Toxicology in the Drug Discovery and Development Process

Therapeutic agents, or drugs, have been used throughout human history. Indeed, the eagerness and willingness to ingest therapeutic substances that would in other circumstances be regarded as poisonous is a distinguishing human trait. All chemical entities, however, are toxic at sufficiently high doses. The therapeutic index (TI) is defined as the ratio of the toxic dose to the therapeutic dose. The term has been used in defining "safety margins" in clinical studies. For nonclinical toxicology evaluations, designed to provide data supporting safety in a clinical trial, the safety term used most often is the margin of safety (MOS). The MOS relates the No Observed Adverse Effect Level (NOAEL; a dose that produces no relevant adverse effects) to the maximum targeted dose in a clinical trial or a therapeutically effective dose in a nonclinical model. In the discussion that follows, the term MOS will be used as an expression of safety. The information about the MOS must be viewed within the context of the nature of the disease to be treated, currently available therapies, and the overall risk/benefit relationship. It is well established that no therapeutic agent is without risk. The identification of the potential risk, and the appreciation of the benefit, are important aspects of the drug development process.

A drug is defined by the World Health Organization Scientific Group as "any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient." The process of drug discovery is wide-ranging, high-risk, multifaceted, expensive, and rewarding. The ultimate goal of drug development is to discover new chemical entities (NCEs) or new biological entities (NBEs) that are safe and effective in treating the targeted condition. The potential toxicity of NCEs and NBEs must be sufficiently defined to allow initiation of clinical trials. Toxicology evaluations have three main purposes: determination of the toxicological spectrum over a broad range of doses in laboratory animals; extrapolation of responses to other species, with particular emphasis on the potential for undesirable effects in humans; and determination of safe levels of exposure. Toxicology studies must be conducted in accordance with regulatory guidelines. In the early 1990s, an effort was initiated to harmonize drug development regulatory guidelines in the European Union (E.U.), Japan, and the United States (U.S.). The International Conference on Harmonization (ICH; see Regulatory Guidelines for Toxicology Profiles, below) has taken the responsibility for providing a set of mutually acceptable regulatory guidelines that will support global drug development. This effort has been largely successful, with studies conducted in the E.U., Japan, and U.S. being generally acceptable for submission in each of the other regions. Toxicology plays a major role in the drug discovery and development process (Gad and Chengelis, 1995; Diener, 1997; Dorato and Vodicnik, 2001).

NEW CHEMICAL ENTITIES (NCEs; SYNTHETIC ORGANIC CHEMICALS)

The use of combinatorial chemistry has provided drug discovery scientists with a large number of leads for potential drug candidates. Once an NCE, or class of NCEs, is identified, the chemical must quickly move through early efficacy testing using in vitro and in vivo models. A comprehensive overview of the international pharmaceutical industry's toxicology testing strategies in relation to clinical development is provided in *The Pharmaceutical R&D Compendium* (Findlay and Kermani, 2000). Indications of potential efficacy for a therapeutic target require the mobilization of additional resources. Defining potential toxicity and drug disposition issues early in the discovery process facilitates decisions on further development of that particular chemical entity. Early drug discovery and development efforts are relatively inexpensive, with the longer-term safety studies and clinical trials being much more capital-intensive. Elimination of an NCE as a potential drug candidate early in the process most efficiently utilizes resources. The new NCE first moves through an abbreviated nondefinitive toxicology profile (early investigative work, pilot studies), usually including studies of up to 2 weeks in rodents and, in some instances, nonrodents. Pharmacologic profiling is often valuable at this early stage to determine pharmacologic effects other than those intended for the therapeutic endpoint—e.g., undesirable

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effects on blood pressure, cardiac activity, or respiration. Provided that the results of the efficacy studies, early investigative toxicology studies, and early definitive toxicology studies are positive, clinical studies of safety, pharmacokinetics, and pharmacodynamics may be initiated. As human clinical trials progress, the NCE will progress through definitive toxicology evaluations that last from 2 weeks to 1 year. Studies of potential reproductive toxicity, and eventually of carcinogenic potential, are conducted. Among the characteristics evaluated for NCEs identified as drug candidates are acute toxic effects, cumulative toxicity, absorption, elimination half-life ($t_{1/2}$), accumulation in deep tissue compartments, milk excretion, teratogenicity, mutagenicity, sensitization and local irritation, and carcinogenicity. The risk of failure associated with one or more of these parameters has been reviewed (Beary, 1997; Findlay and Kermani, 2000).

Approximately 0.01% to 0.02% of NCEs are ultimately marketed as drugs. Even fewer (~0.002%) return a profit to support the development of new therapeutic agents (Fig. 10.3.1). It is estimated that the development of a new therapeutic NCE or NBE can take 6 to 12 years, and costs $0.6 to $1.8 billion. Most pharmaceutical companies are committed to reducing development time, with a target of ≤6 years, while maintaining a focus on product safety (Mullin, 2003).

The information presented in Figure 10.3.1 is historical. The cost of drug development continues to increase, as shown in the data available through the Tufts Center for the Study of Drug Development (2005). The cost of innovation in drug development has been reviewed (DiMasi et al., 1991, 2002). The relative success rate in drug development, as shown in Figure 10.3.1, was confirmed by a recent review in *Drug Discovery and Development* (Koppal, 2004).

By definition, a drug must modify a biological process. While this alteration can have therapeutic benefit, it also carries some degree of risk. The critical role of toxicology in early and late phases of drug development is to determine the level and acceptability of this risk. The initial focus of toxicology is to define the circumstances under which an NCE may produce potential harm and under which no

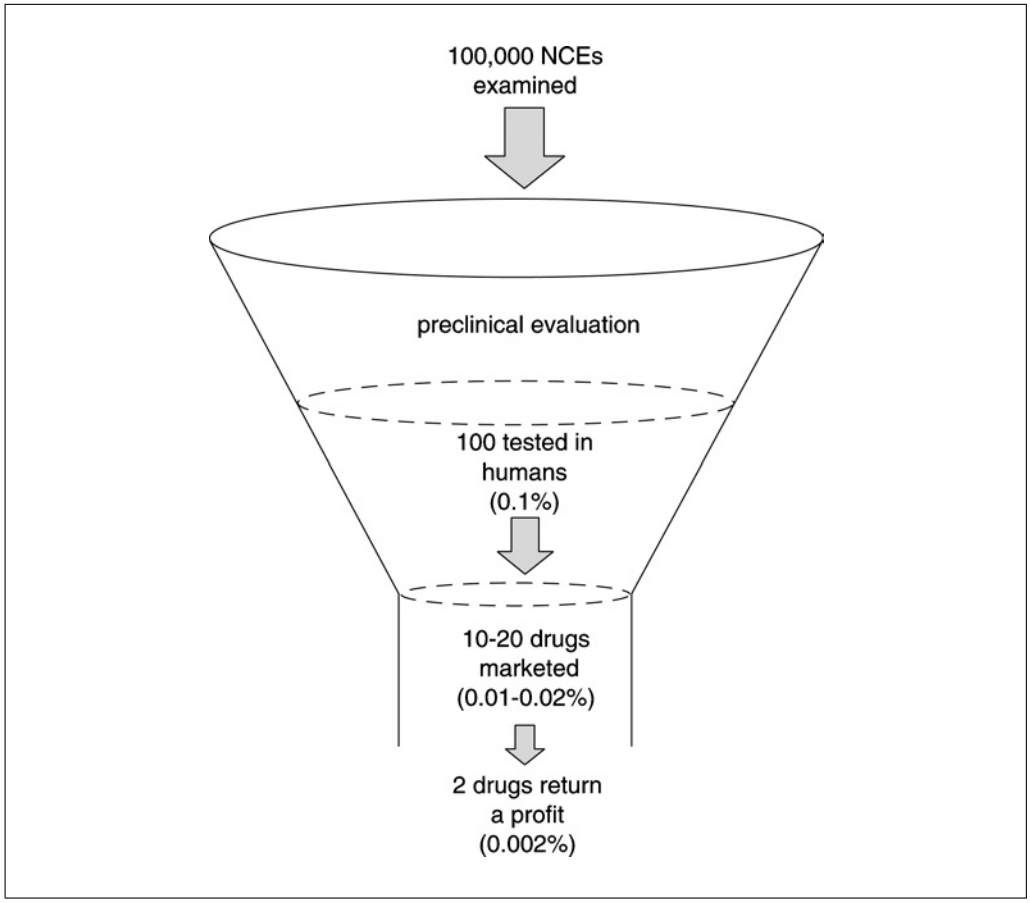

Figure 10.3.1 Attrition rate of new drug candidates in the drug discovery and development process.

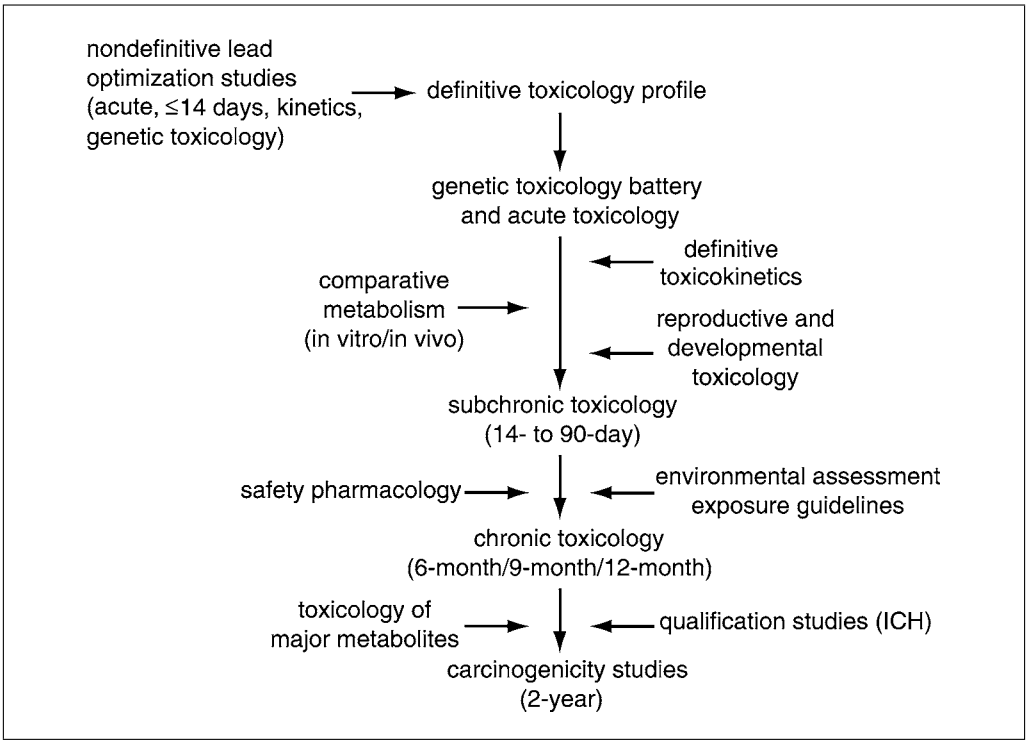

Figure 10.3.2 A typical toxicology profile. Early nondefinitive toxicology studies are designed to identify major safety issues. Timing of the developmental toxicology studies is determined by the inclusion of women of childbearing potential (WCBP) in early clinical trials. Currently the acceptable duration of nonrodent chronic toxicology studies is 9 months. However, if the data indicate a progression of toxicity, a 1-year study may be required by the FDA. Depending on the therapeutic indication, carcinogenicity studies may be conducted after approval (Phase IV) if there is no special cause for concern in that regard.

adverse effects are produced. A typical toxicology profile is shown in Figure 10.3.2.

Toxicology plays an important role throughout the drug discovery and development process (Fig. 10.3.3). During the early discovery process, toxicologists employ rapid, quantitative screening methods, focusing on a limited number of end points. The goal is an early selection of drug candidates with the most acceptable safety profiles. These preliminary screens, including investigations of surrogate markers and in vitro evaluations, are, however, only a prelude to the required comprehensive safety assessments demanded by regulatory agencies. Regulatory requirements, termed Good Laboratory Practices (GLPs), dictate many aspects of study protocols, and must be followed closely for all toxicology studies used in support of a new drug application. The early toxicology studies, conducted during the discovery phase, are not required to be in full compliance with GLPs, although application of the scientific method is expected.

Prior to the initiation of clinical trials, physicians need a toxicity evaluation of the NCE in relevant animal models. Prior to the first human dose (FHD), comparative information, i.e., of human and animal metabolism, is limited to in vitro evaluations using relevant tissue preparations. Such data provide an initial understanding of the relevance of the animal model. The clinician usually needs to know both the effect and the no-effect levels in nonclinical studies, signs and duration of toxic response, progression of the toxic response with duration of dosing, reversibility, target organ(s), and relevance of the nonclinical model to humans. The answers to these and other questions form the basis of the toxicology profile supporting initial and continued clinical trials. In addressing this, the toxicologist should not become embroiled in the political issues of toxicologic and pharmacologic effects. Some equate pharmacology with "good" and toxicology with "bad" effects. Pharmacologic activity can have very severe consequences, and may be considered a toxicity. For example, digitalis glycosides increase the force of cardiac contraction and slow electrical transmission, restoring cardiac rate and rhythm toward normal. The acute toxicity of digitalis glycosides represents extensions of these activities, with nausea, vomiting, slow heart rate, heart block, cardiac arrhythmia, and cardiac arrest. It is important to know whether the observed response is

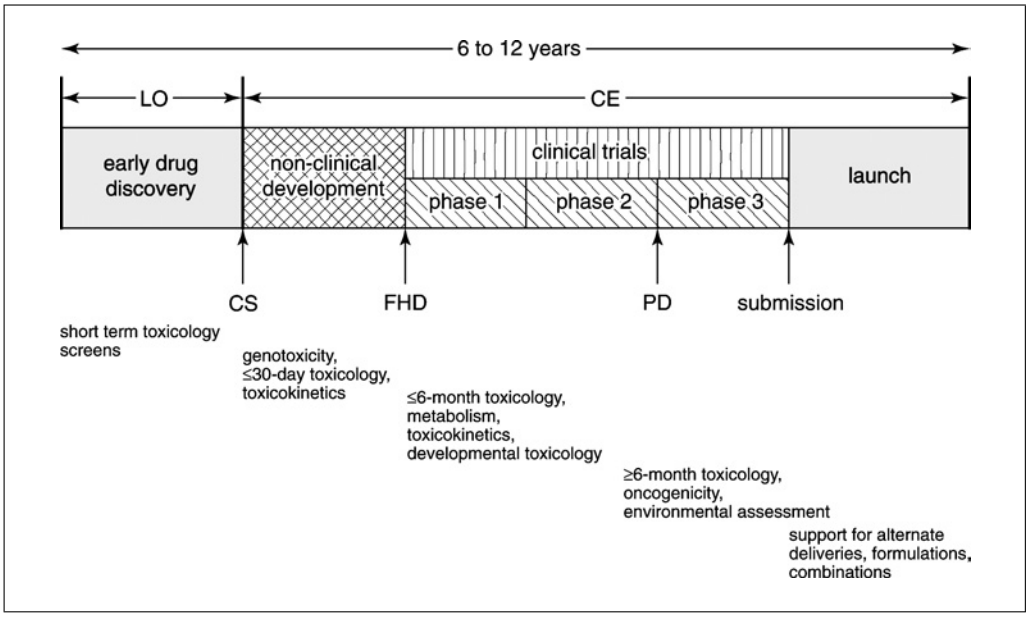

Figure 10.3.3 Duration of toxicology involvement in drug discovery and development showing the major milestones and study types by phase. The goal is to reduce development time while maintaining a focus on nonclinical and clinical safety assessment. Abbreviations: CE, candidate evaluation; CS, candidate selection; FHD, first human dose; LO, lead optimization; PD, product decision.

desirable or undesirable, and, if undesirable, to determine whether it is manageable. Simply classifying a response as expected pharmacology does not satisfy the safety evaluation obligation of the toxicologist.

The major objectives of the toxicology profile change between the early and later discovery phases of the drug discovery and development process. Thus, during the early stages of drug development, the focus is on screening. Definitive toxicology studies are very time-consuming and much more expensive than the nondefinitive screening procedures. Accordingly, the relatively inexpensive, short-term screening procedures are used to eliminate the most toxic compounds early in the development process. The initial screening approaches have a number of inherent limitations: the affected systems may not be fully evaluated, the assay procedures may be inadequate or improperly timed relative to the onset of the toxic response, target-organ exposure may be insufficient, functional evaluations may not be included, metabolic, anatomic, and physiologic differences between species may go unrecognized, and the animal model may not express the same responses as humans (Zbinden, 1989). In the broadest sense, nonclinical safety studies should adequately characterize the toxicity of a new drug candidate in several species, when appropriate, so the clinician can be alerted to potential adverse effects during the initial clinical trials.

