the molecule in large quantities.

Further advances included a centrifuge that enabled Jonas Salk to isolate the poliovirus, which was one of the key steps in developing an early vaccine against polio. Instruments from Beckman have also played important roles in screening large numbers of potential drug candidates to treat diseases such as AIDS and cancer. Additional breakthroughs were oxygen analyzers that monitored air composition in incubators to prevent premature babies from going blind due to oxygen deprivation.

In 1982, Beckman Instruments merged with SmithKline Corporation in a deal that was reportedly worth around US$1 billion. The merger catapulted Beckman and his wife into the category of one of the wealthiest couples in California. As such, it marked a stage in Arnold Beckman's life where he devoted himself increasingly to philanthropy. His earlier efforts in this direction had resulted in the foundation of the Arnold and Mabel Beckman Foundation in 1977, an organization that has contributed more than US$400 million in support of scientific research, medicine and education.

A modest, polite and humble man who valued personal integrity, Beckman's achievements were honoured by many awards including the 2004 Lifetime Achievement Award from the National Inventor's Hall of Fame, the 1988 National Medal of Technology, the 1989 Presidential Citizen's Medal, the 1999 Medal of Science as well as the Public Welfare Medal from the National Academy of Sciences.

Arnold Beckman died on 18th May. He was 104.

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An integrated, multidisciplinary approach for drug safety assessment

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This feature article describes a comprehensive approach in drug safety assessment, with which human drug toxicity is evaluated based on the multiple disciplines of pharmacology, chemistry, drug metabolism and toxicology. Mechanistic understanding of drug toxicity and the determination of potential risk factors are proposed to be key parameters for an accurate prediction of human drug toxicity.

One of the major challenges in drug development is the accurate assessment of human drug toxicity. A review by Lasser *et al.* [1] found that, in the past four decades, 2.9% of the marketed drugs were withdrawn from the market due to severe adverse drug effects, with seven drugs approved and marketed since 1993 and subsequently withdrawn to be associated with over 1000 deaths. Lasser *et al.* [1] also estimated that from 1975–1999, 10.2% of approved drugs required black box warnings to be added after marketing. Examples of the recently withdrawn drugs and drugs that required box warnings are shown in Tables 1 and 2, respectively.

The occurrence of unexpected adverse drug effects after a drug is approved and marketed illustrates that the current practice in safety evaluation, although effective in most cases, allows several drugs with unacceptable safety profiles to be marketed. What is particular disturbing is that drugs with undesirable adverse effects are marketed in spite of the estimated US$800 million and 12–15 years in cost and time required, respectively, to develop a single drug [2]. The liabilities to the pharmaceutical industry of market drug withdrawal are obvious, including losses in resources and time spent in drug development, loss in potential revenues and a compromised public image. There is also an ethical issue of causing harm to the patient population.

There is therefore an urgent need to develop approaches to enhance the accuracy of the prediction of human drug safety.

Why can't we predict human drug toxicity?

According to current practice, drug safety is initially evaluated preclinically in laboratory animals, generally in three animal species such as mouse, rat and dog. Preclinical safety study results are submitted to the US Food and Drug

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Table 1. Examples of drugs that were withdrawn from the market due to unexpected adverse drug effects[a]

Drug (chemical)	Indication	Adverse effects	Year approved	Year withdrawn
Alosetron (Lotronex)	Irritable bowel syndrome	Ischimic colitis	2000	2000
Bromfenac (Duract)	Analgesic	Hepatotoxicity	1997	1998
Cerivastatin (Baycol)	Cholesterol lowering	Rhabdomyolysis	1997	2001
Cisapride (Propulsid)	Gastric reflux	Cardiotoxicity	1993	2000
Dexfenfluramine (Redux)	Obesity	Heart valve damage	1996	1997
Grapafloxacin (Raxar)	Antibiotic	Cardiac arrhythmia	1996	1997
Mibefradil (Posicor)	Calcium channel blocker	Drug–drug interactions	1997	1998
Rapacuronium bromide (Raplon)	Surgery relaxant	Bronchospasm	1997	2001
Troglitazone (Rezulin)	Type II diabetes	Hepatotoxicity	1997	2000

[a]Information obtained from the US FDA webpage: http://www.fda.gov/medwatch

Administration (FDA) for the approval of Investigative New Drug (IND) status for subsequent clinical trials in human subjects.

