The Academy of Medical Sciences | FORUM

Safer Medicines Report

Pre-clinical Toxicology working group report

November 2005

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Executive summary

Overall recommendations

There is one major recommendation from this section on non-clinical toxicology, which is the establishment of a UK Centre of Excellence in Safety Assessment. The subsequent list of key points can largely be achieved under the auspices of such an establishment. In one single exercise this would ensure the future of pharmaceutical company R & D investment in the UK and provide opportunities to change the paradigm of drug development. It could provide the resource and expertise to establish public databases, also to build a resource of expertise and training in new technologies, *in vivo* techniques and risk assessment for the industry and regulators to draw on. It could allow the regulators to have access to research expertise to further their scientific concerns and thus their own understanding. It could act as a vehicle of communication with the public.

It is envisaged that this would be a strategic long term investment with a clear mandate to provide scientific support to the industry and government, in particular with regard to:

- Validation of new techniques
- Establishment of databases of public domain information
- Maintenance of the necessary skill bases/teaching/training
- Communication with the public

It is considered key that all this needs to be concentrated in one place and not diffused around different organisations or it would be subject to conflict between those and would lose the necessary synergy. It would also not give the strong message required to maintain UK Pharmaceutical companies R & D and its skill base and also enhance the regulatory process. It would have far more power with the international regulatory and industrial community to try and change the way we do drug safety evaluation. Without the latter, little will change in the foreseeable future.

In the detail of our Key proposals below we have attempted to address several main themes as follows:

- Challenging the traditional approach -
  - Case-by-case safety programme design
  - Weight of evidence approaches to risk assessment
- More relevant animal models
- Biomarkers
- Discovery and predictive toxicology
- Validation of new approaches and influencing global regulatory authorities
- The skill base for *in vivo* technology, risk assessment and new sciences
- Retention of internal investment in UK Pharmaceutical companies R&D

Key proposals

Better animal models are required that more accurately predict the response of a drug in humans. This may include genetically modified animals which have been demonstrated to better reflect the responsiveness of a class of drugs to humans. Rodent models with human-like ADME characteristics for screening of NCEs should be generated. Also, recommendations could be produced for the creation of custom human target expressors in animal models using transgenics, (conditional) knockouts of animal homologue, RNAi and neutralising antibodies for the expression of humanised pharmacological target-related toxicology.

The use of such alternative toxicity models could be validated through the auspices a UK Centre of Excellence in Safety Assessment and the results could be placed in the public domain.

A longer term objective would be to gain regulatory acceptance of the use of non-standard models for screening and mechanistic toxicology studies and to further promote their use by pharmaceutical companies as a low-risk alternative.

There is an urgent need to develop facilities and create opportunities for training toxicologists. At present there are opportunities within a few universities for specialist training, but there is no major academic organisation in the UK with a comprehensive

knowledge of applied pharmaceutical science. A UK Centre of Excellence in Safety Assessment could provide this and would encourage the development of the necessary skills, both for the pharmaceutical industry but also to provide the resource for the regulatory functions which have to monitor and licence drugs. There should be programmes to encourage the development and application of all analytical and computational tools possibly by extension of toxicologists' training. The UK has to invest in its toxicologists, who need training in 'new' sciences to be able to make best use of these technologies. Once again, a role for the UK Centre of Excellence in Safety Assessment is called for.

There are a number of recommendations for the creation of a repository for information in the UK Centre of Excellence in Safety Assessment. If this was part of a Centre of pharmaceutical science it could cover not only toxicity, but the relationship of pharmacology in experimental animals and humans. This Centre should encourage the development of databases on the concordance of animal and human toxicities effect to the response of drugs with the objective to improve the selection of animal models and enhance the development of new models. An important component would also be the compilation of genome-based information and data generated form the 'omics' technologies. The ultimate benefit would be an improvement of human health risk assessment. There could also be a database to collate both non-clinical and clinical information in support of idiosyncratic ADRs. Ultimately the idea would be to establish a comprehensive database of toxicological and drug safety information under the auspices of a UK Centre of Excellence in Safety Assessment.

Mechanisms need to be established to encourage more open collaboration between regulators and industry to develop paradigms for more rapidly progressing to human clinical ADME, PD, toleration and efficacy studies on the basis of more targeted non-clinical evaluations. This would mean less reliance on standard testing based on current ICH guidelines.

Target-related toxicity may be anticipated from an understanding of animal knock-out, anti-sense and other models or from human genetic disorders causing loss of function. This may be called 'discovery toxicology'. This concept can lead to the acquisition of an early understanding of on- and off-target effects, and chemistry-related effects and can help to ensure that better quality molecules process into candidate selection. Discovery toxicology should be reinforced and be a more accepted, industry-wide philosophy i.e. acquire an early understanding of on- and off-target effects.

The advent of the 'omics' technologies (transcriptomics, proteomics and metabonomics) has offered new opportunities in predictive and mechanistic toxicology. To facilitate the full and appropriate application of 'omics, there should be government funding in partnership with UK industry of 'deep' studies (proof of principle-type studies) to build an understanding of the relationships between 'omics data and conventional toxicology measurements and to further evaluate the potential of these new tools to predict and characterise the potential for adverse effects. These would use a 'systems biology' approach (i.e., with emphasis on the behaviour of the biological system as a whole, rather than on its individual components) to give a more complete overall biological picture. The archival compounds of pharmaceutical companies would be an integral component and inter-platform and inter-laboratory variability would be addressed. The major output from such a study would be the creation of an open, transparent, cross-sector and interactive UK scientific environment for the further development and application of these platforms, such that methods and ideas could be readily shared. This could be another component of the 'UK Centre of Excellence in Safety Assessment'.

The new technologies alluded to above also provide the means for improving our understanding of idiosyncratic human toxicity and adverse drug reactions. Specifically, gene profiling experiments in human hepatocytes with known human hepatotoxins could derive several benefits, including obtaining "proof of principle" that biomarkers relevant to adverse drug reactions can be obtained by this approach, improved understanding molecular mechanisms of human liver toxicity, and providing a basis for comparison with pre-clinical gene profiling studies. Also, dedicated "proteomics" and "metabonomics" effort towards identifying biomarkers in biofluids (serum, urine etc.) will be important for early identification of patients at risk from developing adverse drug reactions.

Also of key importance is ensuring that the learning from large international collaborative efforts, e.g. Eudragene, is properly exploited to reduce adverse

drug reaction incidence from commonly used therapeutic agents. Alongside this, Pharma should be encouraged to devote their own internal effort to understanding causes of adverse drug reactions with their own marketed drugs. This may require some flexibility from regulators to ensure any disincentives for doing this are minimized.

A longer-term goal is captured by the phrase "personalized medicine". It will be desirable for the "polymorphism profiles" for the major drug metabolizing enzymes to be known for individual patients, so that patients at risk from adverse reactions from a particular agent can be identified prospectively, rather than retrospectively.

Chapter one - *Introduction*

1.1 An evaluation the current practice of pharmaceutical toxicology and an attempt to suggest ways of 'changing the paradigm' needs to start with setting the scene. There are some overriding concerns/expectations in the UK with regard to the development of novel pharmaceuticals. These are the not unreasonable public expectation of the highest quality and level of safety, coupled together with the highest standards of animal welfare in the world. If this is put alongside the fact that the process of drug development is carried out in a highly regulated environment, then it is very apparent that there are significant constraints to any dramatic change in the status quo. It must also be appreciated that drugs are developed for global markets and the process is regulated globally, so any UK initiative needs to influence other countries regulatory authorities before any significant changes can occur. There is also a lack of understanding with regard to the fact that it is not possible to have a totally 'safe' drug. The very requirement for biological activity from a novel pharmaceutical to have its pharmacological effect predisposes it to potential on and off target effects that may be adverse. The continuous challenge is to assess the risk-benefit equation in the context of a weight of evidence approach.

1.2 From the perspective of the pharmaceutical company, the cost of developing drugs continues to rise exponentially. This puts pressure on the non-clinical aspects of development to do things quicker and find ways of predicting adverse toxicity as early as possible. The UK has a tremendous record in bringing good drugs to the market but the consolidation of the industry and some of the pressures referred to earlier has resulted in a decline in the availability of the necessary skills in the employment pool. This is a particular issue with regard to *in vivo* pharmacology and toxicology skills and is starting to cause real recruitment shortages for UK Pharmaceutical companies.

