The Three Rs in the pharmaceutical industry: perspectives of scientists and regulators

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Abstract

Six drug regulatory reviewers and 11 pharmaceutical industry scientists were interviewed to explore their perspectives on the obstacles and opportunities for greater implementation of the Three Rs (replacement, reduction, refinement) in drug research and development. Participants generally supported the current level of animal use in the pharmaceutical industry and viewed in vitro methods as supporting, but not replacing, the use of animals. Obstacles to greater use of the Three Rs cited by participants included the lack of non-animal alternatives; requirements for statistical validity; reluctance by industry and regulators to depart from established patterns of animal use; the priority of commercial objectives ahead of the Three Rs; and concern that less animal testing could jeopardise human safety. Opportunities identified for the Three Rs included the development of better animal models including genetically modified (GM) animals; pursuit of more basic knowledge, notably drug action on gene expression; re-use of animals; greater use of pilot studies; using sufficient numbers of animals per test to avoid repeating inconclusive studies; regular review of animal data in regulatory requirements; and following the regulatory option of combining segments of reproductive toxicology studies into one study. In some areas, greater implementation of the Three Rs seemed well aligned with industry priorities, for example, phenotypic characterisation of GM animals and validation of alternative methods. In other areas, wider use of the Three Rs may require building consensus on areas of disagreement including the usefulness of death as an endpoint; the suitability of re-using animals; and whether GM animals and the use of pilot studies contribute to reduction.

Keywords: *animal welfare, drug development,* in vitro *methods, pharmaceutical industry, Three Rs; toxicology*

Introduction

The pharmaceutical industry uses animals to discover and evaluate the pharmacological and toxicological effects of new human medicines. Animal data are used to determine whether to advance a drug to human clinical trials and if so, to estimate safe starting doses and identify clinical parameters to monitor in those trials (ICH 1997). According to the Declaration of Helsinki, animal experimentation, where appropriate, should precede biomedical research in human subjects (World Medical Association 1964) and some use of animals by the pharmaceutical industry is required by drug regulatory agencies.

At the same time, it is generally accepted by society and the scientific community that animals should not be used unnecessarily (eg CCAC 1989). This concern is often acknowledged by support for Russell and Burch's 'Three Rs' (1959, reprinted 1992): replacement (replacing whole animal models with non-whole-animal models), reduction (reducing the number of animals required to a minimum), and refinement (minimising harm to animals in both husbandry and experimental procedures). The Three Rs are widely accepted in the biomedical research community and typically used by Animal Ethics Committees to guide their review of animal care and use protocols.

Many pharmaceutical companies have also adopted the Three Rs in their animal care and use policies (eg Glaxo Smith Kline 2001; Pfizer 2002; Eli Lilly 2004). Similarly, some regulatory agencies have policies that encourage the application of the Three Rs in drug regulatory testing (eg the United States Food and Drug Administration [FDA] 1992). The pharmaceutical industry has been praised for past progress in reducing the number of animals used (eg Stephens *et al* 2001), but wider application of the Three Rs in the industry is still a focus for animal welfare advocacy, for example, in criticisms of the field of toxicology (Rollin 2003) and in efforts to replace dogs as a second test species in pharmaceutical toxicology (Balls *et al* 2003).

Although there has been a great deal of research to develop replacements (eg Balls 1994, 2002) and achieve reduction (eg Festing 1994; Festing *et al* 1998), there is less knowledge of the obstacles to greater implementation of the Three Rs, or opportunities for greater implementation within the pharmaceutical industry. This exploratory study identified favourable areas for increasing implementation of the Three Rs, especially of replacement and reduction, in the pharmaceutical

industry. We interviewed pharmaceutical industry scientists and government regulatory reviewers from North America in order to learn about their perspectives on application of the Three Rs to drug research and development. We asked them to discuss their views on the value of both animal and non-animal data in drug research and development, and to identify what they think are the obstacles and opportunities for greater implementation of replacement and reduction of animal use in the pharmaceutical industry.

Materials and methods

This study received ethical review and approval from the University of British Columbia Research Ethics Board. Six regulatory reviewers and 11 pharmaceutical industry scientists were interviewed during 2003 and 2004 (17 interviews in total). Purposive sampling was used to select participants who (1) used or reviewed animal data when making decisions about human drugs, (2) were based in North America, and (3) were not formally affiliated with advocacy organisations promoting either alternative methods or animal welfare. Participants were found through personal contacts, referrals by colleagues, or referrals by the initial participants. Regulatory reviewers from one agency were found through the agency's public relations department. Participation was voluntary with no remuneration. Four females and 13 males were interviewed; all interviews were confidential.

The regulators were from both the United States FDA and Health Canada. The scientists included toxicologists, pharmacologists and clinical researchers, and therefore had varying types of involvement in the pharmaceutical industry. The average length of professional experience in drug research and development was 24 years, with a range of 8 to > 40 years. Two participants had experience in both industry and regulatory roles, seven had academic experience other than from graduate school, and two of these had remained primarily as academic researchers. Participants' areas of expertise included immunology, medicine, molecular biology, pathology, pharmacology, physiology, toxicology and veterinary medicine, or some combination of these (eg veterinary pathology). Participants also had experience in therapeutic areas that included analgesics, antibiotics, anti-inflammatories, anti-virals, biologics, immune-modulators, ophthalmics, psychiatrics, and therapies for cancer, cardiovascular, central nervous system, and gastro-intestinal diseases.