This standard approach appears to be adequate – toxicity is first defined in three species of animals followed by human trials. Drug candidates with unacceptable toxicity toward laboratory animals and humans should be detected by the preclinical safety trials. Drugs with toxicity only in humans and not in nonhuman animals should be detected in the clinical trials. However, this approach has several pitfalls:

- Human-specific drug toxicity cannot be evaluated in laboratory animals. Species difference in response to toxicants is a well-established phenomenon, with the major reasons being differences in xenobiotic transformation [3] and target cell sensitivity [4]. Animals and humans can also differ in effector pathways to toxic responses.
- The assumption that all drugs that are toxic to humans would be detected in clinical trials might not be true because of technical limitations (only relative noninvasive techniques can be applied), nonrepresentation of the patient population, and limited number of patients in the trials (a major reason for the failure in the detection of rare but severe drug toxicity, such as idiosyncratic drug toxicity).

A comprehensive approach to drug safety evaluation

Classical toxicologists rely on Paracelsus' Principle [5]:

'All things are poison and nothing is without poison. Solely the dose determines that a thing is not a poison.'

This principle of toxicology derived in the 15th century is the cornerstone of today's traditional practice of toxicology. Dose–response relationship is the most important dataset from which safety is determined. For drugs, safety is estimated based on therapeutic index, a ratio of the toxic dose to the dose required for efficacy. It is because of Paracelsus' Principle that toxicologists in general believe that safety can be estimated based on dose–response relationships without a need for mechanistic definition.

It is proposed here that drug toxicity is a complex biological phenomenon, which should be defined based not only on dose–response relationship, but also

Table 2. Examples of drugs recently required to add safety alerts (box warning)

Drug (chemical)	Indication	Adverse effects	Year approved	Year safety warning added
Accutane (isotretinoin)	Severe nodular acne	Pregnancy (interaction with birth control pills); deformity	1982	2003
Arava (leflunomide)	rheumatoid arthritis	Hepatotoxicity	1998	2003
ORLAAM (levoacetyl methadol)	Opiate dependency	Arrythmia	1993	2001
Prandin (repaglinide)	Type II diabetes	Drug–drug interactions	1997	2003
Risperdal Consta (risperidone)	Schizophrenia	Stroke	2003	2003
Viramune (nevirapine)	HIV	Hepatotoxicity	1996	2004

[a]Information obtained from the US FDA webpage: http://www.fda.gov/medwatch/

as a function of pharmacology, chemistry, metabolism, and environmental and genetic risk factors.

Pharmacology

As drugs are developed to be pharmacologically active, it is only logical that one should understand the safety concerns, if any, of the intended pharmacological effects. The toxicological ramification of the interaction of the drug candidate with the intended target in the target and nontarget tissues, and the likelihood of interactions with unintended targets, should be defined. This is especially important for a novel target with little preexisting clinical data.

That pharmacological effects are related to drug toxicity can be observed with anticancer agents. Recently, novel targets have been proposed for anticancer drugs based on the novel discoveries in tumor cell and molecular biology, including molecular controls of cell division, apoptosis, macromolecular processing, invasion and angiogenesis. As many of these molecular targets are also present in nontumor tissues, there is a need to assure that the unintended toxicity toward normal tissue is significantly less than that towards the cancer cells, or at least develop a rationale for why the target remains an appropriate target, even though toxicity to normal tissues is likely to occur (i.e. damage to normal tissue can be monitored and managed).

Another example of pharmacologically related toxicity is a target in the cell signaling pathways that might have myriad cellular functions. Antagonists or agonists to a molecular target to cure a disease or to alleviate certain disease symptoms can lead to undesirable side effects, owing not only to the effects of the agent on the 'normal' functions of the target but also to the interactive cascade of events set off by engagement of the molecular pharmacology target, culminating in organ damage.

An interesting case of unexpected pharmacology-related adverse effect is associated with the biologic infliximab – a monoclonal antibody against tumor necrosis factor, indicated for the treatment of rheumatoid arthritis and Crohn's disease. The inhibitory effects of infliximab on macrophage activation is the mechanism for its desired anti-inflammatory effects. However, diminished macrophage activities cause an increased susceptibility of the patients towards infection [6].

