PORTFOLIO COMMITTEE NO. 2 - HEALTH
INQUIRY INTO USE OF PRIMATES AND OTHER ANIMALS IN MEDICAL RESEARCH IN NEW SOUTH WALES
HEARING – 1 JUNE 2022 SUPPLEMENTARY QUESTIONS TO PROF ANDREW KNIGHT

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1. Your submission references some of the limitations of animal research, and also highlights some of the potential risks due to the biological difference between animals and humans, which can lead to misleading results. Could you please provide some more information on this issue – do you believe we need to be advancing alternatives to animals for the sake of good science?

The poor rates of translation of animal outcomes into human patients and consumers are due both to the animal models themselves, and to the manner in which they are used. Animals differ from humans in multiple relevant ways. Differences in absorption, distribution, metabolism, and elimination pathways or rates, affect *toxico- or pharmaco-kinetics* (i.e., bodily distribution of test compounds). *Toxico- and pharmaco-dynamics* (mechanisms of action and biological effects) may also differ. Jointly these may alter organ systems affected, and the nature and magnitude of those effects (Knight 2011).

Human predictivity is further compromised by the experimental protocols used. Young animals, of single strains and sexes, lacking in biological variably and concurrent human risk factors, such as common comorbidities, become even less likely to predict outcomes of human patients, consumers or workers (Knight 2011).

Many toxicity tests also use *maximum tolerated doses* (above which dose increases become impossible, due to acute, toxicity-related effects), as well as chronic dosing. These factors do maximize sensitivity to toxins. However, these doses can also overwhelm physiological defences that are effective at environmentally realistic doses. As a result, many compounds that would not normally result in toxicity, are falsely indicated as toxic in animal tests, seriously undermining the reliability of any positive results. Human routes of exposure (e.g., inhaled) may also differ from those used in animals, requiring extrapolation between routes of exposure, introducing further uncertainty (Knight 2011).

Laboratory animals also experience stress both chronic and acute, resulting from laboratory environments and procedures. These stressors can alter physiological, hormonal, and immune status, and even behavioural repertoires and cognitive capacities, in ways that may be unpredictable (Balcombe *et al.* 2004, Balcombe 2006, Baldwin and Bekoff 2007).

Additionally, a sizeable body of systematic reviews have confirmed that significant methodological flaws are highly prevalent in most published animal experiments (e.g., Knight, 2019). To date, no systematic reviews have found that a majority of animal studies in any field, were of good methodological quality.

Bias of results occurs when factors systematically alter research outcomes. This may be conscious, but usually results from unconscious factors. Hooijmans *et al.* (2014) described 10 types of bias with potential to influence animal research results. They grouped these into selection bias, performance bias, detection bias, attrition bias, reporting bias, and other sources of bias. Many of these flaws are highly prevalent within animal studies. Common examples include use of apparently arbitrary numbers of animals, rather than statistically justified and significant sample sizes. Failure to use randomisation during allocation to treatment and control groups, and blinding during outcomes

assessment, are also common, as is lack of reporting of basic characteristics of animals used. Percie Du Sert *et al.* (2020) found that randomisation was reported in 30% – 40% of published animal studies, blinding in around 20%, sample size justification in < 10%, and all basic characteristics of animals used reported in < 10% of publications (Macleod *et al.* 2015, Avey *et al.* 2016, Leung *et al.* 2018).

Across a diversity of fields, studies that incorporate the fewest measures to minimize sources of bias, have also reported the greatest treatment effect sizes (e.g., Crossley *et al.* 2008, Vesterinen *et al.* 2010). Accordingly, we can conclude that such apparent increases in effect size, are not real, but are *artefacts*, resulting from flaws in experimental design, conduct or reporting.

In response to such problems, in 2010 Kilkenny and colleagues proposed the *Animal Research: Reporting of In Vivo Experiments* (ARRIVE) guidelines. These comprised a checklist of 20 items, designed to minimise such flaws by ensuring animal research publications include basic information on animal numbers and characteristics, housing and husbandry conditions, and experimental, statistical, and analytical methods employed. Steps to reduce bias were prominent, such as randomisation, blinding, statistical justifications of sample sizes, reporting of exclusion criteria, and of investigator conflicts of interest. Several similar guidelines have been published, but the ARRIVE guidelines are most prominent. Despite their very widespread publication and endorsement by research journals, major funding agencies, and biomedical research organisations, multiple studies have demonstrated that compliance with the ARRIVE guidelines remains poor (Macleod *et al.* 2015, Avey *et al.* 2016. Leung *et al.* 2018, Percie du Sert *et al.* 2020). In response, the guidelines have been simplified into 'essential' and 'recommended' checklists in ARRIVE 2.0 (Percie du Sert *et al.* 2020). It remains to be seen, whether this will improve compliance.