There is concern about the adequacy of the definitive toxicology screening procedures and their ability to protect the public. Opinions vary on the ability of toxicology screening to affect the occurrence of drug toxicity in the human population (Cluff, 1980; Karch, 1980). The magnitude of adverse clinical toxicity seems small, with ~1 per 10,000 patients reported to demonstrate adverse responses (Karch, 1980). A review of the number of NCEs and NBEs introduced in the United Kingdom, the U.S., and Spain from 1974 to 1993 indicates that ~3% to 4% of all drugs introduced during that time were discontinued for safety reasons (Bakke et al., 1995). Although the number of safety withdrawals is low, U.S. companies, or their foreign subsidiaries, have been involved in the majority of cases (Bakke et al., 1995). The ability of the toxicology screening process to prevent adverse clinical events, however, is virtually impossible to evaluate. In some cases, toxicology screening has failed to provide adequate information on potential human risk, since drugs can cause unexpected clinical toxicity. Moreover, it is difficult to predict from animal studies subjective clinical responses such as nausea, dizziness, heartburn, or headache. Zbinden (1991) has provided a list of drug disasters resulting from the failure to conduct adequate toxicity evaluations. The list of drug disasters, however, is balanced by the even larger list of NCEs that would have caused

serious adverse effects in humans had they not been detected and eliminated using appropriate animal experiments. For the most part, data supporting the ability of toxicology screening to prevent human toxicity are generally not reported and remain buried in company files, since data need not be submitted to regulatory agencies if the NCE is canceled prior to human testing. The available information, however, indicates that the majority of NCEs that pass animal toxicity screens are safe in clinical trials (Scales, 1990).

In addition to the NCE, the drug-delivery system to be employed may require safety evaluation. Data must be accumulated indicating the delivery system is safe and effective. Today, an increasing number of new delivery systems such as inhalation devices, oral delivery (for proteins), ocular delivery, depot formulation, and transdermal delivery are under investigation (Gad and Chengelis, 1995; Wolff and Dorato, 1997). DeGeorge et al. (1997) have presented considerations for toxicity evaluations of respiratory drug products. The regulatory requirements for known and novel drug-delivery systems have been reviewed by Weissinger (1990). Updated information is available on the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMEA) Web sites.

NEW BIOLOGICAL ENTITIES (NBEs)

Since 1982 there has been a dramatic increase in the rate of development of drugs produced by recombinant DNA (rDNA) technology. The introduction of modern rDNA technology has allowed large-scale production of proteins that would have been very difficult to produce using classical synthetic techniques. rDNA products are complex, high-molecular-weight substances that may require immunologic, biochemical, or bioassay techniques to quantify the material and assess activity.

When recombinant human insulin was introduced in 1982, there were few regulatory guidelines for addressing the problems associated with testing products of this new technology (Zbinden, 1987). Indeed, the possibility that each biotechnology product might require a uniquely designed safety assessment has been given serious consideration (Stoll, 1987). The current harmonized regulatory guidelines for safety testing of recombinant proteins are reviewed below.

It is important to demonstrate to regulatory authorities that the recombinant protein under development is, in fact, identical to the naturally occurring substance and is devoid of contaminants that could raise safety concerns (Galloway and Chance, 1984). This is especially important today with the reorganization of the FDA, placing NBEs and NCEs under the same reviewing organization. In the approval process for recombinant human insulin, for example, frequent meetings between regulatory and industrial scientists were held to review the manufacturing process, the molecular biology, the hormone purification process, and the clinical trial programs. Such cooperation was critical in facilitating the rapid regulatory approval of rDNA insulin. This illustrates the importance of involving the U.S. Food and Drug Administration (FDA) early in the nonclinical and clinical development plan to facilitate the approval process.

The U.S. Biotechnology Policy (1992) has taken the position that rDNA products per se do not pose an unusual risk to human health or the environment. This is based, in part, on the assumption that the rDNA product is chemically identical to the naturally occurring protein, which is not always the case. Both the regulatory and industry representatives to the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use support the position that nonclinical toxicologic evaluations of rDNA products should be decided on a case-by-case basis (ICH Topic S6; see Internet Resources for information on accessing current ICH topics). The data generated are used to guide the clinical trial and allow judgments on appropriate workplace exposure levels. In this way, scientific judgment, not regulatory dogma, guides the safety assessment process. The role of the toxicologist, therefore, is less routine when dealing with rDNA products than with the more conventional synthetic organic chemicals, although the principal goals are the same when assessing the safety of either class of agents. These include detecting major toxicity, identifying minor toxicity, determining dose response, defining duration of response, evaluating the relevance of the test model, and investigating the mechanism(s) of toxicity.

Toxicity evaluations of rDNA products have established a greater emphasis on the expected pharmacology of the materials. The three areas of concern are intrinsic toxicity, exaggerated pharmacodynamics (anticipated toxicity), and immunotoxicity (Zbinden, 1987). Intrinsic toxicity is defined as undesirable effects having no relationship to the pharmacodynamic properties of the agent. Pharmacodynamic toxicity is defined as an

exaggerated pharmacologic response (e.g., hypoglycemic shock from insulin). Due to the potency of NBEs, the boundaries between pharmacology and toxicology are blurred, making it best to regard effects as desirable or undesirable. Immunotoxicity is related to hypersensitivity reactions, to cell transformations, and to production of neutralizing antibodies resulting in loss of biologic activity. There is presently no consensus on the relevance of animal models of immunotoxicity for rDNA products; some researchers feel the models do not adequately predict human responses, whereas others, such as Graham (1987), suggest this varies case by case. Factors to consider are the similarity to the natural protein, homology across species, immune response in animal models, and production of neutralizing antibodies in nonclinical and clinical studies. Each NBE must be carefully considered on an individual basis.

The rapid regulatory approval of rDNA insulin may have created unrealistic expectations in the biotechnology industry. Two factors facilitate the regulatory approval of rDNA products: therapeutic importance, and the relationship of the rDNA product to an established therapeutic agent. The U.S. FDA has established a "fast-track" approval process for therapeutic agents of critical importance. Even though it is an international body, ICH has also agreed on a case-by-case, scientifically based approach to the approval of rDNA products, although it is likely that many of the requirements for NCEs will still have to be met. Regulatory and industrial scientists continue to ask questions about the existence of subtle changes in the chemical structure of rDNA products that may influence pharmacokinetics, pharmacodynamics, and mutagenicity. Recent information from the Tufts Center for the Study of Drug Development (2005) indicates that the total development times for NCEs and NBEs have been converging since the mid-1980s, although the biotechnology products seem to be enjoying a better approval success rate.

For all potential therapeutic agents, it is appropriate to evaluate the safety of both the parent compound (drug) and known contaminants or residues resulting from the manufacturing and purification processes. The ICH has provided a process to address the discovery of new impurities in bulk drugs (drug substances before final formulation) and formulated drugs that have not been through the toxicology screen (ICH Topics Q3A(R) and Q3B(R); see Internet Resouces for information on accessing current ICH topics).

In addition to evaluating the patient's response to exposure to a new drug, worker exposure and reaction to various end products and intermediates during the manufacturing process may be of concern. Thus, toxicologists must take into account issues related to the therapeutic use of the material, its manufacture, and the effect of the manufacturing process on the environment. In the clinical situation, there is usually a clear therapeutic benefit associated with the use of a new drug. In the workplace, exposure to the drug through the manufacturing process has no therapeutic benefit. Because the workers are not patients, exposure to the new drug substances must be considered on the basis of potential toxicity. In both the workplace and immediate environment, all responses to drug substances must be considered as potentially undesirable, even those that are clearly related to the beneficial pharmacologic effect in clinical situations. Accordingly, the toxicologist must be prepared to address risk perception, risk assessment, and risk management in the clinic, the workplace, and the environment.

MODELS OF DRUG EFFECT

A meaningful safety assessment profile requires the selection of experimental models that best predict human toxicity. To this end, a key assumption of toxicology is that other organisms and biological systems can provide predictive models for effects in humans.

In Vivo

Because of the limited, although growing, knowledge regarding the extensive and complex interactions between a host of cellular and biochemical systems, whole-animal models remain the standard for predicting toxicity in humans (Table 10.3.1). As discussed below, selection of the appropriate species is critical and is based on the following considerations (Wilson and Hayes, 1994):

1. Early studies of comparative metabolism (i.e., in vitro studies with animal and human liver microsomes) or of toxicity observed in animals for which some human information is known or response is expected.
2. Sensitivity to the drug (generally the most sensitive species should be used) and responsiveness of specific organs and tissues.
3. Availability of an adequate historical control database (especially growth parameters, clinical pathology, and histopathology).

Table 10.3.1 Animal Models Traditionally Employed in the Safety Assessment of Pharmaceutical Agents

Assessment	Animal model	Comment
General toxicity	Rodent (rat, mouse); nonrodent (dog, monkey)	Rat and dog are preferred species; other species may be selected based on a closer similarity to humans
Ocular irritation	Rabbit	Draize model
Dermal toxicity/irritation	Rabbit, rat	Draize model
Dermal sensitization	Guinea pig	—
Phototoxicity	Guinea pig, mouse	—
Immunotoxicity	Primarily mouse but also rat	For antigenicity studies, rabbit and guinea pig are also used
Developmental toxicity	Rodent (rat); nonrodent (rabbit)	Mouse is often used as an alternative to rabbit in special cases where rabbit is inappropriate (i.e., antibacterials); dog has been used for neonatal studies
Carcinogenicity	Rodents (rat, mouse)	Rodents have been used because of the relative ease in maintaining a large number of animals over a lifetime (1.5 to 2 years)
Environmental toxicity	Lower organisms (earthworm, *Daphnia*, rainbow trout)	Effects on target species evaluated directly

4. Availability of healthy animals from a reputable supplier.
5. Ability of the facility and staff to provide adequate care and maintenance of animals.

The relevance of experimental animal models in the assessment of risk to humans is an important contemporary issue in toxicology (Dorato and Vodicnik, 2001). Despite the increased use of pharmaceuticals, the incidence of major human toxicity is relatively low (Karch, 1980; Zbinden, 1980), supporting the reliability of nonclinical safety assessment. In a recent survey conducted by the International Life Sciences Institute (ILSI), the concordance of the toxicity of pharmaceuticals in humans and animals was evaluated (Olson et al., 2000; Greaves et al., 2004). Overall, the true positive concordance rate for human toxicities was ~71%. In other words, 71% of human target organ toxicities were predicted by one or more animal species in the same organ system. Of these predicted toxicities, the nonrodent (primarily dog) predicted 21% of all human toxicities, with an additional 7% of human toxicities observed in rodents only (primarily rat), and an additional 36% of human toxicities detected in both nonrodents and rodents, suggesting a considerable overlap in toxicities between species. There was no relationship between toxicities in laboratory animals and those observed in humans in the remaining 29% of human toxicities.

Most of the serious differences observed between animal and human toxicity are related to differences in anatomy and physiology (i.e., metabolism or immune responsiveness) and to differences between the exposure of animals in nonclinical experiments and human clinical exposure (in the quantity, route, and duration of administration). For example:

1. High doses employed in animal testing may be so excessive as to distort the results and thus render the model inappropriate or insensitive for human safety assessment (Slikker et al., 2004a,b).
2. So-called idiosyncratic reactions are difficult to predict because only a small subgroup of subjects are uniquely susceptible, the mechanism is poorly understood or not definable, and/or a dose-response relationship is not apparent.

3. Current pharmaceutical research aimed at developing drugs specific for human therapeutic targets complicate selection of an appropriate model of toxicity.

Examples where animal responses are judged not relevant to human risk include D-limonene, kidney tumors due to male rat–specific α_{2u}-globulin binding protein, atrazine, mammary tumors associated with persistent secretion of estrogen and prolactin specifically in Sprague-Dawley rats, phenobarbital, and thyroid tumors in rats based on quantitative kinetic and dynamic differences from humans (Cohen et al., 2004). Rodent endocrine tumors appear to have little relevance to human cancer risk (Cohen, 2004).

By necessity, human safety assessment is conservative and assumes that, unless proven otherwise, toxicity in animals is relevant to humans and, for purposes of risk assessment, humans can be more sensitive than the most sensitive animal species studied.

In Vitro

In vitro alternatives to whole-animal studies have been developed largely in response to a growing need for rapid, inexpensive screening assays, public concern for the welfare and humane treatment of animals used in biomedical research, and biotechnology advances that support a stronger scientific basis for the toxicologic evaluation process. Apart from the hope that these models may one day provide more definitive insight into potential in vivo toxicity, and thus be more useful in human safety assessment, alternative models have been useful as screens for early detection of adverse properties associated with compounds early in the drug discovery process. Alternative models offer the advantages of requiring small quantities of drug, reduced cost, and increased speed, all of which expedite the drug discovery and development process.

In addition, a variety of in vitro systems have been developed as specific tools to probe and understand discrete mechanisms of toxicity. The final expression of toxicity in humans or animals is typically the integrated summation of extensive and complex cellular and biochemical interactions. Just as the dissection of a complex system into simpler pieces challenges the extrapolation of in vitro models to the intact, integrated organism, simple, well defined in vitro systems allow for the selective isolation, and thus evaluation, of a particular response, thereby aiding in the mechanistic studies of drug effects.

In vitro systems range in structural and biologic complexity from isolated organs to subcellular preparations. A thorough knowledge of the strengths and weaknesses of a given model is critical for establishing the relevance of the results to humans (Table 10.3.2). Systems to evaluate ocular toxicity are the most developed because of concern over the inhumane aspects of the traditional in vivo Draize test. Some in vitro models of toxicity are shown in Table 10.3.3. In most cases, the

Table 10.3.2 Hierarchy of In Vitro Systems to Evaluate Toxicity[a]

Preparation	Some advantages and disadvantages
Tissue preparations	
Isolated, perfused organs	Morphologically identical to organ in vivo; monitoring of function and hemodynamics possible; only short-term use possible
Tissue slices	Tissue architecture and heterogeneity maintained; easily prepared; only short-term use possible
Cells	
Primary cell cultures	Closely related to fresh tissue
Cultured cell lines	Easily obtained and subcultured; origin often ill defined; greater dedifferentiation present
Subcellular preparations	Metabolism absent; easily manipulated; heterogeneous in nature (may be contaminated with other cells)

[a] Adapted from Williams and Rush (1992).

Table 10.3.3 Some In Vitro Models of Toxicity Employed in the Toxicologic Characterization of Pharmaceuticals

Endpoint	In vitro system	Specific observations	Comments
Lethality	Cultured cell systems (mouse lymphoma, hepatocytes)	Cell viability, membrane permeability, metabolic competence	Lack integrative functions of a larger, intact organism
Ocular irritation	Cell systems (>70 systems)	Altered morphology, cytotoxicity (compromised cell adhesion and proliferation, membrane integrity, or cell metabolism), release of inflammatory mediators	Many assays validated on a limited scale
Dermal irritation	Skin organ cultures or cultured cells (i.e., human keratinocytes)	Altered morphology, cytotoxicity, release of inflammatory factors, altered function (i.e., membrane permeability)	May aid in understanding mechanisms of irritation
Toxicity or irritation caused by IV or IM administration	Cultured rat skeletal muscle cells (L6)	Medium creatinine kinase levels	
	Erythrocytes	Hemolysis	
	Limulus amebocyte lysate (LAL) test	Pyrogenicity associated with bacterial endotoxins	
Developmental toxicity	Lower organisms (*Drosophila*, brine shrimp, *Medaka*)	Anatomical, functional, biochemical, and molecular alterations	No acceptable methodology to allow culture of a single mammalian conceptus throughout the entire development period
	Cell or organ cultures		
	Sub/mammalian embryos		
Target-organ toxicity	Isolated organ preparation	Morphologic, observational, functional parameters[a]	
	Tissue/organ culture		
	Cultured cells		
Carcinogenicity	Bacteria, cultured cells	Genetic damage	Assess genotoxicity as a signal of potential carcinogenicity
	Primary/early-passage cells, established cell lines	Neoplastic (morphologic) transformation	Assess promotional activity
	Human tumor cell lines		Screen for anticancer activity

[a]Summarized by Gad (1993) for respiratory, nervous, renal, cardiovascular, hepatic, pancreatic, gastrointestinal, and reticuloendothelial systems.

combined results from a battery of assays, as opposed to a single in vitro test, is used to provide the weight of evidence needed to characterize toxicity. Finally, in vitro techniques have also been helpful in providing information about the comparative metabolism of the agent in humans and laboratory animals commonly employed in toxicity testing.

INCORPORATION OF TOXICOKINETICS INTO THE TOXICITY PROFILE

Exposure Versus Dose

Characterization of dose-response relationships for effects caused by exposure to xenobiotics represents a fundamental goal in the toxicologic assessment of human risk.