A conscientious effort to evaluate pharmacologically related adverse drug effects should enable one to minimize the probability in the selection of a problematic target and to identify management strategies early on to eliminate unexpected postmarketing adverse events. This evaluation is aided by the recent explosion in cell and molecular biology findings, the near-complete definition of the human genome, and the various informatics tools.

Chemistry

Many times during drug discovery, a project is abandoned because the major chemical structure (scaffolding) chosen has undesirable toxicity which cannot be overcome via modification of the side chains. It is therefore important to make sure that chemical structures with a high probability of success are chosen earlier in the program, especially when multiple chemical structures are found positive in the early screening process for efficacy.

One early approach is to evaluate whether the major chemical structure chosen has a history of safety-related problems. *In silico* approaches continue to be developed to correlate chemical structure with toxicity. At the time of writing, it is generally believed that *in silico* approaches are adequate for the prediction of genotoxicity such as the Ames Salmonella/histidine-revision assay, but are not yet applicable for other types of toxicity (e.g. hepatotoxicity and cardiotoxicity) [7,8]. A practical approach is to perform *in vitro* toxicological assays early in drug discovery to allow the selection of the chemical structures with the least toxicological liabilities. Combined use of efficacy screens and *in vitro* toxicity screens enables one to evaluate whether the chemical structures important for efficacy (pharmacophores) can be distinguished from those responsible to toxicity (toxicophores).

Drug metabolism and pharmacokinetics

The relationship between drug metabolism and toxicity cannot be overemphasized. Metabolism-related toxicity is responsible for most adverse drug effects in the liver, the organ where first-pass drug metabolism occurs. Species differences in drug metabolism represent a key reason for species differences in drug toxicity. Pharmacokinetic drug–drug interaction, the effect of one drug on the metabolic clearance of a co-administered drug, is a major mechanism of adverse drug effects. Although organ-specific toxicity can be a function of metabolism, as in the case of the liver, it also can be due to bioaccumulation, as for some CNS toxicants.

An important development in drug metabolism research is the general acceptance of human tissue-derived systems, especially human liver-derived systems, such as liver microsomes and fresh or cryopreserved human hepatocytes, in the evaluation of human drug metabolism. Applications of human-based experimental systems include the evaluation of intestinal uptake, metabolic stability, metabolite identification, and pharmacokinetic drug–drug interactions [9,10]. Drug metabolism data obtained with human *in vitro* systems provide critical safety information, such as the identification of toxification and detoxification

pathways. Drug-metabolism data are routinely used to guide the selection of the most 'relevant' animal species for safety and pharmacology studies based on their similarities to human in metabolism. *In vitro* human systems enable the development of human metabolism data before a drug candidate is administered to humans *in vivo*.

Recently, a consensus is being reached on drug-metabolism properties that appear to occur frequently in drugs with fatal idiosyncratic drug toxicity. These properties include the formation of reactive metabolites, enzyme induction, P450-related toxification pathways and propensity for drug–drug interactions [11–14]. These common properties are consistent with the proposed mechanisms of idiosyncratic drug toxicity and can be used to guide the elimination of drug candidates with high probability of causing idiosyncratic drug toxicity.

Toxicology

Well-designed toxicity studies are key to safety assessment. The commonly applied approach of a battery of genotoxicity assays, and studies with laboratory animals including acute, subchronic and chronic studies, developmental toxicity studies, and life-time carcinogenicity assays are invaluable in the evaluation of drug toxicity. A key in the use of laboratory animals is to use species that are most 'relevant' to human, whenever possible. Key toxicity determinants that are different between the laboratory animals used and humans should be clearly defined to aid data interpretation.

