37

REGULATORY REQUIREMENTS FOR INDs/FIH (FIRST IN HUMAN) STUDIES

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Contents

37.1 Introduction
37.2 Overview of U.S. Regulations
- 37.2.1 Regulations: General Considerations
- 37.2.2 Regulations: Human Pharmaceuticals
- 37.2.3 Regulations: Environmental Impact
- 37.2.4 Regulations: Antibiotics
- 37.2.5 Regulations: Biologics
- 37.2.6 Regulations Versus Law

37.3 Organizations Regulating Drugs in the United States
37.4 Process of Pharmaceutical Product Development and Approval
37.5 Testing Guidelines
- 37.5.1 Toxicity Testing: Traditional Pharmaceuticals
- 37.5.2 General or Systematic Toxicity Assessment
- 37.5.3 Genetic Toxicity Assessment
- 37.5.4 Toxicity Testing: Biotechnology Products
- 37.5.5 Toxicity/Safety Testing: Cellular and Gene Therapy Products

37.6 Toxicity Testing: Special Cases
- 37.6.1 Oral Contraceptives
- 37.6.2 Life-Threatening Disease (Compassionate Use)
- 37.6.3 Optical Isomers
- 37.6.4 Special Populations: Pediatric and Geriatric Claims
- 37.6.5 Orphan Drugs
- 37.6.6 Botanical Drug Products

37.7 International Pharmaceutical Regulation and Registration
- 37.7.1 International Conference on Harmonization
- 37.7.2 Other International Considerations
- 37.7.3 Safety Pharmacology

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37.8 Conclusion
References and Suggested Reading

37.1 INTRODUCTION

The safety of pharmaceutical agents, medical devices, and food additives is the toxicology issue of the most obvious and longest-standing concern to the public. A common factor among the three is that any risk associated with a lack of safety of these agents is likely to affect a very broad part of the population, with those at risk having little or no option as to undertaking this risk. Modern drugs are essential for life in today's society, yet there is a consistent high level of concern about their safety.

This chapter examines the regulations that establish how the safety of human pharmaceutical products is evaluated and established in the United States and the other major international markets prior to initial trials in human beings harmonization requirements between countries has proceeded markedly over the last fifteen years [1].

37.2 OVERVIEW OF U.S. REGULATIONS

37.2.1 Regulations: General Considerations

The U.S. federal regulations that govern the testing, manufacture, and sale of pharmaceutical agents are covered in Chapter 1, Title 21 of the *Code of Federal Regulations* (21 CFR). These comprise nine 6 in. × 8 in. (printing on both sides of the pages) volumes that stack 8 in. high. This title also covers foods, veterinary products, and cosmetics. As these topics will be discussed elsewhere in this book, here we briefly review those parts of 21 CFR that are applicable to human health products and medicinal devices [2].

Of most interest to a toxicologist working in this arena would be Chapter 1, Subchapter A (parts 1–78), which cover general provisions, organization, and so on. The Good Laboratory Practices (GLPs) are codified in 21 CFR 58.

Table 37.1 is a checklist of required data to support the development of a drug (in the general case), as well as the expected time to conduct each study through to an audited draft report.

General regulations that apply to drugs are in Subchapter C (Parts 200–299). This covers topics such as labeling, advertising, commercial registration, manufacture, and distribution. Of most interest to a toxicologist would be a section on labeling (Part 201, Subparts A–G, which covers Sections 201.1 through 201.317 of the regulations) as much of the toxicological research on a human prescription drug goes toward supporting a label claim. For example, specific requirements on content and format of labeling for human prescription drugs are covered in Section 201.57. Directions for what should be included under the "Precautions" section of a label are listed in Section 201.57(f). This included Section 201.57(f)(6), which covers categorization of pregnancy risk, and the reliance upon animal reproduction studies in making these categorizations are made quite clear. For example, a drug is given a

TABLE 37.1 General Case Safety Evaluation Requirements [3][a]

