The Academy of Medical Sciences | FORUM

Safer Medicines Report

Risk-benefit assessment working group report

November 2005

Contents

Summary

Considering the number of safe and efficacious medicines approved during the last 50 years it is reassuring that the number withdrawn because of unacceptable side effects is, by comparison, very small. The reported rate of marketed drug withdrawals has been fairly stable (1) or has declined slightly over the past two decades, down from 3.5% to 1.2% of slightly more than 500 new drugs approved in the USA between 1979 and 1998 (2,3). Given dramatic advances in medical knowledge over the last decade, and the diversity of new drugs currently under development, it is timely to review current practices in the light of these new developments.

This report deals only in broad outline with certain aspects of drug safety and the complex process that is risk assessment. Aspects of risk assessment pertaining to reproductive function, embryo-fetal development, post-natal development and the special needs of children as they relate to drug safety are beyond the scope of this high level review.

In considering the remit of the project "Safer Medicines – new approaches to ensuring the safety of medicines" sponsored by the Academy of Medical Sciences' Forum, the Risk-Benefit Assessment Working Group has identified a number of initiatives that should be undertaken, with government agencies, academia and the pharmaceutical industry working in partnership, to further ensure the safety of future medicines. These initiatives are summarized under each of the recommendations for action that follow, and are further detailed within the text of this report. In brief these initiatives include:

- adoption of "One Medicine" as an operational principle, wherein all disciplines cooperate fully to support an unbroken line of scientific enquiry from discovery through to delivery of a new medicine to patients
- actions to increase public awareness that no medicine is without risk
- steps to improve process and communication at the interface between industry, academia and regulatory scientists
- commitment to an hypothesis-driven, evidence-based approach to drug safety evaluation and risk assessment
- better mechanisms and methods of communicating risk to patients and doctors, and to receive feedback, to include briefings by well informed educators familiar with the drug and the disease
- revisions to existing regulatory guidelines, as indicated by new knowledge, to ensure continuing utility of testing requirements and to encourage development and deployment of modern methods
- harnessing the potential of new technologies and establishing priorities for work to validate new biomarkers for drug and patient safety, and risk assessment
- consultation to establish new paradigms for data analysis including deployment of expert systems in knowledge management, data mining, and systems biology
- expanding the knowledge base to develop and refine predictive physiologically-based, pharmacokinetic and pharmacodynamic modeling and simulation tools to increase precision in risk assessment
- collaborative efforts between industry, academia and regulatory scientists to investigate the mechanistic and constitutive basis of species-specific toxicity or atypical manifestations of toxicity in animals; determine their potential utility as unique tools to investigate certain forms of human toxicity
- consistent standards for characterizing drug safety and risk with transparency of methods and logic applied to risk assessment; publication of outputs to share best practice
- establishing the infrastructure for a National Centre for Safety Assessment to provide focus and leadership to the foregoing initiatives

Among the greatest threats to successfully delivering these initiatives are the challenges relating to: a) managing a burgeoning volume of data generated by large scale screening technologies b) setting the right priorities for translating new information into reliable knowledge c) converting new knowledge into usable methods d) maintaining public confidence that investment in new methods will deliver safer medicines e) maintaining trust and confidence at the

interface between industry and regulatory agencies while managing these uncertainties, and f) evolving a new paradigm for drug safety evaluation and risk assessment wherein active pursuit of knowledge about risk is the new benchmark for establishing the safety of new medicines.

These challenges clearly call for a sustained investment in pharmaceutical R&D and academic research in all the relevant disciplines across the 'One Medicine' spectrum. They also demand a sustained level of inward investment to provide a stream of qualified people with the requisite knowledge and skills needed to maintain the tradition of excellence in UK science and innovation in pharmaceutical R&D.

With intense public attention focused on drug safety at the present time, moving these initiatives forward will progressively enhance the scientific underpinning of drug safety evaluation and risk assessment. The development of better methods and tools with which to characterize, manage and avoid risk of drug adverse effects is key to providing patients with a steady stream of treatments for hitherto difficult and intractable diseases.

Recommendations

Numerous organizations and institutions have the capacity, knowledge and expertise, as well as the administrative infrastructure, required to assess the merits of the recommendations listed below. The membership of professional societies in the UK and Europe, representing the disciplines of Pharmacology, Toxicology, Pathology and various branches of Medicine, along with the respective Colleges, Industrial Associations and the Academy of Medical Sciences can provide valuable impetus to the work required to facilitate implementation of those initiatives deemed worthy of further investment.

Recommendation 1: UK Health and regulatory authorities, in partnership with the UK based pharmaceutical industry, should undertake a variety of initiatives to increase public awareness that some element of risk is endemic to taking a medicine.

- There is a need to review current systems and mechanisms for disseminating information about the safety, risks and benefits of newly approved drugs to patients, health care professionals and the public at large. Mechanisms for receiving patient feedback are needed to gauge the level of tolerance to risk that different patient groups are willing to accept set against the benefits of their medicine. Regulators and industry need to find mechanisms to address net patient benefit-risk such that they are not inappropriately penalized by a litigious system.
- The public must be informed that lifestyle and dietary habits are important in managing risk, and avoiding adverse effects when taking any medicine.
- Approval to market a new drug should be contingent on establishing a panel of educators to provide ongoing educational briefings and updates to patients and health care professionals, these to be administered within the framework of existing Continuous Professional Development programmes.

Recommendation 2: UK and EU Health and regulatory authorities should publish a 'Memorandum of Understanding' endorsing flexibility and innovation in the process of drug safety and risk assessment.

- There is a need to foster a culture of trust, dialogue and transparency between scientists in industry and regulatory agencies. Early engagement in discussion of key issues leading to creation of a risk management plan for a new drug candidate entering development would provide a vehicle to facilitate scientific exchange.
- A Risk Management Plan should be an integral part of all interim regulatory submissions serving as a flexible blueprint throughout the course of developing a new drug candidate with the objective of identifying and predicting potential safety issues on a case-by-case basis. Such investigative work should be hypothesis-driven aiming to prioritise potential risk factors in a manner relevant to the disease target, the drug's mechanism of action, the disease indication, special sub-populations at risk, and overall consideration of risk in relation to potential benefits.
- Actively exploring the boundaries of risk is a vital part of confirming safety, and this requires latitude within the existing framework of regulatory guidelines to employ novel study designs and non-routine testing paradigms in both pre-clinical and clinical phases of drug safety assessment. This also calls for inter-agency dialogue and unification of

administrative processes and communication networks in order to avoid duplication of effort and inconsistency in regulatory decision-making.

Recommendation 3: Representatives of the UK and EU Health and regulatory authorities and representatives of the pharmaceutical industry should jointly review the ICH guidelines with a view to ensuring economies of scale and relevance of current practices to human risk assessment based on accumulated knowledge and access to new technologies.

- While ICH guidelines have proven effective it is timely to consider their applicability within the context of recent advances in technology and investigative capabilities. The new paradigm in drug safety assessment should be hypothesis-driven with emphasis on predicting toxicity and exploring the potential for risk in humans.
- For example, the need for 2 species in general toxicology and carcinogenicity studies, the need for toxicology studies greater than 90 days in duration, and the requirements for pre-clinical studies prior to the first exposure of human volunteers participating in 'Experimental Medicine' studies should be re-assessed in the light of past human experience with all classes of medicine.
- There is a need to facilitate transference and validation of new biomarkers of importance to human risk assessment (so-called bridging biomarkers) from the research laboratory into the clinic. Consensus must be reached on appropriate testing and validation strategies including means of gaining access to archives of chemical structures and relevant data held by pharmaceutical companies without breaching intellectual property interests.
- The possibility of developing new methods to evaluate candidate drugs for toxic effects in lower order organisms should be investigated as knowledge of cellular pathways related to common pathogenic mechanisms are revealed by analysis of large scale gene and protein expression data.

Recommendation 4: Representatives of the UK and EU Health and regulatory authorities, the pharmaceutical industry and academia should convene in scientific session to address the utility of computational 'knowledge management' and advanced 'data mining' tools to detect signals of toxicity or adverse effect in pre-clinical and clinical data.

- There is a need for strategies to review outlier subjects in animal and human studies focusing in particular on temporal, quantitative and qualitative exposure-response relationships in each case for the presence of individual risk factors.
- Greater emphasis needs to be placed on relational analysis of conventional laboratory data for evidence of mutually reinforcing patterns of response that may point to incipient organ system toxicity.
- Resolution and integration of genomic and proteomic data with data derived from measures of conventional endpoints will require a 'systems biology' approach to elucidate complex cellular pathways related to defined modes of toxicity.

Recommendation 5: Representatives of the UK and EU Health and regulatory authorities, the pharmaceutical industry and academia should convene in scientific session to establish guiding principles for use in exposure assessment and dose-response analysis for human risk assessment. There is a need for greater consistency and quality of output.

- The product of this review should be the issuance of guidance notes on the essential principles that should be followed with specific reference to the logic supporting selection of dosimetry parameters, the methods and models employed for inter- and intra-species extrapolation of exposure, and the extent of uncertainty and the potential for error.
- A second output of the review should be publication of non-competitive or anonymised data in the form of case studies illustrating the best examples of robust exposure assessments and dose-response analyses with the objective of sharing best practices.

Recommendation 6: The evolution of *in silico* and *in vitro* methods for prediction of drug kinetics and drug-drug interactions *in vivo* and the incorporation of such data into sophisticated physiologically-based pharmacokinetic models should be encouraged both in virtual mode and for simulation of conditions relevant to patient and disease demographics.

- Standardisation of *in vitro* models for absorption, models of blood-brain barrier transfer under

defined pathological states, standardised screens for enzyme induction, standardization of methods for enzyme inhibition, and integration of *in vitro* ADME studies with cell-based systems for evaluation of toxicity are priorities for further development.

- Additional investment is needed to expand databases of disease prevalence, genetic variability, enzyme/transporter abundancies and interactions in order to refine predictive algorithms for use in real as well as virtual human populations. This facility would enable linkage of physiologically-based PK models to mechanistic pharmaco/toxico-dynamic (PD/TD) models in order to put PK variability into context for projections of efficacious as well as potentially toxic exposures.

Recommendation 7: Representatives of the UK and EU Health and regulatory authorities, the pharmaceutical industry and academia should convene in scientific session to establish guiding principles for risk characterization. There is a need for improvements in transparency, consistency and quality of output.

- The product of this review should be the issuance of guidance notes on the essential elements of a sound mechanism-based, weight-of-evidence approach to risk characterization, where risk is invariably viewed in relation to benefit.
- There is a need to develop consensus on the utility and validation of biomarkers of host response and novel biomarkers of exposure that would permit risk characterization in animals and humans within the confines of a given species, or sub-group of patients, or individual patients. This approach should be considered in the context of other related strategies to deliver personalized medicine.
- Risk-benefit summaries should be published after a drug is approved for marketing in order to share best practice and insights into the basis for regulatory decision-making.

Recommendation 8: Create an organizational matrix to function as a National Centre for Safety Assessment to promote an evidence-based approach to drug safety and risk assessment in the pre-clinical and clinical disciplines.

- A National Centre for Safety Assessment could fulfill several important functions:
  - oversee development and implementation of educational and training programmes
  - provide focus and leadership on issues relating to policy making
  - coordinate clinical and experimental investigations of adverse drug reactions and provide a vehicle for collaboration and sharing of samples with other centres in the US and Europe
  - review gaps in knowledge relating to the cause of idiosyncratic and other forms of unanticipated reactions to drugs in regular use, and institute appropriate actions
  - engage with other institutions internationally to design and populate databases and to establish accessible tissue, cell and serum banks

Recommendation 9: Establish an international programme of collaborative research between scientists in industry and academia, to be funded at least in part by industry, to investigate phenotypic, genotypic, and other constitutive differences that may account for manifestations of species-specific toxicity.

- The principal objectives would be to:
  - elucidate the basis for species differences in toxic response to certain classes of drugs perceived to pose a high risk for humans
  - characterize genetic polymorphisms and allelic variations in toxicology species that may be relevant to the pathogenesis of class-specific or target- specific toxicity
  - accumulate a shared repository of phenotypic and genotypic data from animals in toxicology studies exhibiting atypical responses to various classes of drugs; determine how this information might be used to develop more informative models of human risk
  - increase knowledge of species differences in organ pharmacology and physiology that would contribute to more accurate physiologically-based pharmacokinetic modeling
  - provide evidence to underpin the selection of animal species and/or animal models for

toxicity testing having greater relevance and predictive capacity for human risk assessment

Recommendation 10: Representatives of the major Health and regulatory authorities internationally, and the pharmaceutical industry, should convene to review existing ICH guidelines for assessing carcinogenic hazard with a view to adopting a modified testing strategy in line with contemporary knowledge. The Working Group proposes that in the short term:

- analysis of structure-activity relationships and a battery of genotoxicity tests would eliminate overtly genotoxic chemicals from consideration as new drug candidates, as currently practiced
- a bioassay in one rodent species, the rat, would be used to detect carcinogenic potential other than by direct genotoxicity
- in the event of a tumorigenic effect in the rodent bioassay additional mode of action studies would be conducted to ascertain the significance and relevance of such observations to human risk assessment
- further modifications of testing strategies could be introduced in the longer term in the light of experience and accumulated knowledge and the further evolution of new and advanced methods for assessing carcinogenic hazard

Recommendation 11: Representatives of the UK and EU Granting Authorities, Academic Authorities, Health Authorities, and the pharmaceutical industry should convene to review the training of basic scientists and physicians, both at the undergraduate and postgraduate level.

- there is a need to ensure that the re-supply of scientists and physicians in the future is consistent with meeting the challenges and objectives implicit in recommendations 1 through 10
- appropriate steps should be taken to ensure that medical curricula include courses with a specific focus on drug safety, risk assessment and risk communication

Chapter One - *Introduction*

1.1 The sum of activities required to deliver a new medicine can be likened to those preceding the launch of a manned space mission. Both are complex enterprises imbued with some uncertainty. Progress is conditional on the potential for gain outweighing the risk. Despite all efforts to manage risk rare missions do end in failure, mostly for reasons that are difficult to predict. As with manned space missions so too with a new medicine: complete safety cannot be assured at all times.

1.2 Eight hundred million dollars, eight to ten years, and hundreds of thousands of pages of documentation sum up the cost, time, and effort to demonstrate that a new drug is safe and efficacious for it to gain approval by international Regulatory Agencies (4,5).

1.3 Throughout this long and costly process the incidence, duration and severity of all adverse events are assiduously recorded. After approval is given to market a drug adverse events are recorded by Pharmaceutical Companies and Health Authorities and other agencies around the world. Pharmaceutical companies are obligated by law to provide safety updates on a regular schedule.

1.4 Despite this intensive scrutiny, a small proportion of new medicines, at varying times after approval as marketed products, have been linked with serious adverse reactions causing them to be withdrawn (1,2,3). The overall incidence of serious adverse reactions is difficult to estimate with complete accuracy but one review concludes that between one in thirty and one in sixty physician consultations result from adverse drug reactions (6). The frequency of adverse drug reactions may range from 1 in 1,000 to 1 in 40,000 patients for a particular drug (2,7,8,9). In rare cases adverse reactions may cause death while others are transient and easily remedied. On balance, the record suggests that most of the time, in the majority of cases, existing processes and procedures have delivered efficacious and safe medicines to patients. Whilst it is appropriate to ask whether we can do more to avoid even these rare instances of adverse reactions, it is also important to consider whether the scrutiny of drug candidates has now become so stringent that even potentially safe and beneficial drugs are failing to reach the marketplace, to the ultimate detriment of patients.

1.5 In this report we review certain of the activities connected with the discovery and development of a new drug in order to give perspective and context to the process of assessing risk in relation to benefit. It is clear that successful delivery of safe medicines in the twenty first century depends more than ever before on the exercise of original thinking, innovative application of science and technology, interdisciplinary collaboration and seamless communication between all participants in the enterprise. There is a need to build trust between industry and regulatory agencies such that the safety of each new drug may be addressed on its scientific merits, case-by-case. There is a need to build consensus across Regulatory Agencies on issues of highest priority so that regulatory decisions affecting drugs in development and those on the market are based on objective evidence. Equally, when failure occurs, there must be encouragement and latitude to carry out a full scientific investigation, without punitive consequences, so that by sharing knowledge similar events may be averted in future.

1.6 Finally, the report stresses the importance of using this evidence base as the platform from which to communicate relevant information about a drug's safety to the public and health care professionals.

Chapter two - *Drug safety: perceptions, awareness and expectations of risk*

2.1 Patients elect to take medicines for their ailments on the advice of their physician; many expect their medicines always to be safe and effective. Such patients are not attuned to the possibility that a medicine prescribed by their physician will cause them harm.