Recommendations for scientific animal research

As we've described elsewhere (De Boo and Knight 2008), a multifaceted strategy is warranted to increase the implementation of 3Rs principles, improve the welfare of laboratory animals, and improve the methodological quality of animal research.

Compliance must become mandatory, with 3Rs principles, the ARRIVE guidelines, and other best practice standards, during the design, conduct, and reporting of animal experiments. Such standards should cover animal sourcing, housing, handling, environmental enrichment, socialization opportunities, appropriate use of anaesthetics and analgesics, and of refinement modalities such as non-invasive or *humane endpoints* (the latter being the humane killing of animals early within terminal protocols). Compliance with a range of measures designed to minimize bias and ensure methodological quality, must also become mandatory. Compliance should be necessary for securing ethical approval and research funding; for licensing of researchers, facilities, and experimental protocols; and for publication of subsequent results.

To enable animal researchers and technicians to meet the necessary standards, regular training in 3Rs methodologies, and in the design, conduct, and reporting of animal research, should be universally compulsory. The widespread lack of attention to replacement methods (in favour of refinement methods) must be rectified. And greater efforts must also be made to publish negative results (see below).

To date, compliance with such best practice standards by the animal research community has been demonstrably poor (Leung *et al.* 2018, Percie du Sert *et al.* 2020). To achieve the substantial improvements for both laboratory animal welfare, and human predictivity, that are so urgently needed, widespread change is needed. This would require a willingness and commitment to very significant change, from researchers and their professional associations, regulators, licensing bodies, ethical review committees, funding bodies, and scientific journals.

2. A number of witnesses have given evidence about the medical advances made as a result of animal research. However, you argue in your submission that "evidence indicates that actual human benefit is rarely, if ever, sufficient to justify" animal research. Can you expand on this and explain how you have come to this view?

Advocates of invasive animal research have regularly claimed such research is essential for preventing, curing, or alleviating human diseases (e.g. Festing 2004), with their opponents making counter-claims (e.g. Greek and Greek 2004). However, the most reliable, quantitative information about the utility of such research in advancing human healthcare, comes from *systematic reviews.* A systematic review is "a review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research and to collect and analyze data from the studies that are included in the review. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies" (Moher *et al.* 2009).

Many systematic reviews of animal experiments within various research fields have now examined their utility for advancing human healthcare. Among 20 relevant published systematic reviews located by this author during a previous survey, animal models demonstrated significant potential to contribute toward clinical interventions that were efficacious in human patients, in only two cases, one of which was contentious due to a small sample size. This was despite some of these systematic reviews focusing on those animal experiments most likely to provide human benefit. These included experiments approved by ethics committees on the basis of specific claims that medical advances were likely to result from the animal research; very highly cited animal experiments published in leading scientific journals; and chimpanzee experiments, given that chimpanzees are the species most generally predictive of human outcomes, because they're genetically most similar to humans (Knight 2011).

Seven additional systematic reviews demonstrated poor reliability of animal models in predicting human toxicological outcomes, including carcinogenicity and teratogenicity – the propensity to cause cancer and birth defects, respectively. These are the toxicities of greatest public health concern (Knight 2011). Since then, many additional systematic reviews have yielded similar results (Knight 2019). To date, no published systematic reviews in any healthcare fields appear to have yielded contrary results – that invasive animal research is an effective and efficient tool for the advancement of human healthcare.

3. We have received evidence during this inquiry about human 'lifesaving' research –what percentage of animal research do you think is being conducted that will be human lifesaving or have a good chance of being human lifesaving?

In 2008 Matthews (*J R Soc Med)* assessed the validity of the oft-heard claim that *'Virtually every medical achievement of the last century has depended directly or indirectly on research with animals.'* He found that this had not been, and probably could not be validated, and recommended systematic reviews of the utility of animal models, as the most valuable way to rigorously assess their utility. As noted above, such systematic reviews have consistently demonstrated that the benefits of animal research in advancing human healthcare, are very low. Animal research that achieves major humane healthcare benefits, is very rare indeed.

We must also consider that the financial and scientific resources consumed by animal research, become unavailable to other research fields, that may potentially yield greater human benefits, such as human clinically-focused research, or research focused on preventative healthcare. When financial

and scientific resources are limited, and the lives of patients, consumers and laboratory animals are at stake, we have a responsibility to invest in the research fields most likely to yield public benefits. Other research fields are far more likely to achieve human healthcare advancement, with much lower costs, than animal research.