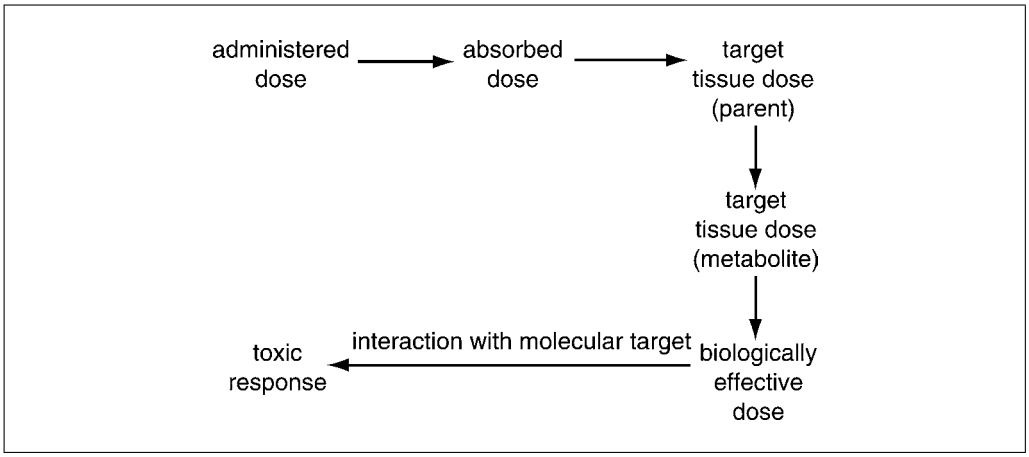

Figure 10.3.4 Representation of the administered dose-response continuum. The biologically effective dose is that which is available to interact with a molecular target.

However, toxicity observed in animal testing is frequently not a linear function of administered dose. Prior to reaching the ultimate site of action, a drug is subject to many dispositional processes (Fig. 10.3.4); thus, characterization of properties relating to absorption, distribution, metabolism, and excretion (ADME) is necessary to maximize the effectiveness of study design and data interpretation.

The primary objective of toxicokinetics is to describe the systemic exposure achieved in animals, its relationship to dose, and the time course of toxicity. Exposure is represented by pharmacokinetic parameters quantifying the local and systemic burden of the parent drug and its metabolites. Exposure is typically characterized by the toxicokinetic parameters C_{max} (peak plasma concentration) and $AUC_{(0\rightarrow t)}$ (area under the concentration curve from time zero to t). Toxicokinetic profiling, based on measurements of plasma drug levels, can provide evidence of absorption and exposure and reveal nonlinearity of ADME processes across doses. It can also aid in selection of dose, treatment regimen, and species for toxicity evaluation, and can be used to support extrapolations across dose and species.

Toxicokinetics based on plasma concentrations should be used with caution in making safety assessments because plasma levels may not reflect the dose of drug contained in tissues or delivered to the site of action (Fig. 10.3.4). For example, some therapeutic agents preferentially accumulate in certain tissues during continued administration through tissue-specific binding and/or induction of new binding sites (Dorato and Vodicnik, 2001). Liposomal formulations can yield very high tissue concentrations, especially in the reticuloendothelial system, with long retention times (Voisin et al., 1990). Finally, it is difficult to measure short-lived reactive metabolites in plasma.

A basic goal of nonclinical safety assessment is the accurate extrapolation of data from laboratory animals to humans to make more accurate predictions of toxicity. Scaling factors represent a means for extrapolations across species, implicitly accounting for differences in pharmacokinetics and pharmacodynamics (*http://www.fda.gov/cder/guidance/5541fnl.pdf*). Conventionally, dose adjustments across species are done on a body-weight basis, assuming an equivalency of dose expressed as mg of test article per kg body weight. However, many important metabolic functions that may be critical determinants of toxicokinetics or toxicodynamics are well correlated with body surface area, which is approximately proportional to $(\text{body weight})^{2/3}$ (Vocci and Farber, 1988; Table 10.3.4). For anticancer agents and antiviral nucleoside analogs, dosing based on body surface area yields better dose-response correlations across species than dosing based on body weight alone. The use of body surface area has important implications for safety assessment. Therapeutic indices based on body surface area are generally more conservative than those based on body weight (Table 10.3.4), and body surface area is the preferred measure of dose for estimation of therapeutic index in the E.U. Even so, there are limitations regarding the use of surface area for interspecies conversions. For example, the metabolic profiles of some drugs do not correlate with overall metabolic rate and therefore surface area (Voisin et al., 1990).

Ultimately, interspecies comparisons are most reliable when pharmacokinetic data

Table 10.3.4 Conversion of Dosage Based on Body Weight (mg/kg) to Dosage Based on Surface Area (mg/m^2)[a]

Species	Weight (kg)	Surface area (m^2)	Factor[b]	Dose (mg/kg)	Dose (mg/m^2)
Mouse	0.02	0.0066	3	100	300
Rat	0.15	0.0250	6	100	600
Monkey	3.00	0.2400	12	100	1200
Dog	8.00	0.4000	16	100	1600
Human	60.00	1.6000	37	100	3700

[a]Dose (mg/m^2) = dose (mg/kg) x factor; from Freireich et al. (1966).

[b]For a mouse no-observed-effect level (NOEL) of 10 mg/kg (30 mg/m^2) and a human clinical trial dose of 0.5 mg/kg, the margin of safety (MOS) based on body weight is 20x; the MOS based on body surface area is 1.6x.

are available, assuming comparable blood-level-response relationships between species (Voisin et al., 1990). The use of conventional toxicokinetic analyses (plasma level C_{max} or AUC) and the development of biologically based mathematical models, wherein determinants of disposition and dynamics are explicitly defined, represent far more accurate and informed approaches than body weight or surface area for extrapolations across species. Conventional toxicokinetic models are purely mathematical descriptions representing a best fit of the data. In contrast, physiologically based pharmacokinetic models are structural, quantitative descriptions of biologic systems. Rather than deriving values for compartments and parameters by mathematically fitting the experimental data, real physiologic structures, such as tissues and organs, and parameters representing biologic processes, such as blood flow and breathing rates, and chemical-specific properties, including tissue/blood partition coefficients and metabolic constants, are precisely defined (Fig. 10.3.5). A multicompartmental biological system can be described by connecting individual tissue compartments in parallel (Fig. 10.3.5). A set of mass-balance differential equations describing the rate of change of the amount of chemical in each compartment can be solved simultaneously to relate exposure concentrations to the amount of drug in blood and tissues.

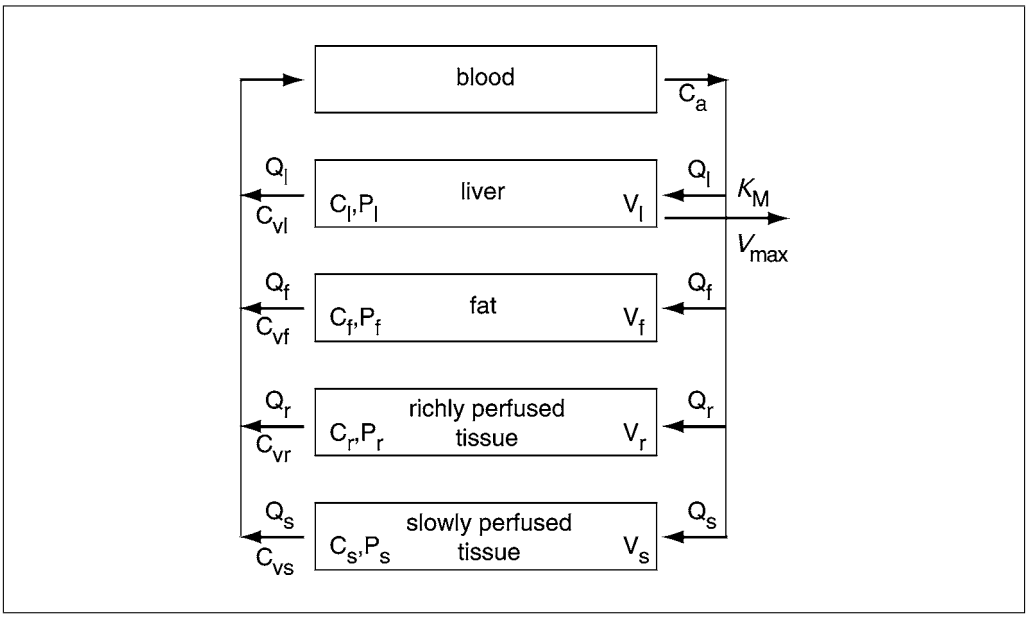

Figure 10.3.5 Schematic presentation of a simple physiologically based pharmacokinetic model. C denotes concentration of the drug, Q denotes blood flow rate, P denotes tissue/blood partition coefficient, and V denotes volume. a, arterial; v, venous; l, liver; f, fat; r, richly perfused tissues; s, slowly perfused tissues. K_M and V_{max} are metabolic rate constants.

Physiologically based pharmacokinetic modeling provides a powerful alternative to traditional methods for predictive extrapolations of dose, route, and species (Clewell and Andersen, 1986; Krishnan and Andersen, 1994). By changing relevant physiologic parameters, or adding appropriate equations to represent input functions for different routes of administration, the same model can be used to describe the dynamics of chemical transport and metabolism in different species or when using different exposure routes or scenarios. Physiologically based pharmacokinetic models have been developed for a number of drugs, including cefazolin, retinoids, nicotine, methotrexate, and thiopental (Dedrick et al., 1973; Tsuji et al., 1985; Mordenti and Chappell, 1989; Plowchalk et al., 1992; Clewell et al., 1997). The need for large amounts of data to fuel these models, however, represents a limitation in their use.

Qualitative and quantitative differences in metabolite profiles are important when comparing exposure and safety of a drug in a nonclinical species relative to humans. If a major metabolite is formed, its exposure-response characteristics may need to be evaluated. The definition of "major" is controversial and currently ranges from 10% to 25% of systemic exposure compared to the parent drug (Baillie et al., 2002; Hastings et al., 2003). At the heart of the issue is determining which human metabolite(s), major or minor, constitute a safety concern, and the extent of toxicologic testing that should be employed to assess that concern.

Maximum Tolerated Dose (MTD)

The premise that toxicologists can predict human risks using animal models is based on two main principles: (1) that there is a basic similarity in biologic structure and function across species, and (2) that exposure of animals to high doses is necessary and valid for identification of potential human toxicity. In carcinogenicity studies, the high dose has traditionally been a maximum tolerated dose (MTD). Experimentally, the MTD should cause no more than a 10% depression in body weight gain and should not elicit toxicity that would be predicted to shorten life span for reasons other than induction of neoplasm. The definition has been expanded to allow MTD selection on the basis of a broader range of biologic information (Bucher et al., 1996). While the MTD is designed to provide a level of toxicity indicative of sufficient chemical challenge to define toxicity, there are drawbacks in interpreting effects that occur only at the MTD and in their extrapolation to low-dose risk assessment for humans. One major complication is the potential for metabolic saturation leading to irrelevant metabolism or clearance.

The use of measured kinetic parameters, such as C_{max} or AUC versus dose, to set doses for carcinogenicity studies is encouraged to ensure an adequate margin between animal and intended human exposure, as discussed by Contrera et al. (1995) and ICH S1C (see Internet Resources); also see below, Regulatory Guidelines for Toxicology Profiles, Carcinogenicity Studies.

Species Specificity

In the ILSI survey, the best concordance between animal model and human response was found for human hematological, gastrointestinal, and cardiovascular toxicities, with the least concordance observed for human cutaneous toxicity (Olson et al., 2000). Nonrodents tend to predict cardiovascular and gastrointestinal toxicity much better than rodents. For anticancer agents, the dog in particular is a strong predictor of gastrointestinal toxicity, whereas the monkey was resistant to vomiting, a common human adverse event. A robust correlation was found between cardiovascular findings in dog and human.

The recent spate of drug withdrawals and updated label warnings for marketed drugs highlights areas of inter- and intra-species differences (FDA Web site; see Internet Resources), but did not in rats and monkeys, the main species used in the toxicity studies, which had much higher rates of drug clearance. Species variability in the expression of drug-induced toxicity may be related to differences in drug disposition associated with bioavailability, protein binding, or formation of reactive metabolites. Species variability in toxicity may also be related to differences in responses associated with receptor number and distribution. Eason et al. (1990) have reviewed a number of instances where animal toxicity studies failed to predict human toxicity due to species differences in metabolism, pharmacokinetics, or receptor activities. FPL 52757, an orally active antiasthmatic drug candidate, caused hepatotoxicity in dog and humans, but did not in rats and monkeys, the main species used in the toxicity studies, which had much higher rates of drug clearance. In contrast, thrombocytopenia induced by amrinone, a cardiotonic drug, was not associated with a marked species difference in pharmacokinetics, but rather with a natural predisposition toward the production

of larger platelets in humans and marmoset. The progestogen lynestrenol causes carcinomas in dogs, but not rats or mice, due to the exquisite sensitivity of canines to the tissue-proliferative effect of progestogens. However, a very high MOS was predicted for humans at very low therapeutic doses based on comparison of pharmacokinetics, tissue receptor concentrations, and receptor binding. Ciprofibrate, a safe and effective hypolipidemic agent in humans, causes gastric and hepatic tumors in rodents. The gastric tumors are likely related to species differences in receptor response, whereas the hepatic tumors are thought to be associated with the unique susceptibility of small animals to drug-mediated generation of oxygen radicals (which is inversely related to body weight). Unfortunately, tienilic acid–induced hepatotoxicity appears to be related to a metabolism-dependent immune mechanism of toxicity. This resulted in several patient deaths even though there were no obvious effects in studies with rats and dogs. Disease state, age, and genetic anomalies and idiosyncrasies within the human population are responsible for many important differences in response to chemicals (Eason et al., 1990). Similarly, the importance of strain as a determining factor in the differential responsiveness of rats to certain chemicals has been reviewed by Kacew et al. (1995).

Selection of the most relevant species for toxicity testing should be based on an understanding of ADME processes affecting the drug disposition, which can be derived from both in vitro tests, such as with liver microsomal preparations, and from in vivo tests. A sensitive and selective assay of the compound in plasma and urine is needed at an early stage of drug development to support absorption and bioavailability studies in animal models. Synthesis of radiolabeled compounds for whole-body autoradiography aids significantly in studies of absorption and distribution. The importance of dispositional characteristics in interspecies extrapolations is a primary reason for determining metabolic and toxicokinetic profiles of a drug for each animal model. The data are then compared with human data to understand the relevance of the nonclinical toxicology findings.

REGULATORY GUIDELINES FOR TOXICOLOGY PROFILES

Toxicological assessment of NCEs and NBEs has been reviewed previously (Gad, 1994; Gad and Chengelis, 1995; Cavagnaro, 1997 Diener, 1997; Dorato and Vodicnik, 2001). Traditionally, regulatory requirements for pharmaceuticals have differed between countries. Recently, however, the regulatory guidelines have been harmonized under the auspices of the ICH. This unique undertaking brought together the regulatory authorities of Europe, Japan, and the U.S., along with pharmaceutical experts from academia and industry, to discuss scientific and technical aspects of product registration. The ICH has compiled a database of internationally acceptable guidelines for the safe and ethical development of pharmaceuticals (Table 10.3.5). The timing of nonclinical studies has been reviewed by Dorato and Vodicnik (2001). Flexibility has been built into the process through the acknowledgment that pharmaceuticals under development for life-threatening diseases such as AIDS-associated conditions and cancer, for which there are no current effective therapies, should be dealt with on a case-by-case basis (Tomaszewski and Smith, 1997; DeGeorge et al., 1998) whereby particular studies listed in the general requirements for registration of a pharmaceutical may be abbreviated, deferred, or omitted. The aim is to speed development of life-saving therapy while providing adequate assurances of safety. In keeping with facilitating the rate of drug development, the FDA has recently published a draft guidance on Exploratory IND Studies (*http://www.fda.gov/cder/guidance/7086fnl.pdf*). This topic has been much discussed in Europe and the U.S. over the past decade (FDA, 1996; CHMP, 2004).

The ICH process includes five approval steps, before a guideline is implemented, in the three principal geographic regions (Table 10.3.6). The ICH has defined the clinical phases of drug development that dictate the various levels of toxicology support. For example, human pharmacology studies (Phase I) correspond to the FHD, and are generally single-dose, dose-escalation, or short-term repeated-dose studies in small numbers of healthy volunteers. Therapeutic exploratory studies (Phase II) are generally small-scale safety and efficacy studies in healthy volunteers and sometimes in patients. Therapeutic confirmatory studies (Phase III) are large-scale, expensive safety and efficacy studies in patients. These definitions fit well with the drug development and approval process in the U.S. (Fig. 10.3.6).