2.2 Drug failures related to safety or efficacy result in widespread and generally negative publicity. This erodes public confidence and trust in the system. Yet, given the millions of prescriptions dispensed each day, adverse reactions to drug treatment are relatively rare and the benefits to human health and society at large are generally well appreciated (10,11).

Challenges and opportunities

2.3 It is unlikely that all risk associated with taking a medicine will ever be eliminated completely. Whilst every effort is taken to minimize harm, some degree of risk is endemic to any therapeutic intervention. There will always be an element of uncertainty in determining the precise level of risk. Balancing risk against benefit is individual to each patient.

2.4 It is important that patients recognise the individual nature of their treatment and the need to report any untoward effects. The present means of communicating this kind of information to patients is inadequate. Patients should be made aware that they all may not experience the same benefit of treatment, and that the greatest benefit and the greatest risk may not be the same for each person; in other words the risk-benefit ratio may be different for the individual compared to the average of all patients in general.

2.5 Patients should be encouraged by their physician to seek up-to-date information about the safety of their medicines and to anticipate that this information may change over the course of time. This would enable patients to participate fully in discussions of possible risk and the hoped-for benefits offered by their treatment.

2.6 Accurate feedback from patients would be of immense value in informing regulators and health care professionals whether they feel the benefit of treatment justifies the risk. This information would help to ensure that regulatory decisions were consistent with the needs of patients.

2.7 Patients should be informed that most adverse reactions to drugs arise from taking the wrong dose, or the right dose at the wrong time, or taking mixtures of medicines at the same time. Patients are largely unaware that alcohol, diet and smoking all add to the risk of their suffering side effects or failing to get the full benefits of treatment. Compliance can be a major determinant of therapeutic outcome. However, the impact of poor compliance varies with the drug. Patients need to be better informed about the importance of compliance for their specific treatment.

2.8 When a new medicine claiming improved efficacy and safety is introduced on to the market physicians and healthcare professionals, particularly those in general medical practice, have little time to assimilate the details contained in the large volume of scientific and medical evidence supporting such claims.

2.9 Pharmaceutical companies, scientists in regulatory agencies, members of the medical community, and the public at large each have a different view of the nature of risk. What is an acceptable level of risk to one group is often unacceptable to another. Failure to achieve consensus on the acceptable level of risk to patients can end development of a promising new drug, or lead to withdrawal of a useful drug from the market.

Recommendations

2.10 UK Health and regulatory authorities, in partnership with the UK based pharmaceutical industry, should undertake a variety of

initiatives to increase public awareness that some element of risk is endemic to taking a medicine.

2.11 Action plans should be developed to achieve the following objectives:

- to increase public awareness and understanding that drug safety is based on a balanced appraisal of risk and benefit, and that patients play a key role in ensuring safe use of medicines,
- to establish formal mechanisms with which to solicit and record patient feedback in order to gauge their level of tolerance to risk. Patient feedback should be subjected to periodic and independent review after approval to market a new drug is granted,
- to conduct a comprehensive review of existing systems and methods of communication used to disseminate information about the safety, risks and benefits of newly approved drugs to physicians, patients, and the public at large, and,
- to establish a mechanism by which a panel of educators is appointed to brief health care professionals and patients about the risks and the benefits of newly approved drugs. Approval to market a new drug should be contingent on establishing such a panel. Educational briefings should be administered within the existing framework of "Continuous Professional Development" programmes.

Chapter three - *Risk assessment and risk management: general principles*

3.1 Risk assessment is a process of gathering evidence to determine the potential of a new medicine to cause undesirable effects in patients under certain conditions of exposure and use.

3.2 The output of a risk assessment defines those conditions that may pose a hazard, either related to the dose, the disease, or an attribute of the patient receiving treatment. In this case regulatory policy seeks to manage risk through labeling a drug with appropriate precautions.

3.3 Safety assessment is principally concerned with setting limits of exposure consistent with the avoidance of harm. In this case regulatory policy adopts the precautionary position that seeks to eliminate risk. Such differences in regulatory policy are believed by some to account for differences in the rates of drug approval by health authorities in various regions of the world (7). There are concerns that such inconsistencies will limit innovation and curtail the development of much needed new medicines without manifest improvement in drug safety.

3.4 The process of risk analysis involves collaboration across several disciplines and agencies. For ease of description it may be broken down into the following constituent activities:

- *hazard identification* – the qualitative and quantitative assessment of adverse effects caused by a new chemical entity using a range of test systems e.g. humans, animals or cells in tissue culture,
- *hazard characterization* – the exploration of mechanisms underlying, or related to, the development of adverse effects in a test system; establishing the relationship between the incidence and prevalence of adverse effects caused by exposure to a range of doses/concentrations of the administered drug,
- *exposure assessment* – the characterization of the pattern of exposure, preferably internal exposure, to a drug with respect to duration, frequency, and intensity of peak and average concentrations in plasma and, where possible, in target tissues using modeling techniques as appropriate, and,
- *risk characterization* – the integration of information obtained in the course of 'hazard identification,' 'hazard characterization,' and 'exposure assessment' in order to estimate the probability of adverse effects occurring in humans under specific conditions of exposure and the identification of any potentially susceptible sub-groups,
- *risk communication* – the translation of all information relevant to risk assessment into clear messages acceptable to regulators and easily understood by patients and writers of prescriptions,
- *risk management* – encompasses all decisions based on the outcome of risk assessment. This may cause termination of a drug in development, limit the dose given to patients in clinical trials or restrict testing to certain patient categories. A marketed drug may be contraindicated in certain patients or may be withdrawn altogether.

Chapter four - *Risk management*

4.1 Risk management embodies all activities and decisions necessary to ensure drug and patient safety. The proceedings of an international workshop sponsored by the Centre for Medicines Research International in 2002 (12) considers the role of risk management strategies in drug development commenting on the strengths and weaknesses of current practices. The current risk management structure for the detection, assessment, and communication of risk is optimized to ensure the safety of new drugs at the time of approval and post licensing when the product is launched on the market (13). However, regulatory scientists recognize the need to formulate new approaches to risk management, particularly during the course of development, where there is a need to explore and predict the potential for risk in addition to compiling evidence of safety (13,14).

Challenges and Opportunities

4.2 The drug discovery and development process is carried out in accord with stringent ethical, legal and regulatory requirements. New drug candidates are subjected to comprehensive testing for efficacy and safety in animals and humans. These investigations are carried out according to standardized testing protocols that meet the regulatory requirements of, amongst others, the three major Health and regulatory authorities in the USA, Europe and Japan.

4.3 Consultation between industry and regulatory scientists to produce a risk management plan earlier in the discovery-development process would promote trust and confidence while, at the same time, supporting innovation in risk assessment. Such a collaborative approach would allow drug development to proceed in a less risk-averse environment and would encourage greater latitude to investigate the nature of adverse effects in a pro-active rather than in a reactive mode. This would introduce a new paradigm into drug development where observations confirming safety as well as investigations relevant to exploring the boundaries of risk would be encouraged. An effective risk management strategy should anticipate and even predict undesirable side effects while at the same time laying down contingencies for managing untoward events. This approach would ameliorate risk to patients in clinical trials but most importantly would allow full exploration of a drug's potential benefits in the disease under investigation.

Recommendations

4.4 UK and EU Health and regulatory authorities should publish a "Memorandum of Understanding" endorsing flexibility and innovation in the process of drug safety and risk assessment within the framework of existing guidelines. A central tenet should be the encouragement of open dialogue with scientists in pharmaceutical companies in the process of creating a Risk Management Plan for a new candidate drug as follows:

- risk management plans should be developed on a "case-by-case" basis in consultation with regulatory scientists. Investigative work should be "hypothesis-driven" consistent with the disease, the patient's needs, the drug's mode of action, its chemical structure, its metabolism and elimination, and the anticipated schedule of dosing,
- the plan should be regarded as a flexible blueprint allowing hypotheses to be tested in order of priority. The plan should describe actions to avoid risk and to manage adverse events should they occur. Plans should be modified in the light of emerging data from animal studies and clinical trials,
- risk management plans should be a requirement of all regulatory applications to progress development of a new drug and should be updated on a regular basis,
- risk management plans should be included in new drug applications such that they are the basis for designing further studies to evaluate

drug safety after approval to market is granted, and,

- regulatory and industry scientists should be encouraged to consult jointly with academic experts and others with relevant expertise to refine risk management plans and to resolve differences of opinion or concerns relating to the interpretation of new experimental data or safety issues arising.

Chapter five - *Risk assessment – the essential elements*

5.1 The objective of risk assessment is to define any conditions of exposure in humans that may result in adverse effects following administration of a drug. It is not the purpose to predict the precise incidence of adverse effects in a particular patient population or to estimate the level of exposure that will most frequently be associated with adverse effects. Although there are a number of largely uncontrolled variables that determine an individual's response to a drug, the one constant in medical practice is the dose administered to the patient.

5.2 In future, with a better understanding of the causes of variability in the response to drugs, it might be possible to assess risk to the individual patient. In an idealized situation this would enable the prescribing physician to adjust the dose or the schedule of dosing according to the patient's metabolic or genetic predispositions as well as the severity or sub-type of their disease (15,16). Thus, in the future, risk assessment and risk management will be an integral part of each treatment regimen with adjustments being made according to the needs of the individual patient.

5.3 The following sections present in broad outline the various activities that underpin the process of risk assessment. Aspects of risk assessment pertaining to reproductive function, embryo-fetal development, post-natal development and the special needs of children as they relate to drug safety are beyond the scope of this high level review. Certain of the principles apply to post-marketing surveillance but this review does not address the specialist aspects of research associated with pharmaco-epidemiology or pharmacovigilance.

Hazard identification

5.4 The primary objective is to characterize the nature and severity of adverse effects that may be elicited by a drug over a range of doses in animals or humans. International regulatory guidelines and articles of the Geneva Convention and the Declaration of Helsinki prohibit exposure of humans to a new drug candidate without prior assessment of risk. To date laboratory animals have been the surrogates of risk for humans who volunteer to participate in clinical trials of hitherto untested drugs. While the search for alternative test systems continues, and progress in certain areas has been possible, it does not appear that cell-based systems will replace all animal use for this purpose in the foreseeable future.

Challenges and opportunities

5.5 Cell culture cannot duplicate conditions in the whole animal that: i) regulate the supply of oxygen and nutrients, ii) facilitate the removal of waste products, iii) maintain blood pressure and flow to organs and tissues. All of these functions are highly integrated in conscious mammalian organisms and they govern the body's response to drugs and other chemicals in the environment.

5.6 However, cell-based systems may be adapted to investigate specific mechanisms of toxicity where these can be studied under the controlled conditions of cell culture. Such *in vitro* methods are used frequently to supplement studies in whole animals.

5.7 Drug development is regulated by guidelines recently agreed under the auspices of the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH). These guidelines have been in effect for slightly more than a decade and stipulate the studies required in animals and humans to secure approval to market a new drug. The time is right to re-examine the requirements detailed in these guidelines in the light of experience of drug safety testing and the availability of new testing methods over the last decade.

5.8 Large-scale screening technologies (genomics, proteomics, metabonomics) and other tools of

molecular biology are providing new insights into mechanisms of toxicity that are increasingly important in drug safety and risk assessment. It is widely acknowledged that harnessing the resolving power of these new technologies in a fashion that delivers valid biomarkers of drug efficacy, safety, and risk is a major challenge for the next decade. The universe of chemical structures, biological targets, evoked pathologies and the range of host responses is seemingly limitless (17).

5.9 Large government funded research agencies such as some branches of the EPA and the NIEHS in the US are heavily invested in these activities and they are engaged with scientists from pharmaceutical and other companies, academia and European agencies in multi-national collaborative projects (18). The challenge is to translate a burgeoning volume of information into usable diagnostic and predictive assays of utility in drug safety assessment. While changes in certain gene families are claimed to correlate with observations of pathology, few if any have yet been validated to a point where they can be used in risk assessment (19). It is also recognized that certain gene changes are prone to artifact arising from variations in experimental procedures (20). There is clearly a need to focus these efforts on the particular needs of drug safety assessment, and UK and EU based scientists and their colleagues in the respective regulatory agencies should be fully engaged in setting priorities for further work.

5.10 With knowledge of human and animal genome sequences now available comparison of coding regions and gene regulatory elements, together with appropriate functional studies, should help in the selection of animal species best adapted to express the biological and/or toxicological activity of a new drug candidate and, therefore, of greatest utility in determining risk to humans. This would extend from considerations of species similarities in metabolic pathways and detoxification mechanisms, as well as any species-specific susceptibility to chemical toxicity or immune mediated adverse effects.

5.11 There is a growing body of evidence suggesting that fewer genes and proteins than hitherto evaluated may be critically involved in regulating cell signalling pathways which control cell replication, programmed cell death and other key functions (21,22); similarly, it is conceivable that host responses to chemical or metabolic stress, reactive intermediates or external environmental agents, may be associated with pathognomonic changes in particular subsets of genes and proteins.

5.12 As better resolution of these critical cellular and molecular pathways is achieved it is possible to envisage that cell-based assays equipped with suitable response elements, target genes and reporter constructs as well as lower order organisms will provide facile and validated testing methods with which to predict specific hazards. For example, *Caenorhabditis elegans* (23), *saccharomyces cerevisiae* (22) or the zebra fish (24) may serve as useful test systems in the future in a manner similar to Salmonella and mouse lymphoma cells used currently for evaluation of genotoxicity (25).

5.13 The concept that key biological processes are regulated by the interaction of chemicals in a defined 'chemical space' with targets in a corresponding 'biological space' suggests that there may be a finite repertoire of chemical structures that elicit activity at specific molecular targets of toxicity. As mentioned above there are many ongoing efforts to validate genomic and proteomic biomarkers employing prototypic toxicants with a known range of toxic effects. This strategy may suffer the disadvantage of discovering markers only of predictable responses and outcomes. There is a need to access more diverse chemical structures and data related to their potency and specificity of action, as well as inactive congeners to serve as negative controls, to validate known and novel biological targets of relevance to toxic mechanisms evoked by pharmaceuticals. Such information will also be of value in developing computer-based quantitative structure-activity relationships for defined endpoints.

5.14 The discovery and development of new drug candidates utilizes all the tools of basic and applied research across the spectrum of disciplines engaged in the process. There is still more opportunity for further integration of

expertise and knowledge between scientific disciplines, still earlier in the discovery-development process, to generate hypotheses and pose questions in a systematic fashion that will increase our ability to forecast and manage risk of adverse effects in patients.

5.15 Regulatory guidelines developed under the auspices of the ICH are largely based on a checklist of tests that must be completed prior to registration of a new drug. While these guidelines provide a good regulatory framework it is important they be interpreted in a manner that will foster innovation and meet the needs of contemporary drug safety assessment programmes.

Recommendations

5.16 A new round of consultation between representatives of the pharmaceutical industry and members of the UK and EU Health Authorities and Regulatory Agencies should be instituted to review the existing ICH guidelines and bring them up to date. In the context of updating current practice the guidelines should:

- promote a "hypothesis-driven" approach to all aspects of drug safety assessment testing strategies on a "case-by case" basis
- endorse investigation of mechanisms of toxicity in animals and adverse events in humans; risk assessment should be evidence-based
- sanction suitable alternative study designs and approaches to data analysis in pre-clinical and clinical development

5.17 In the context of study requirements the consultation should re-examine the following:

- the requirements for pre-clinical testing to support first dose administration of drug to humans in Experimental Medicine studies
- the requirement for 2 species in general toxicology studies and carcinogenicity studies,
- the need for toxicology studies of greater than 90 days duration,
- the criteria for dose selection in general toxicology and carcinogenicity studies

5.18 Consensus should be sought on priorities and mechanisms for funding collaborative validation studies of molecular biomarkers of toxicity

5.19 Validation of molecular biomarkers related to known and novel pathways relevant to mechanisms of toxicity will require access to archives of diverse chemical structures. Active as well as inactive isomers and congeners will be required for adequately controlled experiments. Steps should be undertaken to establish the feasibility of obtaining access to molecules from the archives of pharmaceutical companies without breaching intellectual property interests. The Molecular Libraries initiative in the US provides access to public databases of chemical information with which to probe a diverse range of biological systems (21).

5.20 Opportunities should continue to be sought by UK and EU based scientists to initiate and sponsor basic and applied research with a view to developing alternative *in vitro* screening assays for mechanism-based toxicity related to target and non-target effects. While such assays may only play an adjunctive role in risk assessment within the next 5 years, they may prove to be sufficiently reliable as a means of eliminating less desirable molecules with higher toxic liabilities early in the development process.

Hazard characterization

5.21 Hazard characterization seeks to determine the mode or the mechanism of toxicity caused by a drug in a test system. Most investigations of toxicity carried out currently in drug safety assessment are in fact investigating the mode rather than the mechanism of toxicity, assessed by means of qualitative and quantitative surrogate markers which, by weight of evidence are consistent with a plausible mechanism.