4. You note in your submission that a wide range of alternatives to animal research exist – can you provide some examples that you think are particularly promising or important? Are these alternatives being widely used – and if not, why?

I've previously reviewed 3Rs alternatives in detail (Knight 2011). Replacement alternatives include mechanisms to enhance sharing and assessment of existing data, physicochemical evaluation of test compounds, and computerised modelling of their effects. Advanced tissue cultures include immortalised cell lines (which continue to differentiate indefinitely), stem cells (which can differentiate into other cell types), and organotypic cultures (three-dimensional cell cultures that retain features of the original organ). Tests using bacterial, yeast, protozoal, mammalian, or human cell cultures exist for numerous toxic and other endpoints. Human hepatocyte (liver cell) cultures and metabolic activation systems may allow identification of metabolic pathways (which break down test compounds), and of resultant compounds produced.

Particularly promising are 'human on a chip' systems connecting cell cultures from different organs via microfluidic systems that mimic the circulatory system, allowing assessment of organ–organ interaction. Microarray technology can allow genetic expression profiling of toxins, greatly speeding up their detection, well prior to more invasive endpoints. Surrogate human tissues, e.g., harvested during surgery or childbirth, advanced imaging modalities, and human epidemiological, sociological, and psychological studies, may all increase understanding of illness *aetiology* (causation) and *pathogenesis* (development). Finally, human clinical trials may be enhanced in various ways to increase safety for volunteers, and predictivity for diverse patient populations.

As I've noted previously (Knight 2011), "Non-animal investigative methods cannot, of course, provide answers to all questions about humans, particularly given present technological limitations. However, the same is certainly true of animal models, which have a more limited capacity for further development." Additionally, when human tissues or volunteers are used, these methods may generate faster, cheaper results, that yield superior insights into human biochemical processes, and that are ultimately more reliably predictive for human patients, consumers and workers.

5. Are some researchers continuing to use animals despite these alternatives being available – and if so, why do you think this is happening?

Research careers depend largely on success in scientific publishing, and on gaining external research funding. Currently, there are insufficient incentives, or pressure, from publishers and grant bodies, to use alternatives in favour of animals.

Other key enablers of the status quo include ethics committees that approve animal research without subjecting it to sufficient critical scrutiny (see below).

6. You argue in your submission that we need to subject animal research to "much more rigorous and critical evaluation". What would this look like in practice?

Almost all the animal experiments critiqued within systematic reviews would have been approved by at least one institutional ethics committee obliged to permit only those experiments likely to result in substantial benefits, given the considerable animal welfare, ethical, and financial costs inherent to such research. Although the concept of ethical review is sound, the poor utility of animal research overall in delivering human healthcare advancements or other public benefits, demonstrate that its implementation is currently flawed. This flaw appears to have resulted from an over-reliance on the assumption that animal experiments are likely to be of substantial benefit in advancing these public goals. The approval of large numbers of such experiments despite their questionable merits demonstrates a widespread failure of ethical oversight, adding to previous concerns about the effectiveness of ethics committees in safeguarding laboratory animal subjects (Schuppli & Fraser 2005).

By approving these experiments on the basis of unfounded assumptions about their likely benefits, the ethics committees responsible failed in their duty to society, and to the animals they were charged with protecting. To address this, the likely human benefits of scientific animal use must be scrutinised far more critically than is currently the norm, and more accurately weighed against the animal, human, and financial costs incurred.

It is also clear that rather than relying on assumptions of human utility, we should subject animal experimental models to the same standards of scientific scrutiny currently required for non-animal alternatives prior to regulatory acceptance internationally. Such scientific *validation* has traditionally involved the demonstration in multiple independent laboratories that the test in question is relevant to and reliable for its specified purpose (Balls *et al.* 1995), such as the prediction of a certain outcome in humans. Formal validation should be consistently applied to all proposed animal experimental models, prior to permitting their ongoing use in research aimed at predicting human outcomes.

7. A number of stakeholders have argued that there should be a database or register of 'negative' results from animal research experiments, to avoid duplication. How could this work in practice and why is it so important?

Greater efforts must be made to publish negative results (i.e., studies showing no effect of a test compound). Such studies that fail to show a treatment effect are generally less likely to be published, as they're considered less noteworthy. Systematic reviews aim to locate and appraise all studies concerning the effects of test agents, to draw conclusions about these. However, systematic reviews can generally only locate published studies. The subsequent exclusion of negative results from systematic reviews means that only studies showing positive effects (which may be false) are likely to be found and included. This leads to overestimations of treatment efficacy, and partly explains the widespread failures in human patients, of treatments apparently effective in animals.

