hope that state governments (who are after all responsible for animal welfare legislation in Australia) would pick up many of the programmes implemented under AAWS and take them forward. Only one week later, the Victorian Government responded by announcing that it would be closing down the Bureau of Animal Welfare (BAW) by the end of the current financial year in June.

Both the AAWS and the BAW have been seen nationally and internationally as beacons of knowledge, innovation and examples of how government can effectively promote animal welfare, so I guess describing their demise as 'unfortunate', 'disappointing' or 'regrettable' seems wholly inadequate. What is unfortunate, disappointing and regrettable is the message (or possibly messages) these closures might send to both the general public and the international community.

Other issues aside, if we focus on the implications of these closures on the welfare of animals used in research and teaching, I would have to agree that there still is a very good system of regulation and oversight in place that is both publicly accountable and operating in line with the common government principle of 'user pays'. Equally, I might argue that the AEC system we have in place across Australia and New Zealand is a well-honed model of devolved responsibility that ensures those who have oversight of work using animals are as locally based as possible and therefore well placed to monitor such work effectively. Yet the simple fact that all these measures are also monitored and overseen by the relevant state Government is an important aspect of the systems credibility and ensuring that it is fully supported at the highest levels and this is something I believe we need to ensure will continue. Regrettably, it would seem that one of the key ideals of the AAWS, which was greater consistency of animal welfare legislation across all jurisdictions, is almost certainly going to be a casualty of these closures.

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*The following is a second opinion submitted in response to the original article by Mandy Paterson and a subsequent opinion article by Jeff Schwartz that questioned the use of animals in undergraduate practical classes. Mandy's article can be read here: [ANZCCART News \(2013\) Vol. 26\(3\), pp 3-4](#) and Jeff's opinion here: [ANZCCART News \(2014\) Vol 27\(1\), pp 3-4](#).*

#### **Why undergraduate science students must undertake practical studies involving animals**

*Kathryn L Gatford, Ian Musgrave, Denise Noonan, Susan Hazel, Phillip Hynd, Andrea Yool and Elizabeth Beckett*

A car mechanic who has been taught the theory of car repair but never opened a bonnet is likely to have trouble applying that knowledge and could be a liability rather than an asset when first entering the workforce. A degree in biological sciences, health sciences, or related applied areas is incomplete without hands-on understanding of the dynamics and complexity of living systems. Studies with animals are needed for teaching essential concepts in biology and health. The judicious use of animal models reinforces an appreciation of the value of life and its impressive complexity, while teaching skills that cannot be learned any other way.

Graduates of Science and Applied Science programs leave tertiary educational institutions and enter a workforce where they require skills that can only be gained by using animals in practical classes under direct supervision. Graduates who work as veterinarians, animal scientists, animal technicians and research assistants in pre-clinical studies, or go on to higher degree study, will work directly with animals during their careers. Other science graduates who may not go on to work directly with animals still need an understanding and the capacity to evaluate outcomes obtained in animal experiments, for example, to analyse information required for regulatory approvals of new drugs.

Biological scientists need a practical understanding of research to be able to interpret and evaluate existing knowledge, as well as to competently perform their own research. Evaluating how a drug will work in animals and humans requires progressive evaluation, first *in silico* using computer modelling, then *in vitro* in cells and in whole tissues and subsequently *in vivo* using animals and humans-integrated biological/physiological systems. Science graduates require the skills to undertake and evaluate such research, as recognised by the pharmaceutical industry<sup>1</sup>.

Animal science, animal technology and veterinary science students are required to undertake animal handling, animal husbandry and animal procedures skills training as core components of their respective undergraduate courses. Some may argue that tertiary educators and trainers should never use animals or pre-prepared animal tissue (the opinion expressed by Mandy Paterson in Issue 3 2013 of *ANZCCART News*). Accepting this argument would mean, for example, that veterinary graduates would first practise clinical skills on living animals after they leave university and enter clinical practice. For veterinary students, gaining practical skills in animal handling, surgery, anaesthesia and clinical treatments requires the use of living animals and this is recognised and mandated by accreditation authorities and veterinary registration boards. Put simply, these graduates could not and should not, enter clinical veterinary practice without gaining and demonstrating competency in the appropriate skills.

We fully support the 3Rs and agree that animals should be used in teaching, as in research, only where justified. This is the current practice (and law) in Australia, where animal use must be approved by an Institutional Animal Ethics Committee, as detailed in the *Australian Code for the care and use of animals for scientific purposes* (2013). Science teachers in educational institutions throughout Australia view the animals under their care with responsibility, concern and respect. As discussed by Jeff Schwartz in Issue

1 of *ANZCCART News* this year, the use of animals in teaching is considered by ethics committees on a case-by-case basis. Animal use must be justified and will not be approved or proceed unless it is justified on the basis of evidence that it is necessary to meet the teaching objectives and educational outcomes. Prior to, and during the practical class, the ethics of animal use is explained and all students who use animals in our institution are involved in such discussions.