5.22 Investigations of mechanisms of toxicity, on the other hand, aim to link specific elements in a pathway to the causative stimulus that evokes

cell damage and tissue pathology. The availability of new methods to correlate toxic changes with changes at the level of genes and proteins will enable a more precise determination of mechanisms of toxicity to be made in future.

Challenges and opportunities

5.23 The principal objective of hazard characterization is to determine if the toxicity in animals is a relevant risk to humans, or to ascertain if the adverse event seen in a small sub-set of patients presents a risk to all patients.

5.24 The decision to embark on an investigative programme to determine the importance of a potential hazard to long-term patient safety should be governed by consideration of the perceived level of risk in relation to benefit, as well as the level of unmet medical need, and the availability of alternative treatment options. Not all hazards identified in animals or a sub-set of patients in the course of drug development merit the same level of investment and commitment of resources.

5.25 Investigating the mode of action related to a particular toxicity may adopt a systems-based approach starting with the toxic phenotype in animals or patients with further investigations directed at specific questions and hypotheses. The tools for this purpose are rooted in the basic research laboratory, providing an almost unlimited capacity to deploy conventional and novel methods *in vitro* and *in vivo*.

5.26 A well-structured investigative programme aimed at gaining a better understanding of the mechanistic basis of a particular hazard should also aim to define biomarkers to signal impending risk, thus adding to the quality of risk assessment and the effectiveness of risk management strategies.

5.28 The concept of biomarkers is not new in animal or human medicine. Conventional laboratory values signaling changes in haematology or clinical chemistry parameters outside the normal range are biomarkers of toxicity and are key determinants of safety. There is a need to extract more measurements from these laboratory values by closer scrutiny of minor trends or deviations in individual patient data and relating these changes to dose, or exposure, or disease status. Sporadic, non-dose related, short-lasting deviations outside the normal range, or dose-related trends within the normal range of laboratory values are easily overlooked by statistical analysis of group means. These subtle trends or deviations may hold the key to predicting toxicity or adverse events that, in the past, have arisen unexpectedly either in later clinical trials or after marketing.

5.29 Although technically challenging, it is well recognised that collation and integration of disparate data sets at different levels (primary or derivative) may reveal mutually reinforcing patterns that point to a biological response that otherwise would not be evident by other methods of analysis (26). Integration of disparate data sets of relatively simple laboratory and clinical data such as body weight, red blood cell count, serum glucose, blood pressure, heart rate, etc may reveal evidence of a common pathogenesis related to incipient sub-clinical toxicity. The application to such data of methods developed in the fields of bioinformatics and data-mining may be of value here.

5.30 The repertoire of non-conventional biomarkers is growing. They are capable of signalling organ specific toxicity, for example segmental renal tubular damage (28), isoforms of glutathione-S-transferase (27), and KIM-1, generalized reaction to tissue injury such as fibrosis e.g. collagen type IV (29) or damage to vascular endothelium e.g. von Willebrand factor (30) or vascular smooth muscle e.g. caveolin (31,32). It is widely recognized that validation and qualification of biomarkers for use in risk assessment will be challenging from several vantage points: for example with respect to diagnostic sensitivity and specificity as well as relevance to the mode or mechanism of action underlying the pathogenesis and expression of a particular form of toxic injury (33).

Recommendations

5.31 More effective integration of disparate laboratory data sets from pre-clinical and clinical studies is needed in order to detect subtle signals that would otherwise be obscured by routine statistical analysis.

5.32 Complex analytical tools ranging from 'knowledge management' through to advanced 'data mining' techniques should be deployed to extract maximum value embedded in data derived from measurement of conventional and non-conventional biomarkers of toxicity.

5.33 Individual subjects (animal and human) with subtle trends or minor short lasting deviations in laboratory values should be investigated with reference to temporal, quantitative and qualitative assessment of exposure response relationships, as considered appropriate.

5.34 UK and EU based scientists should develop consensus on the requirements for validation of biomarkers. While the FDA has issued helpful comments (34) on the definitions of biomarkers from a technical and medical use perspective, the level of validation required to qualify a biomarker as a diagnostic aid in post marketing surveillance of efficacy and risk has not been confirmed in formal regulatory guidance notes.

5.35 To utilize and interpret all data effectively in the quest to unravel complex inter-connected cellular pathways operating at different levels will require interdisciplinary applications of computational models and a 'systems biology' approach to confirming the mode and locus of toxicity. Such models should ultimately acquire predictive capacity.

Exposure assessment and dose-response analysis

5.36 Exposure assessment and dose-response analysis relate incremental changes in relevant doses/concentrations of a drug (and/or its metabolites) to incremental changes in function, organization, or structural integrity at the site(s) of action. The interpretation of this relationship is key to risk assessment. The objective in human studies is to achieve a suitably wide margin between the maximum tolerated dose in Phase I volunteers and the projected range of doses required for efficacy in patients in Phase II and Phase III clinical trials.

5.37 For drugs that induce toxicity in animal studies the interval expressed as a ratio of "effect-dose" to "no-effect-dose" is termed the therapeutic index. There are variations in how this index may be calculated but the principle is the same in each case. With a relatively non-toxic drug it may be possible to establish a level of exposure that, without adverse effect, provides an adequate safety margin over exposure at the human therapeutic dose.

Challenges and opportunities

5.38 Dose-response analysis is a central plank of risk assessment for it defines the conditions of exposure under which an adverse effect is likely to occur. A long-standing issue of concern in risk assessment is the practice of extrapolating from high doses causing overt toxicity in animals to lower doses in the pharmacodynamic range, and the validity of conclusions derived from this extrapolation.

5.39 If extrapolation of risk is based exclusively on effects seen at excessively high doses there is a danger that the effect will not relate to the drug's primary mode of action or to relevant mechanisms of toxicity because of non-linear kinetics and saturation of metabolism. This shifts the mode of action to a region of the dose-response curve that bears no relevance to estimating human risk. In effect, the risk assessment is shifted from a "low-dose category" to a "high-dose category". In general the low end of the dose response curve should be regarded as more relevant to human risk assessment. Risk assessment must be based on plausible interpretation of the data otherwise opportunities to fully characterize the therapeutic potential of a new drug candidate may be denied because of unfounded safety concerns (35).

5.40 Prior to the establishment of pharmacokinetics as an integral part of toxicology studies estimation of a "safe" starting dose in humans was based on safety factors whereby the lowest dose not causing toxicity in animal species was divided by 100 to allow for uncertainty in extrapolating from animals to man and for differences in individual sensitivity (36). These calculations were usually based on the external dose in mg/kg.

5.41 Currently, exposure is expressed as the concentration of drug in circulating plasma over time. Peak concentration (Cmax) and the area under the concentration-time curve (AUC) are more frequently used in dose-response analysis. It is a reasonable assumption that Cmax and AUC values for unbound drug reflect concentrations of drug at targets within, or readily accessible to, the vascular compartment. However, they are less accurate measures of drug concentration at targets in deeper less accessible compartments, more especially when toxicity is caused by reactive metabolites. Making the wrong assumptions when comparing Cmax and AUC across species can lead to a miscalculation of the therapeutic index.

5.42 It is important to select the most appropriate exposure metric for use in risk assessment on a case-by-case basis (37). Information about the time course and mechanism of toxicity, the organ/tissue compartment affected, the toxic moiety (parent drug or metabolite, or reactive metabolite), localized exposure concentrations governed by membrane transport (influx or efflux), fractional extraction (liver) or excretion (kidney) of the administered dose are among factors that should guide selection of the most appropriate dose metric.

5.43 Extrapolation across species or between sub-groups of individuals may be prone to error in the absence of data to confirm a common mechanism of toxicity and similarities in potency of response at the target. Species differences in pharmacokinetics and/or metabolism and differences in drug disposition all affect the concentration of drug at the target. These underlying differences in host response and biodisposition are among the main reason why allometric scaling, based on a function of body mass and surface area, is subject to significant error, especially for extensively metabolized drugs.

5.44 Physiologically-based pharmaco/toxico-kinetic (PB-PK/TK) modeling and simulation methods may overcome uncertainties and inaccuracies caused by extrapolating Cmax or AUC values across or within species, or between patient sub-groups (*vide infra*). Iterative comparisons of PB-TK-TD relationships obtained by modeling animal data with corresponding analyses of PB-PK relationships in patients would add weight and accuracy to inter-species and inter-group exposure predictions.

5.45 The utility of any exposure metric in defining a "no-effect" dose for risk assessment is conditional on the mechanism underlying the effect(s) observed being consistent with the existence of a threshold. This presumes a mechanism where there is no effect on the target below a critical concentration. This highlights the importance of understanding the mode or mechanism of toxicity as a basis to support a rational dose-response analysis.

5.46 With the exception of drugs for cancer it is standard practice for drugs that are positive in assays for genotoxicity not to be progressed in development, for it is assumed that they would have the potential to be direct-acting genotoxic carcinogens. They are believed to interact with DNA directly and effectively do not possess a threshold.

5.47 Non-genotoxic carcinogens on the other hand produce tumours through several established modes of action, some related to hormonal imbalance (38), hypo- or hypermethylation of DNA (39), or perturbation of processes regulating cell replication or apoptosis (40,41,42). These non-DNA interactive, indirect modes of action portend a threshold. The existence of a threshold and a clear no-effect dose in the rodent bioassay is used as a basis for risk assessment. In most cases the margin between this notional threshold in the rodent bioassay and the effective therapeutic dose range in humans is sufficient to support the conclusion that the new drug candidate will not

pose a carcinogenic risk to humans. Moreover, there are confirmed examples where the mode of action of tumour induction in rodents is not relevant to humans (see section 11.4), or where therapeutic exposure levels in humans do not evoke the stimulus or response underpinning the induction of tumours in rodents.

5.48 Since there is evidence that tumorigenesis is a multi-step process the availability of a corresponding series of biomarkers that could be used to monitor patients on a continuing basis over time would be a highly significant and positive development, and especially valuable in confirming the safety of new therapeutic classes with novel mechanisms of action.

5.49 Biomarkers of host response are important surrogate indicators of hazard as well as exposure and there are almost limitless opportunities for developing and deploying them in animal studies and clinical trials. Biomarkers of host response might include indicators of oxidative stress, tissue regeneration and repair, inflammation and fibrosis (43,44,45). Biomarkers of exposure might include urinary catecholamines, circulating levels of steroid hormones and ACTH, and urinary metabolites of the administered drug.

5.50 If these biomarkers can be quantified consistently and demarcated in serial rank order at intervals correlating with dose across the pharmacodynamic range then the therapeutic index can be calculated very simply without recourse to plasma drug concentrations or modeling projections, or cross-species or sub-group extrapolations. If these relationships can be shown to be consistent then a therapeutic index can be derived for an individual or a group within a given species. This therapeutic index could well be very similar numerically from one species to the other whereas the ratio of dose or AUC or Cmax may differ widely up or down.

5.51 Industry and regulatory scientists would readily agree that a rational and plausible basis for the analysis of dose-response relationships is key to a successful risk assessment. However, it is evident that the interpretation of these data may differ widely between regulatory agencies in different parts of the world. This may mean that the ability to proceed with development in one situation, or to market a drug in another, may be denied on one continent but approved on another.

Recommendations

5.52 Representatives of the UK and EU Health and regulatory authorities and the pharmaceutical industry should convene in scientific session to review the strengths and weaknesses of current approaches to exposure assessment and dose-response analysis. Conclusions should appear in the form of published guidance notes and principles. The guiding principles should ensure that:

- the analysis is transparent, clearly articulated, and based on a composite analysis of all available data,
- appropriate justification for selection of dosimetry parameters consistent with mode of action, pharmacokinetics and metabolism,
- models and modeling systems are described and properly justified,
- biomarkers of toxicity, host response and exposure are used appropriately to characterise dose-response relationships fully across the dose range, with particular attention to relationships within the pharmacodynamic range, and,
- estimates of potential error and uncertainty in the analysis are presented.

5.53 A second output of the review should be the publication of non-competitive data in the form of case studies illustrating the best examples of dose-response analyses and risk assessment with the objective of sharing knowledge and promoting best practice.

5.54 A concerted research effort is required to discover the basis for constitutive differences between animal species, between animal species and humans, and between subpopulations of humans, with particular reference to genotype, phenotype, organ physiology and pharmacology, and processes affecting bio-distribution of drug in organs and tissues, as they relate to mechanisms of drug toxicity.

Predicting pharmacokinetics, metabolism, and drug-drug interactions

5.56 The expression of toxicity both in animals and humans depends on the concentration of the toxic moiety at the target, the affinity and kinetics of the interaction between the toxic moiety and the target, and the ability of the host to adapt and repair the damage caused. These complex concentration-time relationships between host and drug govern ultimately whether the dose administered is safe or harmful. Clearly, information that predicts when these complex interactions might co-exist materially and temporally to cause tissue damage would be of immense value in predicting risk, managing risk and reinforcing patient safety (46).

Challenges and opportunities

5.57 Early in the development of a new candidate drug, information is sought to determine if it will be adequately absorbed in animals and humans, whether it will exhibit desirable pharmacokinetic and metabolic properties, and whether it is likely to interact with other medications administered concurrently in a manner that could compromise patient safety. It is now possible to obtain much of this qualitative and quantitative information directly using human-derived *in vitro* systems (whole cells, sub-cellular fractions, or expressed enzymes) to help predict the *in vivo* metabolism and kinetics of a drug candidate, as well as providing information on variability between individuals that may lead to adverse drug-drug interactions (46, 47).

5.58 The absorption, distribution, metabolism and elimination (ADME) characteristics of a new drug candidate are similarly studied *in vitro* and *in vivo* in animal species, including those used in toxicology studies. This information is key to optimizing the design of animal toxicity studies with respect to species selection, dose and formulation, dosing regimen and sampling intervals, as well as highlighting potential target organs of toxicity. Analysis of ADME data is an integral part of interpreting the findings in animal toxicity studies and is an essential part of characterizing risk for humans.

5.59 As the liver is the major site of xenobiotic metabolism in mammals, particular attention has been paid to the development of *in vitro* hepatic models. For example, advances in molecular biology techniques have led to the development of expression systems to permit *in vitro* metabolism and interaction studies with specific hepatic xenobiotic metabolising enzymes, including P450 (CYP), flavin-containing monooxygenase (FMO), UDPglucuronosyl-transferase (UGT) and sulphotransferase (SULT) forms (47)

5.60 Other heterologous expression systems have been developed to permit studies with various receptors and transport proteins (48). Extra-hepatic models include intestinal cell lines (e.g. Caco-2) to investigate absorption from the gut.

5.61 Another important and continuing development is the application of *in silico* (i.e. computer based) techniques to all areas of drug discovery and development (49). For example, *in silico* methods have been developed to help design libraries of compounds with appropriate chemical, physical, pharmacokinetic and biological properties.

5.62 **Predicting kinetics**. *In silico* methods, artificial membrane systems and Caco-2 human intestinal cell lines have been developed to evaluate intestinal permeability and metabolism. Heterologous expression systems containing transporter proteins (e.g. P-glycoprotein and organic anion transporter proteins) and high throughput procedures for measuring protein binding have also been developed to evaluate absorption and tissue distribution of orally administered drug candidates.

5.63 Assessment of bioavailability (the fraction of an oral dose entering the systemic circulation from the gut), intrinsic clearance and metabolic stability of a drug candidate is carried out in assays conducted in liver microsomes, other sub-cellular fractions, intact cell systems (hepatocytes and liver slices) and expressed enzymes in conjunction with sensitive analytical techniques (47).

5.64 **Predicting metabolic fate** *In silico* models of metabolism, cultured hepatocytes or liver slices, and expressed enzymes are used to predict the

extent of Phase I (oxidation) or Phase II (conjugation) metabolism; to identify pathways of metabolism; and, to characterize the products of liver metabolism. Using appropriate scaling factors a suitable liver model is applied to estimate intrinsic clearance *in vivo*.

5.65 Information regarding specific enzymes responsible for biotransformation of the drug candidate (generally CYP forms) is obtained by reaction phenotyping studies employing correlation analysis, studies with expressed CYP forms, chemical inhibition and/or inhibitory antibodies (46, 47). This information is important for predicting drug-drug interaction and for identifying drug candidates that are metabolized by enzymes known to exhibit genetic polymorphisms or other inter-individual variability in humans. Other non-CYP dependent enzyme activities may be studied in similar fashion using various liver preparations, or preparations from extra-hepatic tissues.