1.3 However, on a more promising note, there are areas of evolving science that will provide us with better tools to do the job. These include the use of 'omic' technology in both the areas of early 'discovery toxicology' and 'predictive toxicology'. Also the identification of more and better biomarkers, more non-invasive techniques and the possibility of developing more relevant animal models. It is with all of these in mind that the subsequent sections of the Non-clinical Toxicology section were chosen. It is important to discuss the value of the use of animals in the development of pharmaceuticals, but then to put this into context of how their relevance to humans could be enhanced. We then suggest how the omic technologies could be further integrated into safety assessment and how we could exploit genome-based information more. There is room for improvement as to how we assess the non-clinical safety of drugs. There needs to be a better understanding of how to separate on and off target effects and a wider appreciation of the value of using the weight of evidence approach. The increasing use of new approaches to assessing exposure is proving invaluable in this regard.

1.4 To substantially alter the 'check list' approach it will need industry and global regulators to gain confidence in some of these new initiatives and hence allow for more flexibility of approach otherwise little will change. The following text presents some possible future scenarios that could challenge the status quo and the response of 'but we've always done that way'.

Chapter two - *The use of animals in pharmaceutical toxicology*

2.1 Toxicology is one of the oldest applied sciences, stretching back to the early evolution of humans as a hunter-gatherer. It seems likely that, from early time, humans recognised the importance of knowing and passing on knowledge that certain plants should not be eaten. By 2000 years ago, there was considerable knowledge of toxic substances and the use of toxins was well recognised as a means of killing rivals, political or otherwise. Before the 20th Century, it was not uncommon for chemicals, or natural products, to be 'tested' in humans to establish both their efficacy and safety. In the 20th Century, experimental scientists began to consistently test novel molecules in experimental animals before the putative drug was administered to humans. This was done on the basis that at least humans would not be exposed to the most acutely toxic molecules. These studies were carried out because there was a general acceptance that the response of animals should approximate to that of humans. In the 20th Century, this practice was developed and refined to offer the basis of standardised toxicological studies that could predict the safety of the drug in humans.

2.2 The major reasons for the toxicological testing of candidate pharmaceuticals can be summarised as follows:

- To ensure that the first administration of a drug to humans can be carried out safely.
- To identify in experimental animals those adverse effects which should be monitored in subsequent human clinical trials and identify the safe therapeutic margin between those effects and the anticipated therapeutic dose in humans.
- To determine the nature of the metabolism, kinetics and absorption of the drug in animals and humans and thus the relative exposure.
- To evaluate of the genotoxic potential of the drug.
- To evaluate any effects on physiological/ behavioural parameters.
- To evaluate the potential for teratogenicity and any effect on reproduction.
- To evaluate the carcinogenic potential of the drug.

2.3 The ethical and scientific legitimacy of using experimental animals for the safety evaluation of drugs in humans is an issue that has been addressed by many groups in the UK. At one level, it may appear self evident and only common sense that before humans are exposed to a drug, its safety in other species should be established with the intent of avoiding unnecessary harm to humans. However, there are those who believe that either the use of experimental animals is unacceptable, the suffering too great, or that the benefits could be achieved by other means. The ethical considerations cannot be properly covered here. In any event, what would be considered ethically acceptable at the time of writing will certainly be altered by time and geography. Even today, the status of rodents used as experimental animal in UK laboratories is quite different to that in the USA, where in many of the states it is not even necessary to report the number of rodents used.

2.4 From a scientific viewpoint, we need to be sure that the use of animals generates information that can be converted to knowledge that is useful in the development of drugs. It is generally recognised that where possible, *in vitro* tests should substitute for *in vivo* studies. However, it is clear that at present, *in vitro* techniques cannot substitute for the full range of physiological and biochemical processes that occur in the whole animal. For this reason, the ability to carry out *in vivo* experiments is essential. For example, short term and sub-acute toxicity studies need to be carried out before a novel drug can be given to humans for the first time. Perhaps the acid test of this statement is whether any parent would give a recently synthesised white powder to their child, before

it has been shown not to be harmful in experimental animals.

2.5 For chronic toxicities, even less reliability can be placed on *in vitro* studies. However, it is also true that longer-term studies in experimental animals tend to expose, and sometime exaggerate, the responses of humans. Because testing protocols nearly always require the administration of much larger doses of the drug to experimental animals than is given to humans, toxicities are seen in experimental animals, which may never be seen in humans exposed to much lower doses. The paradigm that is used is that in order to evaluate the effect of the drug in a large population of humans, a small number of animals are given very large doses of the drug. In this way it is hoped that the potential for the drug to cause harm can be identified. As stated before, the longer the duration of the study, the more likely the effect of experimental animal will cease to reflect the response of humans.

2.6 At this stage, it is certainly not possible to identify measures that would eliminate the use of experimental animals. However, refinement of studies ought to be possible, but this is not simply a scientific issue. To move away from tests that have been used for many decades, requires certainty that the alternative studies will afford the same degree of protection to humans, compared with those currently used. This in itself is challenging, since the most reasonable way of investigating the relevance of the effects in experimental animals to humans is to reduce the dose administered. This will offend the scientific custom and practice which is to dose the drug at sufficiently high concentration to identify target organ susceptibility and the potential toxicity. Superimposed on this genuine scientific conflict is the problem of litigation which could arise if in the future toxicity studies were determined to be less stringent than those currently used. Moreover, the regulatory process is by its very nature conservative, ensuring that any change in the design of toxicological studies on drugs has a prolonged evolutionary timeframe.

2.7 Those who oppose the use of experimental animals are only too keen to exploit and exaggerate some of the real scientific, legal and regulatory dilemmas, which hinder the rapid evolution of toxicity testing. However, even if these issues could be resolved, the use of experimental animals in toxicity testing would remain an absolute requirement. Even with additional funds to search for alternatives to animal experimentation, it is not plausible to believe that their use could be abandoned in even the medium term. However, this should not deter from the continued refinement of toxicological test, the adoption of well validated *in vitro* tests and the design of more relevant and humane long term studies. Also, the current approach has allowed the development of a large panoply of safe drugs that have cured or improved the quality of life of countless individuals.

Chapter three - *Extrapolation: animals to humans*

Situation – The Issue

3.1 The use of experimental animals to assess the efficacy and toxicity of drugs for their relevance to the human population is predicated on the assumption that experimental animals can be used to predict, in either qualitative or quantitative terms, the likely response of humans. Experimental animals have been used for over 2,000 years to establish basic physiological processes, such as blood circulation or the appearance of various organs in the body, and comparisons between the organs seen in experimental animals with those in humans have given substance to the belief that they can be used, in some manner, to reflect the physiology of the humans.

3.2 The discovery of anaesthetics in the mid-19th Century enabled experimenters to carry out more studies on experimental animals and encourage the use of experimental animals in predicting the response of humans to the administration to pharmacologically relevant substances. Since that time there has been an enormous increase in knowledge, not only of the physiology, pathology and biochemistry of experimental animals, but in the molecular biology and genomic control of a whole range of animal organisms. It is now generally accepted by the scientific community that experimental animals are essential for investigating the complex and integrative biological systems of whole animals and is vital in developing therapies for human disease.

3.3 In toxicity testing, animal models are routinely used, although these have not always proved successful at predicting toxicities in humans. There are several reasons for this, some of which are described later in the sections dealing with idiosyncratic toxicities. Furthermore, the selection of appropriate experimental animals to investigate the pharmacology or toxicology of a drug is vital if the extrapolation to humans is to be made more precise and relevant. However, despite the failure of experimental animals to predict the response in humans in a small number of incidences, there is no doubting their utility in avoiding adverse drug reactions in the human population.

3.4 At present, the range of experimental animals used to investigate the toxicity of drugs has become relatively standard. This is in part due to an acceptance that the current used experimental animals have a record of predicting a significant proportion of those toxicities that will occur in humans, but also true that there is a considerable element of custom and practice which has meant there has been a bias in the use of mice and rats, dogs and on occasions primates, for the study of their extrapolation of drug toxicity to humans. However, the concentration on a small number of animal species has the benefit of using species for which an enormous amount of background data is known on their physiology and biochemistry. This knowledge, taken with our understanding of the physiology and biochemistry of humans, allows a more rational assessment of whether the effects seen in animals can occur in humans, and if they do, to what extent. Certainly when experimental animals are chosen, consideration is usually given to basic parameters, such as pharmacokinetics, pharmacological responses, and a proper understanding of ADME considerations.

