No animals were harmed in the writing of this article

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Increasing animal experiments justify examination of alternatives, writes Andrew Knight, First Veterinary Fellow of the Oxford Centre for Animal Ethics

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Rising laboratory animal numbers

The number of animals used in experiments in Ireland recently reached an unprecedented high. Unpublished Department of Health figures obtained by the Irish Antivivisection Society reveal that the total number of animals used in experiments almost doubled to over 112,800 during 2008. The number of cattle almost doubled to 4,019, while the number of mice used almost tripled to 71,224 when compared to the previous year. Overall, 557 dogs, 456 sheep, 224 pigs, 91 guinea pigs, 68 hamsters, 204 rabbits, and 23,198 fish were subjected to scientific procedures, all of which represented an increase on the previous year (Downes 2009).

Similar increases are occurring internationally. Within the UK, the largest annual increase since 1987 was recently revealed by the Home Office (Home Office 2009). 3,656,080 scientific procedures were conducted on animals in 2008 — 14% more than the previous year. Numbers have risen for seven consecutive years, and are now higher than at any time since the mid 1980s.

Worldwide, around 127 million non-human vertebrates were used for scientific purposes in 2005 — the most recent year for which global figures are available (Knight 2008a, Taylor *et al.* 2008). Two important factors are increasing laboratory animal numbers; namely, the increased use of genetically-modified animals, and the initiation of several large-scale chemical testing programs, which are primarily intended to rectify knowledge gaps regarding the toxicity of chemicals produced or imported into Europe or the US in particularly high quantities (De Boo and Knight 2008).

A scientific controversy

However, invasive animal use within biomedical research and toxicity testing is the subject of increasing public and scientific concern. Compliance with the so-called '3Rs' is now universally recognized as essential to good laboratory animal practice. These include: the *Replacement,* wherever possible, of animal use by non-animal alternatives; the *Reduction* of animal numbers to the minimum possible; and the *Refinement* of animal use, in order to avoid or minimize pain, distress or other adverse effects (Zucco *et al.* 2005, Balls 2009).

Accordingly, the 2008 increase in UK animal experimental numbers was criticised by British Veterinary Association President Nicky Paull and others within the veterinary literature (Dudley 2009). However, such condemnation was by no means universal. Others expressed the opinion that animal experiments have yielded, or have the potential to yield, great societal benefits (Dudley 2009, Henderson 2009), and similar opinions abound within the scientific literature. Some claim that medical progress would be *"severely maimed by prohibition or severe curtailing of animal experiments,"* and that *"catastrophic consequences would ensue"* (Osswald 1992).

The importance of evidence

The prevalence of such opinions within the scientific community may partially explain the inexorably rising numbers of animal experiments. Yet, regardless of the passion with which such views are expressed, or the credentials of those expressing them, such views remain firmly within the realm of opinion. They are not evidence.

In commenting recently in the *British Medical Journal,* Pound and colleagues (2004) noted that clinicians and the public often consider it axiomatic that animal research has contributed to human clinical knowledge, on the basis of anecdotal evidence or unsupported claims. These are inadequate forms of evidence, they asserted, for such a controversial area of research, particularly given increasing competition for scarce research resources. Hence, they called for systematic reviews to examine the human clinical utility of animal experiments. Systematic reviews provide gold-standard evidence, because they examine very large numbers of experiments, selected without bias, via randomisation, or similarly impartial and methodical means.

Systematic reviews of human utility

In recent years, a considerable body of relevant systematic reviews and meta-analyses have been published in peer-reviewed biomedical journals. Of 20 such reviews examining human clinical utility located during a recent, comprehensive search (Knight 2008b), animal models demonstrated significant potential to contribute toward clinical interventions in only two cases, one of which was contentious. Included were experiments approved by ethics committees on the basis of claims they were likely to lead to medical advances, highly-cited experiments published in leading journals, and chimpanzee experiments — the species most generally predictive of human outcomes. Seven additional reviews failed to demonstrate utility in reliably predicting human toxicological outcomes such as carcinogenicity and teratogenicity. Results in animal models were frequently equivocal, or inconsistent with human outcomes.

Given that millions of animal experiments have been conducted to date, links to human clinical advancements will inevitably exist. However, what the evidence clearly establishes is that such links are far too few. Animal experiments constitute a very inefficient means of developing new human clinical interventions, and are insufficiently reliable when predicting human toxicity. Their sensitivity to a wide range of toxins is generally accompanied by poor human specificity, severely limiting the predictive value of positive test results (Knight 2008b).