One semi-structured interview was conducted with each participant, either in person (n = 7) or by telephone (n = 10); approximately 11–13 open-ended, prepared questions were asked. Unplanned questions were also used; therefore, not every participant was asked exactly the same series of questions. The interviews lasted between 25 minutes and 105 minutes and were tape-recorded. Participants were identified by pseudonyms.

Interviews began with participants being asked to describe their background in the pharmaceutical industry and their experiences using animal models. They were then asked if they could describe the following situations: any animal tests that were 'less important'; any occasion where animal data

were 'misleading'; their perspective on the 'use of animal death as an endpoint'; and their opinion on the 'role of *in vitro* data in drug research and development'. Participants were then asked about 'opportunities for decreasing animal use', 'obstacles to decreasing animal use', 'the impact of the regulatory environment on animal use', and 'the role of transgenic animals'. The terms 'Three Rs', 'replacement', 'reduction' and 'refinement' were not used in order to keep the questions as open-ended as possible.

The interviews were transcribed verbatim and analysis of the transcripts occurred concurrently with the data collection. In order to describe and provide insight into the range of views, the analysis focussed on searching for similarities and differences among the participants' responses. Analysis also focussed on how the responses related to the Three Rs in order to provide a framework for discussion. Because questions were similar between interviews, the data was initially sorted and organised using a system of coding (Coffey & Atkinson 1996), based on the interview questions (eg one code was named 'views on animal death endpoints'). As understanding of the data developed, relationships between codes were recognised and more interpretive codes were used (eg 'resistance to change'). The qualitative analysis software program QSR N6 (version 2002 from QSR International Pty Ltd) was used to assist in coding.

The responses presented in this study represent the views and opinions of the participants at the time of the interview and cannot be viewed as either definitively true or false. Quotations from the participants have been used to illustrate the research findings and to allow the reader to more readily evaluate the conclusions that have been drawn. The quotes have been presented mostly verbatim, although they have been 'cleaned' by removing interjections (eg "um", "you know") and by adding punctuation. To maintain anonymity, identifiable quotes have not been used from participants whose identity might be recognised as a result. Gender of the participant, and/or related quotes from the same participant have not been identified, although doing so may have been potentially more interesting for the reader. According to the nature of qualitative methods, this study did not seek to comprehensively or statistically represent the views of all scientists and regulators working in the North American pharmaceutical industry, but rather to identify the range of opinions and perspectives on the issues.

Results

Views on animal use within pharmaceutical research and development

When asked to describe their views on the use of animals in drug research and development, most participants stressed the importance of animal data in determining the safety of new medicines prior to human clinical trials, and generally felt that drug studies in animals were predictive for humans. A greater variety of views were expressed on the following four topics.

1. Appropriateness of animal use

Several scientists and regulators strongly felt that animals were currently used appropriately and at a minimum level.

One scientist affirmed that there was "no superfluous use" of animals and one regulator could not think of any information obtained from animals that was extraneous to the approval of new drugs. Another scientist noted that, compared with basic research, regulatory testing for drugs uses far fewer animals; two others scientists felt that on occasion not enough animals are used. Only one participant (a scientist) expressed the view that animal use was currently "excessive".

2. Difficulties of using animal data

Many participants commented on the difficulties associated with evaluating animal data and selecting animal models. One scientist summarised this with the phrase "all models are wrong, but some models are useful". Another scientist simply said "models can't tell you everything". Participants also referred to the complications of judging the relevance to humans of a toxic effect in animals. In particular, participants observed that one species of animal may be similar enough to humans to model a pharmacological effect, but may not model a toxic effect, and therefore another species may be needed in order to study and model toxicity. Participants also mentioned the level of skill required to both create and properly use some animal models, noting that conflicting results can be generated by different laboratories or by using animals provided by different suppliers. A few participants felt that these difficulties could be further compounded when animal data are interpreted solely by non-clinician scientists (ie those without medical or veterinary training) because these scientists may not have a "whole animal perspective" and may incorrectly attribute clinical observations to a drug effect.

3. Animal death as an endpoint in drug studies

Thirteen participants were asked about their perspective on using animal death as an experimental endpoint: three different viewpoints emerged. Most participants (five scientists and four regulators) did not think that animal death is necessary as an endpoint, and some were adamant that death provides no useful information. One scientist explained:

> "By toxicity, I don't mean that every single animal has to be sick or that you've got animals dying, or major lesions or whatever…. If you had most of the animals, or all of the animals, in the high dose group who lost more than 10% body weight while on study — that would be considered a toxic response, so you don't have to have the mortality".

However, two scientists viewed animal death as a legitimate endpoint for some drug discovery research, and as an unfortunate but unavoidable consequence of some toxicology studies. This view was explained by one of the scientists with reference to sepsis research:

> "Now there are occasions when they do need death as an endpoint. Sometimes there's such a narrow therapeutic margin that they need to find that…. Sepsis by definition is almost always a fatal disease, and a lot of times what they are looking at is 'can we increase survival time?' The other thing about sepsis is it is a very complicated disease, and there's not simple models for it.