Test Requirement (if Approval Sought as Pharmaceutical Ingredient)	Time[b]
Initial Clinical Trial/IND Requirements	
Regulatory Status as a Pharmaceutical Component	
1. Acute toxicity in rodents	6 weeks
2. Acute toxicity in nonrodents	6 weeks
3. Acute parenteral toxicity in rodents	6 weeks
4. Genotoxicity: mutagenicity (bacterial)	4 weeks
5. Genotoxicity: *in vitro* clastogenicity	6 weeks
6. Genotoxicity: *in vivo*	4 weeks
7. Safety pharmacology: CV-hERG[c]	4 weeks
8. Safety pharmacology: CV-Purkinje fiber[c]	4 weeks
9. Safety pharmacology: CV *in vivo*	6 weeks
10. Safety pharmacology: CNS (FOB/Irwin)	4 weeks
11. Safety pharmacology: respiratory—rodent	6 weeks
12. Pivotal/repeat dose in rodents	3–4 months
13. Pivotal/repeat dose in nonrodents	3–4 months
14. Pyrogenicity (if parenteral)	2 weeks
15. Local irritation: ocular	1 month
16. Local irritation: dermal	1 month
17. Immunotoxicity[c]	4 weeks
18. Pivotal/repeat dose in rodents[c]	9 months
19. Pivotal/repeat dose in nonrodents[c]	9 months
20. Reproductive/developmental[c]	6 months
21. Tumorigenicity/carcinogenicity[c]	30 months

[a]All studies described must be performed under GLP.
[b]From study start to draft report.
[c]Not required prior to investigational new drug/first-in-human (IND/FIH) studies but recommended.

Pregnancy Category B if "animal reproduction studies have failed to demonstrate a risk to the fetus." The point here is not to give the impression that the law is most concerned with pregnancy risk. Rather, we wish to emphasize that much basic toxicological information must be summarized on the drug label (or package insert). This section of the law is quite detailed as to what information is to be presented and the format of the presentation. Toxicologists working in the pharmaceutical arena should be familiar with this section of the CFR.

37.2.2 Regulations: Human Pharmaceuticals

The regulations specifically applicable to human drugs are covered in Subchapter D, Parts 300–399. The definition of a new drug is covered in Part 310(g): "A new drug substance means any substance that when used in the manufacture, processing or packaging of a drug causes that drug to be a new drug but does not include intermediates used in the synthesis of such substances."

The regulation then goes on to discuss "newness with regard to new formulations, indications, or in combinations." For toxicologists, the meat of the regulations can be found in Section 312 (INDA) and Section 314 (applications for approval to market a new drug or antibiotic drug or NDA). The major focus for a toxicologist working in the pharmaceutical industry is on preparing the correct toxicology

"packages" to be included to "support" these two types of applications. (The exact nature of these packages is covered later.)

In a nutshell, the law requires solid scientific evidence of safety and efficacy before a new drug will be permitted in clinical trials or (later) on the market. The INDA (covered in 21CFR 310) is for permission to proceed with clinical trials on human subjects. Once clinical trials have been completed, the manufacturer or "sponsor" can then proceed to file an NDA (covered in 21 CFR 314) for permission to market the new drug.

As stated in Section 321.21: "A sponsor shall submit an IND if the sponsor intends to conduct a clinical investigation with a new drug . . . [and] shall not begin a clinical investigation until . . . an IND . . . is in effect." Similar procedures are in place in other major countries. In the United Kingdom, for example, a Clinical Trials Certificate (CTC) must be filed or a CTX (clinical trial exemption) obtained before clinical trials may proceed. Clinical trials are divided into three phases, as described in Section 312.21. Phase I trials are initial introductions into healthy volunteers primarily for the purposes of establishing tolerance (side effects), bioavailability, and metabolism. Phase II clinical trials are "controlled studies . . . to evaluate effectiveness of the drug for a particular indication or disease." The secondary objective is to determine common short-term side effects; hence, the subjects are closely monitored. Phase III studies are expanded clinical trials. It is during this phase that the definitive, large-scale, double-blind studies are performed.