Replacements and alternatives to animal use are already widely used in teaching. Computer simulations and physical models of systems are valuable tools in student learning. Initial training and skills may well be best learned on such systems. For example, animal science, veterinary science and research students who will be collecting blood samples from animals, ideally practise initially on models, progress to using dead animals culled for other reasons and then apply their skills on live animals, refining their skills to minimise impact on living animals. Models however, do not vary in their anatomy or blood vessel locations as live animals do, and neither models nor dead animals react to handling and sampling in the way a live animal will. Practical skills such as animal handling or clinical care cannot be gained without use of animals, which is always under the supervision of trained teachers. Provision of high-quality resources to allow animal numbers in teaching to be reduced through initial training using models is a further issue for institutions with limited resources, and may be a practical way for those with animal welfare interests to assist in reducing unnecessary use of animals in teaching.

We certainly agree with Mandy Paterson that practicals using animals without prior preparation have less value as a learning exercise than those in which students prepare and engage with concepts beforehand. For example, our science students in third year practical classes test fundamental principles of smooth muscle contractility, prepare hypotheses and submit an outline of their proposed experiments prior to conducting their practical using rodent gut segments dissected post mortem. This approach ensures the students understand the underlying theory before undertaking such a practical and that they are able to understand and appreciate the outcomes being tested in real tissues. We are able to use few animals, because one animal provides enough tissue for these practical classes of approximately 30 students. Students work in small groups, ensuring all students have direct contact with the animal tissue and gain “hands on” skills.

Students who have undertaken “hands on” or “wet lab” practicals learn technical competencies and have a better understanding of the skills and techniques

used to generate data than those who perform virtual experiments using a computer simulation. Students with hands-on experience do better in assessments of this knowledge at the end of semester<sup>2</sup>. In an Australia-wide survey of pharmacology student attitudes to practicals, students reported that "wet lab" practicals using animal tissues improved their understanding of theoretical concepts. Furthermore the majority of these students support continued use of animal-based practicals. Practical that use animals or animal tissues allow development of higher level skills in the students, including analysis and interpretation of unpredictable, variable and complex data in real time<sup>2,3,4</sup>. Some students feel that they struggle with hands on practicals and are more comfortable with a simulation in which they can concentrate on one aspect of a concept at a time<sup>5</sup>. Others, however, recognise that they gain more knowledge and are better able to integrate complex physiological concepts after performing the animal experiments<sup>5</sup>. Use of "hands on" as well as theoretical learning addresses the learning needs of more students, by providing kinaesthetic learning opportunities (learning by doing) to complement visual, auditory and read/write learning opportunities<sup>6</sup>.

Although alternatives to animal use are helpful in enhancing the teaching experience, the alternatives that are currently available have significant limitations. Cells and tissue alter their physiology when cultured, making them unsuitable for the testing of basic physiological processes. Organ systems function together in balanced and regulated interactions that cannot be analysed in a cell culture model. Cell culture systems often require the use of fetal calf serum in the culture media, so animals are needed even for cultured cell experiments. Computer stimulations are valuable tools for teaching particular concepts as simplified versions of *in vivo* systems. Because these models are based on existing knowledge, they do not support developing and testing student-driven hypotheses, designed to evaluate novel questions. Running a computer simulation of tissue responses to various agents does not teach students the skills required for handling animal tissue, the intricacies of connecting tissue segments to a measurement systems, nor an appreciation of the variation obtained in measurements when conducting science. The latter is particularly important in developing a good understanding of principles of experimental design, such as use of appropriate controls, and rigorous hypothesis testing. Similarly, the use of animal tissues for courses such as anatomy teaches practical skills in a way that viewing textbook images and videos cannot. The ethical impact of such animal use can and should be minimised by ensuring that these animals are raised ethically, including provision of environmental enrichment, and are killed humanely.

Our "take home" message is not that all teaching must use animals, but that teaching biological science and applied science competencies as well as theory does require the appropriate ethical use of animals in teaching.

<sup>1</sup>S Franz 2003 *In vivo* we trust. *Nature Reviews Drug Discovery* 2:501.

<sup>2</sup>IE Hughes 2001. Do computer simulations of laboratory practicals meet learning needs? *TRENDS in Pharmacological Sciences* 22:71-74.

<sup>3</sup>AW Ra'anani 2005 The evolving role of animal laboratories in physiological instruction. *Advances in Physiology Education* 29:144-150.

<sup>4</sup>RG Carroll 2005 Using animals in teaching: APS position statement and rationale. *The Physiologist* 48:206-208.

<sup>5</sup>RW Samsel, GA Schmidt, JB Hall, LDH Wood, SG Shroff, and PT Schumaker 1994 Cardiovascular physiology teaching: computer simulations vs. animal demonstrations. *American Journal of Physiology* 266:S36-S46.

<sup>6</sup>K Tanner and D Allen 2004 Approaches to biology teaching and learning: learning styles and the problem of instructional selection - engaging all students in science courses. *Cell Biology Education* 3:197-201.