An expert group recently concluded that human-based experimental systems are useful in aiding the prediction of human drug toxicity and that *in vitro* systems with primary cells, especially from human organs, serve as promising experimental systems for the evaluation of human-specific drug properties [9]. Examples of such assays are the use of human blood-vessel endothelial cells in the evaluation of vascular toxicity [15], human hepatocytes in the evaluation of hepatotoxicity [9,10,16], and human kidney proximal tubule cells for nephrotoxicity [17]. A recent development is an integrated multiple organ culture system that integrates cells from multiple organs for the evaluation of drug toxicity [18]. An advantage of *in vitro* experimental systems using primary cells that retain human-specific properties is that the results are likely to be relevant to human. A caveat of the use of *in vitro* systems is that care must be taken to avoid erroneous conclusions due to *in vitro* artifacts and the performance of experiments under physiologically irrelevant conditions (e.g. dose levels that would not be achievable in human *in vivo*), and to fully recognize the limitations of the *in vitro* system [e.g. the differences between *in vitro* and *in vivo* milieu and oxygenation levels; the *in vitro* absence of blood circulation, excretion, multiple organ interactions (which may be improved via the use of the integrated co-culture system, idMOC [18]), and an intact immune system]. For primary cells, one also needs to ensure that individual differences reflect true inherent properties rather than artifacts introduced by the isolation and culturing processes.

Toxicology studies should be performed using an investigative approach. Adverse effects should be further defined mechanistically, using endpoints and experimental systems which might not be routine. *In vitro* approaches using primary cells from human or animal organs, high-content assays such as genomics, proteomics, metabolomics, are examples of experimental tools for mechanistic studies. An example of an application of novel technologies is a recent study with troglitazone, a drug successfully marketed for the treatment of type II diabetes, but withdrawn due to its association with fatal liver toxicity. Troglitazone was found to induce a significantly higher number of gene-expression changes for a battery of toxicologically-relevant genes than the relatively nontoxic structure analogs rosiglitazone and pioglitazone [19]. Based the differences between toxic and nontoxic compounds in their effects on gene expression, one can construct possible mechanisms of toxicity that can be experimentally verified and applied towards the prediction of human effects. These mechanistic studies enable one to make scientific decisions about whether to proceed or not. Although it might appear that additional resources and time are being spent performing the mechanistic studies, the ultimate enhancement of clinical success rate should provide a net benefit to the pharmaceutical industry.

Risk-factor identification

Although it is true that dose is key to toxicity, the dose that is toxic to different individuals might differ because of physiological, environmental and genetic factors. A dose that is nontoxic to a majority of the patient population might be fatal to an individual, owing to one or more of these factors (risk factors). For example, the analgesic acetaminophen is a safe drug but is known to cause fatal hepatotoxicity, especially in individuals who consumed alcohol. Alcohol has been identified as a risk factor for acetaminophen, presumably due to the induction of the metabolic 'toxification' pathway (e.g. cytochrome P450 isoform 2E1), as well as the reduction of detoxifying protective cofactors (e.g. reduced glutathione) [20].

The risk-factor approach in drug toxicity is implied in a recent proposed hypothesis for idiosyncratic drug toxicity, the multiple parameter

hypothesis, which states that the low frequency of idiosyncratic drug toxicity is due to a concurrence of multiple independent events [11]. Based on the hypothesis, the probability for idiosyncratic drug toxicity (P_{idt}) is a product of the following independent probabilities: (a) exposure to the drug (e.g. dose; P_{exp}); (b) inherent biological properties of the drug due to its chemical structure (e.g. the ability to form reactive metabolites; P_{chem}); (c) environmental risk factors (e.g. co-exposure to interacting foods or drugs; $P_{environ}$); and (d) host risk factors (e.g. genetic determinant for drug toxicity; disease conditions predisposing an individual to drug toxicity; P_{host}).

$$P_{idt} = P_{exp} \times P_{chem} \times P_{environ} \times P_{host}$$

A corollary of the multiple parameter hypothesis is that risk factors exist that can dramatically enhance a drug's toxic potential. Individuals in an environment at a specific point in time might have the 'right' combination of risk factors that, if administered a drug with idiosyncratic toxic properties, would succumb to its toxicity.

$$Tox_{individual} = f\,(D, Tox_{inherent}, RF_{total})$$

Where $Tox_{individual}$ is the toxicity of the drug in a particular patient (e.g. dose of drug to cause liver failure) at the specific time of administration; D is the dose of the drug administered; $Tox_{inherent}$ is the inherent toxicity of the drug as related to the chemical structure; and RF_{total} represents a single risk factor as a result of all risk factors, which can be physiological, environmental and genetic factors that the patient has or is subjected to that would enhance toxicity.