The toxicologist's main responsibilities in the IND process are to design, conduct, and interpret appropriate toxicology studies (or "packages") to support the initial IND and then design the appropriate studies necessary to support each additional phase of investigation. Exactly what may constitute appropriate studies are covered elsewhere in this chapter. The toxicologist's second responsibility is to prepare the toxicology summaries for the (clinical) investigator's brochure (described in Section 312.23(a)(8)(ii)). This is an integrated summary of the toxicological effects of the drug in animals and *in vitro*. The U.S. Food and Drug Administration (FDA) has prepared numerous guidance documents covering the content and format of INDs. It is of interest that in the *Guidance for Industry* [4] an in-depth description of the expected contents of the Pharmacology and Toxicology sections was presented. The document contains the following self-explanatory passage: "Therefore, if final, fully quality-assured individual study reports are not available at the time of IND submission, an integrated summary report of toxicological findings based on the unaudited draft toxicologic reports of the completed animal studies may be submitted."

If unfinalized reports are used in an initial IND, the finalized report must be submitted within 120 days of the start of the clinical trial. The sponsor must also prepare a document identifying any differences between the preliminary and final reports, and the impact (if any) on interpretation.

Thus, while the submission of fully audited reports is preferable, the agency does allow for the use of incomplete reports.

Once an IND or CTC/X is opened, the toxicologists may have several additional responsibilities. First, to design, conduct, and report the additional tests necessary to support a new clinical protocol or an amendment to the current clinical protocol (Section 312.20). Second, to bring to the sponsor's attention any finding in an ongoing toxicology study in animals "suggesting a significant risk to human subjects, including any finding of mutagenicity, teratogenicity or carcinogenicity," as described

TABLE 37.2 Composition of Standard Investigational New Drug Application (INDA)[a]

1. IND cover sheets (form FDA-1571)
2. Table of contents
3. Introductory statement
4. General (clinical) investigation plan
5. (Clinical) investigators brochure
6. (Proposed) clinical protocol(s)
7. Chemistry, manufacturing, and control information
8. Pharmacology and toxicology information (includes metabolism and pharmacokinetic assessments done in animals)
9. Previous human experience with the investigational drug
10. Additional information
11. Other relevant information

[a]Complete and thorough reports on all pivotal toxicological studies must be provided with the application.

in 21 CFR 312.32. The sponsor has a legal obligation to report such findings within 10 working days. Third, to prepare a "list of the preclinical studies . . . completed or in progress during the past year" and a summary of the major preclinical findings. The sponsor is required (under Section 312.23) to file an Annual Report (within 60 days of the IND anniversary date) describing the progress of the investigation. INDs are never "approved" in the strict sense of the word. Once filed, an IND can be opened 30 days after submission, unless the FDA informs the sponsor otherwise. The structure of an IND is outlined in Table 37.2.

If the clinical trials conducted under an IND are successful in demonstrating safety and effectiveness (often established at a Pre-NDA meeting, described in 21 CFR 312.47(b)(2)), the sponsor can then submit an NDA. Unlike an IND, the NDA must be specifically approved by the FDA. The toxicologist's responsibility in the NDA/Marketing Authorization Application (MAA) process is to prepare an integrated summary of all the toxicology and/or safety studies performed and be in a position to present and review the toxicology findings to the FDA or its advisory bodies. The approval process can be exhausting, including many meetings, hearings, and appeals. The ground rules for all of these are described in Part A of the law. For example, all NDAs are reviewed by an "independent" (persons not connected with either the sponsor or the FDA) Scientific Advisory Panel, which will review the findings and make recommendations as to approval. MAAs must be reviewed by and reported on by an expert recognized by the cognizant regulatory authority. Final statutory approval in the United States lies with the Commissioner of the FDA. It is hoped that few additional studies will be requested during the NDA review and approval process. When an NDA is approved, the FDA will send the sponsor an approval letter and will issue a Summary Basis of Approval (SBA)(312.30), which is designed and intended to provide a public record on the FDA's reasoning for approving the NDA while not revealing any proprietary information. The SBA can be obtained through Freedom of Information and can provide insights into the precedents for which types of toxicology studies are used to support specific types of claims.