> And we do have sepsis models [where] they say they'll euthanase the animal before it dies, but the truth of the matter is you can't generally get to it fast enough. You'll look at the animal and it looks fine, and an hour later it'll be dead".

Finally, two regulators felt that knowing the dose that causes death in animals is necessary to fully understand a drug. One explained:

> "The object of a toxicology study is to produce toxicity in the animal, and we want to see the spectrum of toxicity. I call it the tox profile; that includes death. Naturally you don't want to take your high dose and kill all your animals, because that's basically a waste, but you want to let some of them die…maybe 20 or 30% of your high dose".

4. Use of genetically modified (GM) animals

Participants were also asked for their thoughts on the use of transgenic animals because of the many welfare problems associated with them (Buehr et al 2003), and because interest in transgenics has been growing in the pharmaceutical industry (eg Burki 1995; Harris 2001). However, when we asked the participants about transgenic animals many of their responses covered GM animals in general; consequently, both transgenic and GM animals are discussed.

Many participants saw use of GM animals as an important research tool in the discovery of disease therapies, hence as "qualitatively useful"; however, there was much less support for GM animals in regulatory testing. One reason was that "a lot of those [GM] animals are not characterised very well" and are typically not thoroughly phenotyped. In toxicology studies, this was believed to make it difficult to distinguish between drug effects and naturally occurring pathologies. One regulator commented that he/she would not make a regulatory decision based on a transgenic model because they are not yet reliable.

Many participants also referred specifically to the attempt to validate the p53 knockout mouse model that was investigated as an alternative to the mouse 2-year carcinogenicity regulatory assay, and was subjected to an international validation trial coordinated by the International Life Sciences Institute (ILSI). This GM animal model has a deletion of one allele of the p53 tumour suppressor gene and such mice develop tumours more quickly than other mice (Robinson & MacDonald 2001). Two regulators in this study were dissatisfied that this and other GM models were embraced too quickly. One regulator expressed concern that the p53 model was adopted for regulatory purposes before the final (negative) results of the validation trial were known:

> "I think we jumped on a band wagon before the model was validated…. ILSI did that lovely project and showed that indeed the positives aren't always positives and the negatives aren't always negatives, so what do you do with the data?"

Participants expressed opposing views regarding whether use of GM animals would reduce or increase animal use by the drug industry. Some participants believed that GM models would promote reduction because they have less genetic variation; therefore, fewer animals would be needed

to achieve statistically valid results. Also, participants speculated that if animals were genetically modified to be closer to the human genome, then results would be more relevant to humans, and perhaps less repetition of studies would be needed. However, other participants believed that use of GM animals would not promote reduction. They remarked on the large number of animals required to create transgenic lines with one scientist noting, "it's gone against the general trend in reduction in the numbers of animals used". These participants also believed that the high degree of variability currently seen in GM animals does not allow smaller numbers to be used in a study.

Views on the use of *in vitro* alternative methods

Participants were asked to describe their views on the role of *in vitro* data in drug research and development. Responses indicated that the participants interpreted the term '*in vitro* data' to include both non-whole-animal and non-animal methods. Responses related to how and when to use *in vitro* methods, limitations of *in vitro* methods and doubts regarding replacement.

How and when to use in vitro *methods*

Almost all participants saw *in vitro* data as being restricted to the role of supporting whole animal data, but most also agreed that *in vitro* work made very valuable contributions to drug research and development, and was as important as the whole animal work. A common perspective was to see *in vitro* assays as a first step in the drug research process, to "pick the most promising ones [drugs] to do pre-clinical animal studies". Similarly, a scientist explained that in toxicology, *in vitro* methods could be used to "knock out the compounds that are potentially toxic…[so] you don't have to carry them forward into the animal". Another scientist explained that "we do all of our early work using these models" and therefore "conserve our animal resources for those compounds [and] those programs where we feel they have the greatest probability of success".

However, other scientists believed that potential drugs should still be tested in animals at an early stage in order to assess whether time and resources should be further invested. Several scientists also noted that there was not always a sequential progression of experiments from *in vitro* to animal studies:

> "I think a lot of times we sort of think of the whole research process as being linear…you synthesise a drug, you go to laboratory tests, you go to animal tests, you go to people, but in fact there is a lot of back and forth".

Limitations of in vitro *methods*

Some participants felt that although *in vitro* tests are very valuable to drug research and development, too much is currently expected of these methods. First, participants commented that a limitation of many *in vitro* methods is their dependence on *in vivo* databases. They explained that this decreased the usefulness of the *in vitro* test for new classes of drugs that have not yet been tested in animals. For example, one regulator cited "structure activity relationships", which attempt to predict a new chemical's biological activity by comparing it to similar chemical structures; however, this requires previous knowledge of how compounds with similar structures affect animal models. Second, the participants raised the difficulties of validation, and the resulting lack of available validated *in vitro* tests as limitations. One scientist described the difficulty of validating alternatives against *in vivo* studies, noting that:

> "With an *in vitro* model you have to validate that model, and 'validate' being a legal term…means that you have to be able to reproducibly, reliably show an endpoint, and that endpoint has to be relevant. Our *in vivo* models have never been validated. We never validated the rat as a predictive species for human toxicity. We never did that, but the rat's the standard, so everything we do now is validated against it. That's a problem".