3.5 Over the last decade or so, there has been a growth in the use of transgenic animals for the investigation of the pharmacology and toxicity of drugs. The use of transgenic animals is still in its growth phase, and although there are literally thousands of transgenic animals available, there is still a paucity of animal models that, in the evaluation of the toxicity of a drug, can take account of the influence of disease status in the response of the animal.

3.6 Studies on the mechanism of toxicity offer the best probability of arriving at a sensible risk assessment on the effect of drug on humans although mechanistic studies are time-consuming and expensive and do not always

guarantee success. Consequently, they are usually reserved for those occasions when there is evidence that toxicity occurs both in humans and experimental animals.

3.7 Other factors have also played a part in the selection of the experimental animal species for study. Clearly regulatory acceptability is important, and operational realities such as the ease of handling, the size of animal, cost of animal housing, are still important. In more recent times the use of sentient species has limited the use of non-human primates, although the societal pressures which impinge on the use of experimental animals is clearly dependent on geography and time.

3.8 A feature of experimental animals that has to be considered is their inherent susceptibility to toxicity. Experience has shown that there are considerable differences in the susceptibility of experimental animals to a given drug and that the toxic effects, especially in cases of chronic toxicity can show marked differences. Although pharmacokinetics and metabolism have been shown to account for the differences between species of experimental animal, there are also differences in response to a given insult, so that the pathology that is seen may differ between species. For example, some animals are much more susceptible to pulmonary fibrosis than others, so an insult to the lung may cause an acute inflammation in the alveolar region in one species and result in a chronic alveolar fibrosis in another. Differences in inherent susceptibility are difficult to predict, although with our increasing understanding of gene expression, it is more likely that the relevance of the effect of a drug in experimental animals will be more easily understood in terms of the effect on humans.

3.9 Another feature, which complicates the interpretation of data generated in experimental animals, is the operational necessity, driven by regulatory requirements, to expose experimental animals to doses up to a maximum tolerated dose (MTD). The concept of using an MTD derives from the idea that to determine the effect of a low dose of a drug on a large population, it is necessary to use a small population of experimental animals and expose them to very high doses. However, the use of an MTD can reveal toxic responses, which will never be seen with human population, if for no other reason that the effects of the drug will be dose limiting in humans, whereas in experimental animals it is sometimes possible to increase the doses to levels never possible in humans. This is especially the case in chronic toxicity where carcinogenic or reproductive endpoints are derived by lifetime exposure to very high doses in experimental animals often bearing no resemblance to the doses seen in humans. Of course, these studies reveal the potential hazard of the drug, but they do not necessarily show whether that hazard will be seen in humans unless the experimental animal is a particularly good model for human toxicity. Conversely, because of greater metabolic activity in rodents (compared to humans) there are occasions when it is not possible to expose rodents to concentrations of a drug given to humans.

Challenges and Opportunities

3.10 There are two types of opportunities that arise from the problem of extrapolating experimental animal data to humans. The first is concerned with the scientific basis for selection and the second is related to the use of information and knowledge about the effects of drugs in experimental animal.

3.11 Scientific basis for the extrapolation of experimental animal data to humans. Because several of these opportunities will be described in more detail in later sections, a brief bulleted point summary of these opportunities will be given.

- Improve the understanding of the pharmacology and mechanism of action and mechanism of toxicity. This is most appropriate when dealing with a new therapeutic class of drugs.
- Improve the use of comparative pharmacokinetics.
- Use the most relevant animal species in terms of ADME profile to humans.

- Compare the systemic exposure between experimental animals and humans and not on an applied dose basis.
- Use the knowledge of bioavailability and other pharmacokinetic differences between species to select dosing regimens in test animals of greater relevance to the clinical situation.
- Use the MTD as a top doses of default, which should be challenged and replaced by dose selection by pharmacodynamic, pharmacokinetic or other scientific, or other credible, basis.
- Consider clinical relevance of toxic effects resulting from metabolites unique to test animals, i.e. as part of an investigation on the mechanism of toxicity.
- Use knowledge of receptor pharmacology to improve the prediction of the effects of experimental animals to humans, i.e. part of mechanism of toxicity.
- Use of experimental animals modified to reflect the human disease under study.

3.12 Information and knowledge management

- Better training in hazard and risk assessment and an appreciation of risk benefit. This is clearly indication and drug specific, and requires experience and sound judgement.
- Improve the use of information and knowledge that are currently available from the safety assessment programmes. This would be greatly helped if there were a Centre of Excellence in pharmaceutical science, which could provide a generalised service to industrial and academic scientists on class specific toxicities and idiosyncratic reactions, for which experience has identified sensible methods of investigation.

Proposals and recommendations

3.13 Again several recommendations in this section will be covered elsewhere. These will be given in brief.

- A selection of genetically modified animals which have been demonstrated to better reflect the responsiveness of a class of drugs in experimental animals to humans.
- Develop facilities and create opportunities for training toxicologists. At present there are opportunities within specific Centres for Excellence for specialist depth, but there is no major academic organisation in the UK with a comprehensive knowledge of pharmaceutical science. To establish such a Centre would encourage the development of skills necessary, both to contribute to the pharmaceutical industry and also to provide the resource for the regulatory functions which have to monitor and licence drugs.
- Create a repository for information and knowledge on the use of experimental animals to assess toxicity. If this was part of a Centre of pharmaceutical science it could cover not only toxicity, but the relationship of pharmacology in experimental animals and humans. This Centre should encourage the development of databases on the concordance of animal and human toxicities effect to the response of drugs with the objective to improve the selection of animal models and enhance the development of new models. The ultimate benefit would be an improvement of human health risk assessment.

Value Added Gains

3.14 The gains from the establishment of a Pharmaceutical Research Centre would be to facilitate the development of the science of drug, design, pharmacology and toxicology, which could serve both the Pharmaceutical Industry and those charged with the licence of drugs. The gain would be a more effective use of experimental animals and an improvement in the quality of data from non-clinical toxicology in terms of its relevance to the human population. This will enhance the eventual development of the drug through its clinical trial phase.

Measurement of Success

- Scientific and regulatory acceptance and novel animal models.

- Reduce number of medicines that fail due to unanticipated toxicity between the first into humans stage and late phase clinical development.
- Reduce number of marketed medicines that are restricted in use or have to be withdrawn due to unanticipated human toxicity.
- Growing professional and public confidence in the safety of new and existing medicines.

Chapter four - *Improving animal models of human toxic response*

Situation – The issue

4.1 The design of non-clinical safety studies has not changed significantly over the last 30 years. In general the evaluation of drug toxicity is established by administering the drug by the appropriate route to both rodents and non-rodents. Although there has been refinement of the toxicological endpoints evaluated in experimental animals, the purpose of the study is essentially to test the null hypothesis (Greaves et al). However, the use of young, healthy animals does not always reflect the potential of the drug to cause toxicity in humans who are likely to be at different ages and health statuses (Boelsterli). Typically two species are used to evaluate the toxicity of the drug to increase the sensitivity of the system (Olsen et al, Tomaszewski).

4.2 There can be considerable diversity in the response of experimental animals to the toxicity associated with a particular drug. Furthermore, even if the toxicity seen in experimental animals can and does occur in humans, there is not always concordance between the effects seen in animals with that in humans. As stated earlier in this report, the proper use of non-clinical toxicology has to be integrated with all the known effects of the drug in order to establish the probability that the toxicity seen in animals will occur in humans at relevant pharmacological doses. A more certain, but time-consuming approach, is the use of mechanism of toxicity studies. These are usually undertaken to establish the precise cause of a particular lesion or effect in the experimental animals. Experience has taught that with an understanding of the mechanism of toxicity, together with an appreciation of the physiology and biochemistry of humans, it is usually possible to establish whether the effect seen in animals can occur in the human population, and if it does, whether the human will be more or less susceptible to the effect seen in experimental animals.

4.3 Because of the differences between experimental animals and humans which can exacerbate or ameliorate the toxicity of the drug, expanded databases are needed to define those specific toxicities which are likely, or unlikely to be, detected using experimental animals. It would be helpful if these databases could be centralised and made publicly available to those interested in studying the toxicity of drugs.

4.4 Recently a HESI/ILSI survey of animal to human extrapolation demonstrated a good (71%), but incomplete concordance of toxicities seen in experimental animals with those identified in clinical trials. There were various reasons for the incomplete concordance between the effects of animals and humans. In part, this may be due to the difference in the dose levels used in non-clinical toxicological studies, versus those seen in clinical trials. More importantly, is that some of the toxicity seen in clinical trials were difficult, if not impossible, to detect in experimental animals, (e.g. Headaches, nausea, tiredness etc (Olson et al)). It appears that about half of the non-clinical toxicities which are detected, are directly, or indirectly related to the pharmacological target of the drug. It is important in non-clinical toxicology to use, where possible, a species which expresses the same drug target as seen in humans.

