Barriers to the Uptake of Human-based Test Methods, and How to Overcome Them[a]

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Summary — Although there is growing concern as to the questionable value of animal-based methods for determining the safety and efficacy of new medicines, which has in turn led to many groups developing innovative human-based methods, there are many barriers to their adoption for regulatory submissions. The reasons for this are various, and include a lack of confidence that the available human-based methods, be they *in vivo*, *in silico* or *in vitro*, can be sufficiently predictive of clinical outcomes. However, this is not the only problem: the issue of validation presents a serious impediment to progress, a particularly frustrating situation, in view of the fact that the existing animal-based methods have never themselves been formally validated. Superimposed upon this is the issue of regulatory requirements, where, although regulators may be willing to accept non-animal approaches in place of particular animal tests, nowhere is this explicitly stated in their guidelines. Such problems are far from trivial, and represent major hurdles to be overcome. In addition, there are a range of other barriers, real or self-imposed, that are hindering a more-predictive approach to establishing a new drug's clinical safety and efficacy profiles. Some of these barriers are identified, and ways forward are suggested.

Key words: *animal alternative, barriers, humanising, in silico, in vitro, in vivo, predictive, replacement, safety, validation.*

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Introduction

It is widely accepted that current test methods for predicting safety issues with medicines and other chemicals in human subjects/consumers are inadequate, and increasing efforts are now being directed toward identifying more-reliable replacements. With a growing realisation that a significant contributor to the poor performance of existing methods is the use of non-human species as the basis of most current tests (1–6), much effort has been directed toward human-based approaches, whether *in vivo*, *in silico* or *in vitro* (7–9). Despite this growing recognition of the shortcomings of current methods, the process of introducing new, potentially more-reliable tests based on human biology, is fraught with difficulty, and researchers face an uphill struggle to get such methods recognised and incorporated into preclinical test regimens (10). This is a worrying situation, and it is important to understand the reasons for it, in order to be able to make necessary progress in improving patient and consumer safety.

The Challenge of Humanising the Process

The transition from any established technology to an alternative one is a difficult process, and any new technology has a hard time in breaking through (11). This is true in any technological area, not least in pharmaceutical research and development. On the question of human *in vivo* testing, it is widely held to be unethical to use humans as experimental subjects in the assessment of new medicine safety and efficacy. However, we must recognise that we are in fact doing exactly that. It is established that in excess of 90% of potential medicines that have successfully passed the preclinical testing process, fail, on the basis of safety and/or efficacy, when evaluated in human subjects. It is clear that human subjects, be they healthy volunteers or patients, are currently the most powerful contributors in the identification of clinical suitability. The obvious failure of animal-based preclinical testing to 'weed out' the unsuitable, leaves the eventual human recipient as the real arbiter on this issue. If we really cannot do any better than this, then we should acknowledge the key role that human subjects play in the process, and consider how best to minimise the possibility of harm to them (12). For example, the use of microdosing, associated with highly sensitive mass spectrometry, has enormous potential to elucidate clinical ADME (Absorption, Distribution, Metabolism and Excretion) issues, and early concerns about extrapolating from sub-therapeutic to

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therapeutic doses have proven to be largely unfounded (13). Another approach that has been generating immense interest in recent years, is the identification of specific biomarkers, the use of which in human subjects could represent a more sensitive way of detecting potential safety/toxicity issues than waiting for the appearance of overt toxicity (14).

In silico methods will undoubtedly contribute increasingly to drug testing paradigms, as significant efforts are being made to develop improved methods, both to predict drug effects in various organs of the human body (15), and, excitingly, also to model the organs themselves (16). However, since these can only be based on what we know, their ultimate value will depend heavily on the availability of the clinical data on which models can be built. Fortunately, the increased awareness of the value of such data has prompted many, but by no means all, pharmaceutical companies to agree to share safety data. We can only hope that, in the not too distant future, the remaining companies currently resisting this forward-looking approach will be persuaded of its value, and will agree to contribute.