Third, many participants described *in vitro* methods as being limited by their lack of biological complexity. As one scientist explained, an *in vitro* result is "taken away from all its normal checks and balances"; therefore, the questions that could be asked of an *in vitro* test were necessarily much simpler than those that could be asked of an animal model. Another scientist described this in terms of the strengths and weaknesses of *in vitro* methods:

> "You can screen a thousand, or ten thousand, or a million compounds and get that yes or no answer. That's the strength. The weakness is that every time we try to ask more questions than that, we are bitterly disappointed by the outcomes. So, for example, you'd say, 'I've got the cell culture; I've got the drug target; I wonder if I can use this to predict cardiovascular safety?' The answer is no!"

Doubts regarding replacement

Participants appeared doubtful as to whether alternative methods could completely replace the use of animals. Several specifically stated that they do not believe that animal models can be replaced entirely. For example, a scientist said, "I think *in vitro* tests are for sure going to be valuable to all concerned parties, but ultimately they are never going to fully supplant the use of animals". Another expressed frustration with the concept that batteries of *in vitro* experiments could be validated as a replacement for trying a drug in an animal:

> "With an *in vitro* model you don't validate it as a replacement for an animal study.… This concept in the eighties was 'if we did batteries of *in vitro* assays we wouldn't have to do animal studies'. That doesn't work. It doesn't work, and in retrospect, I'm surprised now that we really thought it would work, because it doesn't make sense".

Some participants were also not convinced about the usefulness of 'relative replacement' methods (those that involve the use of cells and tissues from animals). One regulator said:

> "I think it's [relative replacement methods] over-relied-on at the moment because I don't think we really know what it means in a lot of instances — Caco–2 cells [permeability assay], some of the p450 hepatocyte cultures. Certainly we've shown that the Purkinje fibre assay is not an effective predictor for the human experience. I think there's lots of examples where perhaps we're jumping in feet first, rather than making sure that it proves effective for extrapolation".

Another regulator noted that *in vitro* methods for predicting long-term toxicological effects are not available:

"Suppose you are looking to see whether or not you get necrosis of the liver. Sure, you can take an isolated liver out and add a high concentration of a compound to it, and see in a period of time whether necrosis occurs. [But] It'll be a different type of area that becomes necrotic…because you don't have blood circulation through the liver.… If you are dealing with compounds intended to be given chronically, you don't have a chronic *in vitro* assay".

Obstacles to replacement and reduction

All 17 participants, both spontaneously and in response to specific questions, identified one or more obstacles to decreasing animal use in the pharmaceutical industry: these have been grouped into seven issues.

1. Lack of alternative methods

Most participants, both regulators and scientists, identified a lack of appropriate alternative methods as an important obstacle. Participants also commented that some alternative methods may be unavailable because industry has difficulty gaining access to human biological material for *in vitro* experiments, and because of the economic costs associated with implementing new alternative technologies. Some also felt that progress in alternatives was not being made. One scientist expressed surprise that there are not more alternatives available "considering all the years we have been working on them".

2. Statistical validity

Many participants identified the requirement for statistical validity as a reason why fewer animals could not be used in regulatory studies. One regulator explained that "you do really have to show that there is a…statistical difference between different groups. It's hard to think of doing less than say 10 animals per group to look at all the different things one looks at in a toxicology study". One scientist felt that he/she was not the appropriate individual to answer questions about reduction, commenting "you're asking a question that really should be addressed to the statistician". Another scientist commented that the application of statistical methods to reducing animal numbers in experiments was hampered by a shortage of statisticians.

3. Regulatory requirements

When participants were asked about obstacles to decreasing animal use, six scientists (but no regulators) identified "regulatory requirements", although some acknowledged that drug companies also played a role in creating and maintaining the regulations, for example, through participation in international regulatory harmonisation processes. Some participants identified the repetition of studies in order to conform to good laboratory practices (GLP) standards (a regulatory requirement) as a source of unnecessary use of animals, in particular for non-human primates. However, only two examples of specific problematic regulations were provided. One scientist criticised the regulatory requirement to have two routes of administration for an acute toxicology study for drugs that will be dosed only orally in human clinical trials (United States Food and Drug Administration 1996). This scientist viewed the requirement as "a bit of a waste" of animals, and expressed frustration that drug companies seem content to just go along with the requirement:

"There are some regulatory agencies in the world that still want to see two routes of administration in the acute studies. So what it comes down to is: many companies, they play the game. They go along and they say, 'this is what the regulatory agencies are going to want; we're just going to go ahead and do the study. We know it doesn't make sense but we're just going to do it'".

Another scientist cited the requirement for two rodent species for carcinogenicity studies, noting:

"It is a big and on-going debate as to whether you gain anything additional by running a second species".

Some scientists also described uncertainty over the expectations of regulators. One scientist mentioned that although a regulatory agency might not specifically require a particular animal study, a particular reviewer may still expect to see it. Similarly, different perceptions were expressed regarding whether non-GLP data were ever acceptable to regulators. Some participants (both scientists and regulators) felt that regulators would accept non-GLP studies on a case-by-case basis if they were carried out with adequate documentation, but one regulator maintained that GLP animal studies are always necessary to protect regulatory agencies from fraudulent data.