5.66 **Predicting variability/polymorphisms**. Genetic polymorphisms, or multiple gene copies, or differences in allelic frequencies in certain drug metabolizing genotypes may account for some individuals being classified as ultra-rapid, extensive or poor metabolisers of drugs or chemicals. These variations may lead to adverse effects or lack of efficacy. These differences may also account for differences between ethnic groups. Differences in the levels of expression of drug metabolizing enzymes may also account for variability between young and old, and between adult and fetus. Variability between populations of humans can be assessed by using well characterised liver preparations from a wide range of donors and from knowledge of the enzymes involved in the metabolism of the drug candidate (47).

5.67 **Predicting drug-drug interactions**. Potentially harmful drug-drug interactions can be caused by induction but more frequently are caused by inhibition of metabolizing enzymes (47, 50). A combination of *in silico* models, high throughput screens and liver microsomes can be used to identify specific CYP forms that may be inhibited by a new drug candidate. The nature of the inhibition, the Ki value and the concentration causing 50% inhibition (IC50) can be determined. These data, coupled with *in vivo* data, enable the risk of harmful drug-drug interaction to be assessed.

5.68 Hepatocyte cultures, or liver slices can be used to confirm induction of CYP forms while cell-based reporter gene constructs can be used to screen chemicals for such activity. For example, high-throughput pregnane-X-receptor ligand binding and activation assays have been developed; this receptor is known to regulate CYP3A4. Cultured hepatocytes can also be used to assess the effects of a drug candidate on other metabolizing enzymes such as UGT forms.

5.69 Besides effects on CYP forms in the liver, drug-drug interactions have also been described involving both inhibition and induction of transporter proteins (48).

5.70 Accurate and useful prediction of the kinetics of drugs and drug-drug interactions *in vivo* prior to the efficient selection and design of confirmatory studies in humans will rely increasingly on *in vitro* data generated with human-based material (tissues, cells, sub-cellular fractions, expressed enzymes/transporters).

5.71 Provided that *in vitro* information is of high quality and is obtained at the appropriate time in the drug development process, its incorporation into sophisticated physiologically-based population PK models (that incorporate patient/disease demographics, physiological, pathological, genetic and developmental variation) should facilitate effective communication between late pre-clinical and early clinical development (51).

5.72 In addition, at earlier stages of development and in drug discovery, confidence in the use of purely *in silico* methods to predict ADME properties (active site modelling, QSAR based on physicochemical and structural properties) of both real and virtual compounds should increase as more experience is gained. In turn, this should help the early differentiation of potential drugs from non-starter compounds likely to fail later on.

Recommendations

5.73 A number of priorities can be identified to support and extend the approach to predicting the kinetics of drugs and drug-drug interactions in humans:

- Maintenance of the supply of human tissue samples while encouraging the development of techniques to use them more efficiently (cryopreservation, immortalisation of cell lines, stem cell research).
- Standardisation of *in vitro* absorption models that address both passive and active processes.
- Standardisation of *in vitro* Blood-Brain-Barrier models that also mimic pathological effects.
- More investment in *in vivo* studies of the role of transporters in drug disposition to put *in vitro* information into perspective and to help to construct rational *in vitro* transporter screens.
- Development of standardised *in vitro* screens for enzyme induction and inhibition.
- Integration of tissue/cellular systems for studying ADME with the evaluation of cellular toxicology.

5.74 Some outstanding issues and needs with regard to *in vitro/in silico – in vivo* extrapolation include the following:

- Expanded data bases on demographics, disease prevalence, genetic variants, enzyme/transporter abundancies and their variances.
- Improved understanding of the interplay between enzymes and transporters and of non-CYP drug metabolism, to extend existing algorithms and models.
- Better communication between large databases and predictive programmes.
- Improved collaboration between and within academia, industry and regulatory authorities in sharing databases and experience on *in vitro-in vivo* extrapolation, PK-PD modelling and clinical trials simulation.
- Rationalisation of IP issues related to access to algorithms, data and databases, without compromise of confidentiality.
- Commitment to dedicate personnel (realignment) to carry out retrospective and prospective evaluation of algorithms and models.
- Commitment to training of skilled modellers. Industry needs to be more pro-active in supporting academia; universities need to upgrade their appreciation of the economic importance of drug development and drug and environmental toxicology (safety sciences).
- Redistribution of expenditure in drug development such that frontloading with high quality *in vitro* data becomes more common, the appropriate information is obtained at the right time and costly clinical studies become more 'confirm' rather than 'learn'.
- Linkage of predictive physiologically-based population PK models to mechanistic PD models to put PK variability into clinical perspective.
- Linkage of predictive physiologically-based population PK models to data on cellular PK and toxicology and complex mechanistic biological models – as part of a systems biology approach to the assessment of drug toxicity.

Risk characterization

5.75 Risk characterization is a key step in the overall analysis of risk. The process involves the integration of data drawn from studies conducted by departments and disciplines that comprise a modern R&D organization. The process utilizes the skills and knowledge of the specialist as well as the generalist.

5.76 There are, on occasions, organizational and philosophical barriers that may impact the quality of the evidence gathered and the decisions emerging from the process of risk characterization. Successful organizations are those that promote interdisciplinary communication and collaboration with equal regard for the technical, intellectual and professional value of each contributing discipline.

Challenges and opportunities

5.77 Fundamental to risk characterization is the integration of data and information arising from three key activities, namely, hazard identification and the determination of the concentration of all chemical entities in test systems and their relevance to the clinical situation, dose-response analysis, and hazard characterization. Under ideal circumstances the assessment of risk in relation to benefit should adopt a weight of evidence approach. In other words, extrapolation of data across systems or species, or within sub-groups of the same species, should always relate the mechanism or mode of toxicity to relevant indices of exposure in order to ensure relevant comparisons are made to support a decision on risk versus benefit.

5.78 Such a balanced weight of evidence approach is not always adopted uniformly and consistently by regulatory agencies. Difficulties arise when the evidence is judged to be insufficient or the methodology flawed, when regulatory policy defaults to attempting to eliminate all possible risk, or when there is failure to agree that the balance between risk and benefit for a given indication is not consistent with patient safety. Given the diversity of drugs and indications under development it is evident that regulatory decisions should be undertaken on a case-by-case basis. There is a need for greater transparency and consistency of practice, both on the part of the pharmaceutical industry and the regulatory agencies, to ensure regulatory decisions are based on sound evidence and rational interpretation.

5.79 It is important to recognize that individual knowledge, institutional memory, clinical experience, and knowledge of therapeutics in a particular indication or disease are paramount in assigning priority and importance to risk factors, and that these must be considered separately for each indication, patient population, and dosage. It is generally agreed that there are no validated models with which to derive objective and quantitative assessments of risk versus benefit that apply universally to all drugs (52). It is virtually impossible for a model to accommodate all the necessary criteria for risk or benefit. Therefore, a case-by-case assessment is clearly the preferred approach.

5.80 Mussen (52) surveyed the European Public Assessment Reports (EPARs) for 33 new drugs, in categories ranging from cancer therapy to Viagra, approved between 1998 and 2000; EPARs summarise drug efficacy and safety and reflect the basis and the grounds for granting regulatory approval to market a new drug. From this limited study Mussen concluded that there was more overall emphasis on efficacy than on safety in the concluding sections of EPARs. He also noted that certain risk factors such as safety in sub-groups, drug-drug interactions, and the generalisability of the safety profile were not discussed extensively in the majority of cases. Possible reasons cited were a lack of guidance in the preparation of risk-benefit conclusions, and the difficulty of writing a summary to meet the needs of a diverse audience of patients and specialists.

5.81 While it is important to preserve operational flexibility in the process of scientific enquiry leading to risk characterization for a new drug candidate, formal guidance on the essential elements of the process is lacking at present. Similarly there is inadequate education and training in the basic scientific elements and principles that underpin the process

Recommendations

5.82 Representatives of the UK and EU Health and regulatory authorities and the pharmaceutical industry should convene to review the strengths and weaknesses of current approaches to risk characterization. The results of this consultation should be published guidance notes and principles to promote consistency of process and quality of output. The guiding principles should ensure that:

- the chosen methodology and the logic supporting conclusions underpinning the risk characterization for the drug under review should be transparent, clearly articulated and inclusive of all relevant data,
- primary as well as secondary risk factors should be identified and prioritised according to disease severity, patient attributes, dose regimen, and conditions of use that may impact the safety of the drug or marketed product,

- a weight-of-evidence, mechanism-based approach to risk characterization should be endorsed thus avoiding regulatory decisions defaulting to a 'de minimis' position; the use of a 'framework' approach to determining weight of evidence should be explored,
- estimates of potential error and uncertainty in the analysis and conclusions should be presented, and,
- summaries of risk-benefit positions and the basis of decisions rendered by regulatory authorities should be published in the open literature when a new drug is approved for marketing; this information should provide the basis for creating communiqués to patients and health care professionals.

5.83 There is a need to consolidate expertise and to focus activities within the UK in order to advance the science and processes connected with risk characterization and risk assessment. This could be achieved by creating an infrastructure to function as a National Centre of Safety Assessment:

- the establishment of a National Centre of Safety Assessment would provide focus and leadership to all efforts aimed at improving the scientific and regulatory aspects of processes that underpin risk characterization, risk assessment and risk management in the UK.
- a National Centre of Safety Assessment would be responsible for development and dissemination of 'best practice' in approaches to risk characterization and risk assessment of drugs.
- a National Centre of Safety Assessment would facilitate access to consultation on scientific and regulatory issues related to risk characterization and risk assessment, both in general terms and on a case-by-case basis.
- the Centre would be well placed to oversee the development and implementation of educational and training programmes designed to strengthen interdisciplinary skills in risk characterization and drug safety assessment.
- a National Centre of Safety Assessment would foster a culture of "One Medicine" wherein the focus of all disciplines engaged in drug safety assessment is on promoting an unbroken line of continuous scientific enquiry from discovery to successful approval and marketing of a new medicine.

False positives and false negatives: implications for risk assessment

5.84 Studies to assess drug safety are conducted in compliance with rigorously controlled protocols in accordance with regulatory guidelines and the various codes of Good Laboratory Practice, Good Clinical Practice and Good Manufacturing Practice. However, even well controlled experimental systems are liable to produce false positive or false negative results that lead to misleading conclusions, so called type I or type II errors in statistics. In the context of predicting risk, false positive results "over-predict" the likelihood of adverse events whereas false negative results "under-predict" such outcomes. Cell-based assays, as well as studies in animals and humans are liable to these shortcomings, especially when data are extrapolated from one test system or species to another.

5.85 Despite these well-recognized shortcomings, animal models are an indispensable resource with which to study the pathogenesis of human disease and to investigate the efficacy and safety of new drug candidates. Given our knowledge of species diversity it is not surprising that they are not perfect replicas able to predict every potential outcome in humans. However, without insights and clues provided by animal models few of the major treatment breakthroughs of the past 50 years would have been possible.

Moreover, there has not been an epidemic of adverse events accompanying the introduction of new prescription drugs during this period. As knowledge of the underlying biology of these systems increases, there is greater understanding of the basis for the false positives and the false negatives, thereby providing greater insight into the true magnitude of any risk.

Chapter six - *Concordance of toxicity in animals and adverse events in humans*

6.1 Although useful perspectives have been gleaned from retrospective reviews of data in company files and regulatory submissions, the latter available through freedom of information, there has not yet been a definitive prospective survey to critically assess concordance of toxicity seen in animal studies with observations of adverse events in humans.

Challenges and opportunities

6.2 Data obtained from animal studies have the greatest impact on drug safety prior to administering the first dose to man. Clinical trials in Phase I, II, and III, are the main sources of data that govern decisions regarding drug safety. Newly emergent pathology in chronic animal studies (6 months or longer), not seen in 30-day studies, is difficult to interpret but may in certain circumstances signal a risk to humans. This pathology may reflect tissue regeneration and repair (fibrosis and/or hyperplasia) in response to ongoing, drug-related, low-grade tissue damage, or may be related to spontaneous age-related changes unique to the species but unrelated to a direct effect of the drug.

6.3 In some cases drug related toxicity may accelerate progression of spontaneous age-related disease in laboratory animals. It is often necessary to investigate the basis of these changes if there is suspicion they may negatively influence the balance of risk versus benefit for humans. It is important therefore to distinguish laboratory animal specific changes (false positives) from changes that may signal a risk to humans (true positive).

6.4 It has been estimated that from 10 to 14% of new drug candidates fail during the pre-clinical phase of testing usually because of toxicity in animals (53). Reasons for withdrawal from development are usually related to several factors, namely:

- severe toxicity affecting the CNS, special sense organs or the haemopoietic system,
- toxicity of major organs without adequate safety margins,
- toxicity related to the drug candidate's primary mode of action without adequate separation of the efficacious and toxic exposures and,
- toxicity whose mechanism is not known.

6.5 Because it is not considered safe to progress these drug candidates into Phase I studies it is not possible to determine if these findings would have been reproduced in humans or whether they were indeed false positive observations.

6.6 It is well recognized that animal studies have been a major factor in ensuring that, of many thousands of drug candidates tested in Phase I studies, only very few have resulted in serious adverse events in human volunteers, and only rare cases of death have occurred (54). With a relatively low withdrawal rate of about 10% of drug candidates from later clinical trials being attributed to adverse events, many related to effects already seen in animals, it appears that animal studies make an important contribution to the safety of patients in clinical trials (55). In other words the incidence of false negative outcomes in animal studies is comfortingly low.

6.7 The results of several published surveys have demonstrated good concordance between the finding of toxicity in animals and the occurrence of similar or complimentary adverse findings in humans (55,56). ILSI/HESI sponsored a retrospective study (56), based on data provided by 12 pharmaceutical companies, to assess the rate of concordance between toxicity in animal studies and adverse events in subsequent clinical trials. The overall concordance rate was 71% when comparing data from rodent and non-rodent species in the aggregate for any given drug. Importantly, 94% of the toxicities in animals occurred in studies of 30 days or less. Drugs for cancer, viral infections and cardiovascular disease showed 80% concordance whereas drugs for endocrine conditions achieved only a 50% concordance

between animal and human data. The human adverse events recorded for the purpose of this survey were generally severe. It appears the majority of non-concordant outcomes were not related to species differences in drug metabolism or pharmacology between animals and humans

6.8 Concordance was greatest for toxicities of the haemopoietic system (80–90%) and least for skin conditions (38%) and abnormalities of liver function (41%). It is estimated that 50% of human adverse reports are related to subjective clinical symptoms (headache, dizziness, anxiety, abdominal pain, nausea, myalgia, lethargy etc). There are no satisfactory means by which to evaluate these effects objectively in animals (57). The need for improved models with which to evaluate functional deficits in the central and peripheral nervous systems, and to assess cognitive function in animals, is addressed in the report of the Safety Pharmacology Working Group.

6.9 A more critical analysis of concordance between observations in animals and adverse events in humans should be possible where criteria for concordance or lack of concordance are agreed in advance. At a minimum it is essential to consider the level of parity between the mode of action of a drug and its associated effects in animals relative to humans so that comparisons are not skewed because of differences in exposures, access to target organs or receptors, genetic predispositions or environmental factors, frequency and timing of dosing, or other constitutive differences that could jeopardize the validity of the comparative analysis. A protocol incorporating these criteria for assessment of concordance is being pursued in a prospectively designed study sponsored by the ILSI/HESI organization.

Recommendations

6.10 Ensure that pre-defined criteria for inclusion/exclusion of data are established in advance for all surveys of concordance between observations of toxicity in animals and humans: the ongoing ILSI/HESI study of concordance appears to have agreed such guidelines in advance.

6.11 Extend the prospective ILSI/HESI concordance study to include compilation of data from 6-months and 1-year toxicology studies in the files of UK and EU Health and regulatory authorities to determine the extent to which data from these studies have impacted decisions affecting human risk and safety in the recent past.

Chapter seven - *Species-specific toxicity and inter-individual variation*

7.1 Toxicity seen only in one or other animal species and seemingly without counterpart in humans is categorized as a "species-specific" phenomenon, but the strength of evidence to confirm or refute this conclusion varies considerably from one situation to another. This uncertainty frequently results in testing a new drug candidate in additional species or strains of animal in an attempt to determine the significance to human risk. However, over the last decade mode of action studies have confirmed that certain tumours in rodents are both strain- and species-specific (see section 11.4) and do not signal a risk to humans.

7.2 Individual animals in a study, or individual patients in a clinical trial, or a post-marketing study of safety or efficacy, may show a transient effect or a trend over time suggesting a possible drug association. Such effects generally occur at very low incidence, are frequently not dose-related, may not deviate outside the normal range and, therefore, are not statistically significant. This pattern of response is often insufficient to conclude definitively that there is a drug-related effect.

Challenges and opportunities

7.3 Despite intensive investigation there are relatively few examples of species-specific toxicity that have been fully elucidated with reference to mode or mechanism of action. Most examples are non-genotoxic rodent carcinogens (58,59). Several cardio-active and vaso-active drugs with different modes of action cause arterial toxicity in different vascular beds in different animal species (60,61). There is often a divergence of opinion regarding the relevance of these findings both for volunteer and patient safety, both from first exposure as well as the concern about exacerbating coincident vascular disease.