The definition of risk factors is aided by mechanistic understanding of the key toxic pathways (e.g. metabolic pathways; primary and secondary toxic effectors). For instance, if toxicity is due to the formation of toxic metabolites, one needs to define potential risks due to individual with enhanced toxification and/or reduced detoxification pathways as well as pharmacokinetic drug–drug interactions that may increase a patient's body burden of toxic metabolites.

Currently, risk factors associated with drug metabolism (e.g. induction of toxifying metabolic activities; reduction of detoxifying activities, polymorphism of metabolic enzymes and pharmacokinetic drug–drug interactions) probably are better defined than risk factors not associated with drug metabolism [21]. Current investigation of nonmetabolic risk factors for established drugs (e.g. inflammation, disease status) should help to define risk factors of new drugs. There is evidence, for instance, that inflammation is a risk factor for drug-induced liver failures [21,22].

Box 1. Examples of toxicologically-relevant questions based on the comprehensive approach to evaluate drug safety

Pharmacology

Are there adverse effects as a result of the desired drug–target interactions?
Is the molecular target present in nontarget organs or tissue? If so, would there be adverse effects due to interactions with the pharmacological target in nontarget tissues?
Are pharmacological drug–drug interactions likely?

Chemistry

Are there known adverse drug effects associated with the major chemical structure (scaffolding)?
Are there chemical side chains with known toxicity?
Can the toxicophore be separated from the pharmacophore?

Drug metabolism and pharmacokinetics

Is the chemical entity biotransformed? If so, is it rendered more (toxification) or less (detoxification) toxic?
Are the human metabolites similar or different from the metabolites formed in laboratory animals? Which animal species is most like human?
How rapidly is the chemical entity cleared?
Is the chemical entity or its metabolites accumulated in specific organs?
Are pharmacokinetic drug–drug interactions likely to occur?

Toxicology

Is there toxicity observed with the chemical entity *in vitro* and *in vivo*?
Is the toxicity associated with the chemical scaffolding or its side chain?
Does drug metabolism or organ distribution contribute to the toxicity observed?
Would there be species differences in toxicity? If so, why?
What are the expected risk factors for toxicity?

Risk factors

Physiological: would specific age, gender, race and disease state enhance toxicity? Is the patient population known to be more susceptible to certain types of adverse drug effects (e.g. hepatotoxicity in the diabetic population)?
Environmental: are there environmental conditions that can enhance toxicity? Are there co-administered drugs or foods that would lead to toxicity due to either pharmacokinetic or pharmacological interactions?
Genetic: are there genetic determinants of susceptibility to drug toxicity? For instance, is there known genetic polymorphism of the toxifying or detoxifying pathways (e.g. CYP2C9; uridine-dependent glucuronosyl transferase) in the human population?

A thorough understanding of risk factors based on known pharmacology, chemistry, drug metabolism, toxic mechanism and patient characteristics will aid key decisions in drug development. An estimation of the probability of human populations with unfavorable risk factors (to enable a go/no-go decision), and the feasibility of the identification of at-risk populations (to enable safe administration of the drug), is information that could be critical to the development of safe drugs.

Conclusion

The proposed comprehensive approach should be a integral part of the drug discovery and development process, from target selection to clinical trials. Examples of scientific questions directing this comprehensive approach are listed in Box 1. A discussion of possible concerns is presented in Box 2. An example of a timeline for the application of the approach is illustrated in Figure 1.