37.2.3 Regulations: Environmental Impact

Environmental Impact Statements, while once important only for animal drugs, must now accompany all MDAs. This assessment must also be included in the Drug Master File (DMF). The procedures, formats, and requirements are described in 21 CFR 2531. This requirement has grown in response to the National Environmental Policy Act, the heart of which required that federal agencies evaluate every major action that could affect the quality of the environment. In the INDs, this statement can be a relatively short section claiming that relatively small amounts will post little risk to the environment. The EEC has similar requirements for drug entities in Europe, although data requirements are more strenuous. With NDAs, this statement must be more substantial, detailing any manufacturing and/or distribution process that may result in release into the environment. Environmental fate (e.g., photohydrolysis) and toxicity (e.g., fish, daphnia, and algae) studies will be required. While not mammalian toxicology in the tradition of pharmaceutical testing, preparing an environmental impact statement will clearly require toxicological input. The FDA has published a technical bulletin covering the tests it may require [5].

37.2.4 Regulations: Antibiotics

The NDA law (safety and effectiveness) applies to all drugs, but antibiotic drugs were treated differently until the passage of FDAMA in 1997. Antibiotic drugs had been treated differently by the FDA since the development of penicillin revolutionized medicine during World War II. The laws applicable to antibiotic drugs were covered in 21 CFR 430 and 431. Antibiotics such as penicillin or doxorubicin are drugs derived (in whole or in part) from natural sources (such as molds or plants), which have cytotoxic or cytostatic properties. They were treated differently from other drugs as the applicable laws required a batch-to-batch certification process. Originally passed into law in 1945 specifically for penicillin, this certification process was expanded by the 1962 amendment (under Section 507 of the Food, Drug, and Cosmetic Act) to require certification of all antibiotic drugs, meaning that the FDA would assay each lot of antibiotic for purity, potency, and safety. The actual regulations were covered in 21 CFR Subchapter D, Parts 430–460 (over 600 pages), which describes the standards and methods used for certification for all approved antibiotics. Section 507 was repealed by FDAMA (Section 125). As a result of the repeal of Section 507, the FDA is no longer required to publish antibiotic monographs. In addition, the testing, filing, and reviewing of antibiotic applications are now handled under Section 505 of the Act like any other new therapeutic agent. The FDA has published a guidance document to which the reader is referred for more details [6, 7].

37.2.5 Regulations: Biologics

Biological products are covered in Subchapter F, Parts 600–680. As described in 21 CFR 600.3(h), "biological product means any virus, therapeutic serum, toxin, antitoxin or analogous product applicable to the prevention, treatment or cure of diseases or injuries of man." In other words, these are vaccines and other protein

products derived from animal sources. Clearly, the toxicological concerns with such products are vastly different from those involved with low molecular weight synthetic molecules. There is little rational basis, for example, for conducting a one year, repeated dose toxicity study with a vaccine or a human blood product. The FDA definition for safety with regard to these products is found in 21 CFR 603.1(p): "Relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered." Such safety consideration has more to do with purity, sterility, and adherence to good manufacturing standards than with the toxicity of the therapeutic molecule itself. The testing required to show safety is stated in the licensing procedures 21 CFR 601.25(d)(1): "Proof of safety shall consist of adequate test methods reasonably applicable to show the biological product is safe under the prescribed conditions." Once a license is granted, each batch or lot of biological product must be tested for safety, and the methods of doing so are written into the law. A general test for safety (i.e., required in addition to other safety tests) is prescribed using guinea pigs as described in Section 610.11. Additional tests are often applied to specific products. For example, 21 CFR 630.35 describes the safety tests required for measles vaccines, which includes tests in mice and *in vitro* assays with tissue culture. Many new therapeutic entities produced by biotechnology are seeking approval as biologics with the results being FDA approval of a Product License Application (PLA). Table 37.3 presents general guidance for the basis of deciding if an individual entity falls under the Center for Drug