7.4 There is a paucity of knowledge regarding constitutive differences between animal species that may explain why some are highly responsive to certain classes of drug while others are more or less refractory. Some of these differences may be related to a lower level of target expression in one species versus another, pharmacokinetic or metabolic differences, or physiologic or genetic differences inherent to the species. There is a need to characterize polymorphic and allelic variations in the genome of animal species showing marked differences in susceptibility to toxicity. Knowledge of the basis for such differences would clarify their significance to human risk assessment.

7.5 Human specific toxicity, in particular drug-induced hypersensitivity reactions have been known to occur at very low incidence without counterpart in animal studies. These so called idiosyncratic adverse reactions have been investigated in patients but often without a suitable animal model with which to elucidate the pathogenesis. There are already examples where low incidence adverse events in clinical trials have been subjected to pharmacogenetic analysis to map the genetic susceptibility of affected patients, and specific polymorphisms have been identified that relate to the functional deficit or adverse event. In the case of Tranilast (treatment for restenosis) a polymorphism in the UGT1A1 genotype (UDP glucuronosyltransferase 1, polypeptide A1) was associated with hyperbilirubinaemia in 40% of affected patients (62). In the case of Abacavir (anti HIV treatment) polymorphisms in two genes (HLA –B57 and TNFalpha –238) both located on chromosome 6 were found to be highly associated with a low incidence hypersensitivity reaction to the drug (63, 64). Further experience will demonstrate if it will be possible to repeat this success as polymorphic determinants of variable response for a larger number and range of novel drug targets are mapped in greater detail.

Recommendations

7.6 UK and EU based scientists in academia and industry should establish a programme of collaborative research funded, at least in part,

by the pharmaceutical industry to explore phenotypic, genotypic and other constitutive differences that may account for manifestations of species-specific toxicity. The objectives of the collaboration would be:

- to discover a rational basis for species differences in toxic responses to certain classes of drugs or modes of action that currently are either denied regulatory approval to proceed into human trials or are considered a high risk for human safety,
- to characterize genetic polymorphisms and allelic variations in toxicology species that may be relevant to assessing the toxic potential of specific drug classes and therapeutic disease targets in general e.g. nuclear or cytoplasmic receptors; such a repository of data could support selection of one rather than two species for toxicology studies,
- to accumulate a repository of phenotypic and genotypic data from animals exhibiting atypical, non-dose related responses to various classes of drugs in toxicology studies as appropriate; such data may reveal polymorphic or allelic variations in certain animals that may have specific utility as sentinels of human risk, and,
- to increase knowledge of species differences in organ pharmacology and physiology that would contribute to more accurate physiologically-based pharmacokinetic modelling.

Chapter eight - *Dose selection*

8.1 Dose selection is one of the critical elements governing the successful completion of animal studies and clinical trials. The exposure (Cmax or AUC) produced by a toxic dose in animals may be greater or less than the exposure produced by a therapeutic dose in patients. It is important that species differences in sensitivity to the primary and secondary actions of the drug or to chemically mediated toxicity be taken into account, for the therapeutic index may be high in the animal species even though blood concentrations are less than in humans. Excessively high doses should not be used in animals in an attempt to achieve a 'safe' multiple of animal relative to human exposure.

8.2 Thus the requirement by regulatory guidelines to use a maximally tolerated dose in animal studies frequently results in toxicity at levels of exposure that may be greater than or less than exposures produced by doses in the human therapeutic range. This may raise unfounded concerns about drug safety.

Challenges and opportunities

8.3 Insufficient attention is given to choosing doses over the lower end of the dose range in animal studies. This is the area of the dose response curve most relevant to human safety and a greater emphasis should be placed in animal studies to quantify drug-related effects at or slightly above pharmacologically active doses (37).

8.4 Selection of the uppermost dose in animal studies is important otherwise there is a danger of producing false positive or false negative results. Administration of oral doses that saturate drug metabolism, protein binding or excretion may cause false positive safety concerns. Such excessive doses can result in non-linear kinetics or produce toxic metabolites, neither of which is necessarily relevant to human exposure within the therapeutic range. However, in rodents with high first pass clearance of orally absorbed drug from the intestine by the liver, it is sometimes necessary to saturate liver clearance mechanisms in order to achieve systemic exposures within range of therapeutic exposures in humans.

8.5 The regulatory requirement that the top dose of low-toxicity drugs in a rodent bioassay should achieve a 25 fold multiple of the human AUC is impractical in the majority of cases especially with relatively low toxicity drugs. High residues of drug in the gut or high concentrations eliminated in bile or urine can cause pathology that is secondary to overdosing.

8.6 The current method for identifying the maximum tolerated dose, although mandated by current regulatory guidelines, should be discouraged as it frequently results in secondary pathology not related to the primary mode of action of the drug. Pharmacokinetics and pharmacodynamic responses of the test species as well as consideration of human exposure to drug at therapeutic doses should be factored in to dose selection in animal studies. (37).

8.7 Observations of toxicity in animals at the maximum tolerated dose elicit considerable variation in the actions taken by regulatory agencies in different countries, especially with regard to the scale and scope of investigations required to absolve the drug from a highly negative risk benefit assessment. This can result in failure to progress a drug in development either because of restrictions on the range of doses that can be tested or because of patient exclusions.

Recommendations

8.8 Representatives of the UK and EU Health and regulatory authorities and the pharmaceutical industry should convene to review regulatory custom and practice over the last decade with a view to revising the requirements and guidelines for dose selection in toxicology studies and minimizing use of the current method for identifying the maximum tolerated dose.

Chapter nine - *Idiosyncratic adverse events*

9.1 Adverse drug events are defined as unintended and undesirable effects of a drug that occur at doses used in humans for prophylaxis, diagnosis or therapy. In the context used here idiosyncratic adverse events are unexpected and occur predominantly after approval of the therapeutic or diagnostic product. Implicit is the assumption of failure, despite extensive investigations, to predict that such events might indeed occur in patients receiving the drug (65).

9.2 Unexpected adverse events related to exaggerated pharmacology, or interaction of the therapeutic chemical or protein with cellular macromolecules, are typically rare, although they may occur from time to time because of unanticipated system failure. In other words, most are predicted in animal studies, studies *in vitro*, or in patients in clinical trials.

9.3 A further category of unexpected adverse event, designated "idiosyncratic," refers to drug-related reactions that appear to be host dependent and typically occur at a frequency of 1:1000 to 1:100,000 patients. They are usually without precedent, either in animal studies or clinical trials, and the mechanism of the reaction, and the reasons for inter-individual differences in susceptibility, are poorly understood.

9.4 Idiosyncratic adverse events present a diverse clinical picture. They have been associated with all systems in the body – the liver, skin, kidney, haemopoietic system, and the immune system being most frequently affected. The same drug may be associated with a different set of symptoms in different patients. In cases of liver injury, symptoms range from mild, asymptomatic changes in serum transaminases to life-threatening liver failure.

9.5 It is often assumed, but without proof, that these idiosyncratic adverse reactions have an immunological basis. Certainly, a second exposure may be more severe and cause death.

9.6 All classes of drugs with diverse mechanisms of action have been found to cause idiosyncratic adverse events. Generally speaking such idiosyncratic reactions are not observed for drugs given at doses less than 10 mg per day, indicating that some form of chemical stress is involved in the pathogenesis.

Challenges and opportunities

9.7 Experience so far would suggest that there are no validated standardized tests either *in vitro* or *in vivo*, of animal or human origin, with which to predict idiosyncratic drug toxicity. At present most idiosyncratic adverse events are detected only after approval to market a drug, and only after very large numbers of patients, much greater than in clinical trials, have been exposed to the drug.

9.8 The challenge is the prediction of idiosyncratic drug reactions in terms of 1) the chemistry of the drug and, 2) the genotype or phenotype of the individual patient in a clinical trial. This begs the question as to when in the course of drug development is it possible to predict the risk of host-dependent drug toxicity. Intuitively one would expect a test system to contain a gene product that is a determinant of host susceptibility to this kind of low frequency event. In the first instance it requires a detailed understanding of the cellular and molecular mechanisms involved. It is important to distinguish between those studies that aim to define the pathogenesis of the reaction and those whose goal is to develop empirical test systems.

9.9 The investigation of idiosyncratic adverse drug reactions to date has largely been undertaken by a small number of academic groups. New processes are required that will support coordination and collaboration between industry, medical practitioners, regulatory authorities and academia in order to achieve the following outcomes.

9.10 **Understanding generic and compound specific mechanisms:** although the detailed

molecular events resulting in idiosyncratic adverse reactions vary between drugs there may exist common aspects of the relevant pathogenic mechanisms that could be identified on the basis of detailed cellular and molecular investigations. New experimental *in vitro* and *in vivo* systems are required for mechanistic studies. The development of *ex vivo* systems incorporating affected patients' cells and transgenic animals provide promising approaches.

9.11 **Availability of biological samples for research:** there is a need to establish a coordinated approach to identifying patients, recording and characterizing idiosyncratic drug reactions, and making available patient data and clinical samples that will facilitate expedited investigations of mechanisms of action. Funding is required to initiate and facilitate data and sample collection. Ethical issues need to he identified, addressed, and resolved.

9.12 **Availability of clinical and experimental expertise:** serious adverse drug reactions lead to immediate discontinuation of drug treatment and may lead to withdrawal of the drug altogether. If investigations to study mechanisms are to be successful they must start without delay. This calls for a high level of efficiency and effectiveness so that timely management of such events can be introduced.

9.13 **Establishment of a national centre for research and information:** consolidation of clinical and experimental capabilities and coordination of operations within a single center of expertise would greatly facilitate basic research into this important area of public health. Such a centre would encourage a greater understanding of idiosyncratic adverse health effects associated with drug exposure as well as serve as a central repository for data, data management and clinical samples. A National Centre for Safety Assessment could serve this function (see above).

9.14 The centre would provide both clinical and laboratory based education in the field and would resource a teaching programme in molecular pharmacology and toxicology allied to human medicine that could be incorporated into the undergraduate medical curriculum.

9.15 **Application of new technologies:** there is a need to develop further those modern pharmacogenomic and toxicogenomic platforms to facilitate a detailed understanding of the heritable and environmental factors that predispose to adverse drug reactions. The 'omic technologies are powerful tools but they require specific fine-tuning for application to particular human health problems, especially those arising from, or associated with, idiosyncratic adverse drug reactions.

9.16 **Patient assessment:** there is a need to understand in greater detail the ways in which acquired, environmental, and dietary factors may act, either alone or in concert, with heritable factors to determine inter-individual differences in susceptibility to adverse drug reactions. Thus techniques are required for simultaneous investigation of patient phenotype and genotype. It is well recognized that these advances are an essential step to achieving the goals of personalized medicines in clinical practice.

Recommendations

9.17 To facilitate progress in these directions, there is a need to determine which drug toxicities were failures of the system and which drug toxicities could not have been avoided with all present knowledge available. This retrospective analysis should guide investment of time and resources into areas of research most likely to enable us in future to avoid idiosyncratic adverse events.

9.18 There is also a need for clinicians, regulators and the general public to develop consensus regarding what level of risk is acceptable, and under what circumstances, especially considering the benefits offered, even by stigmatized drugs, to the majority of patients. It is important not to raise false hopes for, given the diversity of causality and the large numbers of patients who elect to take drugs for their diseases, there will always be a degree of unavoidable risk.

9.19 There is a need for a change of mindset in order to appreciate that idiosyncratic drug toxicity is a

general health problem rather than an obscure medical problem caused occasionally by a drug, with all the responsibility to resolve and understand all its dimensions falling at the door of the pharmaceutical industry.

9.20 To understand and prevent such idiosyncratic reactions fundamental research must have a clinical focus, which requires sufficient experimental material, supported by the latest technologies for understanding drug action. At present such funding is largely directed towards preclinical research.

9.21 National and international databases, and tissue, serum and cell banks are required to provide sufficient clinical material for research. The clinical picture with any adverse drug reaction is variable. Therefore, for idiosyncratic drug toxicity, typically of extremely low incidence, we are faced with the somewhat paradoxical situation that large numbers of patients are required who are accurately characterised clinically, and stratified by bioinformatics.

9.22 In the light of these considerations the key recommendations are:

- to initiate develop and coordinate clinical and experimental investigations of adverse drug reactions,
- to provide a vehicle for collaborations and sharing of samples as well as information with other centres in the US and Europe,
- to initiate a coordinated effort to be funded jointly by the pharmaceutical industry, the Medical Research Council, the Department of Health, NPSA, and the National Health Service.

Chapter ten - *Assessment of carcinogenic potential*

10.1 The principle features, and assumptions, of the rodent bioassay for carcinogenicity were determined in the 1940's and 50's (66,67,68). The process was developed with only limited mechanistic understanding of carcinogenesis, but with the knowledge that exposure to a number of chemicals was associated with the development of cancers in man and experimental animals. Analysis of these data led to the belief that the "majority of all cancer" is caused by chemical or environmental factors (69,70). However, it should be noted that at this time there was a tendency to assume that environmental (which simply meant that the etiological factor was extrinsic) meant chemical. The logical conclusion from these assumptions is therefore self evident: identify carcinogenic potential and cancer avoidance would be possible (70). As a consequence considerable impetus developed to screen chemicals for carcinogenic potential. This is now governed by extensive regulatory requirements for food additives, environmental chemicals, agrochemicals and pharmaceuticals.

10.20 Inherent in the use of animals for carcinogenicity bioassay is the assumption that humans and animals behave in a similar way. In addition, two experimental concepts form the scientific basis on which the animal carcinogenicity bioassay is based.

10.21 The first is the empirical relationship developed by Druckrey (71):

$$\text{Tumour incidence} \propto a\,dt^{n}$$

Where 'd' is dose, 't' is time to tumour incidence, and 'n' is a power term, usually 2, or 3 or even higher.

10.22 The experimental work (mostly in skin using polycyclic aromatic hydrocarbons) which led to this relationship, indicated that time was the most important parameter in determining incidence, and that the tumour incidence was directly proportional to dose. Thus, tumour incidence could be increased or the time to tunour could be decreased by increasing dose, although there was a minimum time before tumours would develop. The second important concept was that carcinogenesis comprises two stages, tumour initiation and promotion. This was developed as an operational paradigm for chemical carcinogenesis in the skin, by Berenblum and Shubik (72,73). Experimental analysis of skin carcinogenesis showed that this first required a short or limited exposure to a chemical that resulted in an irreversible change in the skin, which was called initiation. This needed to be followed by prolonged exposure to a chemical which could acts as a promoter of the initiated cells, the effects of which were reversible for some time. This stage was called promotion. The model had a number of requirements: chemicals that acted as promoters did not act as initiators; initiation had to precede promotion; promotion could be delayed for some time after initiation.

10.23 This general model has been extended to apply to a number of other cancer types. In addition, while first developed as an operational model to describe experimental observations, it has since acquired a mechanistic interpretation although this may not be as well supported as the original operational description. Nonetheless, initiation is now generally taken to imply primary damage to DNA leading to a critical mutation while promotion is taken to mean the epigenetic steps that allow expression of the primary genetic lesion through the acquisition of other heritable genetic changes (74,75).

10.24 The original experimental model has since been modified. Chemicals that can act as complete carcinogens are required to act as both initiators and promoters, although the complete process may take a considerable period of time and the two stages can still sometimes be discerned experimentally. Compounds that do not cause direct damage to DNA may, however, increase tumour incidence because they "promote" cells that have undergone some prior spontaneous

mutation. It has been suggested that particular causes of this may be oxidative damage to DNA; exposure to exogenous carcinogens; reduced repair capacity as animals age (76). It has also to be appreciated that this convenient experimental model is too simplistic and if molecular biology has taught us anything it is that carcinogenesis is a complex process (77,78).

10.25 The evolution in the design of the carcinogenicity bioassay has been greatly influenced by the outcome and experience of the NCI/NTP bioassay programme. The programme was proposed by Michael Shimkin in the1960's who saw the need for a more systematic investigation of chemicals for carcinogenesis. The experimental work was initiated by John and Elizabeth Weisberger (68) who asked the simple question: "How many industrial chemicals, related in structure to reference rodent carcinogens such as 2-AAF and B[a]P, will also prove to be carcinogenic to rodents". The answer turned out to be the majority. As the NTP programme grew, other chemicals, with no structural precedent for carcinogenicity, were added to the growing list of chemicals nominated for bioassay. It turned out that a similar proportion of these agents were also carcinogenic, but that their tumour profile was different to that of the earlier carcinogens (79).