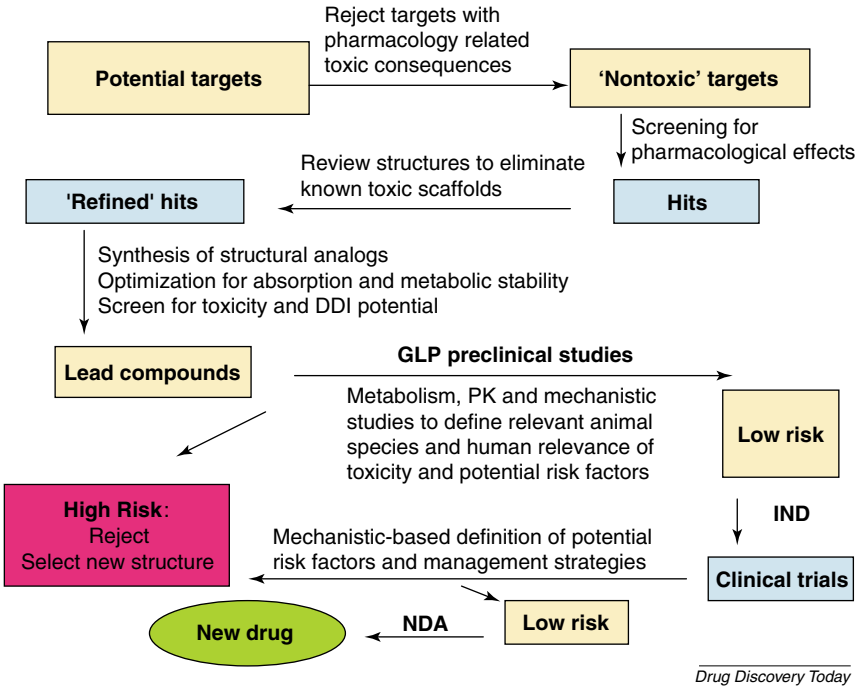

Figure 1. A proposed timeline for the application of the comprehensive approach for drug-safety assessment. The abbreviations used are as follows: DDI: Drug–drug interaction; IND: Investigative new drug application to US Food and Drug Administration (FDA) for clinical trials; NDA: New drug application to US FDA for drug marketing.

This comprehensive approach is best practiced with a team approach, with the team members having in-depth knowledge of the multiple scientific disciplines described. An example of a comprehensive drug-safety evaluation team is one led by a toxicologist, with team members with expertise in pharmacology, chemistry, drug metabolism or pharmacokinetics, genetics and other scientific disciplines (e.g. epidemiology, statistics, medicine), as needed.

The underlying principle for the proposed approach is that the accuracy

Box 2. Possible concerns and rebuttal to the concerns regarding the implementation of the comprehensive approach in toxicological evaluation

Complications with regulatory approval

Current practice: produce the 'cleanest' data package possible for submission to regulatory agencies and avoid experimentations that might 'complicate' the package.

Problems with current practice: data interpretations based purely on standardized, routine tests, without further investigative experimentations, might not allow accurate prediction of human drug safety.

Recommendation: via objective and scientific experimental approaches and data analysis, a drug candidate that is concluded to be safe to humans should rightly receive regulatory approval, and is in fact a more efficient approach than the current approach of a minimum data package and optimistically interpreting adverse data.

Prolonged experimentations

Concerns: as it is difficult for toxicity to be clearly defined, this comprehensive approach might require experimentation, which could escalate, thereby delay the decision-making process.

Rebuttal: additional investigative work early in drug development should be more than compensated for by the decrease in failure rates in the clinic.

NonGLP studies

Concerns: investigative approaches might include experimentations that are not performed under Good Laboratory Practice (GLP) and therefore might not be acceptable for inclusion in the safety submission package for IND.

Rebuttal: although it is possible that some of the key nonGLP studies might need to be repeated as part of the GLP studies, the experimental design can benefit from the initial studies and there should not be unexpected findings in the GLP 'definitive' studies.

of drug safety can be enhanced via a multidisciplinary collaboration to allow a clear understanding of toxicity related drug properties, including pharmacology, chemistry, drug metabolism, toxicology and risk factors. Recent advances in informatics, high-content assays such as genomics, proteomics, metabolomics, *in vitro* molecular and cellular experimental systems, should aid this comprehensive approach to evaluate drug safety.

As drug toxicity is a key determinant of success, secondary only to efficacy, it should be an integral part of drug discovery and development. It is envisioned that the proposed integrated, multidisciplinary approach will enhance the efficiency of drug development by minimizing the probability of the development of drugs with unacceptable toxicity. The apparent additional costs associated with this comprehensive approach, especially resources and time spent on mechanistic studies and risk-factor assessments, should be more than compensated for by the expected enhancement of drug development efficiency, such as the decrease in clinical trial failures due to toxicity and the minimization of market drug withdrawal due to adverse effects.