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## Recent Articles of Interest

### '3D' model could reduce animal testing of asthma and allergy medications

In a recent study scientists reported they have developed a simple "3D" laboratory method to test asthma and allergy medications. The test involves three types of cells from a person's airway and mimics what happens to the airway when the cells are exposed to allergens and bacteria extract. The scientists believe the model has the potential to reduce the need for some animal testing of medications for respiratory conditions.

[http://www.alnmag.com/news/2014/04/3d-model-could-reduce-animal-testing-asthma-and-allergy-medications?et\\_cid=3887253&et\\_rid=454969632&location=top](http://www.alnmag.com/news/2014/04/3d-model-could-reduce-animal-testing-asthma-and-allergy-medications?et_cid=3887253&et_rid=454969632&location=top)

### Experimental cancer drug reverses schizophrenia in adolescent mice

Researchers at Johns Hopkins have been working on an experimental anti-cancer compound which appears to have reversed behaviors associated with schizophrenia and restored some lost brain cell function in mice. Schizophrenia symptoms typically appear in late adolescence and early adulthood and so the findings

in teenage mice are an especially promising step in efforts to develop better therapies for schizophrenia in humans.

[http://www.eurekalert.org/pub\\_releases/2014-03/jhm-ecd032814.php](http://www.eurekalert.org/pub_releases/2014-03/jhm-ecd032814.php)

### **Self-healing muscle grown in the lab**

Researchers at Duke University have grown living muscle in the laboratory that looks and works like the real-thing and when tested was found to be strong and good at contracting as well as being able to repair itself when damaged with a toxin. The muscle has been successfully grafted into mice; however, more tests are needed before it can be used in humans.

<http://www.bbc.com/news/health-26821080>

### **UK research groups pledge openness about animal research**

The United Kingdom has a history of animal-rights extremism including break-ins at laboratories, fire bombs and violent intimidation of researchers, animal breeders, and companies that transport animals. The animal-rights groups have long complained about a "secrecy clause" that prevents details of animal research in the UK being made public and in an effort to put this opposition behind them the Government has released a concordat in openness. This agreement is aimed at helping the public better understand the conditions of animals in laboratories and their importance for medical and biological science. It also commits organizations to increase the visibility of their operations and to report annually on their progress. People for the Ethical Treatment of Animals (PETA UK) are sceptical and believe the concordat allows researchers to decide what to reveal and what to hide from the public. The following two articles discuss the agreement further.

[http://www.nature.com/news/uk-proposes-greater-transparency-on-animal-research-1.15143?WT.ec\\_id=NEWS-20140506#auth-1](http://www.nature.com/news/uk-proposes-greater-transparency-on-animal-research-1.15143?WT.ec_id=NEWS-20140506#auth-1)

<http://news.sciencemag.org/europe/2014/05/u.k.-research-groups-pledge-openness-about-animal-research>

### **Needed: more females in animal and cell studies**

Researchers often avoid female animals because they are considered more variable due to the estrous cycle, however, the National Institute of Health (NIH) hope to change this and are releasing a policy that will require grant applications to balance the sex of animals and cells they wish to study or explain why not. An analysis published earlier this year in *Neuroscience & Behavioural Reviews*, found that male biology was just as variable as that of their female counterparts.

For example, female rats respond differently to stress than males do and also, how males are housed, alone or with others has an impact on results. A neuro-endocrinologist from Smith College in Massachusetts surveyed almost 2,000 animal studies and summarised by saying that there is a "real lack of understanding of human biology" and conclusions are being made about females based on studies that don't support that.

<http://www.sciencemag.org/content/344/6185/679.full.pdf>

### **An end to animal testing for drug discovery?**

The development of "chemosynthetic livers" could drastically alter how drugs are made. These livers use catalysts that act similarly to a specific group of enzymes and so rather than using laboratory animals, researchers could work out the metabolic profiles of drugs by mixing them in test tubes with the livers. So far 50 drugs tested have shown that the catalyst accurately mimics how the human body processes them, however, they are yet to be approved to take the place of animal tests.

[http://www.alnmag.com/news/2014/03/end-animal-testing-drug-discovery?et\\_cid=3830499&et rid=497549351&location=top](http://www.alnmag.com/news/2014/03/end-animal-testing-drug-discovery?et_cid=3830499&et rid=497549351&location=top)

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**ANZCCART**

C/- The University of Adelaide  
South Australia 5005  
Australia

Tel. 61-8-8313 7585 Fax. 61-8-8313 7587

E-mail: [anzccart@adelaide.edu.au](mailto:anzccart@adelaide.edu.au)

<http://www.adelaide.edu.au/ANZCCART/>

or

**ANZCCART New Zealand**

PO Box 598  
Wellington

New Zealand

Tel. 64-4-470 5784 Fax. 64-4-473 1841

E-mail: [anzccart@royalsociety.org.nz](mailto:anzccart@royalsociety.org.nz)

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