In vitro technologies are often criticised on the basis of the seemingly impossible task of modelling the functioning of the whole body through a focus on individual parts. While this concern is clearly not without foundation, it ignores two important facts: a) species differences mean that *in vivo* methods based on non-human biology are inherently unreliable for the purpose of predicting human responses; and b) significant progress has been and is continually being made in the development of more-complex and physiologically more-relevant human-based models, such as human cell systems with high-content output (17–19), which has been given a significant boost by the explosive development of stem cell technologies (20). While it is unlikely that any single *in vitro* approach alone will ever fully mirror an intact human, there is good reason to believe that a suitable combination of such tests, alongside low-risk *in vivo* tests in human volunteers, will ultimately succeed in replacing *in vivo* testing in non-human species.

Paradoxically, one issue that delays the transition from methods based on animal biology to those based on human biology, is the concept of the Three Rs (i.e. *Replacement*, *Reduction* and *Refinement*; 21, 22). While the Three Rs concept has been, and remains, a laudable means of minimising animal suffering, it also somewhat obscures the issue of increasing human relevance. Industry and regulators are inherently conservative, so will naturally opt for the least challenging of the Three Rs — *Reduction* and *Refinement* — at the expense of the considerably more challenging, but infinitely more valuable, *Replacement*. The two more conservative Rs may be ethically desirable, but they do not address the issue of species relevance (23, 24).

Validation

Validation is the process used to demonstrate that a particular test is reliable and relevant for its intended use (25). Clearly, it is essential to ensure that any required test meets these criteria. However, one considerable barrier to the adoption of novel methods is the hugely resource-intensive burden of formal validation, and to date, only a few human-based tests have achieved this status. To make things more complicated, validation does not guarantee regulatory acceptance, nor does regulatory acceptance necessarily require formal validation (26, 27). While the latter is theoretically true, it must be said that few non-animal tests without formal validation have achieved regulatory approval.

Validation itself has two primary components: technical and functional (28). The technical aspect relates to the practicality, robustness, reproducibility and transferability of the method, each of which is crucial if a method is going to have practical application. The functional aspect relates to its ability to do the job, i.e. to identify potential clinical toxicities; it is here that problems arise. How can we establish whether a particular test can identify a toxicity that will occur in human subjects, if we do not know whether any such toxicity will arise? It is a classical circular problem: we need to know the likelihood of the clinical toxicity, so that we can predict the clinical toxicity. There are ways of getting round this problem — for example, by evaluating a new test method with test substances for which human toxicity profiles are already available. Success in predicting the known could be regarded as strong evidence of the utility of the method. Of course, just because a test is successful in predicting known toxicities, does not guarantee that it will be as successful for predicting unknowns, but a strong track record should increase confidence.

The alternative approach, and one that is widely used for formal validation studies, is comparison against an existing 'gold standard' — which, in most cases, is a non-human animal-based method (28). However, in the knowledge that non-human animal-based methods are unreliable, it is hard to see their value as 'gold standard' methods, and this process often results in more questions than answers (29, 30). To fail to validate a test on such a basis is illogical, and thus potentially wastes both time and resources, adding inappropriate delay to the process of humanising safety testing.

Nobody would dispute that validation is essential, but, as acknowledged by ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods; 31), it often serves to prevent, rather than promote, the introduction of

improved methods. It must also be noted that the currently-required animal-based testing regime has never been subjected to validation. Conversely, novel methods are expected to demonstrate a level of performance that is neither expected, nor indeed feasible, from animal-based methods. An obvious solution to this conundrum is that validation needs to become relative, rather than absolute. If a new test, or tests, can be shown to outperform what is currently required, that alone should suffice to ensure the continual and incremental replacement of underperforming tests with better ones, even if they are not yet perfect themselves. Unless this system of gradual improvement (which operates in almost every other sphere of endeavour) is adopted, the perfect will remain forever the enemy of the good.