Animal Welfare Considerations

Animal welfare is a concern for all toxicologists. In addition to ethical considerations,

Table 10.3.5 ICH Guidelines for the Conduct of Nonclinical Studies

Topic	Topic number[a]	Title and contents
Toxicity testing	S4	Single Dose and Repeat Dose Toxicity Tests (Step 5) Recommendation to abandon LD_{50} determination; reduction in duration of longest-term dose toxicity study in rodents from 12 to 6 months
	S4A	Repeat-Dose Toxicity Tests in Nonrodents (Step 5) Reduction of duration of repeat dose toxicity studies in nonrodents from 12 to 9 months
Carcinogenicity studies	S1A	Need for Carcinogenicity Studies of Pharmaceuticals (Step 5) Definition of circumstances requiring carcinogenicity studies, taking into account known risks, indications, and duration of exposure
	S1B	Testing for Carcinogenicity in Pharmaceuticals (Step 5) Need for studies in two species Alternatives to 2-year rodent bioassay
	S1C	Dose Selection for Carcinogenicity Studies in Pharmaceuticals (Step 5) Criteria for selection of high dose
	S1C(R)	Addendum to S1C: Addition of a Limit Dose and Related Notes (Step 5)
Genotoxicity studies	S2A	Genotoxicity: Specific Aspects of Regulatory Tests (Step 5) Specific guidance for in vitro and in vivo tests plus glossary of terms
	S2B	Genotoxicity: Standard Battery Tests (Step 5) Identification of a standard set of assays Extent of confirmatory experimentation
Reproductive toxicology	S5A	Detection of Toxicity to Reproduction for Medicinal Products (Step 5) Specific guidance for testing reproductive toxicity
	S5B(M)	Maintenance of the ICH Guideline on Toxicity to Male Fertility: An Addendum to the Guideline on Detection of Toxicity to Reproduction for Medicinal Products
Toxicokinetics and pharmacokinetics	S3A	Toxicokinetics: Guidance on the Assessment of Systemic Exposure in Toxicity Studies (Step 5) Integration of kinetic information into toxicity testing
	S3B	Pharmacokinetics: Guidance for Repeat Dose Tissue Distribution Studies (Step 5) Need for tissue distribution studies, when appropriate data cannot be derived from other sources
Biotechnology products	S6	Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (Step 5) Nonclinical safety studies, use of animal models of disease and other alternative methods, need for genotoxicity and carcinogenicity studies, impact of antibody formation

continued

Table 10.3.5 ICH Guidelines for the Conduct of Nonclinical Studies (*continued*)

Topic	Topic number[a]	Title and contents
Joint safety/efficacy studies (multidisciplinary)	M3(M)	Maintenance of the ICH Guideline on Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (Step 5) Principles for development of nonclinical testing strategies (addresses full range of studies to support clinical trials for NCEs)
Pharmacology studies	S7A	Safety Pharmacology Studies for Human Pharmaceuticals (Step 5)
	S7B	The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals (Step 3)
Immunotoxicology studies	S8	Immunotoxicology Studies for Human Pharmaceuticals (Step 3)

[a]The most recent information on ICH guidelines can be found on the ICH Web site (see Internet Resources).

Table 10.3.6 Steps in ICH Approval

Step	Action(s)
1	First draft of a "TOPIC" is prepared and reviewed by the Expert Working Group (EWG)
2	Draft is approved by the ICH Steering Committee (SC) and transmitted to the three regional regulatory agencies in the European Union (EU), Japan, and the United States (USA) for formal consultation
3	Comments are collected and exchanged between regulatory authorities; the Step 2 draft is amended and approved by the EWG
4	The final draft is reviewed within the SC and recommended for adoption to the three regulatory bodies of the EU, Japan, and USA
5	Full recommendations are incorporated into domestic regulations according to national and regional procedures

the quality of research depends on the quality of the experimental animal models employed. Strict compliance with federal regulations, which reflect public concerns regarding the use and treatment of laboratory animals in biomedical research, is absolutely necessary.

There are specific regulations and guidelines available to aid scientists in providing adequate animal care. The U.S. Animal Welfare Act (AWA), which is administered by the U.S. Department of Agriculture (USDA), applies to all animals (excluding rodents) used for research purposes and consists of three parts: definitions, regulations, and standards. The AWA includes specific guidelines for the humane handling, care, treatment, and transportation of animals used in research and educational programs, and explicitly defines the minimum requirements for exercising dogs and assuring the psychological well-being of primates. USDA inspectors from the Animal and Plant Health Inspection Service (APHIS) conduct unannounced visits at least annually to inspect physical facilities and to evaluate the training of animal care personnel and the overall care of animals.

The National Institutes of Health (NIH) has published the Guide for the Care and Use of Laboratory Animals (1996), the primary reference guide for animal care and use in the U.S. The Public Health Service (PHS) policy on the humane care and use of laboratory animals lists areas of concern beyond those given in the AWA, which must be satisfied by institutions receiving federal support for research or training involving laboratory animals.

Table 10.3.7 Major Professional Organizations Providing Guidance for the Care and Use of Laboratory Animals[a]

Organization	Abbreviation	Function
American Association for Accreditation of Laboratory Animal Care	AAALAC	Provides a voluntary accreditation program for which institutions may apply. AAALAC accreditation assures compliance with the AWA and PHS policy
American Association for Laboratory Animal Science	AALAS	Provides training materials and programs and offers certification for laboratory animal technical staff
Animal Veterinary Medical Association	AVMA	Major national organization of veterinarians
American College of Laboratory Animal Medicine	ACLAM	Specialty board to encourage education, training, and research in laboratory animal medicine

[a]Abbreviations: AWA, The U.S. Animal Welfare Act; PHS, U.S. Public Health Service.

Included in the AWA regulations and the PHS policy is the requirement that each facility "...operate a program with clear lines of authority and responsibility for self monitoring the care and welfare of such laboratory animals." For this purpose, each institution must establish an Institutional Animal Care and Use Committee (IACUC) that provides oversight regarding animal welfare issues similar to that provided by Institutional Review Boards for clinical trials. Committee members (five) must include a chairperson, a scientist conducting laboratory animal research, an experienced veterinarian, one nonscientist, and a person not affiliated with the facility or institution. The IACUC meets at regular intervals to:

1. Ensure compliance with the Guide for the Care and Use of Laboratory Animals.
2. Inspect the facility every 6 months and provide written reports of the inspections.
3. Review all protocols and procedures for the use of each species.
4. Review or investigate concerns regarding animal care and handling, especially those associated with procedures that may involve pain and distress, such as prolonged restraint or multiple or invasive surgeries.
5. Ensure that adequate veterinary care exists.
6. Verify that staff who care for and use laboratory animals are qualified and trained and that evidence of such training is documented.

The FDA GLP recommendations also contain provisions for the care and use of laboratory animals, including requirements for proper training of personnel (with documentation of that training), animal housing, and separation of species. In addition, various professional organizations provide information and guidance regarding the humane care and treatment of laboratory animals in research (Table 10.3.7). Several professional societies, including the Society of Toxicology, have developed and published position statements on the use of animals in experimentation.

In addition to obtaining study approval from an IACUC, it is the responsibility of each practicing toxicologist to evaluate the necessity of any research performed with laboratory animals and the number of animals necessary to answer a particular question. Furthermore, animals should not be subject to undue pain or distress. U.S., European, and Japanese testing guidelines also recommend that toxicologists design studies to obtain the maximum amount of relevant information from the smallest number of animals (ICH Topic S4; see Internet Resources). For example, determination of an accurate LD_{50} is unnecessary. In addition, doses known to cause marked pain and distress due to corrosive or severely irritant actions need not be administered, even when no mortality has been observed at tolerated doses.

The Code of Ethics of the Society of Toxicology (SOT) states that each member "shall observe the spirit as well as the letter of the laws, regulations, and ethical standards with regard to welfare of humans and animals involved in any experimental procedure" (SOT, 1999). In addition, the Society is committed to what has been termed the principle of the "3 R's" of animal use in toxicologic testing:

Reduction of the numbers of animals used, when scientifically valid and appropriate; Replacement of animals, when possible, for testing; and Refinement of research protocols to allow for the use of less painful or stressful procedures and to improve animals' care (see The Interagency Coordinating Committee on the Validation of Alternative Methods; *http://iccvam.niehs.nih.gov/home.htm*).

Guidelines for NCEs

The ICH has developed a comprehensive document on the scope and duration of nonclinical safety studies to support the conduct of clinical trials worldwide [ICH Topics M3(M) and S6; see Internet Resources; also see Table 10.3.5]. The specific studies included in an NCE toxicology profile depend on a number of factors such as duration of treatment, route of administration, pharmacologic mechanism of action, proposed patient population, and experience with other agents in the same therapeutic class. Animal toxicity testing is conducted in three, or possibly four, phases (Fig. 10.3.6). The FHD is generally supported by short-term (≤1 month) studies. As clinical trials progress, longer-term (up to 6-month) toxicology studies are conducted. The duration of chronic toxicology studies in nonrodents has received a great deal of attention (Contrera et al., 1993). The international consensus supports a 9-month nonrodent toxicology study as the acceptable standard (DeGeorge et al., 1999). However, should the toxicology profile indicate a progression of severity of toxicity or the development of new toxicity with increasing duration, the FDA may require a 1-year nonrodent toxicology study. The majority of toxicologic evaluations occur prior to drug registration. However, based on the therapeutic indication and compassionate-use concerns, some toxicology studies may be conducted after drug approval. For example, the 2-year carcinogenicity studies for Pulmozyme, an inhaled pharmaceutical for cystic fibrosis, were conducted after it was launched (Green, 1994). Following widespread use of a new therapeutic agent, additional toxicology studies may be necessary to examine potential mechanisms of action for unanticipated side effects observed

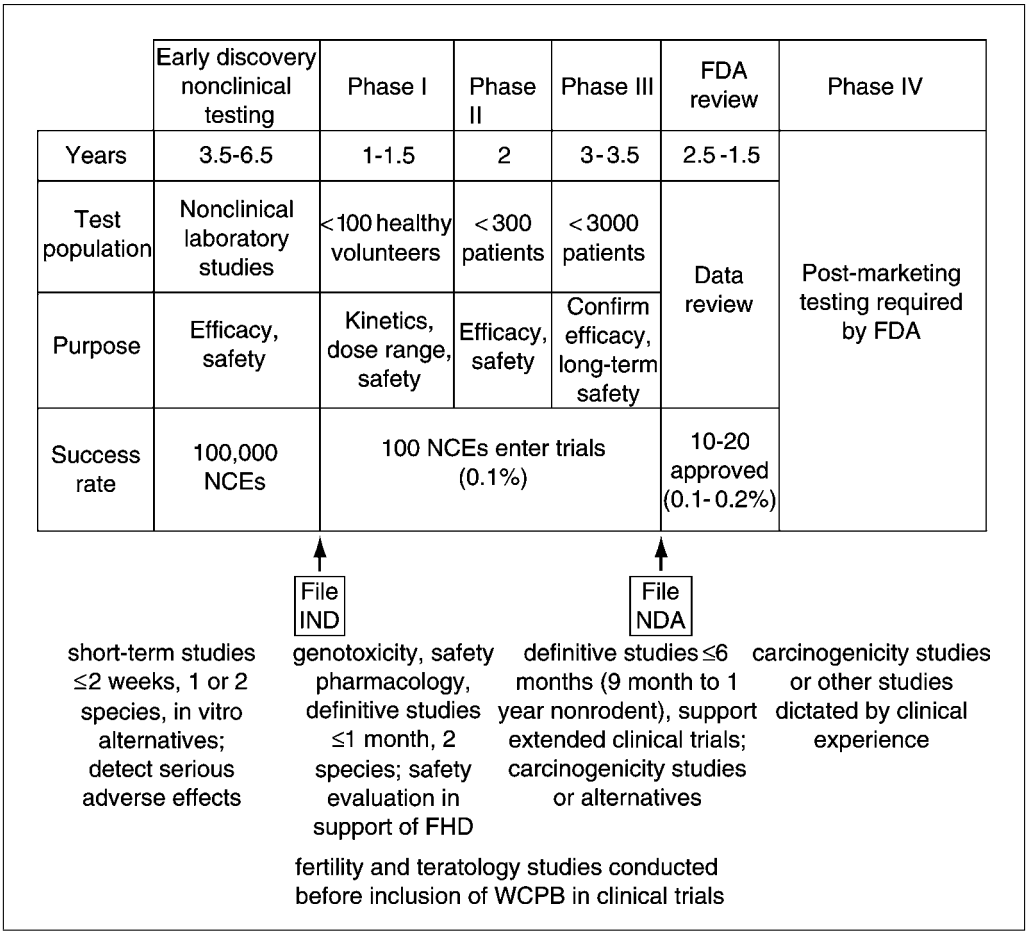

Figure 10.3.6 Schematic of the drug development and approval process in the United States. Similar processes are employed in worldwide pharmaceutical testing and approval. Adapted from Gordon and Wierenga, 1992, and Beary, 1997.

as the patient population increases. The influence of genetic differences, environmental factors, age, patient history, and drug interactions may not have been completely evaluated in the patient population studied before registration. Today, however, the patient population in registration trials is increasing, with regulatory agencies requiring additional testing if new formulations are developed, for new indications, or for the inclusion of patient populations, such as pediatric, that were not covered by the original registration.

As previously indicated, initial clinical trials focus on pharmacokinetics and safety, usually in healthy volunteers, and typically include only one or a few doses. The clinical trials are conducted in a dose-escalation fashion until an acceptable multiple of the anticipated efficacious dose, or toxicity, is achieved. Drug candidates with known, serious toxic potential, such as oncolytics, are initially tested in patient populations.

A major consideration in designing animal studies to support clinical trials is the margin of safety (MOS) between the no-effect (or minimal-effect) level in animals, and the maximum anticipated exposure in clinical trials or nonclinical models (Fig. 10.3.7). Doses selected for animal studies should provide exposure that exceeds the highest anticipated human exposure. It is no longer acceptable to base the MOS on a comparison of administered dose (e.g., mg/kg), as this does not provide adequate information on potential species differences in absorption, distribution, and metabolism (see Toxicokinetic Studies, below). There is no guideline on an acceptable MOS. However, a lower MOS is tolerated for compounds intended to treat life-threatening diseases, especially if they are expected to offer a distinct advantage over current therapies.

No-Observed-Adverse-Effect Level (NOAEL)

The NOAEL is an important concept in development of pharmaceuticals (Calabrese and Baldwin, 1994; Lewis et al., 2002; Dorato and Engelhardt, 2005). It may be considered to be the highest dose/exposure that does not cause biologically important increases in the frequency or severity of adverse effects between the exposed population and the appropriate control. While minimal toxic effects may be observed at the NOAEL, they are not thought to endanger human health or be precursors of serious adverse events.

Lewis et al. (2002) have presented a procedure for determination of an adverse event. This includes applying the following factors:

1. Differentiation of a chance difference from control from a treatment-related effect.
2. Differentiation of a nonadverse effect of treatment from an adverse effect.

This approach fits very well with the position expressed at the first ICH conference that the effect to be determined is the toxicologically relevant effect, i.e., the effect that may endanger human health (Hess, 1991). The evaluation of adverse events leads to a careful evaluation of the toxicologically relevant effects (Fig. 10.3.8).

The ICH has taken major steps to eliminate wide variations in regulatory requirements for the duration of toxicity studies to support clinical trials (Table 10.3.9). It is possible to

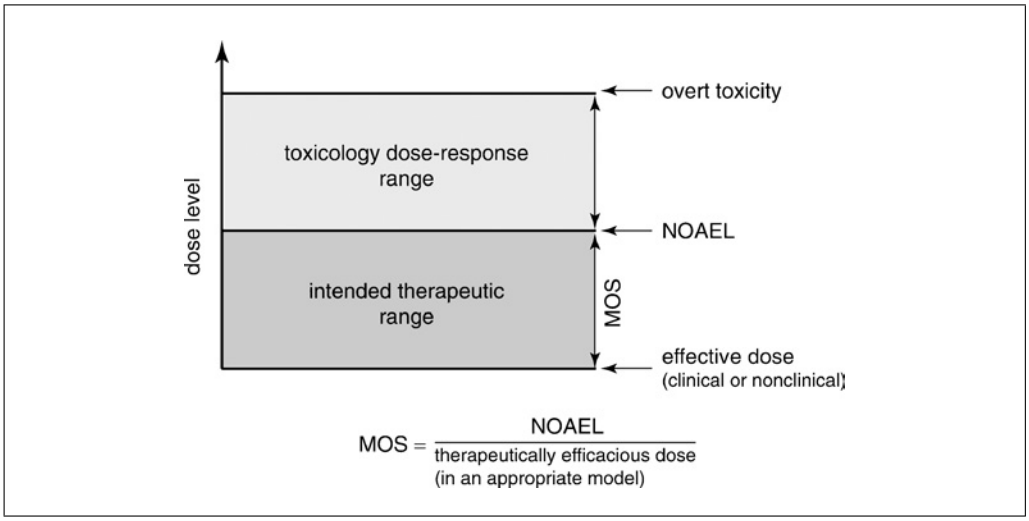

Figure 10.3.7 Relationship of margin of safety (MOS) to toxicity profile. NOAEL, No-Observed-Adverse-Effect Level (NOAEL).