4.5 Rodent models often show differences in absorption, distribution, metabolism and excretion (ADME) profiles (Henderson and Wolf). For example, the BN rat genome has now been published (rat genome consortium) and has shown significant differences from mouse when compared to humans. Genetically modified rats will soon become available which should increase the likelihood of developing pre-clinical rodent models, which will more accurately reflect the response of humans.

4.6 Although the discovery process in most pharmaceutical companies involve scientists

directly investigating the toxicity of a drug, the development of transgenic and genetic models of human disease provide an opportunity for the toxicologists to improve the effectiveness of their studies. Models have been developed to investigate relevant target organ pathology as well as disease pharmacology, for example, in models of diabetes or arthritis. However, with this approach care must be taken to ensure that the response of the experimental animals to the human disease under investigation, offers sufficient similarity, not only in the mode of action of the drug, but also in the response of the animal.

4.7 For regulatory testing requirements, it is usual to use the rat as a primary rodent species for investigation and the dog as a primary species for non-rodent studies. At present there is no routine use of transgenic models, except for some use in the evaluation of carcinogenicity. These models have been developed in the mouse. Initially, it was hoped that the development of transgenic mouse models for carcinogenic testing would obviate the need to carry out a long term study in this species, but so far, it has tended to be used, not so much as an alternative to the life-time mouse study, but as an additional study.

4.8 As stated before, the investigation of mechanisms in toxicity is an increasingly important aspect of the pre-clinical toxicologists approach to assuring the safety of a drug in humans. However, this approach tends to be as a response to an event which is seen either in experimental animals or in clinical trials and demands rapid progress if there is not to be a delay in the licensing of the NCE. As stated above, transgenic mice reflecting the disease or a particular bias of the metabolic predisposition are often used. These have tended to contribute to the hypothesis testing, for example human SNPs. Transgenic mice are available (Henderson, Wolf) which allow more effective ADME studies to be carried out, but also mechanism of toxicity studies help to establish whether the key targets associated with toxicity are directly, or indirectly, related to the pharmacological effect of the drug. Importantly, in a well-controlled mechanistic study, the mediators of toxicity can often be isolated and the relevance of the human situation established. However, this approach requires individuals with a broad knowledge of toxicological processes and the ability to integrate data from molecular biology through to pathophysiology *in vivo*.

Challenges and opportunities – solutions

4.9 In the near term, the limited introduction of knockout and knock-in mouse models that resemble more closely human ADME characteristics will continue. However, there is some reluctance to the use these mice as a routine toxicology species, because of dosing and blood sampling limitations. Also, because of IP issues associated with knockout and knock-in mouse models, costs become a secondary barrier. Nevertheless, there should be a broader use of transgenic animal models and mechanistic toxicology studies with targeted humanised responses. Knowledge of knockout mice pheno-types will assist mechanistic work (Zambrowicz).

4.10 In the longer term development of transgenic rats and possibly other species to compliment mouse model, will become more routine (Mirkes et al). There will be a wider use of reporter models with phenotypic activation of the reporter gene as an indicator of transcriptional gene activation following toxicity (e.g. oxidative stress).

4.11 With the development of animal (usually rodents) with human ADME genes it should be possible to develop a strain for use in routine toxicity screening tests. This should include reproductive toxicity studies conventionally performed in the rat and rabbit. Wider use of transgenic animals in mechanistic research should include the prediction of idiosyncratic responses related to CYP polymorphism.

Proposal and recommendations

- Generate rodent (preferably rat) models with human-like ADME characteristics for screening of NCEs.

- Through industry-academia-regulatory collaboration, validate the use of alternative toxicity models with reference standard toxicants (including chemicals with known species-specific toxicity profiles) and place results in the public domain.
- Gain regulatory acceptance of use of non-standard models for screening and mechanistic toxicology studies and promote use by pharmaceutical companies and chemical industries as a low-risk alternative.
- Identify and create of custom human target expressors in animal models using transgenics, (conditional) knockouts of animal homologue, RNAi and neutralising antibodies for the expression of humanised pharmacological target-related toxicology.
- In collaboration with regulators and industry, develop paradigms for more rapidly progressing to human clinical ADME, PD, toleration and efficacy studies on more targeted non-clinical evaluations, with less reliance on standard testing based on current ICH guidelines.

Value added gains

4.12 Recent advances in understanding of the human and animal genome and together with developments in transgenic methodology allow the possibility of:

- More predictive animal models for screening with ADME characteristics representative of humans for screening and a potential for increased concordance between non-clinical and clinical safety profiles of pharmaceuticals.
- Enable safety evaluation paradigms of pharmaceuticals to move from a standard approach to those that are individually tailored to the pharmacological class of the NME and target patient population.
- Mechanism based minimally invasive toxicity biomarkers identified for human studies.

Measures of success

- Pharmaceutical companies' management and regulatory acceptance of genetically modified animal models in safety assessment screens and mechanistic studies.
- Wider availability of screening mouse and rat strains with "humanised" ADME at an economic price without IP constraints.
- For mechanistic studies, exploit use of customised animal models "for cause" from transgenic / (conditional) knockout animal bank(s) to match human target, disease state and mechanistic pathways. Demonstrate success in use of such models in safety decision making and to gain regulatory and scientific approval.
- Persuade animal welfare interest groups of value (3Rs) of using more appropriate animal models for safety assessment.

Chapter five - *Discovery toxicology*

Situation – the issue

5.1 Approximately 50% of human clinical toxicities that are observed during drug development are target-related effects of pharmacology. A similar proportion of pre-clinical toxicities are considered to be "on-target" effects. The remainder of the toxicities in animals and humans are "off-target" effects related to the chemical structure of the new molecular entity (NME) and/or its metabolites. Toxicity (non-clinical plus clinical) now exceeds lack of efficacy and poor ADME characteristics as a major cause of compound attrition during drug development.

5.2 With the explosion of novel targets, biomedical scientists often lack basic information of the target's biological role, its regulation of expression, tissue distribution and primary contribution to human disease (as opposed to altered expression due to host responses). However, target-related toxicity may be anticipated from an understanding of animal knock-out, anti-sense and other models or from human genetic disorders causing loss of function. This may be called 'discovery toxicology'.

5.3 This concept can lead to the acquisition of an early understanding of on- and off-target effects, and can help to ensure that better quality molecules process into candidate selection. The challenges and opportunities of this approach will now be discussed in terms of both target and chemistry-related activities:

Challenges and opportunities – solutions

Target related:

5.4 Target related toxicities of an unacceptable nature can be evaluated in target validation (TV) using:

- Knockouts and transgenics (mouse and rat) (humanised models may need animal homologue to be knocked out; embryolethal genes will need crelox or other conditional knockouts)
- Neutralising antibody (for cell surface receptors)
- Anti-sense or RNAi *in vivo* (subject to effective delivery)
- Reference compounds (if available but intellectual property issues may prevent access)

5.5 Using this approach, the potential for toxicity can be evaluated early in target validation prior to expensive chemical lead generation investment. The later identification of such toxicities can jeopardise an entire project that is focussed on a given target.

5.6 Target distribution studies can also point to likely target organs and species risk factors. Human polymorphisms of the drug target that may modify toxicity and idiosyncratic responses need to be assessed.

Chemistry Related:

5.7 If a greater chemical diversity was built into candidate drug series then that would allow safe substitutes in a timely manner. At present, project chemists may focus on only one chemical series early in lead optimisation. However, most pharmaceutical companies have significantly expanded their compound libraries and the diversity of structures contained within will increase both efficacy, but also toxicity hits. Identification of the toxicophore region of NMEs or their metabolites and their molecular target(s) can help in the redesign of molecules to reduce toxicity. At present SAR studies can be too narrow in terms of the chemistry explored. A better understanding of toxicities related to the chemistry of NMEs would benefit from improved informatics for Quantitative Structure Activity Relationships (QSAR) and larger

databases. Ideally these would include safety pharmacology and receptor binding data.

5.8 In addition, metabolic profiling could be improved and CYP humanised models *in vitro* and *in vivo* need to be used to model the patient exposure to the correct metabolites as well as parent drug.