TABLE 37.3 Product Class Review Responsibilities

Center for Drug Evaluation and Review
Natural products purified from plant or mineral sources
Products produced from solid tissue sources (excluding procoagulants, venoms, blood products, etc.)
Antibiotics, regardless of method of manufacture
Certain substances produced by fermentation
Disaccharidase inhibitors
HMG-CoA inhibitors
Synthetic chemicals
Traditional chemical synthesis
Synthesized mononuclear or polynuclear products including antisense chemicals
Hormone products
Center for Biologics Evaluation and Review
Vaccines, regardless of manufacturing method
In vivo diagnostic allergenic products
Human blood products
Protein, peptide, and/or carbohydrate products produced by cell culture (other than antibiotics and hormones)
Immunoglobulin products
Products containing intact cells or microorganisms
Proteins secreted into fluids by transgenic animals
Animal venoms
Synthetic allergens
Blood banking and infusion adjuncts

Evaluation and Research (CDER) or the Center for Biologic Evaluation and Research (CBER) authority for review.

The International Conference on Harmonization has published its document *S6 Preclincial Safety Evaluation of Biotechnology-Derived Pharmaceuticals*. The FDA (both the Center for Drug Evaluation and Research, and the Center for Biologics Evaluation and Research jointly) has published the document as a *Guidance for Industry* [8, 9].

A current list of regulatory documents available by email (including the most recent PTCs, or points to consider) can be found at DOC_LIST@A1.FDA.GOV.

37.2.6 Regulations Versus Law

A note of caution must be inserted here. The law (the document passed by Congress) and the regulations (the documents written by the regulatory authorities to enforce the laws) are separate documents. The sections in the law do not necessarily have numerical correspondence. For example, the regulations on the NDA process is described in 21 CFR 312, but the law describing the requirement for an NDA process is in Section 505 of the FDCA. Because the regulations rather than the laws themselves have a greater impact on toxicological practice, greater emphasis is placed on regulation in this chapter. For a complete review of FDA law, the reader is referred to the monograph by Food and Drug Law Institute [2].

Laws authorize the activities and responsibilities of the various federal agencies. All proposed laws before the U.S. Congress are referred to committees for review and approval.

37.3 ORGANIZATIONS REGULATING DRUGS IN THE UNITED STATES

The agency formally charged with overseeing the safety of drugs in the United States is the FDA. It is headed by a commissioner who reports to the Secretary of the Department of Health and Human Services (DHHS) and has a tremendous range of responsibilities. Drugs are overseen primarily by the CDER (although some therapeutic or health care entities are considered biologics and are overseen by the corresponding CBER). Figure 37.1 presents the organization of CDER. The organization of CBER is shown in Fig. 37.2.

Most of the regulatory interactions of toxicologists are with the two offices of Drug Evaluation, which have under them a set of groups focused on areas of therapeutic claim (cardiorenal, neuropharmacological, gastrointestinal and coagulation, oncology and pulmonary, metabolism and endocrine, antiinfective and antiviral). Within each of these are chemists, pharmacologists/toxicologists, statisticians, and clinicians. When an INDA is submitted to the offices of Drug Evaluation, it is assigned to one of the therapeutic groups based on its area of therapeutic claim. Generally, it will remain with that group throughout its regulatory approval "life." INDs, when allowed, grant investigators the ability to go forward into clinical (human) trials with their drug candidate in a predefined manner, advancing through various steps of evaluation in human (and in additional preclinical or animal studies)