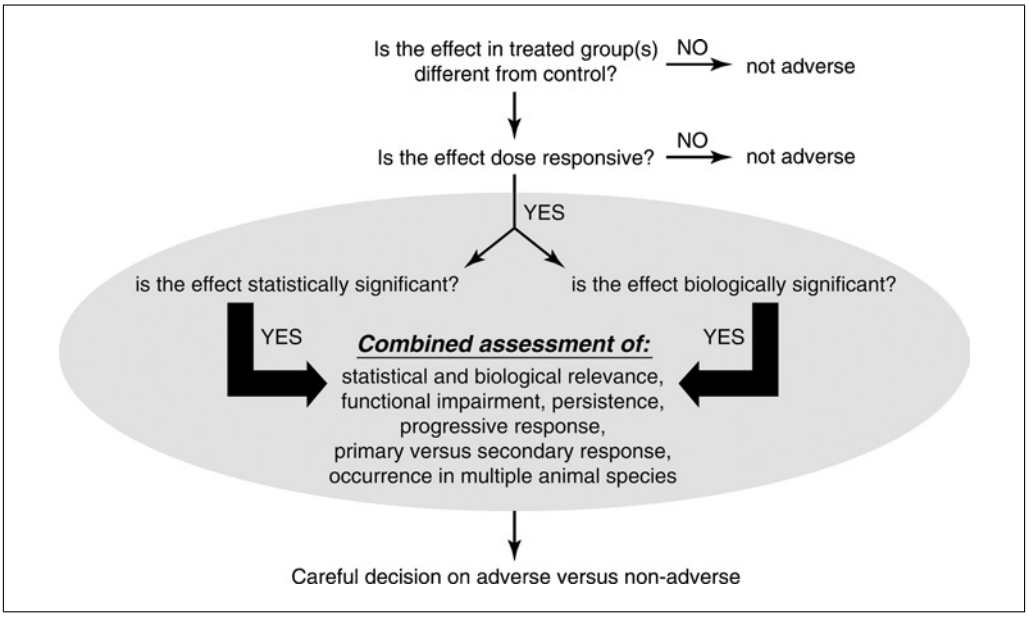

Figure 10.3.8 Approach to classifying toxicology study results as adverse or non-adverse, showing the considerable gray area (modified from Lewis et al., 2002).

discuss the duration of toxicology studies with regulators and, depending on the particulars of the test substance in question, modify these recommendations.

Safety Pharmacology

The ICH Topic M3(M) (see Internet Resources) recognized safety pharmacology as an important facet of the toxicology profile. These studies assess effects on vital functions such as the cardiovascular, central nervous, respiratory, and renal systems, and should be conducted prior to human exposure (*UNIT 10.1*). Evaluations may be conducted as additions to planned toxicology studies, or separately. There are geographical differences in the extent of the safety pharmacology tests, which are generally performed prior to initiation of clinical trials. Properly performed safety pharmacology studies provide valuable information that complements more traditional toxicological evaluations. The knowledge gained from these studies adds mechanistic information and functional evaluations to the toxicology profile. Safety pharmacology provides crucial information for the selection of NCEs during the early discovery process, the design of toxicology studies, and the design of safety monitoring in clinical trials (Lumley, 1994; Proakis, 1994).

The ICH process now includes select pharmacology guidance in the series of safety guidelines (Table 10.3.5). ICH Topic S7A (see Internet Resources) addresses the definition, objectives, and scope of safety pharmacology studies, as well as studies needed prior to Phase I clinical trials and for marketing approval. ICH Topic S7B(R) (see Internet Resources) provides recommendations for nonclinical studies to address the potential for QT interval prolongation and guidance on an integrated risk assessment. ICH Topic S8 (see Internet Resources) provides general guidance and recommendations primarily for nonclinical studies of immunosuppression induced by low-molecular-weight drugs. While the guidance is titled "Immunotoxicity," it deals primarily with immunosuppression.

Genotoxicity

Genotoxicity is defined as the ability of an agent to damage DNA or alter DNA sequence in such a way as to cause mutation. The most serious effects of these mutations are neoplasms, inheritable neoplasms, or birth defects. In vitro tests for the evaluation of mutations and chromosomal damage should generally be conducted prior to the first human dose (Table 10.3.8), and the entire battery of tests should be completed prior to Phase II. Genotoxicity analysis entails in vitro and in vivo tests designed to detect compounds that induce direct or indirect genetic damage by various mechanisms. The suspicion that a compound may induce heritable effects is considered to be just as serious as the possibility that it may induce cancer. The standard battery of tests recommended by ICH consists of a gene mutation assay in bacteria, an in vitro test of chromosomal damage, or an in vitro mouse

Table 10.3.8 Standard Genetic Toxicology Test Battery[a]

Genetic toxicology test	Purpose
Ames bacterial mutation assay	Gene mutation in bacteria
Mouse lymphoma assay (MLA) Chinese hamster ovary (CHO) chromosomal aberration assay	In vitro evaluation of chromosomal damage
Micronucleus test (MNT)	Evaluation of in vivo chromosomal damage in bone marrow polychromatic erythrocytes

[a]ICH Topics S2A and S2B (see Internet Resources).

lymphoma thymidine kinase (TK) assay, and an in vivo test of chromosomal damage using rodent hematopoietic cells. Additional evaluations may be necessary to confirm a negative result.

The conduct of genotoxicity studies on biotechnology products has been an area of much discussion, with the case-by-case approach being generally accepted. Gocke et al. (1999) presented a flow scheme for conducting genotoxicity studies for compounds that interfere with DNA synthesis, that interfere with growth regulation, that have been modified with use of reactive agents, or that are produced by unusual methods.

Acute, Subchronic, and Chronic Toxicology

The toxicity evaluation of most pharmaceuticals includes tests in each of these three categories. While the nature and duration of the clinical trial generally dictates the nature of the toxicity evaluation, it is advisable to discuss the approach with the appropriate regulatory agency before initiating the studies.

Acute toxicology testing generally entails single-dose studies with a 14-day observation period, whereas subchronic tests are multiple-dose studies usually lasting from 2 weeks to 6 months. Chronic toxicology involves multiple-dose studies of ≥6 months. A list of the parameters commonly evaluated in toxicology studies has been presented by Dorato and Vodicnik (2001). In general, a toxicology profile represents a series of building blocks with the knowledge from previous studies, or the knowledge from other agents in the same therapeutic class or of similar chemical structure, resulting in the addition or deletion of parameters from a study protocol. The duration of nonclinical studies in support of clinical trials of various duration are shown in Table 10.3.9.

Acute toxicology studies are generally conducted in two species, both of which can be rodents, prior to the first human dose (ICH Topic S4; see Internet Resources). This is generally

Table 10.3.9 International Guidelines for the Duration of Animal Toxicology Studies Necessary to Support Clinical Trials of Various Duration [from ICH Topic M3(M); see Internet Resources][a]

Clinical trial duration	Toxicology duration to support Phase I, II (E.U.), and Phase I, II, III (U.S. and Japan)		Toxicology duration to support Phase III (E.U.) and marketing (all regions)	
	Rodents	Nonrodents	Rodents	Nonrodents
Single dose	2 weeks	2 weeks	—	—
≤2 weeks	2 weeks	2 weeks	1 month	1 month
≤1 month	1 month	1 month	3 months	3 months
≤3 months	3 months	3 months	6 months	3 months
>3 months	—	—	6 months	chronic
≤6 months	6 months	6 months	—	—
>6 months	6 months	chronic	—	—

[a]Assessment of reversibility may be necessary in 3- or 6-month toxicology studies. Carcinogenicity studies are not required prior to clinical trials, and may be conducted post-approval for some indications unless there is a cause for concern.

accomplished by conducting single-dose studies and observing the animals for 14 days. A well designed dose-escalation study in two rodent species, or one rodent and one nonrodent species, is also acceptable. Requirements for single-dose studies and the elimination of the classic LD_{50} determination were harmonized at the first ICH (D'Arcy and Harron, 1992). For single-dose toxicity studies, assessments of both the intended clinical route and a parenteral route are required, unless the only intended clinical route is parenteral.

The FDA has published revised guidelines on single-dose toxicity testing as part of the implementation of the ICH Safety Working Group consensus (FDA, 1996). This represents an area of continued regional difference, since the FDA guide applies only to the U.S. Recently, the Committee for Medicinal Products for Human Use has published a position paper on nonclinical safety studies to support a single microdose in a clinical trial (CHMP, 2004). This position paper is similar to the FDA approach, but slightly more restrictive. The FDA allows the use of a single-dose toxicity study to support a single-dose Investigational New Drug (IND) application for screening drug development candidates in clinical trials. The FDA Screening IND approach is designed to quickly identify drug development candidates in a clinical setting. Clinical trials are supported by toxicology studies of $\leq$2 weeks, and perhaps single-dose toxicology studies. Additional support is provided by a limited genetic toxicology package such as the Ames assay to test for bacterial mutagenic potential, an assay for clastogenesis, a limited safety pharmacology package, and close interaction with the FDA reviewer. This approach requires a clear clinical plan with established decision points. Another advantage to this approach is that multiple compounds can be tested under the same IND. The first human dose is a critical event in the development of a new therapeutic agent. The single-dose acute nonclinical studies in support of the screening IND are designed to assess dose response, pharmacokinetics, tolerability, and bioavailability (Choudary et al., 1996). They also include clinical pathology and histopathology evaluations both at an early time and at the end of the study for the identification of maximum effect and recovery. Because the clinician is interested in disabling and potentially life-threatening acute responses, the single-dose toxicity approach is more useful if the studies focus on functional changes. The more traditional toxicology approach to acute studies (less interim evaluation, or focus on function) is recommended if the aim is to progress smoothly and rapidly into multiple-dose clinical trials (Choudary et al., 1996). One option is to use the acute toxicology studies to screen compounds quickly in the clinic, whereas another is to plan for success and screen compounds in nonclinical studies, using more traditional approaches to move rapidly from the first human dose to multiple-dose clinical trials. The FDA has published a draft guidance on Exploratory IND Studies (*http://www.fda.gov/cder/guidance/7086fnl.pdf*), which evaluates possibilities for rapid entry to limited clinical trials.

Repeated-dose toxicity studies are generally conducted in two mammalian species, rodent and nonrodent. The duration of these studies is typically equal to, or greater than, the duration of the clinical trial, up to the maximum duration recommended (ICH Topic S4A; see Internet Resources). In some cases, clinical trials may extend beyond the duration supported by the repeated-dose toxicity studies. This is true primarily when there is a significant therapeutic advantage to the test substance and a lack of adverse effects observed clinically. Strong regional differences still exist in the recommendations for conducting nonrodent toxicology studies. The E.U. and Japan are satisfied with 6 months as the longest duration for nonrodent toxicology studies, whereas the FDA takes the position that 6-month studies are not sufficient to address potential adverse effects (Contrera et al., 1993). Therefore, there is an international consensus that rodent studies of 6 months and nonrodent studies of 9 months are acceptable for a tripartite development plan (ICH Topic S4A; see Internet Resources).

Local tolerance studies should be conducted prior to the first human dose (FHD; ICH Topic M3(M); see Internet Resources). Assessment of local tolerance should be conducted using the clinically relevant route of administration and may be evaluated in the context of other toxicology studies.

Toxicokinetic Studies

The importance of characterizing systemic exposure when designing studies and when interpreting and understanding the clinical relevance of nonclinical data cannot be overstated. Currently, there are no U.S. regulations that define the scope and extent of toxicokinetic studies needed to support nonclinical safety studies. Individual experiments are performed based on scientific merit, and are decided on

an individual basis. The ICH has published a guideline to aid in understanding the application of kinetics to toxicity studies (ICH Topic S3A; see Internet Resources). Toxicokinetic studies may be an integral part of nonclinical toxicity studies or may be conducted as separate, supportive studies. In general, toxicokinetic studies should be performed according to GLP regulations in conjunction with drug safety studies.

As discussed earlier, the primary objective of toxicokinetics studies is to define systemic exposure in animals along with the relationship of such exposure to the dose level and time course of the toxicity study. Secondarily, kinetic analyses relate exposure to toxicology findings and contribute to the assessment of the relevance of these findings to clinical safety. They also support the choice of species and treatment regimen in nonclinical toxicity studies and provide information needed to design subsequent studies.

In toxicokinetic studies, the matrix of choice (e.g., blood, plasma, excreta, or tissues) should be sampled frequently enough to permit estimation of the exposure without interfering with normal conduct of the study or causing undue physiologic stress to the animals. The doses chosen for toxicokinetic evaluations should be based on those used in the single- and multiple-dose toxicology studies. Typically, samples are collected from animals in all dose groups, but those from controls are discarded without analysis. However, a draft guidance recently issued by EMEA recommends assaying levels of test substance in samples from control animals as well to assess the impact of potential contamination (EMEA, 2000).

At some point, kinetics should be characterized in each sex, using the minimum number of animals needed for definitive data. Although toxicokinetic analyses focus on measurement of the parent drug, knowledge of metabolite concentrations is especially important when the test substance is a prodrug, when it is biotransformed to active metabolite(s), or when it is extensively metabolized such that measurement of metabolite is the only practical way of establishing exposure. Species differences in protein binding, tissue accumulation, receptor properties, and metabolic profiles, as well as in the antigenicity of biotechnology products, should also be considered when interpreting exposure data.

The toxicokinetic strategy to support alternate routes of exposure should be based on the pharmacokinetic properties of the substance when it is administered by the intended route. If exposure is not substantially greater or different by the new route, additional toxicology studies may focus on local toxicity.

The ICH guideline (ICH Topics S3A and S3B; see Internet Resources) also provides specific direction on developing kinetic strategies for single- and repeated-dose toxicity studies, genotoxicity studies (demonstration of systemic exposure may be appropriate for negative in vivo studies), carcinogenicity studies (dietary administration should have confirmation of exposure), and reproductive toxicity studies (assessment of pharmacokinetics in pregnant or lactating animals, analysis of concentration in milk, and analysis of fetal exposure).

Single-dose tissue distribution studies are required in regulatory submissions worldwide and are generally considered to provide sufficient information to support a preclinical safety assessment program. However, under some circumstances repeated-dose distribution studies should be considered (ICH Topic S3B; see Internet Resources). These circumstances include the following: when the estimated half-life of elimination in tissues significantly exceeds that in plasma and is greater than twice the dosing interval; when steady-state levels determined in repeated-dose studies are not as predicted from single-dose kinetics; when histopathologic changes occur that would not have been predicted from short-term toxicity or single-dose distribution studies; and when the drug is being developed for site-specific, targeted delivery. Study duration from 1 to 3 weeks is generally adequate for repeated-dose drug disposition studies. Analysis of parent drug and/or metabolites in the target tissue should be considered, especially in cases of extensive tissue accumulation or targeted delivery. Overall, the timing and design of repeated-dose tissue distribution studies should be determined with each agent individually.

Reproductive Toxicology

Studies of potential adverse effects on fertility, fetal development and behavior, and fetal toxicity should be conducted to support the populations chosen for a clinical trial. Sutherland (1996) reviewed guidelines for reproductive toxicology studies. The FDA published a draft guidance that describes an integrative approach to assessment of concerns about human reproductive and developmental toxicities (see Integration of Study Results to Assess Concerns About Human Reproductive

Table 10.3.10 Regional Differences in the Timing of Reproduction Toxicity Studies to Support the Inclusion of Women of Child-Bearing Potential (WCBP) in Clinical Trials

Region	Requirements
Japan	Female fertility and embryo-fetal development must be completed before inclusion of WCBP using birth control in clinical trials
E.U.	Embryo-fetal development must be completed prior to Phase I Female fertility must be completed prior to Phase III
U.S.	WCBP may be included in early, carefully controlled clinical trials prior to the conduct of reproduction toxicity studies, provided adequate precautions are taken Female fertility and embryo-fetal development must be completed prior to Phase III

and Developmental Toxicities; *http://www.fda.gov/cder/guidance/4625dft.pdf*). A variety of nonclinical information, such as general and reproductive toxicity, toxicokinetics and metabolism, and clinical information are systematically considered to evaluate the potential to increase the risk of an adverse developmental or reproductive outcome in humans.

In the U.S., there is an interest in the early inclusion of women in clinical trials, particularly for new therapies targeted at the treatment of life-threatening diseases. The FDA guideline for the study of gender differences has effectively lifted the previous ban on the inclusion of women of childbearing potential (WCBP) in early clinical trials. The new FDA guideline allows the inclusion of WCBP in early clinical trials prior to the conduct of fertility and teratology studies. Despite protection provided by the informed consent process, the pharmaceutical industry is concerned with legal liability should a pregnancy occur during a Phase I clinical trial. Therefore, the conduct of developmental toxicity studies may be moved to a much earlier point in the drug development process. The view of 41 pharmaceutical companies representing the E.U., Japan, and the U.S. on the ideal approach to the timing of reproduction and developmental toxicity studies has been published by Parkinson et al. (1997).