Regulation

Another barrier to the adoption of non-animal methods relates to perceived regulatory requirements. There is a widespread perception among manufacturers that regulatory authorities require animal data, whereas, in fact, what they actually require is a degree of assurance that a particular substance will not cause harm. They do not necessarily mandate by what means that assurance is provided, only that it is logical and has some practical basis (32). There is a pressing need for a clearer understanding of actual regulatory requirements in the relevant industries. Consultation with the regulatory authorities before embarking on a regulatory submission, rather than presenting them with a *fait accompli*, is strongly advised, in order to avoid conducting animal tests that were simply not required. However, many companies do not do this, relying instead on advice from regulatory consultants, who tend to be more conservative than the regulators themselves, and often recommend a 'belt and braces' approach, believing that this will increase the likelihood of approval (H. Stemplewski [MHRA], personal communication).

It must be mentioned that the regulators are not entirely without fault, often by virtue of excessive conservatism in their response to non-animal approaches (33), and they can also be guilty of giving out ambiguous messages. In statements on the issue, they may actively encourage the increased use of non-animal tests (34), but this is not necessarily supported by their written guidance. For example, the FDA's Investigational New Drug (IND) and Investigational Device Exemption (IDE) regulations give the FDA the flexibility to accept non-animal test methods (NATMs), such as *in vitro* studies or prior experience with the drug or biological product in humans, when appropriate (35, 36). However, despite this stated willingness to accept NATMs when they are at least as valid as other methods, the FDA has not modified the text of its regulations. The current regulations clearly suggest a requirement for animal testing. For example:

— New Drug Application (NDA) Records and Reports: "To acquire necessary data for determining the safety and effectiveness of long-term use of such drugs, *extensive animal and clinical tests are required as a condition of approval*" (37).

— IND Investigator's Brochure: "A summary of the pharmacological and toxicological effects of the drug in animals and, to the extent known, in humans" (38).

— Application Technical Sections: "A description and analysis of each clinical pharmacology study of the drug, including a brief *comparison of the results of the human studies with the animal pharmacology and toxicology data"* (39).

The fact that companies perceive the full repertoire of animal tests as being required by the regulators, even if this is not always the case, discourages their adoption of NATMs, which are viewed as an additional and inessential expense: "Increasing the use of alternative methods is advised by the law, but it is not mandatory and nobody wastes time on anything that is not essential" (40). Dr Derek Knight, Senior Scientific Advisor to the European Chemicals Agency, made an impassioned plea at the EPAA (European Partnership for Alternative Approaches to Animal Testing) Conference in November 2014: "Regulators, please, please communicate your regulatory R&D requirements to the regulated community" (41).

Unfortunately, the regulatory acceptance of a human-based approach by one regulatory authority does not guarantee similar acceptance by others, and as the pharmaceutical industry is global in nature, even companies enlightened with regard to the inclusion of human-based data in their regulatory submissions, may feel obliged to include animal data, in order to gain access to the widest market. Despite the fact that harmonisation between authorities in different territories is of the greatest importance, and industry's admitted efforts to expedite the process, achieving harmonisation is both a lengthy and difficult process (23, 27, 42, 43). However, through the efforts of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), some progress is being made (44).

Other Barriers

Even validation and regulatory acceptance do not guarantee success. There are further self-imposed

Table 1: Examples of self-imposed barriers to the adoption of non-animal methods

Type of barrier	Example statement
Entrenched views	"…this is the way I've always done it, and I see no reason to change now."
Vested interest	"Animal studies are advantageous for us and we would not risk abandoning any of them."
Complacency	"I don't know anything about alternatives, I'll just carry on doing things the way I always have."
Scepticism	"I really don't believe that *in vitro* and *in silico* alternatives will ever be able to replace *in vivo* animal tests."
Lack of 'courage'	"I'm concerned that use of alternatives may result in expensive delay in getting my drug on the market, and may lay me open to possible litigation if things go wrong."