4. Human safety concerns

Many participants identified the need to protect human safety as an obstacle to replacing or reducing animal use. Regulatory agencies in particular were perceived to be responsible for protecting public safety and ensuring the safety of medicines. One scientist explained:

"I think regulatory agencies take a longer period to adjust to changing science.… They have to be convinced because they are on the hot seat of having to protect human safety, and so…the weight of the scientific evidence has to grow very strong in order for them to change their mind. I see that as one of the major obstacles".

Similarly, when speaking of why *in vitro* alternative methods are not readily adopted by regulatory agencies, one regulator explained that "we really need to be assured that this method is adequate, and that is the issue for us and why we probably do not move as quickly as we'd like, because we need that extra assurance". The same regulator also noted that public opinion polls have confirmed the preference of society to have regulatory agencies remain conservative when it comes to evaluating new medicines (although the participant did not identify the relevant opinion poll).

Two scientists touched on the sense of personal responsibility they had felt when drugs they worked on ended up having toxic effects in humans. One described that it felt "really scary" to find out they had gone into clinical trials with a drug that had "the potential to cause harm" in spite of having tested it appropriately in animals. Another explained their reaction

when a drug was found to have serious side effects after it was approved for use in the general population:

> "There's a sense of ownership and a sense of pride when the drug gets out to market and is successfully used to treat human diseases and conditions. And then when you find out that the drug you developed — that you thought was non-toxic and efficacious — ends up killing people, it's kind of a devastating blow".

5. Resistance to change

A number of participants used the phrase "resistance to change" when discussing obstacles to decreasing animal use. Regulatory agencies were perceived by scientists to be resistant to change, in part because of their mandate to protect human safety, as discussed earlier. Some scientists also perceived that resistance to change was related to regulators' reliance on precedents. One scientist complained that regulators adopted a "box-checking" mentality when reviewing new drug submissions, just checking if certain tests were completed instead of reviewing whether the tests were scientifically relevant. This scientist believed that not all tests were necessary all the time, and that drugs should be evaluated more on a case-by-case basis.

Both scientists and regulators perceived that drug companies are resistant to change. Drug companies were also described as relying on precedents. Referring to LD50 tests, one scientist explained that companies "got stuck on a lot of these tests where they just do things because historically that's the way it had been done". One regulator felt that the industry's resistance to change was due to the comfort level of the existing condition: "That is how they've always done it and they've been successful.… That's what they are comfortable with, so that's what they are going to use".

This was echoed by one scientist who commented:

> "Most of the drug companies do have kind of a set pattern that they follow for the development of a drug, and so this gets back to the original question of 'are they doing studies they don't have to do?' Well yeah, because they do pattern how they develop a drug, and they just find it easier to say, 'Ok we're going to go ahead and we're actually going to run this drug through mice and run it through rats and put it through dogs, [and] we'll put it through monkeys'. And then they just go ahead and do that in sequence without really giving a lot of thought about does it make sense".

Another scientist noted a division in thinking in the industry regarding the adequacy of current methods for drug evaluation in animals:

> "I think that there are two camps.… There's your group of people who believe that what we're doing now [in animal studies] is sufficient and we don't need to ask any more questions. And there's a group who believe that what we're doing now is Neanderthal and we're not even now asking the right questions, let alone coming to the right conclusions. So…it's really polarised".

A few participants felt that resistance to change would be overcome only in response to external pressures. For example, two participants (one regulator and one scientist) felt that only political pressure would force the wider adoption of alternatives to animals. A few scientists also remarked on the effectiveness of animal care committees to "push" scientists to consider alternatives.

6. Commercial goals

Several scientists pointed to the commercial nature of pharmaceutical research and development as an obstacle to replacement and reduction. One scientist expressed frustration that attempts to use as few animals as possible may be compromised by timelines and difficulties in scheduling studies:

> "Too many times what I see is this overlapping of studies where…you don't have all the information that you need to actually go in and properly set up the next level studies. And that continues all the way through the development process, and the excuse that's always given is 'We've got corporate timelines.… We've got to meet what our shareholders want. They want us to get this drug to market…'".

Similarly, another scientist spoke of how responding to changing business pressures can sometimes cause scientists to rush into animal experiments:

> "Often there's a situation where things happen very quickly. Priorities change and someone's identified a candidate drug from somewhere, and we want to test it — we want to test it fast. We don't have experience with the model…[but] we'll throw the compound in there to just start, and we may end up with nothing really, because we don't really understand the model very well and the result you get is virtually uninterpretable".

One scientist observed that protecting a company from litigation may prompt some companies to do the maximum amount of animal safety testing regardless of whether there is a scientific basis:

> "At least in the US, where people will sue you at the drop of a hat, I mean that's a huge obstacle because we're not willing to take any risks".

Some scientists questioned whether it is the responsibility of drug companies to develop and validate alternatives and commented that it may be particularly difficult for small companies. As one observed, the development, validation and implementation of alternatives is very expensive and the cost is not really part of a drug company's core business:

> "It's expensive to look for an alternative way.… It's not just being creative and coming up with a method. Really we have to validate it, so if you're going to replace something you need to make sure your replacement is at least as good as, if not better, than what you're replacing.… It's very resource intensive.… And why would you? You might as well run a 30-day rat study instead".