5.9 Significant toxicity is seen with chemical perturbations of a finite number of biological systems e.g. DNA, mitochondria, ion channels, redox systems, proliferation/apoptosis signals, membrane effects, transcription/translation inhibition. New tools may enable these to be modelled in high throughput screens.

Proposals and recommendations

5.10 For target related toxicology, early target evaluation could include:

- Study of knockouts in mice and now rats, administration of antibody neutralising cell surface targets, effects of RNAi and/or antisense administration, generation of toxicity phenotypes indicative of target-related toxicity.
- Target tissue distribution studies in all tissues in humans and toxicology species could alert toxicologists to potential target organs and help choose appropriate animal models for toxicology.

5.11 For chemistry related toxicity, front load *in silico* and *in vivo* assays for:

- DNA and genotoxicity (including DNA adducts, use of repair deficient models).
- Mitochondrial respiration to cover TCA cycle, electron transport, cytochrome C-apoptosis.
- Ion channel actions of physiological importance, e.g. in cardiac conduction, neuronal activity, renal function.
- Oxidant damage from redox cycling, glutathione depletion etc.
- Cell membrane damage e.g. haemolysis.
- Inhibition of cell proliferation and induction of apoptosis.
- Inhibition of transcription and translation.

Value added gains

- Improved target validation for toxicological liabilities will allow target-related. Toxicities to be addresses early in lead identification (or earlier if reference compounds, knockouts etc available)
- For chemistry related toxicity, front loading of high throughput screens can be used to select structural series where toxicity can be avoided by project without loosing target opportunity

Measures of success

- Less attrition from target identification through to lead optimization and beyond.

Chapter Six - *The 'omics technologies*

6.1 This section addresses the issues, opportunities and barriers associated with the application of evolving 'omics technologies (genomics, transcriptomics, proteomics and metabonomics) to the pre-clinical safety evaluation of new medicines.

Situation – The issues

6.2 The development of 'omics technologies has provided exciting new opportunities to enhance the way new drugs are evaluated for safety in a pre-clinical setting. These new technologies can be defined as follows:

- Genomics (transcriptomics, gene expression profiling) – the large-scale measurement of changes in gene expression
- Proteomics – the measurement of changes in protein expression level, localization, activity or post-translational modification
- Metabonomics – the measurement of changes in metabolite levels in tissues and biological fluids

6.3 Collectively, these technologies can be used to measure holistically drug-induced alterations in individual transcripts, proteins, metabolites or biological pathways and, therefore, can be used alongside more conventional approaches to define rapidly mechanisms of toxicity and potential relevance to man. Furthermore, the 'omics technologies are well-suited to the identification of biomarkers of toxicity. The identification of changes in transcript, protein or metabolite levels that precede the onset of toxicity in test species may lead to the development of short-term assays for endpoints that are currently long-term. Ultimately, this will reduce the reliance on endpoint assays, refine animal testing procedures and will accelerate the identification of potential adverse effects. Similarly, these expression changes may also provide the basis for the establishment of novel *in vitro* assays for toxicity that are rapid and require smaller quantities of test compound, thereby allowing toxicity to be addressed earlier in the drug discovery process, when test compound is limiting, and reducing the rate of attrition at later stages (also see section 4).

6.4 An exciting application of these novel 'omics technologies is the identification of biomarkers that can bridge between pre-clinical species and man. For example, it is hoped that transcript, protein and metabolite biomarkers that precede significant toxicity in test species may be used equally in clinical studies to identify early indications of adverse effects in patients. Protein and metabolite markers in plasma and other biological fluids are of particular significance in this context.

Challenges and opportunities

6.5 While the 'omics technologies have enormous potential to enhance the safety assessment of new medicines, it must be recognised that the widespread use of these tools is in its infancy, and many challenges must be met before their full potential can be realised. Prominent among these challenges is understanding the relationships between changes in the levels of transcripts, proteins and metabolites and conventional toxicology parameters (e.g., histopathology and clinical pathology). These relationships can only be defined empirically, through the conduct of experiments that incorporate sufficient compound doses, time points and appropriate controls. These so called "deep" studies will allow the identification of transcripts/proteins/ metabolites that play a direct role in mediating toxicity, and will allow them to be discriminated from, for example, those changes that reflect an adaptive response to a test compound. Unfortunately, the high expense often associated with the use of 'omics technologies has largely inhibited the execution of "deep" studies, with most published studies employing limited doses and time points.

6.6 Another significant issue that remains to be addressed is the inherent variability that exists

in the data generated by 'omics tools. This is especially relevant for genomics (transcriptomics) and reflects the rapid development of microarray-based technologies. The ongoing ILSI-HESI committee on the Application of Genomics to Mechanism-Based Risk Assessment" has addressed this variability through a series of cross-laboratory experiments and has identified a number of sources through which data variability can arise, including differences in gene annotation, sample preparation and data analysis (see http://hesi.ilsi.org). Efforts to identify and eradicate (or control) data variability must continue, if these technologies are to reach their full potential.

6.7 Among the most promising applications of genomics in the prediction of the potential for a compound to elicit toxicity relies on a technique known as "expression fingerprinting". Central to this technique is the assumption that toxicants that operate through similar mechanisms will induce comparable changes in the expression of transcripts/proteins/metabolites in tissues or cultured cells. Hence, the overall pattern (or "fingerprint") of change induced by a test compound can be used to diagnose the potential for, and mode of, toxicity (Afshari et al., 1999; Orphanides, 2003). The use of patterns of change, rather than single well-defined biomarkers, presents additional challenges: (1) the relationship between the mechanism of toxicity and the transcripts/proteins/metabolites that comprise the pattern may not be apparent and (2) the identification of diagnostic patterns often requires sophisticated computational algorithms that are not widely available.

6.8 The holistic nature of the 'omics tools can result in the generation of extremely large volumes of data. For example, a single genomic (transcript profiling) experiment can generate in excess of 10 million data points. The requirement to store these data in a format that facilitates efficient retrieval and analysis has driven the development of databases tailored to the storage of 'omic data (e.g., the HESI-EBI Toxicogenomics database (http://hesi.ilsi.org) and the NIEHS Chemical Effects on Biological Systems database (http://cebs.niehs.nih.gov); also see Section 6). It is important that UK scientists engage fully in the continued development and population of 'omics databases. This will require the training and development of interdisciplinary, UK-based scientists with skills in toxicology and computational biology.

6.9 Many of the drawbacks of utilising 'omics technologies in the toxicology setting relate to the vast outlay required in terms of capital expense and requisite expertise. Unsurprisingly therefore, for economic reasons, the onus of development of most of the technologies related to toxicology in the UK, lies with industry and a few select institutions. This in itself is means that in the UK at least, there is only a very limited pool of scientists with appropriate expertise.

6.10 A major hurdle to overcome is the general scepticism amongst the wider scientific community regarding the introduction of 'omic tools in toxicology, coupled with a general resistance to change. This is due, in part, to the perception that 'omic tools have yet to make a significant impact in pre-clinical toxicology, as well as from the high expectations associated with these new approaches. It should be stressed however that, for the time being, 'omic tools will be complementary (and not an alternative to) current approaches. Within the pharmaceutical industry, there is concern that some 'omic data cannot currently be interpreted unambiguously and this, coupled with the high expense associated with these tools, has inhibited the widespread application of the 'omics to the safety assessment of new drugs.

Proposals and recommendations

6.11 In order to facilitate the widespread application of 'omics technologies, the funding of "deep" experimental studies, incorporating sufficient compound doses and time points such as to allow the links between 'omics data and conventional endpoints to be established, should be encouraged. This may involve interdisciplinary and cross sector collaboration. These studies must be of sufficient complexity (dose response, multiple time-points) and must be supported by consistent and comprehensive

conventional toxicological assessments (histopathology, clinical pathology). An important aspect of such an undertaking is access to the appropriate drugs/compounds, including those associated with adverse effects and compounds with favourable safety profiles. These studies would also be used to help define inter- and intra-platform/laboratory variability, and how it can be managed effectively.

6.12 The major output from such a study would be the creation of an open, interactive and transparent scientific environment in the UK for the assessment, development and application of the 'omic technologies in pre-clinical safety evaluation. Considerable effort should go into improving methods for individual protein and metabolite identification (publicly available databases), as well as comprehensive gene annotation (especially of non-human pre-clinical species), so as to encourage the computational analysis of 'omics data by biochemical pathway and/or biological network, rather than solely by individual component changes. Although most of the necessary analytical chemistry skills have been developed, they are not always readily available to toxicology initiatives. Allied to this would be the development of computational tools for the analysis and storage of 'omics data couple with the training of appropriately-skilled scientists.