Source: MacLachlan (45).

barriers to adoption, some of which are rather more insidious (see Table 1). Such reactionary positions as these, are, of course, encountered in all walks of life, but they are highly frustrating. Unless real regulatory action is taken, they can only be overcome by clear demonstrations of the advantages to be gained from NATMs, including reduced costs and increased accuracy, reliability and speed.

A clear example of the above problems is the continued unnecessary and inappropriate use of the unreliable and highly contentious Draize tests for detecting potential ocular and skin irritancy. The Draize tests are acute toxicity tests that were originally devised in 1944, in order to test cosmetics for irritancy (46), but subsequently became widely used to determine the possible eye and skin irritancy of chemicals in general and topically-applied drugs. As early as 2005, the FDA stated publicly that Draize testing was no longer required for skin and ocular irritancy in preclinical test packages for regulatory applications. While stopping short of issuing formal guidance to industry, the FDA has published posters, made public presentations, and communicated with sponsors during Pre-IND meetings, that Draize tests are not required. However, of the 137 drugs approved by the FDA between the years 2011–2014, 24% were tested for skin and eye irritancy, and of these, the Draize test was used in 94% of all skin irritation and 60% of all eye irritation tests (see Table 2). And what is worse, of these drugs for which Draize testing was conducted, 76% were for systemic, not topical, administration — i.e. no irritancy testing of any kind was necessary. Thus, despite the legal requirement to consider non-animal methods before embarking on animal tests, and the FDA's pronouncement that they no longer require Draize testing, the toxicologists concerned carried on regardless, and they even used the test for drugs where there was no obvious skin or eye irritancy issue. The lack of negative consequences for companies who continue to perform unnecessary tests that are not recommended, provides no incentive for change. If tests are no longer required, their use should be challenged by regulators, not passively tolerated.

Various attempts have been made to illustrate, and in some cases to quantify, the inappropriate use of animal-based test methods. Carvalho and colleagues performed a study based on citation analysis to investigate the contribution that animal-based test methods have made in advancing clinical studies of ADHD (47). Systematic analysis showed that, despite a considerable number of animal-based studies of ADHD, they have made, at most, a minimal impact on research into the clinical condition. Similarly, citation analyses have reached very much the same conclusions for many other disease areas, including anorexia and bulimia (48), and stroke and head injury (49). A citation analysis of more than 1,000 animal studies, reported over a 12-year period at three German universities, showed that none of them led to any new therapies or had any clinical impact (50). Furthermore, citations of these studies declined to zero after 17 years, illustrating their lack of long-term impact (51). Citation analysis of 749 studies in chimpanzees showed that half were never cited at all, and of the 27 that went on to clinical application, none made an essential contribution, or in most cases, any contribution at all, toward the development of the described human treatment (52). Another study revealed that even the most highly cited animal studies published in the leading scientific journals, only translated to human clinical application in 10% of the cases, leading the authors to caution patients and physicians about extrapolating the findings of even highly-cited animal research to the treatment of human disease (53).

Table 2: The use of the Draize Tests for skin and eye irritancy testing for new molecular entities submitted for NDA and BLA review between 2011–2014

	2011	2012	2013	2014	2011–2014
Total NMEs	30	39	27	41	137
SIT performed	5	8	7	13	33
Draize used for SIT	5	8	6	12	31
SIT (% Draize)	100%	100%	86%	92%	94%
SIT (% total NMEs)	17%	21%	27%	32%	24%
EIT performed	4	8	8	10	30
Draize used for EIT	2	5	4	7	18
EIT (% Draize)	50%	63%	50%	70%	60%
EIT (% total NMEs)	13%	21%	30%	24%	22%

EIT = eye irritancy testing; NME = new molecular entities; SIT = skin irritancy testing.