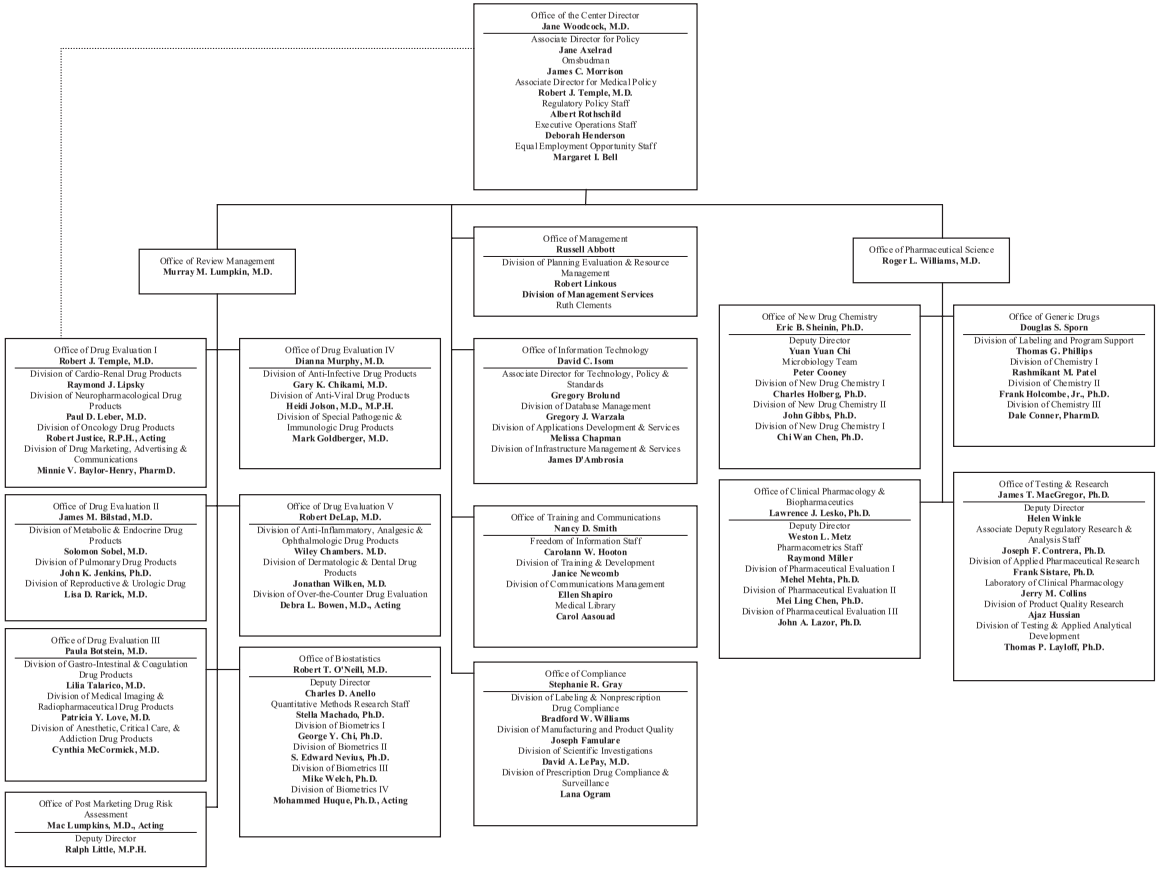


FIGURE 37.1 Organizational chart for CDER.

