Alternative Action

This review argues that an adherence to animal testing methods (ATMs) in drug development and testing is not scientifically justifiable. The burgeoning field of non-ATMs is better placed to achieve this, and an urgent move towards their greater adoption by industry is both warranted and imperative.

Non-ATMs spare significant numbers of animals from pain and distress, are typically less costly and time-consuming, and may require a lower investment of personnel and other resources, compared to ATMs. Most importantly, they often have more predictive value and specificity for the human condition. Examples of the superior value of non-animal tests include the embryonic stem-cell test for embryotoxicity, new assays for skin corrosivity, in vitro tests for cancer causation and drug efficacy/toxicity at the US National Cancer Institute (NCI), and microdosing technologies.

ATMs – many in use for decades – have never been validated. Despite this, a bias persists towards them and, with no true gold standard available, they have typically been used as the default against which non-ATMs are judged. In vitro tests may appear inferior to ATMs without actually being so: for example, human tissue-based in vitro methods may not show high correlation with animal tests because they correlate better with the human response, which is, after all, the ultimate goal. Discrepancies demonstrate not any inferiority of in vitro alternative methods, but rather the scientific fallacy of animal test results as the standards of reference.

Alternative Methods

As of April 2013, 44 alternatives to animal tests have been validated by the European Centre for the Validation of Alternative Methods (ECVAM). For example, these methods have reached a status in which a final opinion of the ECVAM Scientific Advisory Committee and/or an ECVAM recommendation exist. Of these, 19 have achieved international regulatory acceptance by adoption at the Organisation for Economic Cooperation and Development (OECD), and a further nine methods have been accepted by the European Directorate for the Quality of Medicines and HealthCare (EDQM)/European Pharmacopoeia.

The methods adopted at OECD are in vitro methods permitting the identification of corrosive substances, skin and severe eye irritants, skin phototoxicity, skin penetration and genotoxicity, as well as a refinement and reduction of the acute oral toxicity test. Those accepted by EDQM deal with the potency and safety testing of vaccines and assessment of pyrogenicity.

In the US, the Interagency Coordinating Committee on the Validation of Alternative Methods has validated fewer methods, indicating a need for greater harmonisation. This need is further illustrated by the favourable impact of harmonisation on reducing animal use in drug development and safety testing worldwide, by means of guidelines from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the Test Guidelines Programme of the OECD.

Costs and Problems

Extrapolating research results across species is a tenuous enterprise. Animal test data are compromised by species differences in anatomy, organ structure and
function, toxin metabolism, chemical and drug absorption, and mechanisms of DNA repair. These differences are compounded by additional variables related to experimental animal demographics and husbandry.

Problems repeatedly manifest when attempting to apply animal data to human diseases and drug responses. Examples include: hormone replacement therapy for women; development of HIV protease inhibitors: Vioxx and other COX-2 inhibitors; teratology studies; harmful effects of smoking; non-steroidal anti-inflammatory drugs; antibiotics; anti-virals; antidepressants; and cardiovascular medications. Many harmful and ineffective drugs have tested safe and effective in animal studies. Conversely, many safe and beneficial human drugs would not survive animal testing today because of severe or lethal toxicities in some species.

The most recent analysis reports that 94 per cent of drugs that enter clinical trials following animal testing fail to achieve marketing approval, and the failure rate is at least 95 per cent for cancer drugs (1). Of the remaining drugs that are approved, half are withdrawn or relabelled due to severe or lethal adverse effects which are not detected during animal testing. Levels of discordance between results from animals and humans range from 67-96 per cent.

**Human Responses**

Entire fields of translation science have demonstrated the failed paradigm of animal testing to predict human responses. All of the more than 85 preventive and therapeutic HIV/AIDS vaccines successful in non-human primates failed in human trials. More than 4,000 studies report the efficacy of more than 700 treatments in animal models of stroke; however, none of the approximately 150 of these tested in humans has shown clinical benefit. The entire field of cancer immunotherapy animal research has failed to produce even one successful therapeutic cancer vaccine. Dozens of human clinical trials have failed due to toxicities, or lack of efficacy, after animal tests showed cures, mitigation, or prevention of diseases such as: diabetes mellitus; spinal cord injury; multiple sclerosis; and psychiatric disorders.