6.13 Ultimately, the UK has to invest in its toxicologists, who need training in 'new' sciences to be able to make best use of these technologies. This may be addressed through professional accreditation training programs to ensure widespread appreciation of such platforms and their use in the development of safer medicines. A UK Centre of Excellence could fulfil the role of conducting the studies described above and the training of toxicologists in the application and interpretation of 'omics technologies.

Value added gains

- Fewer candidate molecules would fail during later stage development, thereby minimising the potential for clinical toxicity.
- Improved confidence of discovery scientists in the use of 'omics tools to predict and define the potential for adverse effects.
- Improved understanding of toxicological mechanisms will lead to enhanced interpretation of conventional *in vivo* toxicological data.

Measures of Success

- Reduction in attrition rate due to late stage toxicology in relation to smarter attrition at the pipeline stage (i.e. Pre-first time into human).
- Metrics on the number projects that have benefited from the application of 'omics technologies.

Chapter Seven - *Exploiting genome-based information: knowledge management*

Situation – the issues

7.1 With the sequencing of the entire genomes of humans and those of the majority of model species used in toxicology (i.e., mouse, rat, dog and primate), drug safety assessment is beginning to benefit from the fruits of the post-genomic era of medicine. New 'omics technologies – such as transcriptomics, proteomics and metabonomics – allow the effects of drugs on biological systems to be assessed rapidly and holistically, providing novel opportunities to identify and characterise the potential for adverse effects earlier in the development of a drug. However, the full exploitation of these new tools will require the concurrent development of computational resources capable of housing the resulting data in a manner that will allow it to be interrogated efficiently, and that will permit relationships between animal toxicology data and clinical drug safety data to be ascertained.

7.2 Information generated by genomic sequencing, together with the rapid development of technologies for detecting and quantifying mRNA, proteins and metabolites (termed transcriptomics, proteomics and metabonomics, respectively), are expected to contribute significantly to the development of safer medicines. However, these new tools generate unprecedented volumes of complex, multivariate data: for example, a typical transcriptomics experiment generates over a million data points. There is a need to store these data in a manner that will allow them to be interrogated in the context of established toxicology and drug safety data. The most appropriate solution is he construction and maintenance of a flexible, publicly accessible database (or series of databases) that can be drawn upon by industry, academia and the regulatory agencies. Presently, there is limited activity in this area: the CEBS (Chemical Effects on Biological Systems) database developed by the US NIEHS (National Institute for Environmental and Health Sciences; http://cebs.niehs.nih.gov) and the toxicogenomics database developed by EBI (European Bioinformatics Institute) in collaboration with ILSI-HESI (http://hesi.ilsi.org) are two examples of databases designed to manage data derived from these new technologies. No UK-based initiatives exist.

Challenges and Opportunities – The Solutions

7.3 The long-term objective must be to establish a comprehensive database of toxicological and drug safety information. In addition to the appropriate storage and management of data derived from 'omics technologies, there is also an urgent need to collate and organise the wealth of data that exists on the toxicological and safety properties of drugs. Therefore, the database should also be capable of housing the following types of data:

- Histopathology data and other "conventional" toxicology measurements
- Clinical pathology measurements
- Chemical QSAR data
- Information on strain-specific differences in response in test species
- Established adverse drug reactions and their mechanistic basis
- Reference maps for toxicologically relevant molecular pathways and processes
- Single Nucleotide Polymorphism (SNP) database (human polymorphisms and their functional consequences, including potential effects in adverse drug reactions)

7.4 The emphasis of the database must be not only on data storage, but more importantly, on the housing of data in a form that will allow relationships to be established between data of different types. In this respect, the database may

be used as the starting point for hypothesis-based research and information-based decision making. For instance, a researcher may begin by interrogating the database for genes whose expression is altered by exposure to a given drug and may then wish to identify the molecular pathways these genes operate within, the genetic polymorphisms that exist in the human population, the functional consequences of these polymorphisms, and whether these polymorphisms are associated with established adverse drug reactions. The database query system must, therefore, be flexible, such that a researcher can enter the database by searching for any of the data types listed above.

7.5 Construction and maintenance of the database will require substantial effort, which should not be underestimated. Significant funding and a dedicated resource of computational biologists will be required. Efforts should begin with a survey of existing and planned databases. It may be feasible, for example, to integrate a UK database effort with existing European or US activities. It will also be important to establish at the outset the potential value of a database of this kind across the industry.

7.8 A number of potential obstacles to the successful implementation of the database exist. Among these are:

- The subjective nature of some of the data (e.g., the assessment of histopathology should follow standardised criteria and be peer-reviewed)
- The proprietary nature of some of the information – companies may be unwilling to deposit and make available data on proprietary compounds in the fear that data may be misinterpreted or intellectual property may be compromised. Consequently companies may decide not to participate in the database

Proposals and recommendations

7.9 To establish a comprehensive database of toxicological and drug safety information. (see chapter 3) under the auspices of a UK Centre of Excellence in Safety Assessment.

Near term proposal:

- An information gathering exercise:
  - Determine interest in a database project among industry, academia and the regulatory authorities
  - Commission a cross-sector survey in how toxicological information – including 'omics data – is currently managed (e.g., which "safe harbour" databases are used) and the level of satisfaction with current systems.

Longer-term proposal:

- Based on the outcome of the information gathering exercise above, decide on whether to go ahead on UK/European database programme and the form it should take.

Value added gains

- The database would represent a "one-stop shop" for all 'omics data and would include information on drug toxicology and safety.
- The construction and implementation of the database would foster collaboration between pharmaceutical companies

Measures of success

- A large number of pharmaceutical companies committing to the construction of the database
- A significant number of datasets being submitted – including those relating to proprietary compounds
- Significant use of the database (number of website "hits") by industry, academia and the regulators
- Use of 'omics data and the database in weight-of-evidence drug submissions

Chapter eight - *Adverse drug reactions and idiosyncratic toxicology*

Situation – the issues

8.1 It could be argued that the topics of Adverse Drug Reactions (ADRs)/Idiosyncratic Toxicology and Pharmacogenetics (PGx) do not sit easily under the auspices of Pre-Clinical Toxicology, since historically the focus of these has been exclusively clinical. Also historically, there has been limited liaison between the disciplines of pre-clinical and clinical drug safety. However, the development and implementation of new technologies for genetic and genomic analysis are beginning to impact on both these areas, and therefore offer the opportunity for closer interaction.

8.2 This section addresses the issues, opportunities and barriers associated with the application of these evolving technologies to drug safety issues in the pre-clinical setting.

8.3 A distinction should be made first of all between idiosyncratic toxicity and adverse drug reactions. The former occur very rarely, and characteristically involve some kind of inflammatory or hypersensitivity-type reaction. "Non-idiosyncratic" ADRs tend to be more frequent, are not generally characterized by hyperimmune-type reactions, and can be manifest in a number of ways although hepatic effects are commonplace. These continue to be a significant problem, both to the healthcare system where there are thousands of hospital admissions per year, and to the pharmaceutical industry where the application and therefore usefulness of many drugs is limited by drug-related toxicity In fact, a 2004 study conducted in Liverpool concluded that 6% of all hospital admissions were related to ADRs, and the annual burden for the NHS is £466 million annually. Non-idiosyncratic ADRs are generally of unknown aetiology/mechanism and cannot be predicted. They then tend to become evident as a class effect of a drug and attempts are made to go back to experimental animals to find a predictive model. Once this has been established, where possible, the response in humans is no longer an unpredictable ADR. The answer may not always be in the genotyping, but the focus of this section will be to address that aspect as one of the most promising avenues.

8.4 Whilst our understanding of the basis of many ADRs is limited, it is clear that most if not all have a significant genetic component that provides a rationale for improving our knowledge of the mechanisms underlying ADRs. Indeed, there is significant steer from regulatory authorities for drug sponsors to conduct research in this area. Although up to now the pre-clinical safety community has been largely focused on animal toxicity issues in early drug discovery, it is perceived as desirable that human toxicity issues should be addressed early in the "pipeline" as well. The concept of "frontloading" toxicity testing into early drug discovery is being realized largely as a consequence of the advent of new genetic/genomic technologies, and since these approaches are able to cross species boundaries, in principle at least it is feasible to consider both animal and human safety early in the lifetime of a drug discovery programme.