Scientific limitations of animal models

The reasons underlying the poor human predictivity of animal models are increasingly understood. Their limitations include: differences between species and genders — with subsequent effects on toxico- and pharmacokinetics (the study of bodily distribution, particularly absorption, distribution, metabolism and excretion), or pharmacodynamics (the study of mechanisms of action, and drug effects). They commonly include the use of unrealistic doses and exposure durations; the loss of biological variability or predictivity, resulting from the use of in-bred strains, young animals, restriction to single genders, and inadequate test group sizes; the lack of co-morbidities or other human risk factors; and stress-related physiological or immunological distortions (Hartung 2008a-b, Matthews 2008).

Interspecies variations in P450-dependent mono-oxygenases, for example, are well established (Guengerich 2006). These constitute the major family of xenobiotic metabolising enzymes — that is, enzymes catalysing the metabolism of foreign compounds, such as drugs or toxins. Their major purpose is the generation of nontoxic blood-soluble metabolites suitable for renal or other elimination. Interspecies differences in metabolic pathways, rates and products, may decrease efficacy or increase toxicity of test compounds, and are a key cause of high clinical trial failure rates during pharmaceutical development (DiMasi *et al.* 2003). Within the US, for example, only 8% of all drugs progressing to human trials after demonstration of safety in animal studies gain licensing approval by the Food and Drug Administration (Pippin 2008), usually because concerns arise about human toxicity or efficacy.

Non-animal alternatives

Fortunately, a rapidly growing range of non-animal alternatives exist, which were recently reviewed in detail (Knight 2008c). These include mechanisms to enhance the sharing and assessment of existing data prior to conducting further studies, and physicochemical evaluation and computerised modeling. The latter include the use of structure-activity relationships (which predict biological activities such as toxicity, on the basis of molecular substructures or other chemical moieties), and expert systems. Such systems seek to mimic the judgment of expert toxicologists by using known rules about factors affecting toxicity, in combination with physicochemical or other information about a specific compound. They make predictions about toxicity and related biological outcomes, such as metabolic fate.

Microorganisms, higher plants, minimally-sentient animals from lower phylogenetic orders and early developmental vertebral stages are all sometimes used, although the 'harvesting' and use of embryonic and foetal forms can pose substantial ethical problems in their own right.

A variety of tissue cultures, including immortalised cell lines (including neoplastic cell lines), embryonic and adult stem cells, and organotypic cultures, are also available. The ability of stem cells to differentiate into a wide variety of tissue types offers exciting potential for the future replacement of dysfunctional tissues. However, the harvesting of embryonic stem cells can be ethically contentious, and substantial regulatory restrictions exist in many regions.

In vitro assays utilising bacterial, yeast, protozoal, mammalian or human cell cultures exist for a wide range of toxic and other endpoints. These may be used individually, or combined within test batteries — which increases the sensitivity of the battery to toxins of different types. The generation of toxic metabolites by the liver — the main metabolizing organ — is a key cause of toxicity, and so human hepatocyte cultures and metabolic activation systems may be used to assess metabolite activity and organ-organ interaction.

Identification of genes that are up- or down-regulated by cellular exposure (potentially *in vitro*) to toxins of a certain type, may allow toxin detection in a fraction of the time required for more traditional, invasive endpoints, such as those resulting in organ damage or death. This developing field is termed 'toxicogenomics.' Microarray technology ('gene-chips') allowing examination of the activity of hundreds of genes simultaneously are being developed to facilitate such genetic expression profiling.

Enhanced human clinical trials utilising microdosing, staggered dosing, and more representative study populations and durations would all increase the safety for volunteers, and the predictivity for diverse patient populations. The use of surrogate human tissues, advanced imaging modalities, and human epidemiological, sociological and psychological studies, may all increase understanding of illness aetiology and pathogenesis, and facilitate the development of safe and effective pharmacologic interventions.

Particularly when human tissues are used, non-animal models may generate faster, cheaper results, more reliably predictive for humans, whilst yielding greater insights into human biochemical processes.

Conclusions

Ever-increasing numbers of animal experiments within Ireland and many other countries indicate the necessity for considerably greater awareness of, and compliance with, the principles of the 3Rs. Considerably more stringent compliance with relevant animal welfare legislation requiring the consideration or use of alternatives could — and should — become a prerequisite of research funding, ethics committee approval, and publication of results (Knight 2008c). Increased compliance with the 3Rs would be likely to improve research quality and the robustness of results, result in reduced timeframes and resource consumption,

and jointly benefit consumers, industry and laboratory animals. Combinations of different 3Rs strategies may also have synergistic effects, improving both scientific outcomes and animal welfare (De Boo & Knight 2008).

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