10.26 A decade of mechanistic studies confirmed that there are many ways for a chemical to increase the tumour incidence in rodents, only some of which appear relevant to humans (80). This view of relevance is not universally accepted; some still consider that any increase in tumour incidence induced by a chemical, in either rats or mice, is of immediate relevance to humans. Indeed it has been argued that the value of the rodent bioassay lies in the fact that there is no assumption of mechanism of cancer development (81).

10.27 Although there has been much discussion of the relevance of the testing procedures they are still based on a dual strategy:

1) assessment of genotoxic potential

2) assessment of carcinogenic potential through life-time studies in rodents

10.28 The data from such studies may be supported by investigative studies aimed at determining mode of action and relevance to human exposure (dose, metabolism, etc.)

10.29 Experimental data are then set against potential human exposure and a risk evaluation is carried out. This process may differ between pharmaceutical and other situations such as environmental or work-place exposure. In either case assessment is made against potential benefit but the weighting may be different in different situations.

Current Position on Genotoxicity Tests

10.30 Over the past 30 years there have been several perceived roles for genetic toxicity assays in the hazard definition/risk assessment process. Initially it was assumed that the Salmonella bacterial mutation assay could single-handedly predict potential rodent carcinogens, and by implication, potential human carcinogens. Considering that the rodent and human carcinogens known at that time were potent, due mainly to the fact that they had been capable of detection using limited rodent bioassays or small human epidemiological studies, it was not surprising that the large majority (> 90%) of them were also damaging to DNA (genotoxic) and mutagenic to bacteria. Further, the mutagenicity of such carcinogens could usually be easily rationalised in terms of their chemical structures being reactive to DNA, either directly or following predictable metabolism (structurally alerting).

10.31 However, with more refined and detailed assessments of carcinogenicity came two realisations. First, that not all agents defined as genotoxic *in vitro* are carcinogenic to rodents, and secondly, that approaching 50% of newly defined carcinogens were devoid of genetic toxicity. The latter problem has been addressed in two distinct ways. First, it was assumed that these non-genotoxic carcinogens are, in fact mutagens incapable of detection using current assays. This has led to the development of ~100 new mutagenicity assays for the detection of alleged 'crypto-mutagens'. Alternatively, there was the viewpoint that

such instances are representative of a growing group of non-genotoxic carcinogens whose detection must rely on biological activities other than mutagenicity. This latter view is the one that currently has general acceptance and is the principle on which most testing and risk assessment are done today.

10.32 All candidate drugs are now routinely assessed for genetic toxicity, as described later herein. Except in cases of overwhelming potential benefit, in practice usually valuable anti-cancer agents, agents with intrinsic genetic toxicity are not developed further. All candidate drugs are submitted to lifetime rodent carcinogenicity bioassays as part of their development, and irrespective of the fact that only agents devoid of genetic toxicity are submitted to bioassay, a large number of new candidate drugs produce positive results when bioassayed for rodent carcinogenicity, 40% in an analysis published in 2001 (82). This would be an unacceptable rate of attrition – either the existing genetic toxicity assays are lacking, or the rodent bioassay is sensitive to aspects of chemical toxicity independent of genetic toxicity and of limited or no relevance to humans (see Section 10.44).

10.33 The term 'non-genotoxic carcinogen' is still contested in some quarters, but it is now generally accepted that some chemicals can increase cancer incidence in rodents and humans by virtue of properties they posses beyond simple DNA-reactivity. Perhaps the most striking and explicable examples of non-genotoxic carcinogenesis are the human carcinogenicity of the immunosuppressive agent cyclosporin A, and the carcinogenicity to the mouse uterus of the synthetic oestrogen diethylstilbestrol and the phytoestrogen genistein. The carcinogenicity of the last two chemicals is clearly related to their intrinsic estrogenic activity, as evidenced by the fact that equal carcinogenic incidences were observed when each was tested at equi-estrogenic doses, as defined by the mouse uterotrophic assay (83). However, most apparent non-genotoxic carcinogens are not associated obviously with an alternative mechanism of carcinogenic action. The main challenge facing safety assessment of drugs thus becomes to recognize a range of possible markers of non-genotoxic carcinogenesis, such that these can be used to supplement genetic toxicity data when predicting carcinogenic potential.

Current use of genetic toxicity assays

10.34 Due to the unavoidable and insoluble problems faced when mutagenicity assays were attempted to be used to predict *all* classes of chemical carcinogen, attention has turned over past years to gaining international consensus on three more justifiable and practical uses for these assays, as discussed below.

10.35 **For the definition of genetic toxicity:** this is the most secure use of genetic toxicity assays, because used in this way they are defining an activity *per se*, not providing data to be used to predict another biological activity. The clearest consensus statement on this matter is provided by the testing strategy reported by the International Program for Chemical Safety (84). This strategy involves assessment of chemical structure and genetic toxicity *in vitro*. The assays used in this phase of testing involve bacterial mutation and mammalian cell mutation tests – the precise mammalian cell assays to be used being left undefined. However, both the IPCS strategy, and that of most regulatory bodies, implies the need for both gene mutation assays and cytogenetic assays. Great progress has been made on refining these assay protocols in order to reduce the incidence of false results.

10.36 Chemicals devoid of genetic toxicity *in vitro* are assumed by most investigators not to pose a hazard *in vivo*. However, some authorities require the conduct of a rodent bone marrow cytogenetic assay as part of preliminary screening. Agents found to be genotoxic *in vitro* are either assumed to present a potential hazard, or they are assessed *in vivo* to establish their genetic toxicity to rodents. The use of rodent assays for unscheduled DNA synthesis (UDS) or DNA fragmentation (e.g., the Comet assay) means that most testing strategies end with a statement of genetic toxicity (i.e.

damage to DNA), as opposed to mutagenicity (i.e. heritable change).

10.37 The recent introduction of transgenic rodent gene mutation assays (such as BigBlue™ rats and mice, and MutaMouse™ mice) means that the mutagenicity of chemicals in any tissue of a rodent may now be assessed. However, these assays have not replaced the more established assays mentioned above due to uncertainties regarding their sensitivity, their relatively high cost, and the current absence of internationally agreed regulatory guidelines for their conduct. Currently, these assays are most often used to assess mechanism of action of tissue-specific rodent carcinogens.

10.38 **For the prediction of somatic and germ cell mutagenicity:** germ cell mutagenicity is a major toxic endpoint for chemicals. This is usually assessed only for males via the use of the rodent dominant lethal, heritable translocation, or specific locus assay. These assays are demanding of resources and cannot be conducted routinely in most laboratories. To this end great reliance is placed on the fact that to date, all rodent germ cell mutagens are also active as somatic cell mutagens, as detected using the IPCS strategy. About 30 rodent germ cell mutagens are currently known, all of which are detected by the standard rodent bone marrow micronucleus assay – and no examples exist of a confirmed human germ cell mutagen.

10.39 **For the prediction of genotoxic carcinogenesis:** had Bruce Ames entitled his 1974 paper 'Genotoxic carcinogens are mutagens', instead of 'Carcinogens are mutagens' the situation would have not altered over the succeeding years. However, the few carcinogens tested by Ames that were not mutagenic to Salmonellae (sodium saccharin, thiourea, etc) slowly grew to the point where close on half of all rodent carcinogens defined by the late 1990s were not mutagenic to Salmonellae. However, genotoxic carcinogens, whose mechanism of action involves perturbation of the genetic integrity of the host animal, as the direct result of the agent's ability to alter the integrity of host DNA, can be reliably detected using the test strategy outlined above.

10.40 **The problem of false positive predictions of carcinogenicity:** not all agents found to be genotoxic *in vitro* are genotoxic *in vivo*. This usually requires additional experiments in rodents before concluding on the genetic toxicity of an agent. Most sources of truly artifactual positive results *in vitro* have been eliminated by protocol refinements (controlling pH, osmolarity etc), and it is probable that most instances of 'false positive' genetic toxicity results represent failures of the assays to anticipate pharmacokinetic (PK) or pharmacodynamic (PD) (e.g. host response to initiation) factors associated with the agent in rodents. This is a problem common to all *in vitro* assays and cannot be remedied without recourse to animal experimentation or the advent of accurate modelling of PK and PD factors.

Current Bioassay Practice

10.41 The standardized version of the bioassay requires that two rodent species are exposed from early adulthood to the test chemical for a large proportion of their natural lifespan (75%). Groups of 50 or 60 animals are given the test compound by an appropriate route. There may be 3 or 4 dose levels with one or more control groups. The top dose is generally set at the Maximum Tolerated Dose and lower doses at geometric intervals. Earlier designs may have only two dose levels: the MTD and 1/2 MTD. For pharmaceutical agents the lower dose may be a dose level that results in an exposure, as measured by the AUC, that is close to the exposure seen in patients at therapeutic doses. On occasion the top dose may be set at some large multiple (e.g. 25) of the therapeutic exposure (AUC) (see Section 8).

10.42 The current practice for animal bioassays is formalized in various national and international guidelines and for pharmaceuticals has been harmonized under the ICH guidelines (85).

10.43 There has been much discussion over the utility of the rodent bioassay in the process of carcinogenesis assessment. The significance of testing at the MTD has been questioned

because of the considerable physiological disturbances that may occur at this dose level (80). Furthermore, the kinetics of the compound may also be quite different to that at lower doses, with saturation of metabolic pathways. In addition, extrapolation from experimental ranges to human exposure levels often requires assumptions regarding the dose response relationship that are, by definition, not verifiable by experimental evidence. The carcinogenic response may differ between species and between different strains: aflatoxin B_1 causes liver cancer in rats when given in the diet at a few parts per million whereas the mouse is non-responsive at 1000 times this level (86); dimethylbenzanthracene causes mammary tumour development in Sprague-Dawley rats but not in the Wistar strain (87). Such differences may reflect differences in metabolism or differences in interaction with a number of other risk factors such as hormonal status and are evident, as indicated, even for genotoxic agents.

10.44 Species and strain difference in response are even greater for non-genotoxic agents. In many cases increased tumour incidence caused by non-genotoxic carcinogens in rodents, and in at least one case in the dog, have been shown to be strain, sex or species specific and therefore, it is claimed, not relevant for human risk assessment (87). Recently, detailed mode of action analysis with systematic comparison of the key events and of pharmacokinetics between experimental species and humans has provided support for this claim, in several instances (88,89).

10.45 The need for both the rat and the mouse in bioassays has been questioned regularly. In formulating its guidelines, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) evaluated available data on the testing of pharmaceuticals for carcinogenicity in mouse and rat bioassays. The conclusion reached was that the mouse contributed little in the evaluation of carcinogenic potential of pharmaceuticals to humans and that there would be little loss of sensitivity by omitting studies in the mouse (85). However, the consensus position adopted was that pharmaceuticals should be assessed for carcinogenic potential in a rat bioassay together with an additional *in vivo* test for carcinogenicity. The second test could be either a short or medium-term *in vivo* rodent test or a long-term test in a second rodent species. In practice this has meant either a transgenic mouse model or a mouse bioassay (90,91). In several subsequent studies, it has again been concluded that the mouse provides no added value in the evaluation of the carcinogenic potential of pharmaceuticals (92).

10.46 For non-pharmaceutical agents human exposure usually results from environmental contamination or work place exposure. Usually the exposure levels are much less than those set in the bioassay, and standardized procedures for estimating human risk have been developed, although these differ amongst regulatory authorities. Human risk can be estimated on the basis of identification of carcinogenic hazard and the extrapolation of response based on the dose response curves and estimates of human exposure. Some assessment of that risk is then made which may be called "risk characterization" (93).

10.47 Opposing views have been taken on the utility of using chronic animal data in assessing human risk. Thus Gio Batta Gori wrote in 2001: "It should be apparent beyond doubt that presently no science is available for the translation of chronic animal test data into objective forecasts of human cancer risk" (94). This contrasts with the more optimistic view expressed by Maronpot, Flake & Huff (95), who argued that useful data could be obtained from animal studies. They argued that there are sufficient similarities in the anatomy, physiology and carcinogenic process between man and animals for studies in animals to have relevance for man. Their argument, however, was based on the identification of hazard rather than an estimate of risk and in either event for the majority of animal carcinogens there is no corroborating data in man. There is excellent concordance between known human carcinogens and animal data (81); however, the converse is not true; there

are many thousands of animal carcinogens for which there is no concordant human data (94).

10.48 There are two possible reasons for this lack of concordance: 1) the compounds are not carcinogenic in man or 2) the conditions under which they are carcinogenic in rodents do not apply to human exposure: a further possibility is that some of these compounds are carcinogenic in man but the tumour incidence is too low to detect in feasible studies. Nevertheless, it is unlikely that that we will ever be allowed to expose groups of 50 humans, held in cages separated by sex, fed diets in excess of need to doses of a test article that can be barely tolerated for between the age of 15 and 65!

10.49 These difficulties let to alternative models: mice were developed with genetic modification that incorporated changes considered important in the carcinogenic process. It was thought that these animals would give a more consistent response in a shorter time. Recent ICH guidance indicates that one long-term rodent bioassay supported by additional evidence, such as that obtained using a transgenic mouse model, may be acceptable and give improved risk assessment. (91). However, it is unclear which mechanisms of carcinogenicity were expected to be detected by each of these new models; this allows constant retro-justification of the results obtained when testing 2-year NTP bioassay carcinogens (96). Despite early hopes, none of the available transgenic rodent carcinogenicity bioassay models (97) has matured to be reliable and capable of general adoption.

10.50 The full expression of a cancer phenotype requires changes in oncogenes, tumour-suppressor genes and so called stability genes such as those involved in DNA repair and in mitotic recombination and chromosomal segregation (78). If these general principles hold, then it would be more appropriate to define the conditions under which these alterations might exist in order to improve risk assessment. It is recognized, however, that rodents, and their cells, may behave in a different way to humans and human cells, which is an additional confounding factor when assessing human risk from animal data (98).

10.51 What would be reasonable to expect from the rodent bioassay and is there a better way of assessing carcinogenic risk to man? If the question is: 'does this chemical increase the tumour incidence in any of the available rodent bioassay models' then recourse to all of those models will be needed in order to define a non-carcinogen. If the question is: 'does this chemical present an obvious cancer hazard to exposed humans' then limited and focused studies can lead to an adequate answer. Balancing the adequacy of answers with the rate of testing of chemicals is obviously a matter of judgment, but such judgment is rarely discussed.

Current Understanding of Carcinogenesis

10.52 Before discussing an alternative strategy, there are a number of general points that should be considered:

- Pattern of tumour types and incidence differ between animal species and between strains. Eighty percent of the tumours that arise in man are carcinoma while 80% of tumours in some strains of mice are sarcoma, although the life-time risk of developing cancer is about the same in man and rodents (30%). (70,98)
- Smoking is a major confounding factor in tumour development in man
- Diet plays an important role in cancer development in man and animals (70,99,100)
- Standardised incidence rates show up to 100- fold variation in certain cancers in different geographical regions (IARC World Cancer Reports, 2003)

10.53 Cumulative data in both man and animals show that cancer results from the progressive accumulation of a number of traits related to a limited number of molecular functions: proliferation, differentiation and death (75,77). These functions may be modified by alterations in the function of three broad gene

classes: tumour-suppressor genes, stability genes and oncogenes (78). There are over a hundred genes that may be associated with one or other of these broad classes; not all are needed and the sequence of action and the detail of their interaction are poorly understood. Some steps in the carcinogenic process, and perhaps even the occurrence of tumours in an individual, are stochastic events, and hence not reliably predictable other than on the basis of probability theory (101).

10.54 The complex nature of any assessment that has to be made in assessing risk may be illustrated by the case of estrogens and the role they play in influencing the incidence of cancer at several sites in man (102). Epidemiological data indicate that prolonged unopposed estrogen exposure may double the risk of endometrial cancer (103). Obesity is also an important risk factor in post-menopausal women and it has been postulated that the increased risk is associated with the conversion of androstendione to estrone in adipose tissue (104,105). Similarly the risk of breast cancer is increased in post-menopausal women in whom estrogen levels are raised (106). Animal studies have also convincingly shown that estrogens play an important role in the induction and promotion of mammary tumours (107,108). Furthermore, both medical and natural Selective oEstrogen-Receptor Modulators (SERMs) appear to have a profound affect on the development of breast cancer although the relationship is complex and may depend on genetic and other predisposing factors (109,110).

10.55 SERMs may exhibit different estrogenic and anti-estrogenic effects in different tissues and in different hormonal states (110). There is a general consensus that estrogens may play a promotional role in carcinogenesis by increasing proliferative activity thus increasing the risk of critical mutations occurring or "fixing" those that have already occurred (111). However, a number of oxidative metabolites of estrogens have been reported, which bind to DNA, and that may result in mutation and initiation of the cancer process in breast tissue (112). Against this, it is possible to inhibit the carcinogenic response to estrogens with anti-estrogens, without any change in covalent binding to DNA (113).