8.5 Being able to do this effectively will have far-reaching benefits, not only in terms of patient health, but also for the public perception of the pharmaceutical industry as being able to delivery safer therapies. Another obvious benefit is the huge financial savings from a reduction in the number of drug withdrawals and late-stage compound attrition, and from litigation associated with ADRs.

8.6 Notwithstanding these important potential benefits, it is important to manage expectations about what can be achieved and in what time scale. There are issues outside the technical and scientific that require resolution before significant progress can be made.

Challenges and Opportunities

8.7 Once a predictive animal model and thus mode of action has been ascribed to an ADR, then the genetics can be established to allow the population to be screened for susceptible individuals. Idiosyncratic reactions and ADRs occur in only a small proportion of the individuals receiving a particular drug, which immediately raises the problem of acquiring sufficient numbers of cases to analyse, since pharmacogenetic experiments need to have sufficient "power" to unequivocally ascribe a particular effect to a particular genotype – thus, a large number of "normal" unaffected individuals are also required. Therefore, a co-ordinated effort requiring a large number of participating centres is the best approach. One such effort is the Eudragene collaboration, funded by the European Union, which is aimed at establishing a case-control DNA collection for studying the genetics of ADRs (see below).

8.8 As with any investigation of this type ethical issues need to be considered, particularly now that there is heightened public concern around the use of human material for experimentation. Unfortunately, the application of more rigorous ethical standards has made pharmacogenetic studies more difficult to design and carry out. Patient participation is always optional, and consent can be withdrawn at any stage resulting in the destruction of the samples. A move to a more flexible approach is desirable, for example in exploratory (i.e. non-diagnostic) work the samples could be anonymised or coded, so that findings would not be reported back to the individual. Whether or not this would encourage participation remains to be assessed.

8.9 Another key consideration is the exploitation of new information on the genetic basis of ADRs. There are logistic obstacles to taking new mechanistic information and making it available for patient screening at the hospital bedside or in the GP's surgery. For example, the anticoagulant warfarin has a narrow therapeutic range and ADRs are relatively common, notwithstanding the known role of the polymorphic drug metabolising enzyme CYP2C9 in warfarin metabolism. To encourage pre-clinical science input into ADR mechanism determination, further examples of the clinical application of such information need to be "showcased".

Proposals and recommendations

8.10 The collaboration/co-operation between pre-clinical and clinical safety is presently not very extensive. To improve this situation, the impetus for change needs to come from both areas.

8.11 Hitherto, the pre-clinical initiative of front-loading safety assessment into early drug discovery programmes has been with a view to reducing project attrition due to unanticipated animal toxicity. One of the tools that are assisting this process is gene profiling using animal tissues, mainly rat, and the creation of gene expression-based predictive models for toxicity. Such predictive models have been developed by commercial organisations, although their utility is still being evaluated. As some of these predictive models are *in vitro*-based, e.g. using primary hepatocytes, it would be feasible to perform parallel experiments using human hepatocytes to determine the extent of overlap of toxicity marker genes between the two species. In this way toxicity biomarkers could also be identified, although gene expression biomarkers are of limited use in the clinic unless they can be detected in blood cells. Non-invasive biomarkers from the urine or plasma would be more useful in this regard, and metabonomics and proteomics could be key technologies for their identification.

8.12 The use of genomics also has the potential for identifying which cellular pathways are associated with toxic effects, so from a pharmacogenetic perspective could be useful for identifying candidate genes for polymorphism analysis. Initial studies could be performed with drugs that have a "clean" pre-clinical profile but which are associated with liver ADRs in certain patients, for example the anti-diabetic drugs.

8.13 Prior to candidate drug nomination, information is usually available on the

metabolic profile and route of metabolism of compounds from studies using human hepatocytes and microsomes. Indeed, this information is available for most currently marketed drugs (as with the example of warfarin already quoted), but this does not impact at present on whether a particular medicine is appropriate to prescribe for a particular individual. Of course, this is because "polymorphism profiles" of the major drug metabolising enzymes (P450's, GSTs, etc.) for patients are not available. Indeed, the associations between genetic polymorphisms and ADRs are at present established retrospectively.

8.14 Where a drug is metabolised by a particular enzyme that is known to be highly polymorphic, it is anticipated that there will be large inter-individual differences in exposure based on the recipient's genotype. In such cases, it would be desirable to be able to screen for patients with a "slow metabolism" phenotype that may predispose them to ADRs, particularly where drug interactions due to polypharmacy are significant. This would require a framework for the collection of personal genetic data, and the exploitation of such data towards the personalised medicine goal. There are significant issues around patient reluctance and compliance here, as well as ethical issues, which will need addressing before significant progress can be made. A separate challenge is to be able to perform pharmacogenetic screens at the "point of use".

8.15 Already there are known a large number of ADRs associated with marketed drugs, and the recently established Eudragene collaboration seeks to establish underlying genetic associations with a subset of these. Six ADR types have been prioritised for initial focus, based on the fact that these types cause serious easily diagnosed effects not related to the disease for which the drug was prescribed. These are:

1. Myopathy caused by cholesterol-lowering drugs (statins)
2. Agranulocytosis caused by thyroid drugs and sulphasalazine
3. Tendinitis caused by fluoroquinolone antibiotics
4. QT prolongation caused by a number of classes of agents, including anti-arrhythmics and anti-psychotics
5. Liver injury caused by anti-diabetic drugs
6. Neuropsychiatric effects caused by mefloquine anti-malarials

8.16 There are 15 participating centres in 11 countries, including the UK. At least 500 cases of each ADR will collected together with an equal number from healthy volunteers, with all DNAs available to participants. Progress with this needs to be monitored carefully by both the pre-clinical and clinical safety communities, to establish whether there is any learning that can be applied earlier in the drug discovery/development process, for example whether any of the polymorphisms identified are related to other drug classes, or have been associated with any toxicities identified pre-clinically.

8.17 In spite of the potential for improving our knowledge of ADRs through genetic and genomic approaches, pharmaceutical companies show a general reluctance to apply these methods early when ADRs are observed following initial human exposure. A change of mind-set is required to bring to bear both pre-clinical and clinical safety expertise, in drafting plans for sample acquisition and mechanistic investigation.

Value added gains

- Reduction in the numbers of ADRs would significantly reduce the burden on the health care system; while at the same time would boost public confidence in the pharmaceutical industry's ability to deliver safe as well as effective medicines.

Measures of success

- Reduction in the numbers of ADR patients seen by GPs and/or admitted to hospital – should be straightforward to monitor this.

- Reduction in the numbers of marketed drugs withdrawn due to adverse events not predicted from pre-clinical toxicity testing. Similarly, a reduction in the number of times a drug programme fails due to toxicity at the "first dose to humans" stage, following a clean pre-clinical safety profile, is a useful indicator that the relationship between pre-clinical and clinical safety is improving.

Chapter nine - *Technical, regulatory and commercial constraints*

Situation – the issue

9.1 In this chapter we have attempted to cover a number of technical, regulatory and commercial issues, which restrain the effective use of experimental animals for the pre-clinical evaluation of drugs. Although these issues are often independent of each other they combine to create a culture which means that the most scientifically rationale and perhaps meaningful approach to the pre-clinical evaluation of drugs is not always undertaken. The trend is sometimes to substitute the most scientifically robust approach for the conventions (ICH standardisation) which set specific hurdles for the registration and development of the drug, which, although not optimised, are highly predictable and encompass well understood commercial risks. This predictability of the process provides comfort to the industry since even if it is not scientifically optimal it provides a degree of certainty for what must be achieved.

9.2 As stated previously, the paradigm which drives much of pre-clinical toxicity testing is the exposure to high doses of experimental animals for short durations in order to represent the exposure of humans to low doses over long periods of time. To some extent this approach has been based on tradition, established in major countries involved in the licensing of drugs. The rationale is not without merit. High doses given to experimental animals attempts to compensate for the fact that usually relatively small number of animals are used in comparison to the number of humans who will be exposed to the drug. Also, this paradigm provides the toxicologist with an understanding of the potential adverse effects of the drug at extremely high dose levels; although the development of toxicity at extremely high dose levels often bears no relationship to the toxicities or adverse effects that may be seen with much lower doses over long periods of time. In some cases misleading conclusions result.

9.3 The use of a maximum tolerated dose (MTD) may cause toxicities resulting from alteration of normal physiology and biochemistry, not indicative of the toxic effect relevant to human risk at much lower dose levels. The development of toxicities irrelevant to man can lead to further investigations, often costly, in terms of the use of experimental animals and/or opportunity cost.