Scientists disagreed over whether attempts to save money within the company would encourage less animal use. One scientist did not think that the expense of animal use would deter use because "drug companies have lots of money". In contrast, another scientist identified economics as a potential influence to decrease animal use:

> "People are no longer going to be paying the price that they are right now for our drugs, and…animal testing is very, very expensive. Anything that we can do in vitro, in a computer model, is way cheaper. And again, when we're screening…tens of thousands of drugs…we can't

be running them all through animal tests…[and] the sooner we decide [a drug] isn't going to work, the more money we've saved".

7. Development of patent extension products

Finally, two scientists observed that the drive to extend existing product patents (rather than create novel medicines) also results in use of animals, but for possibly less benefit. One explained:

> "There will be more attempts to come up with slightly improved versions of existing drugs, and yet those slightly improved versions still have to go through the entire development process. So in that sense, maybe it's not so much that there are going to be more animals used, but they're going to be used less profoundly".

Suggested opportunities for greater implementation of the Three Rs

When asked to identify opportunities for decreasing animal use in the pharmaceutical industry, two participants (one regulator and one scientist) responded that they did not feel that too many animals were being used, and therefore could not think of ways to reduce animal use. Responses from the other 15 participants identified 10 possible opportunities.

1. Ask more basic questions

Several scientists felt that better use of animal models would be achieved if toxicology studies sought more basic information, such as changes in gene expression caused by a drug ("predictive toxicogenomics"), in addition to phenotypic information, such as tissue damage. One scientist explained:

> "I think that the animal models that we use are a lot more predictive than we give them credit for because we are not asking the right questions.… For example, you can see the effects of a carcinogen on the liver in seeing the induction of DNA damage-repair genes without ever seeing tumours".

2. Develop and select better animal models

Several participants felt that by developing or selecting better animal models, less repetition of experiments would be required; hence, animal use would be reduced. One scientist noted that in regulatory experiments "the way you minimise the use of animals is to select the appropriate species to begin with". Similarly, a regulator felt that drug companies "should be doing a better job of looking at the physiology and the anatomy and basic physiological parameters that these animals have" in order to improve their selection of species.

3. Use pilot studies

Some scientists suggested carrying out pilot studies — that use smaller numbers of animals — to select which drugs should progress to large GLP toxicology studies. One scientist explained:

> "I really do believe that this approach of doing pilot and definitive [GLP] studies actually does, in the long term, reduce animal numbers. It may seem initially like it's increasing because of the repetition, but because of the volume of drugs that we're screening…it allows us to not have to run definitive [GLP] studies on a lot of our compounds".

4. Re-use animals, as appropriate

Participants suggested reduction strategies that involved some re-use of individual animals. Some scientists proposed altering animal study designs so that they are similar to human clinical drug trials. One scientist suggested developing models where "an animal is used for a series of investigations" to examine different drugs in sequence. In order to avoid killing some of a study's animals at each data collection time-point, another scientist suggested doing "longitudinal studies in the same animal" so that fewer animals would be needed to achieve the desired sample size.

5. Use enough animals to avoid repeating inconclusive experiments

Several participants also explained that it is equally important that sufficient animals are used in a study to make the experiment statistically meaningful and therefore acceptable from a regulatory point of view. This was felt to contribute to reduction in the long term by avoiding the repetition of inconclusive studies. These participants felt that, in general, industry scientists erred on the side of not using enough animals and that this resulted in the repetition of experiments.

6. Reproductive toxicity guideline

One regulator noted that the regulatory guidelines for reproductive toxicity studies (ICH 1994) provide an opportunity to reduce animal numbers, but that this is rarely used by drug companies:

> "In rat reproduction studies you can use rats for your Segment One studies, where you look at the fertility and ease of…animals copulating and stuff like that, or for the Segment Two study, you can do that separately, where you look at organogenesis, or Segment Three which is…pre-birth and post-birth. They can be combined into one, so you can do dosing throughout…[and] rather than using 60 animals for reproductive toxicology studies you get to use 20 and combine all three of the segments".

7. Regular review of animal data and regulatory requirements

Regular review of existing animal data was proposed by both scientists and regulators as an opportunity for decreasing animal use and a practice that should be routinely followed by both drug companies and regulatory agencies. Many participants mentioned that drug companies should develop correlations between *in vitro* and *in vivo* data for their own compounds. Through actively looking for correlations, one scientist felt that some use of animals to study the pharmacology of drugs with well validated receptor-targets could be replaced with *in vitro* methods:

> "You may say, 'Well, I don't really need to use an animal model anymore. I have enough information based on clinical findings and I know that my compound exhibits the right *in vitro* profile and the right pharmacokinetic profile, that there really isn't much point in taking it into an animal [disease] model. I have enough information from other sources'".

A few participants expressed the view that regulatory agencies should review their requirements more frequently to see if information was still needed or used in assessing

new drug safety. For example, one regulator pointed to the need to review "what is being done currently and figuring out…how large the studies and how long the [future] studies should be based on past experience". This regulator suggested selecting a "cut-off" number of animals and reviewing how much additional information is provided by use of animals exceeding this cut-off number.

8. Require companies to publish certain data

One scientist suggested that a legal requirement for companies to publish some of their data could lead to less duplication of work.

9. Use historical control groups

The use of historical control groups was suggested by one scientist as a method to reduce the number of animals used; however, one regulator specifically expressed distrust of studies using historical controls.