Undue emphasis by funding review boards on animal-based studies over more-relevant human-based studies has been noted by many researchers (22, 45, 54, 55). This is not helped by the fact that researchers are far more expert in the areas of their work than regulators, and can thus have an advantage in expressing the perceived advantages of their particular animal-based technology; a phenomenon known as 'informational asymmetry' (56). A pioneer of evidence-based medicine, Dr David Sackett, firmly believed that 'experts' were an impediment to progress and change (57). As long ago as 1911, a Nobel Laureate, Maurice Maeterlinck, observed that: "At every crossroads on the path that leads to the future, tradition has placed ten thousand men to guard the past".

The phenomenon of 'technological lock-in' (where the superior long-term path is not necessarily the path chosen) applies strongly to the continued default use of animal testing, even where NATMs would be advantageous. There is also a strong case that animal testing is subject to institutional, psychological and behavioural lock-in, as explained by Frank (58). An important message of this paper is that, when an inferior technology has become locked-in, there is no reason to expect the path to self-correct. Therefore, intervention is necessary to 'de-lock' or change paths, in order to overcome the many factors contributing to entrenchment against change.

Perhaps the greatest barrier to the replacement of animal tests is the legal protection that they afford to pharmaceutical companies in litigation regarding adverse drug reactions (ADRs). It is therefore imperative to increase awareness of the fallacy of such protection: unpredictive tests do not protect patients, and should no longer protect companies who continue to use them, when more-predictive methods not reliant on interspecies extrapolation are available.

Stimulating Change

To break down the barriers to change, it must first be more widely acknowledged that current methods are unsatisfactory in terms of predictive value, time and expense. The issues of excessive time and expense associated with current animal-based methods in drug development are now reasonably well acknowledged, but their poor predictive performance less so. There are many sources of evidence for this, including:

— the high rate of failure of drugs in clinical testing and therapeutic use (59);

— the lack of association between clinical toxicities and correlates in preclinical animal tests, to the extent that, in one study, animal tests missed 81% of the serious side-effects of 43 drugs that went on to harm patients (60); and

— the lack of contribution of research based on animal models to clinical research (47, 55, 61–64).

As Professor John Ioannidis has observed: "It is nearly impossible to rely on most animal data to predict whether or not an intervention will have a favourable clinical benefit–risk ratio in human subjects" (65).

The consequences of continued reliance on inadequate tests include the exposure of patients and research volunteers to unacceptable risks and frequent harm. Many clinical trials are now conducted in India, where hundreds die each year in trials for Western medicines (2,644 between the years 2005–2012; 66). ADRs kill 197,000 people in the EU each year, costing €79 billion (67). In the USA, in 2011, prescription drugs were associated with serious or disabling injuries in two to four million people, including 128,000 deaths (68). Of course, animal tests are not the sole cause of ADRs, but it cannot be denied that more-predictive

tests would prevent many toxic medicines from reaching patients. The scale of the problem necessitates urgent action to improve the performance of preclinical safety testing.

In our view, the most important contribution that can be made is to ensure that human biology is given absolute priority throughout drug development and testing. Although the pharmaceutical industry is making moves in this direction, the absence of regulatory pressure for change is allowing progress to occur at a glacial pace. There are precedents that show that deadlines create tremendous impetus for change, such as the EU Cosmetic and REACH regulations. Mandatory targets and time limits are called for, if we are serious about reducing the ever-increasing burden of death and disability caused by ADRs. In order to realise the potential of a human-based approach, we must continue to research and refine human-based tests, improve and accelerate validation, educate researchers, regulators and insurers about the limitations of extrapolating between species and the advantages of a human-focused approach, clarify, pro-actively communicate and enforce official guidelines, and, most importantly, set timelines for action. Many organisations and individuals have called for all of these things for many years, but without clear milestones and firm deadlines, the "paradigm shift from the use of experimental animals… toward the use of more efficient *in vitro* tests and computational techniques", as called for in the seminal 2007 report *Toxicity Testing in the 21st Century* (69), will continue to be postponed indefinitely.

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