The ICH guideline on reproductive toxicity (ICH Topic S5A and 5B; see Internet Resources) does not address the inclusion of WCBP in early clinical trials. Men may be included in Phase I and II clinical trials before any male fertility studies. Histologic evaluation of male reproductive organs in repeated-dose toxicity studies provides an assessment of potential effects on male fertility. In Japan, unlike the U.S. and E.U., male fertility studies have been performed prior to inclusion of men in clinical trials. Histopathologic evaluation of male reproductive organs in 1-month repeated-dose studies is now recommended in Japan. In the U.S. and E.U., a 2-week repeated-dose study is sufficient for this purpose. Male fertility studies must be completed before Phase III clinical trials.

Women not of childbearing potential may be included in clinical trials without an evaluation of reproductive effects, provided that a careful histopathologic evaluation of female reproductive organs was conducted in repeated-dose toxicity studies. There are currently regional differences in the timing of reproductive toxicity studies to support the inclusion of WCBP in clinical trials (Table 10.3.10). In all geographic regions, however, female reproduction studies and a full genotoxicity battery should be completed before including in clinical trials WCBP not using birth control or whose pregnancy status is unknown. There is general agreement that before including pregnant women in clinical trials, all reproduction toxicity studies and a complete genotoxicity battery should be conducted. Safety data from previous human exposure is also required.

Carcinogenicity Studies

Carcinogenicity studies are not generally required in advance of clinical trials unless there is concern about a class effect relevant to humans, evidence of preneoplastic lesions in repeated-dose toxicity studies, long-term tissue retention resulting in local reactions, or structural features suggesting carcinogenic risk (ICH Topic S1A; see Internet Resources). Carcinogenicity studies may be completed after NCE approval if the test substance is under evaluation for serious life-threatening diseases. The current FDA

position on peroxisome proliferation–activated receptors (PPARs) requires carcinogenicity studies, in some cases before entering efficacy clinical trials.

Carcinogenicity studies should be conducted for any NCE with an expected clinical use of at least 6 months (ICH Topic S1A; see Internet Resources). They should also be conducted for NCEs intended to be used frequently but intermittently in the treatment of a chronic or recurrent disease, such as anxiety or allergy. While the FDA has traditionally required a 3-month time frame for carcinogenicity studies, 6 months has generally been required in the E.U. and Japan. Carcinogenicity studies are usually not required for oncolytic agents intended for treatment of advanced cancers.

As a result of questions about their utility in identifying therapeutic agents that pose a carcinogenic risk to humans, the use of 2-year rodent carcinogenicity studies is being reevaluated. ICH discussions indicate that the rat would be the preferable species for carcinogenicity studies in the absence of any evidence favoring the mouse (ICH Topic S1B; see Internet Resources). Alternatives to the 2-year study have been proposed, such as initiation-promotion assays or assays using transgenic or neonatal rats. The choice of an alternative method should be based on the degree to which the information is of value in assessing risk. Given the debate on this issue, it is advisable to discuss the selection of alternative methods with the appropriate regulatory agency prior to initiating the study.

Important issues in dose selection for oncogenicity studies have been addressed by ICH [ICH Topics S1C and S1C(R); see Internet Resources]. Selection of the maximum dose in carcinogenicity studies is based on one of the following: maximum tolerated dose (MTD), the dose expected to produce minimal toxicity over the course of the carcinogenicity study (namely a $<10\%$ decrease in body weight gain, target organ toxicity, alteration in clinical pathology); 25-fold AUC rodent/human ratio; saturation of absorption; dose-limiting pharmacodynamics (i.e., hypotension, decreased blood clotting time); or maximum feasible dose (i.e., 5% of the diet). When there is no evidence of genotoxicity, the highest dose may be set at 1500 mg/kg. The maximum recommended human dose is $\leq$500 mg/day, and the rodent/human AUC is $\geq$10 [ICH Topic S1C(R); see Internet Resources].

The middle and low doses should provide additional information for the evaluation of risk. Consideration of dose linearity, saturation of metabolic pathways, therapeutic index, pharmacodynamics, specific animal physiology, threshold effects, and unpredictability of the progression of toxic effects should be included in the selection of the middle and low doses for preclinical carcinogenicity evaluations.

Alternatives to the traditional 2-year in vivo study have been developed. In most cases, a rat 2-year study and a mouse alternative study are conducted. The available alternative models include the $rasH_2$ mouse and the p53 knockout mouse. The $rasH_2$ mouse is preferred for testing nongenotoxic compounds while the p53 knockout is useful for evaluating genotoxic compounds. MacDonald et al. (2004) reviewed the general utility of the seven alternative models of carcinogenicity testing identified by the Alternatives to Carcinogenicity Testing (ACT) technical committee of the Health and Environmental Sciences Institute (HESI), a part of the International Life Sciences Institute (ILSI). In addition, a discussion of data gaps and regulatory perspectives across the U.S., Europe, and Japan are provided. Alternative assay results should not be considered in isolation, but rather should be included with other data pertaining to risk assessment (MacDonald et al., 2004). Cohen (2004) presented another alternative to carcinogenic risk evaluation where the focus is on exposure to a chemical rather than on the 2-year rodent bioassay.

An historical perspective of industry experience with alternative models is available from Ashton et al. (1999).

Toxicology Studies to Support Clinical Trials in Pediatric Populations

There is little information about the use in pediatrics for most therapeutic agents. As a result, the FDA has proposed a new regulation requiring pediatric studies for certain NCEs and NBEs. The E.U. Committee for Proprietary Medicinal Products (CPMP) has also concluded that specific age-dependent differences in pharmacokinetics, pharmacodynamic responses, process of growth and development, and specific pathology require that therapeutic agents be tested in the target age group. The ICH has recommended that pediatric clinical trials be supported by repeated-dose toxicity studies of an appropriate duration, all reproductive toxicity studies, and the full battery of genotoxicity tests. These studies should be concluded before initiating pediatric clinical trials [ICH Topic M3(M); see Internet

Resources]. Due to the potential extended duration of treatment, carcinogenicity studies must be considered prior to the initiation of long-term pediatric clinical trials.

The performance of nonclinical studies in juvenile animals may also be necessary if previous toxicology evaluations and human safety data are insufficient, or suggest a possible risk. The FDA provides advice on the role and timing of animal studies in the safety evaluation of drugs intended for pediatric use. This is especially true regarding irreversible serious adverse effects that cannot be adequately, ethically, or safely assessed in pediatric clinical trials (*http://www.fda.gov/cder/guidance/5541fnl.pdf*). Juvenile toxicology studies should precede long-term exposure in pediatric subjects unless there is minimal, usually adult, clinical data to support initiation of pediatric studies.

SPECIAL ISSUES FOR BIOTECHNOLOGY-DERIVED NBEs

A biotechnology NBE product is a naturally occurring or structurally modified polypeptide, protein, DNA, or RNA product, produced in cell lines or by transgenic animals, that is used for therapeutic, prophylactic, or diagnostic purposes. Advances in rDNA technology have made it possible to produce a number of highly purified species-specific NBEs. A major question in evaluating NBEs relates to the appropriate nonclinical safety studies necessary to support clinical trials. Safety evaluation of biotechnology products is not addressed by ICH Topic M3(M) (see Internet Resources). Some feel that the emphasis on differences between NCEs and NBEs is overplayed. In a broad sense, NCEs and NBEs are governed by identical principles, with no fundamental difference in the approach to developing either class of materials. Early industry strategies for nonclinical safety studies of biotechnology products have been reviewed by Griffiths (1999). The current regulatory approach to biotechnology products (ICH Topic S6; see Internet Resources) allows for customization of the protocols with respect to the agent under investigation. This flexibility has led to perceived inconsistencies in regulatory requests for safety evaluations. In fact, companies have conducted studies with NBEs more suitable for NCEs without adequate scientific justification, and have thereby reset regulatory expectations on the extent of biotechnology safety profiles. Pharmaceutical companies in the E.U., Japan, and U.S. have noted several areas of concern relative to safety testing of NBEs (Griffiths et al., 1997). Included are identification of relevant animal models, implications of antibody formation on study duration, relevance of genotoxicity, reproductive toxicity and carcinogenicity studies, and use of alternative testing approaches, such as those involving animal models of disease, transgenic animal models, and homologous proteins.

The prevailing opinion is that nonclinical safety evaluations with species-specific proteins may do little more than reveal enhanced pharmacodynamic properties, rather than predict the potential for toxicity. Indeed, the potential for toxicity related to immunologic responses, such as altered clearance or sustained blood levels, may not be relevant for human risk assessment. The toxicology profile of an rDNA product should indicate that it has no adverse effects other than those specifically related to the expected pharmacodynamics, such as hypoglycemia with insulin and insulin analogs. Safety evaluation of rDNA products should focus on the clinical dose range rather than on exaggerated toxicity (Bass and Scheibner, 1987).

Emphasis has been placed on the chemical characterization of the rDNA product to establish that it is identical to the naturally occurring protein, after which the nonclinical safety evaluations may be appropriately abbreviated. It must be recognized that rDNA products containing amino acid sequences that differ purposefully from the native protein to increase potency, duration of action, or solubility will require a more comprehensive toxicology profile. This situation was apparent with the FDA recommendations for nonclinical safety studies with analogs of gonadotropin-releasing hormone (GnRH; Raheja and Jordan, 1994). GnRH analogs, either agonists or antagonists, were modified to increase biologic potency, duration of action, and solubility, and, in some cases, to decrease toxicity. Because the GnRH analogs were originally developed for treatment of prostate cancer, they were subjected to a less rigorous toxicology program than is usual for nonchemotherapeutic NBEs and NCEs. The current focus with these agents on less serious conditions such as fertility disorders, and the modifications in the structure of the native compound, have made it necessary to examine them in a more traditional way (Table 10.3.11). Comparison of the toxicology profiles for biosynthetic human insulin (BHI) and its analogs (Table 10.3.12) is also instructive in this regard. The toxicology profile for BHI was, at the time, unconventionally

Table 10.3.11 FDA Recommendations for Toxicology Profile for Gonadotropin-Releasing Hormone (GnRH)[a]

Study type	Recommendations
Acute toxicology	Rodent, appropriate route Nonrodent (optional)
Subchronic and chronic toxicology	Rodent, appropriate duration and route Nonrodent, appropriate duration and route
Genetic toxicology	Genetic toxicology not required by FDA DMEDP[b] recommends genotoxicity testing of GnRH analogs (full battery)
Reproduction toxicology	Follow ICH Harmonized guideline Demonstrate reversibility in fertility effects GnRH agonists and antagonists are known to interfere with ovulation and spermatogenesis
Carcinogenicity	Due to chronic therapy and chemical dissimilarity with native GnRH, DMEDP[b] recommends long-term carcinogenicity studies
Special studies	*GnRH analogs are known to release histamine* In vitro histamine release assay Local inflammation and intradermal sensitization (guinea pig) Blood pressure (rat) Hemodynamics (dog) Edematogenic and vascular permeability test (rat) *Antigenicity in species used in long-term toxicology studies* Neutralizing antibodies Changes in pharmacokinetics

[a]Material evaluated in toxicology studies should have the same impurity profile as material intended for market.
[b]Division of Metabolism and Endocrine Drug Products, US FDA.

short, because the pharmacology of insulin was well known and there were substantial data supporting the chemical identity of the biosynthetic product with the naturally occurring protein (Dorato and Vodicnik, 2001). The toxicology profiles of rDNA products, and in some cases their analogs, differ considerably from the more comprehensive nonclinical safety profiles of traditional NCEs such as omeprazole (Table 10.3.13).

The regulatory environment for biotechnology products is outlined by the ICH guideline on safety evaluations of biotechnology products (ICH Topic S6; see Internet Resources). There is overall agreement that conventional safety testing paradigms may not be suitable for evaluation of NBEs. The overall objective of ICH Topic S6 is a flexible, individualized approach to nonclinical safety evaluation. The test material used in definitive safety studies should be comparable to the material proposed for clinical studies, and the impact of process changes, such as the impurity profile, must be evaluated as the changes occur. Biotechnology products similar to well established therapeutic agents may require a less extensive toxicology profile. While specialized test systems necessary for the evaluation of biotechnology products may not be fully compliant with GLP requirements, these studies may still be used to support regulatory submissions.

Animal models and alternative systems are a serious consideration when designing tests for NBEs. Relevant animal models are necessary based on species specificity of the biotechnology products. While relevant species may be defined as those that express the desired pharmacologic response, it must be realized that the toxicity of concern may be distinct from the desired pharmacology, an issue that must be addressed in justifying the toxicological approach. Usually, two relevant species are required for the toxicology profile, with only one needed for long-term

Table 10.3.12 Toxicology Profile for Biosynthetic Human Insulin (BHI) and a BHI Analog

Study type	BHI	BHI analog
Acute toxicology	Mouse, and rat s.c.	Rat, s.c. and i.v.
	Dog, s.c.	Dog, s.c. and i.v.
	Monkey, i.v.	
Subchronic toxicology	Rat, 30-day oral	Rat, 30-day s.c.
	Dog, 30-day oral	Dog, 30-day s.c.
	Dog, 30-day i.v.	
Chronic toxicology	—	Rat, 6-month s.c.
		Dog, 12-month s.c.
Genetic toxicology[a]	Ames assay	Ames assay
	Unscheduled DNA synthesis	Unscheduled DNA synthesis
	Sister chromatid exchange	Mouse lymphoma assay
	Mouse lymphoma assay	Chromosome aberration
		Mouse micronucleus test
Reproductive and developmental toxicology[b]	—	Rat, male fertility, s.c.
		Rat, Segments I, II, and III, s.c.
		Rabbit Segment II, s.c.
Immunotoxicology	Guinea pig sensitization	—
	Rat, immunotoxicology	
	Rat, dermal toxicology	

[a] BHI genetic toxicology conducted prior to 1981.

[b] Abbreviations: i.v., intravenous; s.c., subcutaneous. Segment I, fertility and general reproduction performance; Segment II, embryo-fetal toxicity (teratology); Segment III, perinatal/postnatal development.

toxicology studies if short-term studies show comparable toxicity profiles across species. Transgenic animal models of disease, or other alternative models, may be used, although background information on disease processes in these animals is usually lacking. It has also been suggested that homologous proteins may be evaluated, particularly when immunogenicity prevents study of the human protein in animals. The production process, range of impurities, and pharmacologic mechanisms may, however, differ from those of the human protein designed for clinical trials.

It is important to define the potential interaction between a new drug and immune system function, since many human proteins are immunogenic in animals. The development of any adverse effects, activation of complement, the effect of the antibody response on kinetics and dynamics, and the emergence of new toxicity must be characterized. If interpretation of the safety study is not compromised by the presence of antibodies, then no special significance should be ascribed to the antibody response. It is recommended, however, that tests of potential immune system involvement be included in the evaluation of subchronic and chronic toxicity. Because the immune system is conserved across species, laboratory rodents provide a reasonable model for the evaluation of potential effects on the human immune system, assuming there are no dramatic differences in kinetics between the two species (Selgrade et al., 1995). The FDA has issued a Guidance for Industry, Immunotoxicology Evaluation of Investigational New Drugs (*http://www.fda.gov/cder/guidance/4945fnl.pdf*). The five major areas of immunotoxicology identified are immunosuppression, immunogenicity, hypersensitivity, autoimmunity, and adverse immunostimulation. The EMEA (2000) has also published its position on immunotoxicity. The major difference between it and the FDA

Table 10.3.13 Toxicology Profile to Support the Registration of Omeprazole in the United States

Study type	Recommendations
Acute toxicology	Mouse, oral and i.v. Rat, oral and i.v. Dog, oral
Subchronic toxicology	Mouse, 3-month oral Rat, 2-week and 1-month i.v., 3-month oral Dog, 1-month i.v., 3-month oral, 3-month oral with 3-month recovery
Chronic toxicology	Rat, 6-month oral, 3- and 6-month oral with 2-week to 6-month recovery Rat, 2-year study in female rats to examine gastrin-dependent variables Dog, 1-year oral with 4-month recovery Dog, 5-year oral (ongoing at time of submission)
Genetic toxicology	Ames *Salmonella*/mammalian microsomes test Mouse lymphoma forward mutation assay Mouse micronucleus test Mouse chromosome aberration assay Rat liver DNA damage assay
Reproductive and developmental toxicology[a]	Rat, Segment I oral Rat, Segment II oral Rabbit, Segment II oral Rat, Segment III oral Rat, Segment III, oral extended
Carcinogenicity studies	Mouse, oral 78-week Rat, oral 104-week Rat, oral 104-week in female rats

[a]Segment I, fertility and general reproductive performance; Segment II, embryo-fetal toxicity (teratology); Segment III, perinatal/postnatal development.

document is the mandatory requirement of functional tests by EMEA (Dean, 2004). Moreover, the FDA guidance supports a weight-of-evidence approach, as opposed to the tier approach of the EMEA involving a standard set of tests for each new drug candidate (Hastings, 2002). In all cases, histopathologic evaluation of lymphoid organs and tissues is considered important for identifying potential immunotoxicity (Kuper et al., 2000). While most animal models are thought to be adequate for evaluating immunotoxicity of NCEs, they are thought to be inadequate for evaluation of the potentially immunologically significant differences in the human response to rDNA products. An exception is the rhesus monkey, which has been shown to accurately predict the human immunogenicity of several rDNA products (Zwickl et al., 1991).