Developmental toxicity studies in animals show considerable discordance between different species, and are not predictive of human response. The lethal dose 50 test, in which the dose of a test substance required to kill 50 per cent of the test animals is calculated, was used for decades but has now been classed as ‘no longer admissible’ by at least two regulatory bodies. It was also notoriously unreliable, and there have been better non-animal alternatives available for years. Notably, acute toxicity data from animals has not been used to either terminate drugs from development or to set doses in the first clinical trials in humans.

Adverse drug reactions (ADRs) are one of the biggest causes of premature death in developed countries in spite of extensive animal safety testing. ADRs were directly responsible for nearly seven per cent of all hospital admissions, and one in every 300 deaths of hospitalised patients in 1998 – making them the fourth or fifth biggest killer in the US. In addition, ADRs are the cause of at least 70,000 deaths and cases of serious disability in England each year, putting them just behind heart attacks and stroke. In 2004, 6.5 per cent of UK hospital admissions involved an ADR – accounting for four per cent of hospital bed capacity and an annual cost to the UK National Health Service of £466 million (£706 million/$847million).

Recent examinations of animal trials have shown that: they may occur concurrently with or even after human studies; the results are often conflicting; there is poor correlation of cancer risk between assessments carried out by the US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer; and transgenic animal models frequently fail to duplicate human symptoms characteristic of many conditions, and do not enable scientists to elucidate the molecular processes underlying those diseases.

**ATM Replacement**

As well as the benefits to human health and animal welfare, there are economic advantages to ATM replacement. For example, the DakDak test (used to measure the efficacy of sunscreens in preventing skin damage) can provide data for five or six products at less than half the cost of testing one product in animals.

The current gold standard for testing a chemical to determine if it is carcinogenic is the rodent bioassay, which takes up to five years from planning to evaluation and review, at a cost of up to more than $4 million per substance. In vitro screening allows companies to identify promising test compounds in a cost-and-time-efficient manner before progressing to expensive human trials. Non-ATMs save on various costs associated with animal methods, including animal procurement, equipment, maintenance and husbandry, and hazardous waste disposal.

Moreover, costly legal claims against companies that rely heavily on animal data are becoming more commonplace. For example, the pharmaceutical company Merck and Co, Inc set aside $5 billion for lawsuit settlements, after improper reliance on animal tests to show that its painkiller Vioxx was safe for humans, while GSK set aside $6 billion for similar purposes. Given current reliance on animal preclinical studies and the frequency with which new pharmaceuticals damage human patients, the industry urgently needs testing methods that minimise its fiscal risks.

From an animal welfare perspective, factors resulting in the inherent suffering of animals used in testing include the manner in which animals are housed, transported and handled. Common laboratory routines have been shown to cause pronounced stress that can influence test results, and links between such stress and the development of behavioural stereotypes (which are
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Support for Non-ATMs

The public is uncomfortable with animal experimentation and testing, particularly when it involves pain and distress, or the testing of non-essential products. According to polls: a majority of the British population opposes invasive and/or pain-causing procedures on non-human primates (NHPs), whatever the proposed benefit to humans; and across Europe 81 per cent of the public believes experiments causing pain or suffering to NHPs should be banned (2-4). A US poll showed that 75 per cent of people disapproved, and most strongly disapproved, of experiments that subject animals to severe pain and distress, while 60 per cent of respondents opposed research and testing that cause even moderate pain and distress (5).

Testing accounts for the vast majority of animals reported in the highest categories of pain and distress, underscoring the importance of replacing animal use in regulatory toxicity testing. Prevailing sentiments suggest widespread public support for the adoption and use of replacement methods. Broad scientific support for non-animal methods is demonstrated in the growth and diversification of such methods, and in the number of scientists and scientific organisations supporting them.

Impact on Drug Development

Acute toxicity tests using human cells are at least 25 per cent more predictive of human toxicity than animal tests, and are being improved. The NCI has developed and implemented superior non-ATMs for carcinogenicity, anti-HIV drug efficacy and certain categories of cell toxicity.