References

1 Lasser, K.E. *et al.* (2002) Timing of new black box warnings and withdrawals for prescription medications. *J. Am. Med. Assoc.* 287, 2215–2220

2 Dimasi, J.A. *et al.* (2003) The price of innovation: new estimates of drug development costs. *J. Health Econ.* 22, 151–185

3 Easterbrook, J. *et al.* (2001) A comparison of aroclor 1254-induced and uninduced rat liver microsomes to human liver microsomes in phenytoin O-deethylation, coumarin 7-hydroxylation, tolbutamide 4-hydroxylation, S-mephenytoin 4'-hydroxylation, chloroxazone 6-hydroxylation and testosterone 6beta-hydroxylation. *Chem. Biol. Interact.* 134, 243–249

4 Volpe, D.A. *et al.* (1996) Myelotoxic effects of the bifunctional alkylating agent bizelesin on human, canine and murine myeloid progenitor cells. *Cancer Chemother. Pharmacol.* 39, 143–149

5 Borzelleca, J.F. (2000) Paracelsus: herald of modern toxicology. *Toxicol. Sci.* 53, 2–4

6 Centocor Dear Health Professional Letter October 5, 2001.

7 Hulzebos, E.M. and Posthumus, R. (2003) (Q)SARs: gatekeepers against risk on chemicals? *SAR QSAR Environ. Res.* 14, 285–316

8 Zmuidinavicius, D. *et al.* (2003) Progress in toxinformatics: the challenge of predicting acute toxicity. *Curr. Top. Med. Chem.* 3, 1301–1314

9 MacGregor, J.T. *et al.* (2001) *In vitro* human tissue models in risk assessment: report of a consensus-building workshop. *Toxicol. Sci.* 59, 17–36

10 Li, A.P. (2001) Screening for human ADME/Tox drug properties in drug discovery. *Drug Discov. Today* 6, 357–366

11 Li, A.P. (2002) A review of the common properties of drugs with idiosyncratic hepatotoxicity and the "multiple determinant hypothesis" for the manifestation of idiosyncratic drug toxicity. *Chem. Biol. Interact.* 142, 7–23

12 Boelsterli, U.A. *et al.* (1995) Idiosyncratic liver toxicity of nonsteroidal antiinflammatory drugs: molecular mechanisms and pathology. *Crit. Rev. Toxicol.* 25, 207–235

13 Williams, D.P. and Park, B.K. (2003) Idiosyncratic toxicity: the role of toxicophores and bioactivation. *Drug Discov. Today* 8, 1044–1050

14 Uetrecht, J. (2002) N-oxidation of drugs associated with idiosyncratic drug reactions. *Drug Metab. Rev.* 34, 651–665

15 Schleger, C. *et al.* (2004) Development of an *in vitro* model for vascular injury with human endothelial cells. *ALTEX* 21 (Suppl 3), 12–19

16 Li, A.P. *et al.* (1999) Cryopreserved human hepatocytes: characterization of drug-metabolizing enzyme activities and applications in higher throughput screening assays for hepatotoxicity, metabolic stability, and drug-drug interaction potential. *Chem. Biol. Interact.* 121, 17–35

17 Li, W. *et al.* (2003) Use of cultured cells of kidney origin to assess specific cytotoxic effects of nephrotoxins. *Toxicol. In Vitro* 17, 107–113

18 Li, A.P. (2004). A novel cell culture tool. U. S. P. T. O. application number 10/751983.

19 Kier, L.D. *et al.* (2004) Applications of microarrays with toxicologically-relevant genes (tox genes) for the evaluation of chemical toxicants in Sprague Dawley rats *in vivo* and human hepatocytes *in vitro*. *Mutat. Res.* 549, 101–113

20 Schiodt, F.V. *et al.* (2002) Influence of acute and chronic alcohol intake on the clinical course and outcome in acetaminophen overdose. *Aliment. Pharmacol. Ther.* 16, 707–715

21 Boelsterli, U.A. (2003) Diclofenac-induced liver injury: a paradigm of idiosyncratic drug toxicity. *Toxicol. Appl. Pharmacol.* 192, 307–322

22 Roth, R.A. *et al.* (2003) Inflammation and drug idiosyncrasy–is there a connection? *J. Pharmacol. Exp. Ther.* 307, 1–8