9.4 The factors, which contribute to the difficulty in the interpretation of experimental data include:

- At high dose levels the kinetics may be non-linear.
- Metabolites that are only formed at high dose levels in animals, may cause toxic effects which would not occur at low doses.
- The detoxification mechanisms may be over-whelmed with high dose levels, which would not occur at lower doses.
- Bioavailability of the compound may be different at higher dose levels than low dose levels, especially if physiological, biochemical or pathological effects occur at high doses, which do not occur at low.
- There is at best, patchy, scientific and regulatory consensus on the use of top dose levels in toxicity studies, other than the MTD (or maternal toxicity in the case of reproductive toxicity studies).

Challenges and opportunities – solutions

9.5 The real challenge is to develop animal testing paradigms that reduce the number of false positive results, but retain our ability to identify adverse effects which will occur in humans exposed to drugs in clinical practice. This can be achieved, at least in part, by insisting on a scientific justification for the selection of dose levels, without relying on the use of the MTD, except as a default position. It is important that

the pharmacodynamic response in test species should be identified and characterised. This in turn, relies on good pharmacokinetic data and an understanding of the likelihood that saturation kinetics will not occur in relevant metabolic pathways. It means that the doses of drugs given to experimental animals pre-clinically have to be reviewed in the light of the results from clinical trials. In other words, pre-clinical toxicology may have to be supplemented by toxicity studies initiated following human exposure.

Proposals and recommendations

9.6 Only through a greater understanding of the mechanisms of disease to be treated can the most relevant animal models be identified and used. It is vital to use the emerging technologies of genomics, proteomics and metabonomics to establish a greater understanding of the patho-biochemistry of the disease to be treated. On the basis of this information, the advantages or shortcomings of particular animal models can then be more accurately assessed. Nevertheless, it seems improbable that new animal models of disease will rapidly emerge that will mimic the situation in man. It is therefore important that the mechanism of toxicity, as well as the mechanism of disease, is also understood in the experimental animals, so that this knowledge can be used to make rational judgements as to whether the mechanism of toxicity seen in experimental animals is likely to occur in the diseased patient. The reliance on data from experimental animals also needs to be interpreted on the basis of an understanding of the physiology and biochemistry of humans. Even so, the differences between experimental animals and man cannot always be predicted.

Value added gains

9.7 The gains of introducing a more scientifically based and pre and post-clinical toxicity studies will mean that some irrelevant toxicities will not detract from a consideration of human risk. The reliance on more mechanistic data, both in terms of the process of disease in the animal model, or the mechanism of toxicity where these are undetected, will lead to a more solid and meaningful interpretation of the data and increase the probability that the toxicities that are relevant to man will be assessed in clinical trials. Finally, the requirement for a more mechanistic approach to the evaluation of toxicity will necessitate the training and development of toxicologists who are focussed on an understanding of toxic mechanisms. This will improve the quality of thinking and outcome of pre-clinical toxicity studies and will in turn help to ensure that the UK has well trained experts in mechanisms of toxicity.

Measures of success

9.8 Ultimately, the outcome that really matters is facilitating the marketing of pharmaceuticals that are both effective and do not generate adverse drug reactions. Only over the long term will the trend of this achievement become obvious. Also, and importantly for the development of the Pharmaceutical industry in the UK will be the acceptance that the quality of science in toxicology is comparable with the natural sciences and that this in turn will encourage the best and brightest scientists to view toxicology as a rewarding and important area to invest their time and energy.

Chapter ten - *Regulatory and commercial constraints*

Situation – the issues

10.1 There are a number of organisational issues which cause regulatory and commercial aspects of drug licensing to reduce effective pre-clinical drug development. From a UK perspective the move to internationally harmonised guidelines limits the influence of UK interested parties in the development of new guidelines. In short, there is a dilution of the influence of the UK Committee in Safety of Medicines, which hitherto had been generally recognised as one of the most scientifically based and effective Committees associated with drug licensing.

10.2 The development of a global consensus approach to guidelines has significant advantages in ensuring that industry understands the hurdles that have to be overcome. However, with the consolidation of a consensus approach under the aegis of the EU, for example, new alternative testing methods require international validation and regulatory acceptance. This is often time consuming and can become bureaucratic and diverts attention from the essence of the scientific evidence most relevant to a comprehensive assessment of the drug although this has the merit of ensuring that regulatory guidelines change slowly and reflect well established and validated scientific understanding, the requirement for consensus can, and does, add to the delay in the development of new guidelines.

10.3 It is obvious the development of new tests takes a considerable length of time and that the advances need to be valid, reproducible and there provides concordance of the results between animals and humans. Furthermore, pro-active mechanisms of toxicity studies are time consuming and unpopular since the pharmaceutical industry needs to obtain licences as quickly as possible in order to recoup their research and development investment through sales under patent cover. The industry does invest in mechanistic studies reactively, provided the financials support this investment, and the time element is not too prolonged. Furthermore, there is a concern that with the development of new tests, they often do not substitute for old tests, but become additive to what is already done. For example, it is not yet clear whether the investment, costed at approximately $32M by the pharmaceutical industry, in the evaluation of alternative methods for detecting carcinogens, using transgenic mice has proved successful (ILSI/HESI reference). The cost of this exercise alone indicates why there is reluctance to invest in new tests, since the outcome is often neutral, or worse, additive to what is already required for the regulatory process.

10.4 It has to be acknowledged that the current approach to pre clinical testing is an amalgam of the results of best practice over many years, the absence of alternative *in vivo* or *in vitro* tests and an increasing awareness of the need to reduce the number of animals and the range of species used in research laboratories. Furthermore, to compensate for the small number of animals used, high doses of the test drug are administered in the hope this will identify potential hazards that result when large numbers of humans are exposed to low levels of drug. It has proved difficult to move from this paradigm despite the knowledge that the pharmacokinetics of drugs can be non-linear over wide dose ranges and metabolites formed at high doses might not be relevant to low dose treatment. Detoxification, bioavailability and lack of concordance of effects between experimental animals and man all argue for changes in approach identified in previous chapters.

Challenges and opportunities

10.5 The challenge is to change the present culture of thinking about regulatory toxicity and replace it with the concept of science-driven toxicology. This will require greater dialog between pharmaceutical companies and regulatory authorities to ensure that the decisions on how a drug should be tested meets the stringencies of

the regulatory authorities, but affords the opportunity for more flexibility and open scientific evaluation of drugs. This case-by-case approach will only work if there is an acceptance by both regulators and industry project manager that the interpretation of the data has to reflect best scientific practice and that no study in experimental animals can predict with certainty the outcome when a drug is given to humans. In short, industry and the regulators have to work together to develop appropriate guideline studies that are tailored to an individual drug or drug class.

10.6 To facilitate this, there needs to be a Centre of Excellence in Pharmaceutical Sciences, which can provide academic input based on an in depth understanding of the development of drugs and the likely testing paradigms. This would improve the opportunities for training in toxicology and a continuing education of existing toxicologists. It would provide a FORUM for discussion, debate, argument and hopefully agreement, between regulators and industrial toxicologists on innovative ways to improve pre-clinical toxicology. Ultimately, it would develop a larger pool for recruitment of high quality scientists for both industry and regulatory agencies.

Value added gains

- Non-clinical test programmes would be designed on a scientific rational on a case-by-case process, rather than a strict adherence to guideline studies.
- Reduction in high dose toxicity findings in animals that are irrelevant to humans.
- Improved interpretation of existing animal data by integrating assessment with chemical data.
- The establishment of a Centre of Excellence in Pharmaceutical Science would lead to the recruitment training and continuing education of high quality toxicologists to meet the needs of industry and regulatory authorities. It would also improve the dialog between industry and the regulatory authorities, since there would be a common understanding of the approach to pre-clinical toxicity testing and hopefully expedite the drug development process.

Measures of success

10.7 The measure of success that really matters is a rapid development of effective drugs that do not cause significant adverse drug reactions, or at least being able to identify and exclude individual at risk patients where benefits are great. The likelihood that this will happen will be aided if there is effective dialog between industry, academia and regulatory authorities on the development and acceptance of better and novel animal models. However, there is desire to identify the relevant toxicities as early as possible and that the risk assessment for humans will be supported by high quality science, often underpinned by an understanding of the mechanism of toxicity and its relevance to the disease state in question. Finally, it is important that the UK is seen as a role model for how effective pre-clinical drug development can be achieved. This is vital if the licensing authorities in other parts of the world are to be persuaded to accept this approach.

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