10. Refinement

Although the participants were not asked directly about refinement, several participants commented on it. Refinement was acknowledged by many participants to be a reasonable and achievable goal and "really our biggest opportunity". Suggestions for refinement included developing less invasive methods of evaluating animals, for example, "put the animals in a scanner instead of sacrificing their brain"; using animals that are more suited to confinement, for example, "you can keep a pig happier in confinement than you can a monkey"; and using pain control methods to "try and reduce pain and suffering of the animal in some of these tests".

Interestingly, several participants considered the use of GM animals as a refinement. They believed that because some GM animal studies could be of a shorter duration than non-GM studies, refinement would be due to fewer in-study deaths because "you don't lose as many on study when the study's shorter". Using pilot toxicological studies was similarly described as a refinement. One scientist explained that with pilot studies the overall number of animals that suffer serious toxic side effects is minimised because those drugs don't progress to larger GLP studies that use more animals.

Discussion

This study did not find two distinct clusters of responses — one from industry scientists and one from regulators. Instead, most views were shared by one or more members of each group, and a wide range of perspectives existed among all participants; although a few points were raised only by the scientists, such as the role of commercial goals and regulatory agencies' resistance to change as obstacles to reform. However, overall there was no evidence of large differences between regulators and pharmaceutical industry scientists that would need to be resolved for progress in the Three Rs to be made.

Areas of agreement between scientists, regulators and Three Rs advocates

Many of the opportunities identified in this study have also been discussed in the Three Rs literature (eg Tweats 2000;

Combes *et al* 2002; Richmond 2002; Stephens *et al* 2002). In particular, the views of participants agreed with those of Three Rs advocates in four main areas.

First, participants clearly acknowledged the need for better models because animal data are not without problems. For example, animals may not reflect human responses; an animal may model pharmacokinetics but not toxicology; and individual animals vary in their responses. Therefore, the pharmaceutical industry, like animal advocates, sees a need for improved animal and non-animal methods to replace less effective animal models.

Second, participants identified improvements to the current use of animals as opportunities to reduce the numbers of animals used. These included routine reviews of animal data, asking questions at the level of gene expression, and more careful species selection. These approaches are also advocated by the Three Rs community (eg Balls *et al* 2000). These strategies may be particularly well aligned with the pharmaceutical industry as they may also contribute to improved scientific quality of drug studies.

Third, participants identified the difficulty of validation and the lack of validated alternatives as key problems for expanding the use of alternative methods. Validation of alternative methods is necessary if pharmaceutical companies and regulatory agencies are to adopt them. Therefore, validation is a priority for Three Rs advocates (Balls *et al* 1995) and is supported by the pharmaceutical industry and regulatory agencies in North America. For example, validation activities for regulatory purposes in the United States are coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), a multi-agency organisation that includes the FDA (ICCVAM 2005). Although alternatives used for regulatory purposes must undergo a stringent validation process (Snodin 2002), validation for in-house use — a less onerous form of validation — could allow companies to replace animals in drug development (Clark 1994).

Fourth, some participants commented on the need to better understand the phenotypes of GM animals. These comments generally related to trying to improve the scientific value of GM animal tests, for example, by allowing investigators to distinguish between the effect of the genetic modification and actual effects produced by the drug. Improved phenotypic characterisation of GM animals has also been identified by animal advocates as a method to improve animal welfare. Given that some types of genetic modifications can cause welfare problems (Buehr *et al* 2003), phenotypic characterisation of GM animals could help to reveal any special needs or problems, including the animal's handling and housing needs (Jegstrup *et al* 2003). Therefore, characterising GM animals would promote refinement in using these animals, as well as possibly allowing reduction.

In addition to these broad opportunities for Three Rs, participants also made two specific suggestions for reduction: removing the acute toxicity testing requirement for two routes of administration if the drug only has one, and using

the option in the regulatory guideline for reproductive toxicity that permits combining three sections into one study.

Areas for consensus building

Participants also raised five contentious points where consensus would need to be reached in order for the Three Rs to be implemented more widely. First, a few participants saw the re-use of animals as an opportunity for reduction, and suggested structuring animal studies in a manner similar to human clinical trials. Traditionally, the re-use of animals has not been promoted by animal welfare advocates because of the potential to increase harm to individual animals, such as pain or stress from handling. Re-use has also been hampered by the small size of mice and rats, as these animals can provide only limited blood samples and need to be sacrificed to examine tissues. However, re-use is more commonly carried out with larger animals such as dogs (Broadhead *et al* 2000) and with the advent of newer and less invasive methods of analysis, such as telemetry and imaging technologies (Stephens *et al* 2002), re-use of smaller animals may become more feasible. Nonetheless, effects on individual animal welfare must be considered before this type of approach can be advocated; hence re-use may need to be judged on a case-by-case basis by weighing the different options to achieve the least overall harm (Russell & Burch 1959, reprinted 1992).

Second, some participants suggested that the use of GM animals could contribute to reduction, but other participants asserted that GM animals could increase the numbers of animals used overall. Similarly, in the Three Rs literature both perspectives have been expressed (eg Buehr *et al* 2003). Current methods of GM production involve a large increase in animal use, but a good model, once developed, could reduce the numbers of animals required. Furthermore, newer methods of GM production may prove more reliable, and so might produce GM models more efficiently (Schuppli *et al* 2004).