There are a number of specific study considerations for biotechnology products. Safety pharmacology studies, which may be incorporated into single-dose toxicology protocols, are recommended to evaluate functional effects on major physiological systems. A recovery period should be included in repeated-dose toxicology studies. If the effect of the test material is prolonged, animals should be monitored until reversibility is demonstrated. An attempt should be made to understand the influence of binding proteins on the pharmacodynamics of the biotechnology product and on the assay used to determine exposure. Exposure to the test material should be defined, and the effect of immune-mediated clearance on toxicokinetics understood. The need for reproductive toxicology studies is dependent on the product, the clinical indication, and the patient population. An additional consideration is the need to evaluate immune function in neonates.

Because conventional genotoxicity studies are inappropriate for biotechnology compounds, they need not be conducted, although

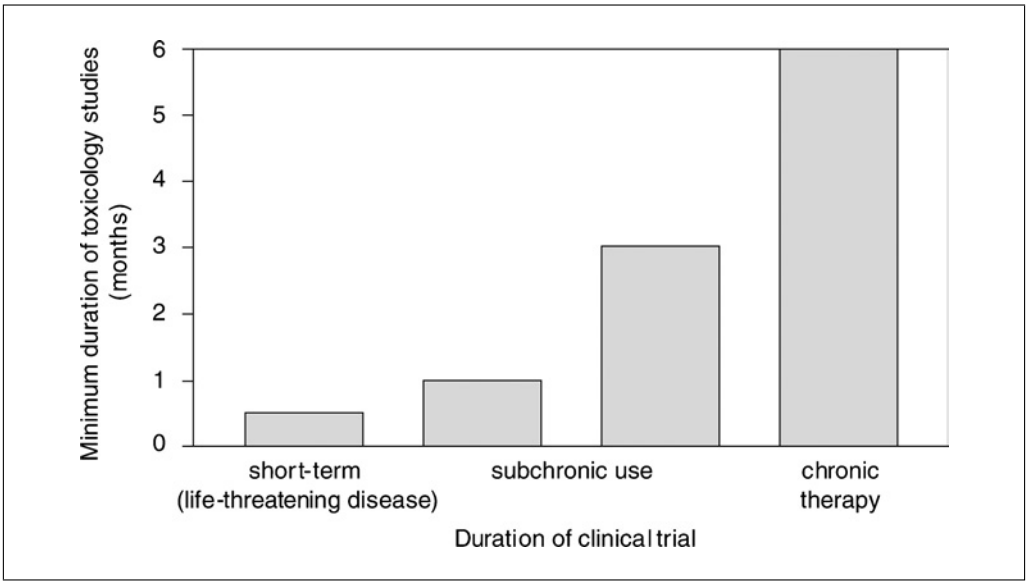

Figure 10.3.9 International guidelines for the duration of animal toxicology studies (in relevant animal species) necessary to support clinical trials with NBEs (from ICH Topic S6, 1997; see Internet Resources).

process contaminants may need to be evaluated in this way. Depending on the duration of the clinical trials, the nature of the patient population, and the biologic activity of the agent, it may be appropriate to evaluate carcinogenicity in a single rodent species. Carcinogenicity studies with recombinant peptides, proteins, or hormones must be seriously considered if there is uncertainty about whether the test substance is identical to the native agent or significantly different in pharmacodynamics (species effects), if there are purposeful structural modifications to promote activity (e.g., increase potency or $t_{1/2}$), or if exposure is significantly greater than at physiologic levels of the native material (ICH Topic S6; see Internet Resources and CPMP, 2001).

The duration of the repeated-dose toxicology studies should be based on the duration of the clinical trials (Fig. 10.3.9), and the route of exposure used in the toxicology studies should be relevant to the intended human studies. Use of a specialized drug delivery system will necessitate additional safety assessment. Toxicokinetic evaluations should be included when feasible.

As regulatory agencies have agreed that there is no standard approach to the safety evaluation of biotechnology-derived products, a case-by-case approach is utilized. This requires that both sponsors and regulators provide sound scientific rationales for their position. However, this attitude regarding the safety evaluation of NBEs may be affected by the FDA merger of the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER). Toxicology studies with biotechnology products should be designed to answer specific scientific questions and not be used solely to fulfill regulatory requirements. The unique pharmacologic aspects of species-specific proteins must be taken into consideration. Pharmaceutical scientists are encouraged to work closely with regulators in the design of repeated-dose toxicology studies of biotechnology-derived products. A comprehensive background on the history of Safety Evaluation of Biotechnology Products has been published by the Centre for Medicines Research International (CHMP, 1998).

IND SAFETY REPORTS

The U.S. has a specific requirement for the rapid reporting of effects noted in continuing toxicology studies with compounds being studied in clinical trials. Particular attention must be paid to this requirement as therapeutic agents continue their development, and when IND compounds are used in discovery studies as positive controls or for the investigation of new indications. The Guideline provides for a 15-calendar-day reporting requirement for the observation of any finding from tests in laboratory animals possibly caused by the drug that suggests a significant risk for humans, including findings of mutagenicity, carcinogenicity, and teratogenicity (FDA, 2004). The ICH has also included a recommendation for rapid notification of regulatory authorities on

major safety findings from recently completed animal studies (ICH Topic E2A; see Internet Resources).

SUMMARY AND FUTURE APPROACHES

In the coming years, toxicologists will be asked to develop more rapid, less expensive, and more reliable ways to predict human toxicity with a focus on safety assessment. There are several areas of particular interest with regard to the evolving role of toxicology in the discovery and development of pharmaceuticals. Toxicogenomics, which is the application of genomic concepts and technologies for the study of adverse effects of chemicals, represents a particularly powerful technology that has the potential to revolutionize preclinical safety assessment. A review by Suter et al. (2004) provides an especially good overview of the field and its applications to drug discovery. The principal tool is the cDNA microarray or "chip" that allows simultaneous monitoring of thousands of genes. Analysis of gene expression patterns following exposure to a drug can provide insight into mechanisms of toxicity by the identification of "toxicity-related gene-expression profiles," predict compound classifications by comparing profiles for unknown compounds with those known to be associated with toxicities, and provide information of value in developing new, specific, and sensitive biomarkers of toxicity.

In 1999, the ILSI/HESI organization formed the Genomics Committee to develop a scientific program to address issues, challenges, and opportunities afforded by toxicogenomics (Pennie et al., 2004). Through this international collaborative effort, numerous toxicogenomic studies were designed to define relationships between gene expression profiles and conventional toxicity endpoints for a number of known hepato-, nephro-, and genotoxicant compounds. Part of the ILSI/HESI effort is also directed towards validation of the technology and understanding the sources of biologic and technical variability. The results demonstrate that genomic profiles can discriminate between classes of compounds and some toxicities. The intent is to make these data available through public toxicogenomic databases.

Other new technologies available to the toxicologist include proteomics, the analysis of protein expression patterns, and metabolomics, the qualitative and quantitative evaluation of endogenous metabolite profiles in tissues and excreta (Nicholson et al., 2002; Lindon et al., 2004). Characterization of gene and protein expression, combined with metabolomic analysis, is an important step in advancing mechanistic and predictive toxicology.

Regulatory agencies are eager to facilitate the advancement of these research tools, creating mechanisms for submission and review of such data, such as the FDA Guidance on Pharmacogenomic Data Submissions (*http://www.fda.gov/cder/guidance/6400fnl.htm*), and have made it possible to consult with the agency (Interdisciplinary Pharmacogenomics Review Group, IPRG) on the utilization of these techniques. Pharmacogenomic data are required if they are being used for making decisions about clinical trials or for supporting scientific arguments relating to mechanism of action, or if they constitute a "known and valid" biomarker. Biomarker data, or data of an exploratory nature, can be submitted voluntarily without regulatory impact. While the growing acceptance of pharmacogenomic technologies by the scientific and regulatory communities is encouraging, their utility for risk assessment remains unclear because of a lack of a thorough understanding of the biologic and toxicologic relevance of the findings.

The importance of toxicokinetics in study design, understanding of dose-response relationships, and extrapolation of data from experimental animals to humans is critical to the meaningful safety assessment of drugs. Physiologically based pharmacokinetic modeling can provide a powerful alternative to traditional methods for extrapolations of dose, route, and species (Clewell and Andersen, 1986; Mordenti and Chappell, 1989; Krishnan and Andersen, 1994). Routine application of these models is currently limited by their relative complexity, the data-intensive nature of model parameterization, such as in obtaining independent measures of physiologic and physicochemical parameters and biochemical rate constants, and the difficulty in validating the models. Decisions to use physiologically based pharmacokinetic models or simpler models should be made on an individual basis. With regard to regulatory considerations, these models have been more widely applied to risk assessment of environmental exposure than to risk assessment of new drugs.

The use of genetically altered animals in drug discovery and development has advanced significantly over the last decade. These animals are engineered to overexpress a foreign gene or to remove or replace a specific gene or gene sequence. Information obtained

from gene knockout and/or gene-addition transgenics, such as p53± mice, has been increasingly applied as a screen for, or alternative to, traditional bioassays for carcinogenicity (Contrera, 1998). Gene knockout mice lacking expression of certain drug-metabolizing enzymes, such as cytochrome P450 isozymes or epoxide hydrolases, were designed to explore the role of biotransformation in acute toxicity and chemical carcinogenesis (Gonzalez, 2002). Transgenic mouse models are being used to probe mechanisms of action and safety implications of therapeutics, although caution must be exercised when interpreting these data (Bolon, 2004).

rDNA technology, hybridoma technology, and protein engineering are having profound effects on the development of new, therapeutically useful molecules, including naturally occurring human peptides, hybrid proteins, and vaccines. Predictive human safety assessment for these products using animal models can be problematic because of the highly species-specific nature of the receptors. However, as more rDNA products are evaluated, it appears that relevant toxicology findings can be demonstrated. For example, Meyers and Hayes (1993) observed that species-specific effects are often quantitative (the same responses occur in different species, but at different doses) rather than qualitative (different responses in different species). Another case in point is shown by the toxicity profile of antisense oligonucleotides, which degrade or inhibit translation of target mRNA in a complementary, sequence-specific manner. Major toxicities of antisense oligonucleotides have been largely attributable to chemical modifications or chemical structure, and are independent of nucleotide sequence (Jason et al., 2004). Current regulatory guidelines promote individual, scientifically based approaches for the toxicologic evaluation of biologics for safety assessment. This less structured approach has fostered heightened collaborative interactions between pharmaceutical and regulatory scientists.

Significant progress has been made in harmonizing regulatory guidelines for nonclinical safety studies, with many areas of agreement on the approach to developmental and reproductive toxicology studies. There remain, however, some regional differences between the E.U., Japan, and the U.S. in this regard. With a focus on safety, an element of common sense must be employed in the conduct of nonclinical toxicology studies in support of clinical trials.

Literature Cited

Ashton, G.A., Griffiths, S.A., and McAuslane, J.A.N. 1999. Industry experience with alternative models for the carcinogenicity testing of pharmaceuticals. Centre for Medicines Research International, Surrey, U.K.

Baille, T.A., Cayen, M.N., Fouda, H., Gerson, R.J., Green, J.D., Grossman, S.J., Klunk, L.J., LeBlanc, B., Perkins, D.G., and Shipley, L.A. 2002. Drug metabolites in safety testing. *Toxicol. Appl. Pharmacol.* 182:188-196.

Bakke, O.M., Manocchia, M.A., deAbajo, F., Kaitin, K.I., and Lasagna, L. 1995. Drug safety discontinuations in the United Kingdom, the United States, and Spain from 1974 through 1993: A regulatory perspective. *Clin. Pharmacol. Ther.* 58:108-117.

Bass, R. and Scheibner, E. 1987. Toxicological evaluation of biotechnology products: A regulatory viewpoint. *Arch. Toxicol. Suppl.* 11:182-190.

Beary, J.F. III. 1997. The drug development and approval process. *In* New Drug Approvals in 1997. Pharmaceutical Research and Manufacturers of America, Washington, D.C.

Bolon, B. 2004. Genetically engineered animals in drug discovery and development: A maturing resource for toxicologic research. *Basic Clin. Pharmacol. Toxicol.* 95:154-161.

Bucher, J.R., Portier, C.J., Goodman, J.I., Faustman, E.M., and Lucier, G.W. 1996. National Toxicology Program studies: Principles of dose selection and applications to mechanistic based risk assessment. *Fundam. Appl. Toxicol.* 31: 1-8.

Calabrese, E.J. and Baldwin, L.A. 1994. Improved method for selection of the NOAEL. *Regul. Toxicol. Pharmacol.* 19:48-50.

Cavagnaro, J.A. 1997. Considerations in the preclinical safety evaluation of biotechnology-derived products. *In* Comprehensive Toxicology, Vol 2: Toxicological Testing and Evaluation (I.G. Sipes, C.A. McQueen, and J. Gandolphi, eds.) pp. 291-298. Elsevier Science Publishing, New York.

Centre for Medicines Research International. 1998. Safety Evaluation of Biotechnology-derived Pharmaceutical: Facilitating a scientific approach (S.A. Griffiths and C.E. Lumley, eds.). Kluwer Academic Publishers, London.

CHMP. 2004. Position paper on nonclinical safety studies to support clinical trials with a single microdose. 23 June. CPMP/SWP/2599/02Rev1.

Choudary, J., Contrera, J.F., DeFelice, A., DeGeorge, J.J., Farrelly, J.G., Fitzgerald, G., Goheer, M.A., Jacobs, A., Jordan, A., Meyers, L., Osterberg, R., Resnick, C., Sun, C.J., and Temple, R.J. 1996. Response to Monro and Mehta proposal for use of single-dose toxicology studies to support single-dose studies of new drugs in humans. *Clin. Pharmacol. Ther.* 59:265-267.

Clewell, H.J. and Andersen, M.E. 1986. Dose, species, and route extrapolation using physiologically based pharmacokinetic models. *In* Pharmacokinetics in Risk Assessment, Drinking

Water and Health, Volume 8, pp. 159-182. National Academy Press, Washington, D.C.

Clewell, H.J., Andersen, M.E., Wills, R.J., and Latriano, L. 1997. A physiologically based pharmacokinetic model for retinoic acid and its metabolites. *J. Am. Acad. Dermatol.* 36:S77-S85.

Cluff, L.E. 1980. Is drug toxicity a problem of great magnitude? Yes! *In* Controversies in Therapeutics (L. Lasagna, ed.) pp. 44-50. W. B. Saunders, Philadelphia.

Cohen, S.M. 2004. Human carcinogenic risk evaluation: An alternative approach to the two-year rodent bioassay. *Toxicol. Sci.* 80:225-229.

Cohen, S.M., Klaunig, J., Meek, M.E., Hill, R.N., Pastoor, T., Lehman-McKeeman, L., Bucher, J., Longfellow, D.G., Seed, J., Dellarco, V., and Fenner-Crisp, P. 2004. Evaluating the human relevance of chemically-induced animal tumors. *Toxicol. Sci.* 78:181-186.

Contrera, J.F. 1998. Transgenic animals: Refining the two-year rodent carcinogenicity study. *Lab. Anim.* 27:30-33.

Contrera, J.F., Aub, D., Barbehenn, E., Belair, E., Chen, C., Evoniuk, G., Mainigi, F., Mielach, F., and Sancilio, L. 1993. A retrospective comparison of the results of 6 and 12 month non-rodent toxicity studies. *Adverse Drug React. Toxicol. Rev.* 12:63-76.

Contrera, J.F., Jacobs, A.C., Prasanna, H.R., Mehta, M., Schmidt, W.J., and DeGeorge, J. 1995. A systemic exposure-based alternative to the maximum tolerated dose for carcinogenicity studies of human therapeutics. *J. Am. Coll. Toxicol.* 14:1-10.

CPMP. 2001. Points to consider document on the non-clinical assessment of carcinogenic potential of Insulin analogues. 15 Nov. CPMP/SWP/372/01.

D'Arcy, P.F. and Harron, D.W.G. 1992. Proceedings of the First International Conference on Harmonization, Brussels, 1991 (pp. 183-184). Queen's University Press, Belfast, U.K.

Dean, J.H., 2004. A brief history of immunotoxicology and a review of the pharmaceutical guidelines. *Int. J. Toxicol.* 23:83-90.

Dedrick, R.L., Zaharko, D.S., and Lutz, R.J. 1973. Transport and binding of methotrexate in vivo. *J. Pharm. Sci.* 62:882-890.

DeGeorge, J.J., Ahn, C.H., Andrews, P.A., Brower, M.E., Choi, Y.S., Chun, M.Y., Du, T., Lee-Ham, D.Y., McGuinn, W.D., Pei, L., Sancilio, L.F., Schmedt, W., Sheevers, H.V., Sun, C.J., Tripathi, S., Vogel, W.M., Whitehurst, V., Williams, S., and Taylor, A.S. 1997. Considerations for toxicology studies of respiratory drug products. *Regul. Toxicol. Pharmacol.* 25:189-193.