10.56 The estrogen receptors are members of the nuclear-receptor superfamily that plays an important role in cell differentiation and proliferation (114). As well as estrogen receptors it includes androgen, retinoic acid, retinoid X, vitamin D, constitutive androstane and per-oxisome proliferator-activated receptors (110) and it would not be surprising if modification of these receptors influenced the incidence of a variety of tumours in the long term. However, the complexity of their interaction is further confounded by the activity of transcriptional co-activators and co-repressors that may modify the response of ligand-receptor interactions in different tissues (115). In addition, such receptors can show marked tissue and species difference in their levels of expression, e.g. PPARα This makes extrapolation across tissues and between species difficult.

10.57 The epidemiological data, observations of cancer in man and experimental data support the view that cancer morbidity rates may be influenced by environmental factors. It is now generally considered that the term 'environmental factors' should not be taken to equate only to man-made chemicals, but to gross aspects of diet, to infectious agents and to chemicals that are either natural in origin or produced by cooking practices. Nonetheless the view that identification of specific chemical-risk for carcinogenicity is the primary means of preventing cancer still largely persists and remains the cornerstone of present testing policies including the testing of drugs.

10.58 It is unlikely that an overtly genotoxic chemical (other than perhaps an anti-cancer drug) would be developed as a pharmaceutical in the present regulatory environment. The problem that we face is that there are multiple potential epigenetic mechanisms that might lead to increased cancer and some of these may be species and organ specific. In both rats and mice tumours that have arisen because of non-genotoxic mechanisms and have been preceded by hyperplasia have usually occurred at sites subject to a high background

Table 1. Epigenetic modes of action in rodent carcinogenesis

Liver	Enzyme induction	altered foci	Adenoma/carcinoma
Kidney	alpha-2u-globulin	Hyperplasia	Adenoma/carcinoma
Mammary	Prolactin elevation	Hyperplasia	Adenocarcinoma
Thyroid	TSH elevation	Hyperplasia	Adenoma/carcinoma
Pancreas	Trypsin inhibitors	Hyperplasia	Adenoma/carcinoma
Stomach	Proton pump/H2 antagonist	Hyperplasia	Carcinoid tumours
Testis	LH elevation	Hyperplasia	Leydig cell tumours
Salivary	β2-agonist	Hyperplasia	Adenoma/carcinoma
Muscle	β2-agonist	Hyperplasia	Leiomyoma

incidence (116). In these cases it has been argued that the process is not relevant in assessing risk to man because of a threshold above therapeutic exposure or because of a mechanism that is not applicable to man (117). Some of these are listed in table 1.

10.59 Without prior knowledge of outcome in man it seems prudent to make some assessment of potential carcinogenicity in man from experimental data. The question is what is the appropriate experimental system. The bioassay has been criticized because of cost and of the uncertainty in whether the observations that are made are relevant to man: induction of liver tumours is common in rodents but this is not a common tumour type of man in western industrialised countries. It has been proposed that better mechanistic understanding would improve predictivity and risk assessment and this is encapsulated in the concept of evaluating mode of action of a carcinogenic effect (118). In some instances, as indicated, it may be sufficient to show that the process, as observed in rodents, has no relevance to man: examples of this are the induction of α_{2u}-globulin-associated kidney tumours in male rats and thyroid tumours that result from perturbation of thyroid hormone status (117,118,119). In other instances, the mode of action may prove relevant, but clear thresholds for key events in humans can be identified (89). However, these notable examples are the exceptions and in most cases in which mode of action has clearly been defined it is generally for potent genotoxic carcinogens. Detailed analysis for butadiene, vinyl chloride and benzene has been reported by Albertni et. al. (119), and the analysis clearly shows how this kind of data may be used to further characterize the dose response and assist in the extrapolation of the risk to man.

10.60 Additional features that have been associated with experimental carcinogenesis are: increased oxidative stress; hormone or hormone-like effects; perturbation in the immune system and changes in apoptotic rates in preneoplastic lesions (altered foci in the liver). While it is probable that some of these factors are important in the genesis of cancer in man, the magnitude of the risk is uncertain as even in animals dose-response relationships are poorly documented and, in man, largely unavailable other than in the case of hormonally mediated disease (120).

10.70 Detailed analysis of the relationship of hepatic carcinogenesis to prechronic liver lesions (121) for over 80 chemicals in the NTP bioassay programme, showed that hepatocellular hyperptrophy gave a good prediction of carcinogensis in both rat and mice. This was improved if increase in liver weight was included, however, in both cases the false positive rate was high and markedly so when liver weight was added. Interestingly the concordance between results of the Salmonella mutation assay and carcinogenicity in the liver was poor, supporting the view that the majority of rodent hepatocarcinogens acted through a non-genotoxic mechanism.

10.71 These data are of interest in that they clearly demonstrate that the induction of tumours in rodents is complex and that the relative risk of each of the potential contributing mechanisms

is difficult to quantify. Nonetheless it is clear that epigenetic processes are relevant in assessing risk for cancer in man. Detailed mode of action analysis can help in evaluation of the potential risk of rodent hepatic carcinogens to humans. We have previously alluded to the complex issue of hormonally mediated cancer but even in the case of smoking-induced pulmonary cancer in man epidemiological data would suggest that epigenetic mechanisms also play an important role (120).

Recommendations

10.72 There are many safeguards built in to the drug discovery processes that mitigate the risk of causing cancer in humans. Drugs are designed to interact with well-characterized, disease-modifying molecular targets, the dose is known, and the exposure is controlled, treatment is under medical supervision, and there is always intended benefit to offset risk. The strategies and methods used to date to avoid introducing drugs that might pose a carcinogenic hazard to humans appear to have met their objective. It appears timely therefore to propose improvements to current testing strategies in line with current knowledge and understanding. Modifications to the testing strategy in the short term would include a tiered approach to assessing carcinogenic hazard as follows:

- overtly genotoxic new drug candidates would be identified, with the exception of DNA-reactive drugs for cancer, and eliminated from development prior to the conduct of the conventional rodent bioassay. This would be accomplished by structure-activity considerations and by a battery of recommended genotoxicity tests.
- the bioassay, or any suitable *in vivo* test for carcinogenicity, would be directed at detecting carcinogenic potential other than by direct genotoxicity. Given concern over the interpretation of the conventional animal bioassays, it is proposed to limit this to a single study conducted in a suitable rat strain. The reasons are as follows:
    - there is generally considerably more biochemical and metabolism data in the rat
    - the particular sensitivity of the mouse to the development of hepatic tumours and this often confounds interpretation
    - conducting a long-term rodent study would allow the detection of novel carcinogenic mechanisms that require the whole animal: additional assessment would then be made on a case-by-case basis.
- additional assessment of risk would be conducted on a tissue by tissue basis looking at:
    - proliferative, antiproliferative and apoptotic drivers
    - oxidative stress, genetic damage and repair
    - general effect on the immune system.
    - hormone or hormone like effects
    - pertubation in metabolic function
- the use of genetically modified animals should be on a for-cause basis in order to further characterise mechanism.

10.73 Further modifications to testing strategies in the longer term could be introduced in stepwise fashion based on experience, accumulated knowledge and the evolution of new and advanced methods for assessing carcinogenic hazard, for example:

- as understanding of the modes of action of non-genotoxic carcinogens increases, it may prove possible to develop more focused, shorter-term studies both to detect and to exclude potential human carcinogens and thus eliminate the need for a lifetime study in rodents. This would be based on analysis of the type of precursor effects listed above, augmented as appropriate with novel biomarkers identified in multi-parametric assays (e.g. proteomics). Compounds negative in such an assay would be considered to pose no carcinogenic risk to humans. Compounds that were positive may be tested further, for example in a

lifetime bioassay, to confirm the findings. The decision to proceed with a lifetime rodent bioassay would be based on considerations of dose, duration of exposure, clinical indication and potential benefits.

- this information should be collected in a systematic way with a clear emphasis on dose response and threshold effect. Genomics, proteomics and other of the newer technologies may be used in order to add additional mechanistic understanding rather than for screening. These data would be used to assess mode of action in respect to a positive bioassay result and secondly for extrapolation to man.
- data from animals show that for genotoxic carcinogens the site of carcinogenicity may differ between species. Furthermore, some non-genotoxic modes of action appear site and species specific and so extrapolation across species is difficult, although site concordance for human-relevant carcinogens will be greater, because of the specificity of the mechanism. This is confounded by the relative ease of transformation of animal cells when compared with human cells that further magnifies the complexity for the extrapolation of risk. We therefore propose that greater emphasis should be given to identifying conditions that would enhance the risk for the common human tumours as it is among this group that marginal changes in risk would have the greatest affect. In man both obesity and inflammation may affect tumour incidence at a number of sites. The mechanism is not understood but may be mediated by cytokines or hormones or by changes in local metabolism leading to oxidative injury, the very targets at which many drugs under development are aimed.

Conclusions

There has been continuous improvement in all aspects of drug safety assessment over the past several decades. However, given experiences of unexpected drug withdrawals in recent years, it is timely to review regulatory processes and industry procedures with a view to seeking improvements. Although there appears not to be a need for radical changes to the overall process, there are ways in which new methods and different ways of working, particularly at the interface between regulatory agencies and industry, could effect improvements in risk assessment and drug safety. If these changes are introduced incrementally they will have a significant impact on the ability of pharmaceutical companies and regulators to deliver safer medicines to patients. We hope that some of the recommendations presented in this review may provide further impetus for discussion of the issues.

References

1. Bakke OM, Manocchia M, de Abajo FJ, Kaitin KI, Lasagna L (1995)
Drug safety discontinuations in the United Kingdom, the United States, and Spain from 1975 through 1993: a regulatory perspective.
Clin Pharm Ther 58, 108–117

2. Fung M, Thornton A, Mybeck K, Wu JH, Hornbuckle H, and Muniz E (2001)
Evaluation of the characteristics of safety withdrawal of prescription drugs from worldwide pharmaceutical markets – 1960 to 1999.
Drug Information Journal 35, 293–317

3. Friedman MA, Woodcock J, Lumpkin MM, Shuren JE, Hass AE, and Thornpson LJ (1999)
The safety of newly approved medicines. Do recent market removals mean there is a problem?
JAMA 281, 1728–1734

4. DiMasi JA, Hansen RW, and Grabowski HG (2003)
The price of innovation: new estimates of drug development costs.
J Health Econ 22, 151–185

5. Dickson M, and Gagnon JP (2004)
Key factors in the rising cost of new drug discovery and development.
Nature Drug Discovery 3, 417–429

6. Stephens MDB (2004)
Drug products withdrawn from the market for safety reasons.
In: Stephens' Detection of New Adverse Reactions: Fifth Edition, pp1–91. Eds. John Talbot and Patrick Waller. John Wiley and Sons Ltd.

7. Towler C (2002)
Risk management: the new paradigm.
In: Risk Management: the role of regulatory strategies in the development of new medicines, pp11–18. Eds. Anderson C, McAuslane N, and Walker SR. Centre for Medicines Research International, Institute for Regulatory Science, Epsom, UK

8. Lumley CE, Walker SR, Staunton N, and Grob PR (1986)
The under-reporting of adverse drug reactions seen in general practice.
Pharmaceutical Medicine 1, 205–212

9. Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ, Farrar K, Park BK, Breckenridge AM (2004)
Adverse drug reactions as a cause of admission to hospital: prospective analysis of 18,820 patients.
Brit Med J 329, 15–19

10. Silcock J and Pritchard C (2003)
To Heal and harm: an economic view of drug safety.
Office of Health Economics, London

11. OHE (2003)
Compendium of Health Statistics.
London: Office of Health economics

12. Workshop review (2002)
Risk Management: The role of regulatory strategies in the development of new medicines.
Eds. Anderson C, McAuslane N and Walker SR. Centre for Medicines Research International, Institute for Regulatory Science, Epsom, UK

13. Raine J (2002)
Visions from a European perspective: the MCA.
In: Risk Management: The role of regulatory strategies in the development of new medicines, pp 43–50. Eds. Anderson C, McAuslane N, and Walker S R. Centre for Medicines Research International, Institute for Regulatory Science, Epsom, Surrey

14. Lonngren T (2002)
Factors influencing risk management in the EU.
In: Risk Management: The role of regulatory strategies in the development of new medicines, pp59–65. Eds. Anderson C, McAuslane N and Walker SR. Centre for Medicines Research International, Institute for Regulatory Science, Epsom, UK

15. Roses AD (2002)
Genome based pharmacogenetics and the pharmaceutical industry.
Nature Reviews: Drug Discovery, 1, 541–549

16. Lindpainter L (2002)
The impact of pharmacogenetics and pharmacogenomics on drug discovery.
Nature Reviews: Drug Discovery 1, 463–469

17. Lipinsky C and Hopkins A (2004)
Navigating chemical space for biology and medicine.
Nature 432, 855–865

18. Robinson DE, Pettit SD and Morgan DG (2003)
Use of genomics in mechanism based risk assessment.
In: Toxicogenomics pp194–203. Eds. Inoue T and Pennie WD. Springer-Verlag, Tokyo

19. Ulrich R and Friend SH (2002)
Toxicogenomics and drug discovery: will new technologies help us produce better drugs?
Nature Reviews: Drug Discovery 1, 84–88

20. Hughes TR, Marton MJ, Jones AR, Roberts CJ et al. (2000)
Functional discovery via a compendium of expression profiles,
Cell 102, 109–126

21. Dobson CM (2004)
Chemical space and biology.
Nature 432, 824–828

22. Thomas RS et al (2001)
Identification of toxicologically predictive gene sets using cDNA microarrays.
Mol. Pharmacol. 60, 1–6

23. Jones AK, Buckingham SD, and Sattelle DB (2005)
Chemistry-to-Gene screens in Caenorhabditis Elegans.
Nature Reviews: Drug Discovery 4, 321–330

24. Spitsbergen JM and Kent ML (2003)
The state of the art of the Zebrafish model for toxicologyand toxicologic pathology research – advantages and current limitations.
Toxicol. Pathol. 31, Suppl. 62–87

25. MacGregor JT, Casciano DA and Muller L (2000)
Strategies and testing methods for identifying mutagenic risk.
Mutat. Res. 455, 3–21

26. Searles DB (2005)
Data integration: challenges for drug discovery.
Nature Rev Drug Discovery 4, 45–58

27. Kilty C, Doyle S, Hassett B and Manning F (1998)
Glutathione-S-transferases as biomarkers of organ damage: Applications of rodent and canine GST enzyme immunoassays.
Chem Biol Interact 111–112, 123–135

28. Han WK, Bailly V, Abichandani R, Thadhani R and Bouventre JV (2002)
Kidney injury molecule-1 (KIM-1): a novel biomarker for human proximal tubule injury.
Kidney Int 62, 237–244

29. George DK, Ramm GA, Walker NI, Powell LW and Crawford DH (1999)
Elevated serum Type IV collagen: a sensitive indicator of the presence of cirrhosis in hemachromatosis.
J Hepatol 31, 47–52

30. Newsholme SJ, Thudium DT, Gossett KA, Watson ES, and Schwartz LW (2000)
Evaluation of plasma von Willebrand factor as a biomarker for acute arterial damage in rats.
Toxicol Pathol 28, 688–693

31. Arnal JF, Dinh-Xuan T, Pueyo M, Darblade B and Rami J (1999)
Endothelium derived nitric oxide and vascular physiology and pathology.
Cell Mol Life Sci 55, 1078–1087

32. Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B et al (2001)
Loss of caveolae, vascular dysfunction and pulmonary defects in caveolin-1 gene-disrupted mice.
Science 293, 2449–2452

33. International Life Sciences Institute – Health and Environmental Sciences Institute (2002)
Subcommittee on the development and application of biomarkers of toxicity.
November 4–5, Washington DC

34. US FDA (2004)
Draft Guidance for Industry: Pharmacogenomic data submissions.

35. Gehring PJ, and Blau GE (1977)
Mechanisms of carcinogenesis: dose response.
J Env Path & Toxicol 1, 163–179

36. Moloney D (1993)
Toxicity tests in animals: extrapolating to human risks.
Env Hlth Perspectives 101, 1–24

37. Morgan DG, Kelvin AS, Kinter LB, Fish CJ et al (1994)
The application of toxicokinetic data to dosage selection in toxicology studies.
Toxicol Pathol 22, 112–123

38. Capen C (2001)
Overview of structural and functional lesions in endocrine organs of animals.
Toxicol Pathol 29, 8–33

39. Goodman JI and Watson RE (2002)
Altered DNA methylation: secondary mechanism involved in carcinogenesis.
Ann Rev Pharmacol Toxicol 42, 501–525

40. Swenberg JA, Richardson FC, Boucheron JA, Deal FH, et al (1987)
High- to low- dose extrapolation: critical determinants involved in the dose response of carcinogenic substances.
Env Hlth Perspectives 76, 57–63