Third, some participants saw the apparent repetition of studies to a GLP level as a source of increased animal use, although other participants viewed this practice as a way to both reduce overall numbers by decreasing the number of large GLP studies that are conducted and to refine experiments by subjecting fewer animals to severe toxic effects. Some participants also felt that animal studies did not always need to be conducted to GLP standards, but at least one regulator felt that GLP was the only acceptable standard; therefore there is a need to create consensus on these points. Pilot studies may well reduce animals and minimise overall harm if they are followed by a judicious switch to GLP at a certain point to avoid or minimise repetition. Similarly, non-GLP studies may be acceptable for regulatory agencies under specific circumstances; clarification of these circumstances would be useful.

Fourth, two regulators expressed the opinion that determining the lethal dose is important to understanding the full toxicological profile of a new drug, and two scientists viewed death as a legitimate endpoint in some drug discovery research and as an unavoidable consequence of some toxicological studies. Death is generally not a legislated regulatory requirement (Richmond 2002; Stokes 2002) except for some biologics (eg vaccines), and these views run counter to common calls for humane endpoints. If the belief in the usefulness of death as an endpoint is widespread among regulators and scientists, it could detract from efforts to refine animal use in drug research and development; hence, consensus is needed on where, if ever, death is useful.

Fifth, complete replacement of animals with non-animal testing methods was not accepted by participants in this study as a reasonable or achievable goal whereas *relative* replacement was more supported. Participants generally viewed data obtained from *in vitro* methods as always requiring follow-up confirmation by tests in whole animals. This apparent reluctance to pursue replacement on the part of industry scientists and regulators could hinder progress in the Three Rs, particularly if alternatives are equated only with non-animal methods (Zurlo 2000). Therefore, there is a need to create consensus on what are realistic goals for replacement so that the pharmaceutical industry, regulators and the Three Rs community can pursue the same objectives. Probably much can be achieved in relation to *relative* replacement, even if participants are correct in the strongly held view that complete elimination of animal studies is not feasible.

Other obstacles to the Three Rs

Concern for human safety was identified as an obstacle to reducing and replacing animal use in regulatory tests. Human studies, although proposed as a replacement by the Three Rs community (eg Balls 2002), were never mentioned as an opportunity. Participants felt that a move away from animal-based testing could put human safety at risk. This concern draws on past tragedies with unsafe medications that were either not tested, or not adequately tested, on animals (eg Gad & Chengelis 1995; Schechtman 2002). Studies showing how animal studies have helped to predict human safety risks (eg Broadhead *et al* 2000; Olson *et al* 2000) may further support this view. For scientists or regulators who perceive that any move away from animal tests may increase the risk to humans, protecting human safety presumably justifies the costs to animals; hence the Three Rs, with their emphasis on decreasing use of animals, may be seen as incompatible.

Another obstacle is that many scientists and regulators did not appear to perceive their companies or agencies as having a mandate to pursue the Three Rs. The objective of a drug company is to achieve commercial success with new medicines and any substantial diversion of resources for Three Rs purposes, without a clear commercial benefit, may not be acceptable. Patent extension products, which from a Three Rs perspective may seem to duplicate animal studies, benefit the company as they extend the financial returns on a drug. Because pharmaceutical companies must also follow the guidelines of regulatory agencies, some participants felt that the industry could not take the lead in implementing the Three Rs. The mandate of regulatory agencies is to protect human safety; hence they too may feel that a

diversion of resources to pursue the Three Rs is inappropriate. Moreover, because some participants identified problems over clarity between regulators and the pharmaceutical industry, industry may be unclear as to how regulators will evaluate data from alternative methods and are therefore reluctant to use them for regulatory purposes (O'Connor 1997). Here again, progress will require more consensus on who has a mandate to pursue the Three Rs and how the work should be funded. In this case, greater acceptance of the Three Rs may only be achieved through legislation or pressure from the public.

Conclusions and implications for laboratory animal welfare

Most of the regulators and scientists interviewed in this study could identify opportunities for greater implementation of reduction and refinement within the North American pharmaceutical industry, for example, by following the regulatory option of combining segments of reproduction toxicology studies into one study. Some of the opportunities identified by participants may result in both scientific improvements and improvements to animal welfare, for example, the phenotyping of GM animals, routine review of animal data and more careful species selection. Participants also identified other opportunities for reduction and refinement that are more contentious; these include re-using animals in multiple studies to achieve reduction, and the use of pilot studies to reduce animal use overall. In such cases, there is a need for consensus-building and perhaps the development of guidelines specifically for the pharmaceutical industry to assist in the implementation of the Three Rs.

This study found substantial obstacles to the implementation of replacement. Although participants viewed *in vitro* data as valuable, they did not see the complete replacement of animals in drug development and testing as either feasible or desirable. In particular, participants were concerned that pursuit of the Three Rs might jeopardise human safety, and they did not appear to perceive that their organisations have a mandate to pursue the Three Rs. In this case, greater implementation of replacement may require the development of realistic goals for replacement in the pharmaceutical industry, a clearer sense of who has a mandate to pursue the Three Rs, and consensus-building on the relationship between the Three Rs and human safety.

Acknowledgements

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