DeGeorge, J.J., Ahn, C.H., Andrews, P.A., Brower, M.E., Giorgio, D.W., Goheer, M.A., Lee-Ham, D.Y., McGuinn, W.D., Schmidt, W., Sun, C.J., and Tripathi, S.C. 1998. Regulatory considerations for preclinical development of anticancer drugs. *Cancer Chemother. Pharmacol.* 41:173-185.

DeGeorge, J.J., Meyers, L.L., Takahashi, M., and Contrera, J.F. 1999. The duration of non-rodent toxicity studies for pharmaceuticals. *Toxicol. Sci.* 49:143-155.

Diener, R.M. 1997. Safety assessment of pharmaceuticals. *In* Comprehensive Toxicology, Vol. 2: Toxicological Testing and Evaluation (I.G. Sipes, C.A. McQueen, and J. Gandolphi, eds.) pp. 269-290. Elsevier Science Publishing, New York.

DiMasi, J.A., Hansen, R.W., Grabowski, H.G., and Lasagna, L. 1991. Cost of innovation in the pharmaceutical industry. *J. Health Econ.* 10: 107-142.

DiMasi, J.A., Hansen, R.W., and Grabowski, H.G. 2002. The price of innovation: New estimates of drug development costs. *J. Health Econ.* 22:151-185.

Dorato, M.A. and Engelhardt, J.A. 2005. The No-Observed-Adverse-Effect-Level (NOAEL) in drug safety evaluations: Use, issues, definitions. *Regul. Toxicol. Pharmacol.* 42:265-274.

Dorato, M.A. and Vodicnik, M.J. 2001. The toxicologic assessment of pharmaceutical and biotechnology products. *In* Principles and Methods of Toxicology (A.W. Hayes, ed.) pp.243-283. Taylor and Francis, Philadelphia.

Eason, C.T., Bonner, F.W., and Parke, D.V. 1990. The importance of pharmacokinetic and receptor studies in drug safety evaluation. *Regul. Toxicol. Pharmacol.* 11:288-307.

EMEA. 2000. Note for guidance on repeated dose toxicity. *In* Guidance on Immunotoxicity. CPMP/SWP/1042/99 Corr. Appendix B.

FDA. 1996. Guidance for industry: Single dose acute toxicity testing for pharmaceuticals. 26 Aug. 61FR43934.

FDA. 2004. IND Safety Reports. 21CFR312.32, Subpart B—Investigational New Drug Application (IND). Revised April 1, 2004.

Findlay, G. and Kermani, F. 2000. The Pharmaceutical R&D Compendium: CMR International/SCRIP's Complete Guide to Trends in R&D. CMR International and PJB Publishers Ltd., Surrey, U.K.

Freireich, E.J., Gehan, E., Rall, D., Schmidt, L., and Skipper, L. 1966. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Chemother. Rep.* 50:219-244.

Gad, S.C. 1993. In vitro/in vivo toxicology. *In* Biotechnology and Safety Assessment (J.A. Thomas and L.A. Meyers, eds.) pp. 97-129. Raven Press, New York.

Gad, S.C. 1994. Safety Assessment for Pharmaceuticals. Van Nostrand-Reinhold, New York.

Gad, S.C. and Chengelis, C.P. 1995. Human health products: Drugs and medical devices. *In* Regulatory Toxicology (C.P. Chengelis, J.F. Holson, and S.C. Gad, eds.) pp. 9-49. Raven Press, New York.

Galloway, J.A. and Chance, R.E. 1984. Human insulin rDNA: From rDNA through FDA. *In*

Proceedings of the Second World Conference on Clinical Pharmacology and Therapeutics (L. Lemberger and M.M. Reidenberg, eds.) pp. 503-520. American Society for Pharmacology and Experimental Therapeutics, Bethesda, Maryland.

Gocke, E., Albertini, S., Brendlen-Schwaab, S., Mullen, L., Suter, W., and Wurgler, F.E. 1999. Genotoxicity testing of biotechnology-derived products. *Mutat. Res.* 436:137-156.

Gonzalez, F. 2002. Transgenic models in xenobiotic metabolism and toxicity. *Toxicology* 181-182:237-239.

Gordon, C.V. and Wierenga, D.E. 1992. The drug development and approval process. New Drug Approvals (PMA), January. Pharmaceutical Manufacturers of America, Washington, D.C. Graham, C.E. 1987. Overview: The industry position. *In* Preclinical Safety of Biotechnology Products Intended for Human Use (C.E. Graham, ed.) pp. 183-187. Alan R. Liss, New York.

Greaves, P., Willliams, A., and Eve, M. 2004. First dose of potential new medicines in humans: How animals help. *Nat. Rev. Drug Discov.* 3:226-236.

Green, J.D. 1994. Pharmaco-toxicological expert report: Pulmozyme (rhDNase) Genentech *Hum. Exp. Toxicol.* 13:1-42.

Griffiths, S.A. 1999. Pharmaceutical company strategies for designing nonclinical safety programs for products of biotechnology. *Drug Inf. J.* 33:933-938.

Griffiths, S.A., Ashton, G.A., McAuslane, J.A.N., and Lumley, C.E. 1997. Non-clinical safety testing of products of biotechnology: Issues of concern. Society of Toxicology Annual Meeting, Cincinnati, Ohio.

Guide for the Care and Use of Laboratory Animals, National Research Council (revised). 1996. National Academy Press, Washington, D.C.

Hastings, K.L. 2002. Implications of the new FDA/CDER immunotoxicology guidance for drugs. *Int. Immunopharmacol.* 2:1613-1618.

Hastings, K.L., El-Hage, J., Jacobs, A., Leighton, J., Morse, D., and Osterbert, R.E. 2003. Letter to the editor. *Toxciol. Appl. Pharmacol.* 190:91-92.

Hess, R. 1991. Repeated dose toxicity: Industry perspective. *In* First International Conference on Harmonization (P.F. D'Arcy and D.W.G. Harron, eds.) p. 197. Brussels.

Jason, T., Koropatnick, J., and Berg, R. 2004. Toxicology of antisense therapeutics. *Toxicol. Appl. Pharmacol.* 201:66-83.

Kacew, S., Ruben, Z., and McConnell, R.F. 1995. Strain as a determinant factor in the differential responsiveness of rats to chemicals. *Toxicol. Pathol.* 23:701-714.

Karch, F.E. 1980. Is drug toxicity a problem of great magnitude? Probably not. *In* Controversies in Therapeutics (L. Lasagna, ed.) pp. 51-57. W.B. Saunders, Philadelphia.

Koppal, T. 2004. Toxicogenomics Warns of Drug Dangers. *Drug Discovery & Development.* July 1, 2004.

Krishnan, K. and Andersen, M.E. 1994. Physiologically based pharmacokinetic modeling in toxicology. *In* Principles and Methods of Toxicology (A.W. Hayes, ed.) pp. 149-188. Raven Press, New York.

Kuper, C.F., Herlema, J.H., Richter-Reichelm, H.B., and Vos, J.G. 2000. Histopathologic approaches to detect changes indicative of immunotoxicity. *Toxicol. Pathol.* 28:454-466.

Lewis, R.W., Billington, R., Debruyne, E., Garner, A., Lang, B., and Carpanii, F. 2002. Recognition of adverse and nonadverse effects in toxicity studies. *Toxicol. Pathol.* 30:66-74.

Lindor, J.C., Holmes, E., and Nicholson, J.K. 2004. Toxicological applications of magnetic resonance. *Prog. Nucl. Mag. Reson. Spect.* 45: 109-143.

Lumley, C.E. 1994. General pharmacology, the international regulatory environment, and harmonization guidelines. *Drug Dev. Res.* 32:223-232.

MacDonald, J., French, J.E., Gerson, R.J., Goodman, J., Inovu, T., Jacobs, A., Kasper, P., Keller, D., Lavin, A., Long, G., McCullough, B., Sistare, F.D., Storer, R., and van der Laan, J.W. 2004. The utility of genetically modified mouse assays for identifying human carcinogens: A basic understanding and path forward. *Toxicol. Sci.* 77:188-194.

Mordenti, J. and Chappell, W. 1989. The use of interspecies scaling in toxicokinetics. *In* Toxicokinetics and New Drug Development. (A. Yacobi, J.P. Skelly, and V.K. Batra, eds.) pp. 42-96. Pergamon Press, Elmsford, N.Y.

Mullin, R. 2003. Drug development costs about $1.7 billion. *Chemical and Engineering News.* Dec. 15, p.8.

Myers, L.A. and Hayes, T.J. 1993. Safety assessment and biotechnology products. *In* Biotechnology and Safety Assessment (J.A. Thomas and L.A. Myers, eds.) pp. 23-36. Raven Press, New York.

Nicholson, J.K., Connelly, J., Lindor, J.C., and Holmes, E. 2002. Metabonomics: A platform for studying drug toxicity and gene function. *Nature Rev.* 1:153-161.

Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., van Deun, K., Smith, P., Berger, B., and Heller, A. 2000. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol.* 32:56-67.

Parkinson, C., Thomas, K.E., and Lumley, C.E. 1997. Reproductive toxicity testing of pharmaceutical compounds to support the inclusion of women in clinical trials. *Hum. Exp. Toxicol.* 16:239-246.

Pennie, W., Pettit, S.D., and Lord, P.G. 2004. Toxicogenomics in risk assessment: An overview of

an HESI collaborative research program. *Environ. Health Perspect.* 112:417-419.

Plowchalk, D.R., Andersen, M.E., and deBethizy, J.D. 1992. A physiologically based pharmacokinetic model for nicotine disposition in the Sprague-Dawley rat. *Toxicol. Appl. Pharmacol.* 116:177-188.

Proakis, A.G. 1994. Regulatory consideration on the role of general pharmacology studies in the development of therapeutic agents. *Drug Dev. Res.* 32:233-236.

Raheja, K.L. and Jordan, A. 1994. FDA recommendations for preclinical testing of gonadotropin releasing hormone (GnRH) analogues. *Regul. Toxicol. Pharmacol.* 19:168-175.

Scales, M.D.C. 1990. Relevance of preclinical testing to risk assessment. *BIRA J.* 9:11-13.

Selgrade, M.K., Cooper, K.D., Devlin, R.B., van Loveren, H., Biagini, R.E., and Luster, M.I. 1995. Immunotoxicity: Bridging the gap between animal research and human health effects. *Fundam. Appl. Toxicol.* 24:13-21.

Sipes, I.G., McQueen, C.A., and Gandolphi, J. (eds.) 1997. Comprehensive Toxicology Vol. 2: Toxicological Testing and Evaluation. Elsevier Science Publishing, New York.

Slikker, W., Anderson, M.E., Bogdanffy, M.S., Bus, J.S., Cohen, S.D., Conolly, R.B., David, R.M., Doerrer, N.G., Dorman, D.C., Gaylor, D.W., Hattis, D., Rogers, J.M., Setzer, W., Swenberg, J.A., and Wallace, K. 2004a. Dose-dependent transitions in mechanisms of toxicity. *Toxicol. Appl. Pharmacol.* 201:203-225.

Slikker, W., Anderson, M.E., Bogdanffy, M.S., Bus, J.S., Cohen, S.D., Conolly, R.B., David, R.M., Doerrer, N.G., Dorman, D.C., Gaylor, D.W., Hattis, D., Rogers, J.M., Setzer, W., Swenberg, J.A., and Wallace, K. 2004b. Dose-dependent transitions in mechanisms of toxicity: Case studies. *Toxicol. Appl. Pharmacol.* 201:226-294.

SOT. 1999. Animals in research: The importance of animals in the science of toxicology. http://toxicology.org/publicoutreatch/air/air.html.

Stoll, R.E. 1987. The preclinical development of biotechnology-derived pharmaceuticals: The PMA perspective. *In* Preclinical Safety of Biotechnology Products Intended for Human Use (C.E. Graham, ed.) pp. 169-171. Alan R. Liss, New York.

Suter, L., Babiss, L.E., and Wheeldon, E.B. 2004. Toxicogenomics in predictive toxicology in drug development. *Chem. Biol.* 11:161-171.

Sutherland, M.F. 1996. New guideline for preclinical testing of novel medicines for their effects on reproduction: Clarity or confusion? *Reg. Toxicol. Pharmacol.* 23:300-303.

Tomaszewski, J.E. and Smith, A.C. 1997. Safety testing of antitumor agents. *In* Comprehensive Toxicology, Vol 2: Toxicological Testing and Evaluation (I.G. Sipes, C.A. McQueen, and J. Gandolphi, eds.) pp. 309-290. Elsevier Science Publishing, New York.

Tsuji, A., Nishide, K., Minami, H., Nakashima, E., Terasaki, T., and Yamana, T. 1985. Physiologically based pharmacokinetic model for cefazolin in rabbits and its preliminary extrapolation to man. *Drug Metab. Dispos.* 13:729-739.

Tufts Center for the Study of Drug Development. 2005. Global review of pharmaceutical industry. Tufts CSDD, Boston.

U.S. Biotechnology Policy. 1992. *Nature* 356:1-2.

Vocci, F. and Farber, T. 1988. Extrapolation of animal toxicity data to man. *Regul. Toxicol. Pharmacol.* 8:389-398.

Voisin, E.M., Ruthsatz, M., Collins, J.M., and Hoyle, P.C. 1990. Extrapolation of animal toxicity to humans: Interspecies comparisons in drug development. *Regul. Toxicol. Pharmacol.* 12:107-116.

Weissinger, J. 1990. Pharmacology and toxicology of novel drug delivery systems: Regulatory issues. *Drug Saf.* 5:107-113.

Wilson, N.H. and Hayes, J.R. 1994. Short-term repeated dosing and subchronic toxicity studies. *In* Principles and Methods of Toxicology, 3rd edition (A.W. Hayes, ed.) pp. 649-672. Raven Press, New York.

Wolff, R.K. and Dorato, M.A. 1997. Inhalation toxicity studies. *In* Comprehensive Toxicology, Vol. 2: Toxicological Testing and Evaluation (I.G. Sipes, C.A. McQueen, and J. Gandolphi, eds.) pp. 269-290. Elsevier Science Publishing, New York.

Zbinden, G. 1980. Predictive value of pre-clinical drug safety evaluation. *In* Proceedings of the First World Conference on Clinical Pharmacology and Therapeutics (P. Turner, C. Padghan, and A. Hedges, eds.) pp. 9-14. Macmillan, London.

Zbinden G. 1987. Biotechnology products intended for human use, toxicological targets and research strategies. *In* Preclinical Safety of Biotechnology Products Intended for Human Use (C.E. Graham, ed.) pp. 143-159. Alan R. Liss, New York.

Zbinden, G. 1989. Improvement of predictability of subchronic and chronic toxicity studies. *J. Toxicol. Sci.* 14:3-21.

Zbinden, G. 1991. Predictive value of animal studies in toxicology. *Regul. Toxicol. Pharmacol.* 14:167-177.

Zwickl, C.M., Cocke, K.S., Tamura, R.N., Holzhausen, L.M., Brophy, G.T., Bick, P.H., and Wierda, D. 1991. Comparison of the immunogenicity of recombinant and pituitary human growth hormone in Rhesus monkeys. *Fundam. Appl. Toxicol.* 16:275-287.

Key References

Chengelis, C.P., Holson, J.F., and Gad, S.C. (eds.). 1995. Regulatory Toxicology. Raven Press, New York.

Comprehensive overview of regulatory issue in toxicology.

Dean, 2004. See above.

Immunotoxicology in drug development.

Dorato and Vodicnik, 2001. See above.

Overview of the drug development process and the role of toxicology.

Dorato and Engelhardt, 2005. See above.

Overview of NOAEL in safety assessment.

FDA. 2006. Guidance for industry, investigators, and reviewers: Exploratory IND studies. January. *http://www.fda.gov/cder/guidance/7086fnl.pdf.*

Important reference on exploratory IND studies.

Hayes, A.W. (ed.) 2001. Principles and Methods of Toxicology. Taylor and Francis, New York.

Comprehensive overview of toxicology principles and methods.

Lewis et al., 2002. See above.

Adverse effects in toxicology studies.

Sipes, I.G., McQueen, C.A., and Gandolphi, J. (eds.) 1997. Comprehensive Toxicology Vol. 2: Toxicological Testing and Evaluation. Elsevier Science Publishing, New York.

Comprehensive review of the toxicology profile for a potential drug.

Findlay and Kermani, 2000. See above.

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http://www.emea.eu.int

Current information from The European Medicines Agency (EMEA).

http://www.ich.org

Current information on ICH Topics (regulatory guidelines) and the structure of ICH.

http://www.access.gpo.gov/su_docs/

U.S. Government Printing Office listings of available documents—e.g., Code of Federal Regulations.

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