To address this, it should be a requirement of the licencing of studies, that both study protocols and results are made publicly available for scrutiny in a database(s) of animal research undertaken. This would allow negative results to be located, and included within, systematic reviews.

8. What developments have occurred in other countries to improve welfare and reduce the number of animals in experimentation, that Australia could learn from? What changes in this spaces are happening elsewhere that Australia should adopt?

European Union

EU legislation improves transparency through measures such as publication of non-technical project summaries and retrospective assessment. The development, validation and implementation of alternative methods is promoted through measures such as establishment of a Union reference laboratory for the validation of alternative methods supported by laboratories within Member States and requiring Member States to promote alternative methods at national level. The European Commission ALURES Statistical EU Database on the use of animals for scientific purpose database has been created to increase transparency in animal research and includes data on animals bred but not used for scientific purposes.

Further, animal experimentation is now an issue for parliamentary debate. The governments leading the way in this respect are the UK and the Netherlands,
where several parliamentary debates have recently taken place. E.g.:

- https://www.youtube.com/watch?v=SgKckhxhq8w (fast forward to 11 minutes and 30 seconds)
- https://debatgemist.tweedekamer.nl/node/28272

UK

Three licences are required by the Animals in Scientific Procedures Act before research on animals is permitted:

- personal licence for each person carrying out procedures on animals
- project licence for the programme of work
- establishment licence for the place at which the work is carried out

What this means is that when a scientist applies for a licence to conduct animal experiments, they must prove to ASRU that there is no scientifically satisfactory non-animal-based procedure that can be used instead of an animal experiment. This has raised the barrier to obtaining an ASPA licence somewhat.

The UK has two tiers of regulation: local and national. Projects must be approved by an institution's Animal Welfare and Ethical Review Body (AWERB), who will also advise the principal investigator on matters relating to animal welfare and the 3Rs. The project must then be approved by the Home Office, who will carry out a harm/benefit analysis to assess whether the expected benefits outweigh any possible adverse effects to the animal.

The Scientific Procedures Act 2012 revision has also enshrined the concept of the development of 'alternatives' as a legal requirement. The wording in ASPA 2012 reads:

20B Alternative strategies
(1) The Secretary of State must support the development and validation of alternative strategies.
(2) In particular, the Secretary of State must—
(c) take such other steps as the Secretary of State considers appropriate to encourage research into alternative strategies;
(d) ensure the promotion of, and dissemination of information about, alternative strategies.
(3) The Secretary of State may make grants to any person concerned with the development, promotion or validation of alternative strategies.

Netherlands

The Dutch government announced its plan to phase out toxicology tests for chemicals, food ingredients, pesticides, veterinary medicines, and vaccines by 2025. Their Transition Program for Innovation without the use of animals sets out the means to achieve this through collaboration between the science, health care, government and business communities.

US

US Senators Cory Booker and Rand Paul have introduced the FDA Modernization Act to end animal testing mandates that demand experimental drugs must be tested in animals before they are used on humans in clinical trials. This bill allows an applicant for market approval for a new drug to use methods other than animal testing to establish the drug's safety and effectiveness. Under this bill, these alternative methods may include cell-based assays, organ chips and microphysiological systems, sophisticated computer modelling, and other human biology-based test methods. This legislation has passed through the Senate.

In the interests of transparency, The USDA Animal Care Public Search Tool allows members of the public to search for:

- A list of persons licensed or registered under the Animal Welfare Act
- Inspection Reports
- Animal Welfare Enforcement Actions
- Teachable Moments
- Research Facility animal use annual reports

National Centres for Validation of Alternative Methods

These exist in numerous foreign nations, e.g.:

- BraCVAM – BRA: Brazilian Center for Validation of Alternative Methods.
- CaCVAM - Canadian Centre for the Validation of Alternative Methods
- ECVAM - The European Centre for the Validation of Alternative Methods.
- ICCVAM - the Interagency Coordinating Committee on the Validation of Alternative Methods (U.S.).
- NKCA – The National Knowledge Centre on Alternatives to Animal Experiments (Netherlands).
- JaCVAM - Japanese Center for the Validation of Alternative Methods.
- NC3RS – National Centre for the 3Rs (UK)
- SKoCVAM - the Korean Center for the Validation of Alternative Methods.
- Swiss 3R Competence Centre
- ZEBET - the Centre for Documentation and Evaluation of Alternatives to Animal Experiments (Germany).

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