Three-dimensional cultures of human liver cells are providing improved prediction of drug metabolism, while a fully human modular immune in vitro construct for efficacy testing of potential vaccines has been developed “in response to the recurrent failure of animal vaccine protection studies to accurately predict human trial results” (VaxDesign). Human tissue approaches “are often superior to extrapolation from animal data”, and were shown to detect the adverse reactions caused by Cox-2 inhibitor drugs such as Vioxx, which caused over 320,000 heart attacks and strokes and killed tens of thousands or more, despite cardioprotective results in preclinical tests using monkeys. Culture systems for infectious hepatitis C virus now exist, enabling groundbreaking human-specific research and obviating the need to infect NHPs.

The results from human microdosing trials have revealed that concerns about their accuracy and applicability are overstated, and that the technique was over 80 per cent predictive of human pharmacokinetics for a panel of 25 drugs tested – a far higher prediction rate than provided by animal tests. Microarrays can identify which genes have been damaged when human cells have been exposed to test substances, giving a human-specific indication of toxicity. They have been used with complementary technologies in hundreds of successful experiments to date – for example, to classify diverse types of chemicals and human tumours, to derive prognoses for breast and ovarian cancer patients, to select breast cancer patients for appropriate follow-up chemotherapy, and to obtain human efficacy and tolerability data for new drugs.

Examples of drugs whose development owes much to the use of human cells and computer modelling include anti-HIV protease-inhibitor and nucleoside-analogue drugs, some of which are known to have properties that vary wildly between animal species and that can be particularly dissimilar in NHPs, and the Novartis leukaemia drug Glivec that was designed and developed in vitro.

Way Forward

It is clear that the current paradigm – centred as it is on animal-based approaches – is not delivering effectively. The public – which needs new drugs to treat or prevent many illnesses and diseases – is being let down and the pharmaceutical industry – which needs to generate profits in order to develop those successful new drugs – is struggling to do so.

Scientific bodies are in agreement: in the last few years, the US National Academy of Sciences and the National Research Council published a keystone report calling for transforming toxicology “from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin” (6). This process is already happening.
with programmes such as the EPA's Tox21 agenda in which the Food and Drug Administration (FDA) now participates – an extension to the ToxCast project which is a multidisciplinary collaboration utilising the High Throughput technologies of the National Institutes of Health Chemical Genomics Center (7,8).

Other examples include the AXLR8 initiative, and the work of the “transatlantic think tank for toxicology” (9, 10). Additionally, these in vitro methods are profitable. The in vitro sector of Charles River Labs, the world's largest supplier of animals for research, grew 10 per cent in 2011, compared to overall company growth of 0.8 per cent, and in 2012 in vitro sales were the only portion of the Research Models and Services division to increase, thereby offsetting losses.

Several approaches would be immediately beneficial from a regulatory perspective: stronger industry guidance to discourage submission of unnecessary animal test results; standardisation of reviewer practices so animal tests are not requested; global harmonisation of implementation practices; development of regulatory agency policy for the designation and adoption of 'scientifically satisfactory' replacement methods; making maximum use of human drug databases to eliminate the perceived need for animal testing, and adoption of more available non-animal methods.

Still, even more needs to be done: a real paradigm shift is urgently required, in which all stakeholders move their focus away from animal use and toward superior, more human-relevant and predictive, quicker, cheaper technologies that will deliver safer and more effective drugs in greater numbers, with accompanying greater profits.

Conclusion

The time has never been more appropriate for industry, regulatory and policy changes in the use of animals for preclinical drug testing. Regulatory preferences for non-ATMs will help drive industry to develop them further, as markets for such methods will exist. The time is ripe for industry to work with – rather than against – animal protection groups: to open the door for these tools that can get drugs to market more quickly, safely and profitably. However, as long as regulatory and industry guidelines and practices do not provide a mandate or even a timeline and a plan to achieve this, there is little incentive to change from the current suboptimal preclinical testing methods. Therefore, progress is stymied and the potential for greater corporate success missed.

The industry is persisting in shooting itself in its collective foot, clinging to expensive and inefficient ways that can only reach inadequate ends. The use of humane and scientifically sound alternatives is vital to improve the accuracy of preclinical testing, minimise the approval of hazardous drugs and devices, decrease pain and suffering to animals, reverse the decline and lack of recent successes of the pharmaceutical industry, and increase human safety and industry profitability.

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References


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