41. Green S (1995)
PPAR: a mediator of peroxisome proliferator action.
Mutat Res 333, 101–109

42. Thompson CB (1995)
Apoptosis in the pathogenesis and treatment of disease.
Science 267, 1456–1462

43. MacGregor JT, Farr S, Tucker JD, Heddle JA et al (1995)
New molecular endpoints and methods for routine toxicity testing.
Fund Appl Toxicol 26,156–173

44. Aardema MJ and MacGregor JT (2002)
Toxicology and genetic toxicology in the new era of toxicogenomics: impact of "-omics" technologies.
Mutat Res 499, 1–16

45. Chandler M and Spain M (2004)
Pitfalls to using small animals in preclinical testing are being eliminated.
Preclinica 1, 245

46. Tucker GT, Houston JB, and Huang S-M (2001)
Optimizing drug development: strategies to assess drug metabolism/transport interaction potential – towards a consensus.
Brit J Clin Pharmacol 52, 102–117

47. Bjornsson TD, Callaghan JT, Einolf HJ, Fischer V et al (2003)
The conduct of in vitro and in vivo drug-drug interaction studies: A Pharmaceutical Research and Manufacturers of America (PhRMA) perspective.
Drug Met Disp 31, 815–832

48. Ayrton A and Morgan P (2001)
Role of transport proteins in drug absorption, distribution and excretion.
Xenobiotica 31, 469–497

49. Roberts SA (2001)
High-throughput screening approaches for investigating drug metabolism and pharmacokinetics.
Xenobiotica 31, 557–589

50. Park BK, Kittteringham NR, Pirmohamed M, and Tucker GT (1996)
Relevance of induction of human drug-metabolizing enzymes: pharmacological and toxicological implications.
Brit J Clin Pharmacol 41, 477–491

51. Rostami-Hodjegan A and Tucker GT (2004)
'In silico' simulations to assess the 'in vivo' consequences of 'in vitro' metabolic drug-drug interactions.
Drug Discovery Today: Technologies 1, 441–448

52. Mussen F (2002)
The use of models for benefit-risk assessment.
In: Risk Management: the role of regulatory strategies in the development of new medicines pp19–27. Eds. Anderson C, McAuslane N and Walker SR. Centre for Medicines Research International, Institute for Regulatory Science, Epsom, Surrey, UK

53. Kennedy T (1997)
Managing the drug discovery/development interface.
Drug Dev Today 2, 436–444

54. Monro A and Mehta D (1996)
Are single-dose toxicology studies in animals adequate to support single dose of a new drug in humans?
Clin Pharmacol Ther 59, 258–264

55. Greaves P, Williams A, and Eve M (2004)
First dose of potential new medicines to humans: how animals help.
Nature Rev Drug Disc 3, 226–236

56. Olson H, Betton G, Robinson D et al (2000)
Concordance of the toxicity of pharmaceuticals in humans and animals.
Toxicol Pathol 32, 56–67

57. Zbinden G (1991)
Predictive value of animal studies in toxicology.
Reg Tox and Pharmacol 14, 167–177

58. McClain RM (1994)
Mechanistic considerations in the regulation and classification of chemical carcinogens.
In: Nutritional Toxicolgy. pp273–304 Eds. Kotsonis FN, Mackey M and Hjelle J. Raven Press, NY

59. Cohen SM (1998)
Urinary bladder carcinogenesis.
Toxicol Pathol 26, 121–127

60. Kerns WD and Schwartz LW (Eds.) (2004)
Drug-induced vascular injury – a quest for biomarkers.
Report of the Expert Working Group on Drug-Induced Vascular Injury. Tox Appl Pharmacol,

61. Louden C and Morgan DG (2001)
Pathology and pathophysiology of drug-induced arterial injury in laboratory animals and its implication on the evaluation of novel chemical entities.
Pharmacol Toxicol 89, 158–170

62. Danoff TM, Campbell DA, McCarthy LC, Lewis KF et al (2004)
A Gilbert's syndrome UGT1A1 variant confers susceptibility to tranilast-induced hyperbilirubinemia.
Pharmacogenomics Journal 4, 49–53

63. Roses AD (2002)
Genome-based pharmacogenetics and the pharmaceutical industry.
Nature Reviews: Drug Discovery 1, 541–549]

64. Hetherington S et al (2002)
Genetic variations in HLA-B region and hypersensitivity reactions to abacavair.
Lancet 359, 1121–122

65. Pirmohamed M, Breckenridge AM, Kitteringham NR and Park BK (1998)
Adverse drug reactions.
Brit Med J 316, 1295–1298

66. Berenblum I (ed) (1969)
Carcinogenicity testing. A report of the panel on carcinogenicity of the cancer Research Commission of UICC, Vol 2,
International Union Against Cancer, Geneva

67. Weisburger EK (1981)
Techniques for carcinogenicity studies.
Cancer Res. 41, 3690–3694.

68. Weisburger EK (1983)
History of the bioassay program of the National Cancer Institute.
Prog. Exp. Tumor Res. 26, 187–201.

69. Epstein SS (1979)
The Politics of Cancer.
Anchor Books, New York

70. Roe FJ (1989)
What is wrong with the way we test chemicals for carcinogenic activity.
In: Advances in Applied Toxicology. Ed. Dayan AD and Paine AJ. Ch 2, pp. 2–17. Taylor and Francis Ltd, London.

71. Druckrey H (1967)
Quantitative aspects in chemical carcinogenesis.
In: Potential Carcinogenic Hazards from Drugs. Ed. Truhaut R. UICC Monograph Series Vol 7, pp. 60–78. Springer-Verlag, New York.

72. Berenblum I and Shubik P (1947)
A quantitative approach to the study of the stages of chemical carcinogenesis in the mouse skin
Br. J. Cancer Res. 1, 383–391

73. Berenblum I and Shubik P (1949)
The persistence of latent tumour cells induced in the mouse's skin by a single application of 9,10-dimethyl-1,2-benzanthracene.
Br. J. Cancer Res. 3, 384–386.

74. Foulds L (1954)
The experimental study of tumor progression: a review.
Cancer Research 14, 327–339.

75. Hanahan D and Weinberg RA (2000)
The hallmarks of cancer.
Cell 100, 57–70.

76. Evans MD, Dizdaroglu M and Cooke MS (2004)
Oxidative DNA damage and disease: induction, repair and significance.
Mutation res. 567, 1–61

77. Hahn W and Weinberg RA (2002)
Modelling the molecular circuitry of cancer
Nature Reviews: Cancer 2, 331–341

78. Vogelstein B and Kinzler KW (2004)
Cancer genes and the pathways they control
Nature Medicine 10, 789–798

79. Fung VA, Barrett JC and Huff JE (1995)
The carcinogenesis bioassay in perspective: application in identifying human cancer hazard.
Environ. Health Perspect. 103, 680–683.

80. MacDonald JS and Scribner HE (1999)
The maximum tolerated dose and secondary mechanisms of carcinogenesis.
In: Carcinogenicity. Ed. Kitchin KT. Ch 3, pp. 125–144. Marcel-Dekker Inc, New York.

81. Huff J (1999)
Value, validity and historical development of carcinogenesis studies for predicting and confirming carcinogenic risk to humans.
In: Carcinogenicity. Ed. Kitchin KT. Ch 2, pp. 21–123. Marcel Decker Inc, New York.

82. Snyder RD and Green JW (2001)
A review of the genotoxicity of marketed pharmaceuticals.
Mutat Res 488, 151–169

83. Newbold RR, Banks EP, Bullock B and Jefferson WN (2001)
Uterine adenocarcinoma in mice treated neonatally with genistein.
Cancer Research 61 4325–4328.

84. Ashby J, Waters M D, Preston J, Adler I D, Douglas G R, Fielder R, Shelby M D, Anderson D, Sofuni T, Gopalan H N B, Becking G and Sonich-Mullin C (1996).
IPCS Harmonisation of methods for the prediction and quantification of human carcinogenic/mutagenic hazard, and for indicating the probable mechanism of action of carcinogens
Mutat. Res., 352, 153–157

85. ICH (1995)
Proceedings of the Third International Conference (1995) on Harmonization,
Yokohama, Japan, December, 1995.

86. Busby WF and Wogan GN (1984)
Aflatoxins,
Chemical Carcinogens, Volume 2, Ed. Searle C. American Chemical Society Monograph 182, pp. 945–1136. American Chemical Society, Washington, DC.

87. King, RJB (2000)
Chemical and Radiation Carcinogenesis.
In: Cancer Biology 2 ed. Ch 7, pp. 96–119. Prentice Hall, Edinburgh

88. Cohen SM (1998)
Cell proliferation and carcinogenesis.
Drug Metabolism Reviews 30, 339–357.

89. Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J and Patton DE (2003)
A framework for human relevance analysis of information on carcinogenic modes of action.
Critical Reviews in Toxicology 33, 591–653.

90. ICH (1997)
Guidance for Industry. S1B Testing for Carcinogenicity of Pharmaceuticals.

91. Van der Laan J-W and Spindler P (2002)
The in vivo rodent test system for assessment of carcinogenic potential.
Regulatory Toxicology and Pharmacology 35, 122–125.

92. Van Oosterhout JP, Van der Laan JW, De Waal EJ, Olejniczak K, Hilgenfeld M, Schmidt V and Bass R (1997)
The utility of two rodent species in carcinogenic risk assessment of pharmaceuticals in Europe. Regulatory Toxicology and Pharmacology 25, 6–17.

93. Grasso P and Price S (1999)
The cancer bioassay. In: Carcinogenicity.
Ed. Kitchin KT. Ch 1, pp. 1–19. M Dekker Inc, New York.

94. Gio Batta Gori (2001)
The costly illusion of regulating the unknowable risk.
Reg. Toxicology and pharmacology 34, 205–212.

95. Maronpot RP, Flake G and Huff J (2004)
Relevance of animal carcinogenesis findings to human cancer prediction and prevention.
Toxicologic Pathology.32, 40–48.

96. Pritchard JB, French JE, Davis BJ and Haseman JK (2003)
The role of transgenic mouse models in carcinogen identification.
Environmetnal Health Perspectives 111, 444–454.

97. Ashby J (1997)
Identifying potential human carcinogens – the role of genetically altered rodents.
Toxicologic Path. 25, 241–243.

98. Rangarajan A and Weinberg RA (2003)
Comparative biology of mouse versus human cells: modeling human cancer in mice.
Nature Reviews: Cancer 3, 952–959

99. Leakey JEA, Seng, JE and Allaben WT (2004)
Influence of body weight, diet and stress on aging, survival and pathological endpoints in rodents: implications for toxicicty testing and risk assessment.
Regulatory Research Perspectives. 4(2), 1–28

100. Bingham S and Riboli E (2004)
Diet and cancer-the European prospective investigation into cancer and nutrition.
Nature reviews: Cancer 4,206–215

101. Root-Bernstein RS and Bernstein MI (1999)
A simple stochastic model of development and carcinogenesis.
Anticancer Research 19, 4869–4876.

102. Henderson BE and Feigelson HS (2000)
Hormonal carcinogenesis.
Carcinogenesis. 21, 427–433.

103. Weiss NS and Szekely DR and Austin DF (1976)
Increasing incidence of endometrial cancer in the United States.
N Engl. J Med. 294, 1259–1262

104. Potischman N, Hoover RN, Brinton LA, Siiteri P, Dorgan JF, Swanson CA, Berman ML, Mortel R, Twiggs LB, Barrett RJ, Wilbanks GD, Persky V and Lurain JR (1996)
Case-control study of endogenous hormones and endometrial cancer.
J.Natl. Cancer Inst. 88, 1127–1135.

105. Calle EE and Kaaks RF (2004)
Overweight, obesity and cancer: epidemiological evidence and proposed mechanism.
Nature Reviews: Cancer 4, 579–591.

106. Zeleniuch-Jacquotte A, Shore RE, Koenig KL, Akhmedkhanov A, Afanasyeva Y, Kato I, Kim MY, Rinaldi S, Kaaks R and Toniolo P (2004)
Postmenopausal endogenous oestrogen, androgen, and SHBG and breast cancer long-term results of a prospective study.
Br. J. Cancer 90, 153–159.

107. Dao TL (1981)
The role of ovarian steroid hormones in mammary carcinogenesis.
In: Hormones and Breast Cancer. Eds. Pike MC, Siiteri PK and Welsch WC., (Banbury Report No 8) pp. 281–295. Cold Spring Harbor Laboratory Press, New York.

108. Allred CD, Allred KF, Ju YH, Clausen LM, Doerge DR, Schantz SL, Korol DL, Wallig MA and Helferich WG (2004)
Dietary genistein results in larger MNU-induced, eostrogen-dependent mammary tumors following ovariectomy of Sprague-Dawley rats.
Carcinogenesis 25, 211–218

109. Powles TJ (2004)
Anti-oestrogenic prevention of breast cancer-the make or break point.
Nature Reviews: Cancer. 2, 787–794

110. Sporn MB and Suh N (2004)
Chemoprevention: an essential approach to controlling cancer.
Nature Reviews: Cancer 2, 537–543.

111. Feigelson HS and Henderson BE (1996)
Estrogens and breast cancer.
Carcinogenesis 17, 2279–2284.

112. Rogan EG, Badawi AF, Devanesan PD, Meza JL, Edney JA, West WW, Higginbotham SM and Cavalieri EL (2003)
Relative imbalance in estrogen metabolism and conjugation in breast tissue of women with carcinoma: potential biomarkers of susceptibility to cancer.
Carconogenesis 24, 697–702

113. Liehr JG, Sirbasku DA, Jurka E, Randerath K, Randerath E (1988)
Inhibition of estrogen-induced renal carcinogenesis in male Syrian hamsters by tamoxifen without decrease in DNA adduct levels.
Cancer Research 48, 779–783.

114. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M and Chambon P (1995)
The nuclear receptor superfamily the second decade.
Cell, 83, 835–839.

115. Katzenellebogen BS and Katzenellebogen JA (2002)
Defining the "S" in SERMSs.
Science 295, 2380–2381.

116. Hardisty JF (1985)
Factors influencing laboratory animal spontaneous tumour profiles.
Toxicologic pathology 13, 95–104.

117. Ettlin RA and Prentice DE (2002)
Unexpected tumour findings in lifetime rodent bioassay studies-what do we do.
Toxicology Lett. 128,17–33.

118. Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM and Younes M (2001)
IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis.
Regulatory Toxicology and Pharmacology 34, 146–152.

119. Albertini R, Clewell H, Himmelstein MW, Morinello E, Olin S, Preston J, Scarano L, Smith MT, Swenberg J, Tice R and Travis C (2003)
The use of non-tumor data in cancer risk assessment: reflections on butadiene, vinyl chloride, and benzene.
Regulatory Toxicology and Pharmacology 37, 105–132

120. Clayson DB and Kitchin KT (1999)
Interspecies differences in response to chemical carcinogens.
In: Carcinogenicity. Ed. Kitchin KT. Ch 29, pp. 837–880. M Dekker Inc, New York.

121. Allen DG, Pearse G, Haseman JK and Maronpot RR (2004)
Prediction of rodent carcinogenesis: an evaluation of prechronic liver lesions as forecasters of liver tumours in NTP carcinogenicity studies.
Toxcicologic Pathology 32, 393–401

Abbreviations

2-AAF: 2-acetyl aminofluorene
ACTH: adreno-cortical trophic hormone
ADME: absorption, disposition, metabolism, elimination
AUC: area under the concentration-time curve
B(a)P: benzo[a] pyrene
Cmax: maximal concentration
CNS: central nervous system
CYP: cytochrome P
DNA: deoxyribonucleic acid
EPA: Environmental Protection Agency (USA)
EPAR: European Public Assessment Report
EU: European Union
FDA: Food and Drug Administration (USA)
FMO: Flavine-containing monoxygenase
HESI: Health and Environmental Sciences Institute (USA)
HIV: human immuno-deficiency virus
HLA: human lymphocyte antigen
IARC: International Agency for Research on Cancer (France)
ICH: International Conference on Harmonisation of Technical Requirments for the Registration of Human Pharmaceuticals
ILSI: International Life Sciences Institute
IPCS: International Program on Chemical Safety
MTD: maximum tolerated dose
NCI: National Cancer Institute (USA)
NIEHS: National Institute of Environmental Health Sciences (USA)
NTP: National Toxicology Program (USA)
PB-PD/TD: physiologically-based pharmaco/toxico-dynamic (models)
PK: pharmacokinetic
PPAR: peroxisome proliferator-activated receptor
QSAR: quantitative structure-activity relationship
R&D: Research and Development
SERM: selective (o)estrogen receptor modulator
SULT: sulphotransferase
TNF: tumour necrosis factor
UDS: unscheduled DNA synthesis
UGT: UDP glucuronosyl transferase
UK: United Kingdom