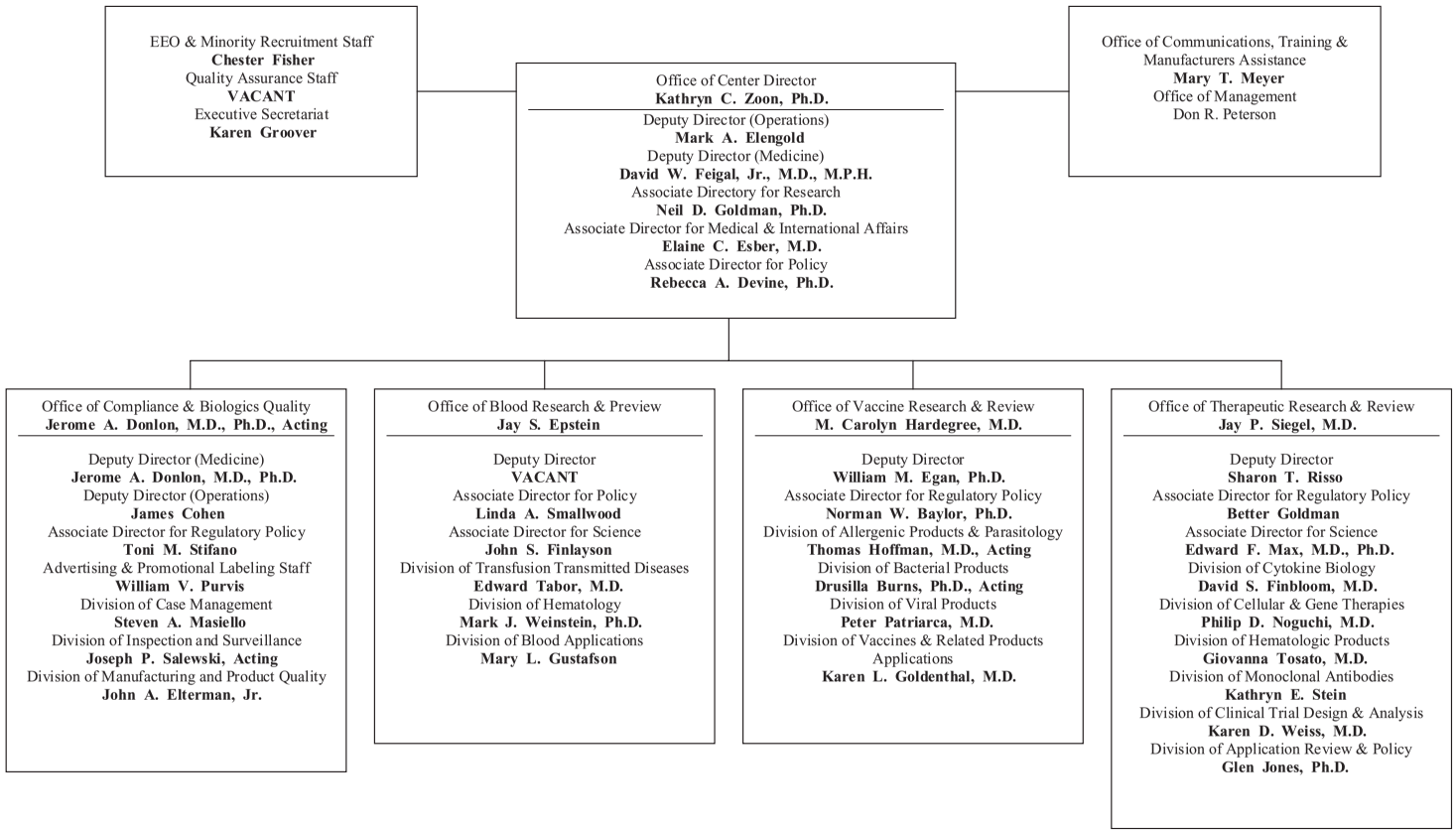


FIGURE 37.2 Organizational chart for CBER.

until an NDA can be supported, developed, and submitted. Likewise for biological products, the PLA or other applications (INDA, IND) are handled by the offices of Biological Products Review of the CBER.

For drugs, there is at least one nongovernmental body that must review and approve various aspects—the USP (United States Pharmacopeia, established in 1820)—which maintains (and revises) the compendia of the same name, as well as the National Formulary, which sets drug composition standards [10]. This volume sets forth standards for purity of products in which residues may be present and tests for determining various characteristics of drugs, devices, and biologics.

37.4 PROCESS OF PHARMACEUTICAL PRODUCT DEVELOPMENT AND APPROVAL

Except for a very few special cases (treatments for life-threatening diseases such as cancer or AIDS), the safety assessment of new drugs as mandated by regulations proceeds in a rather fixed manner. The IND is filed to support this clinical testing. An initial set of studies (typically, studies of appropriate length by the route intended for humans are performed in both a rodent (typically rat) and a nonrodent (usually a dog or a primate)) are required to support Phase I clinical testing. Such Phase I testing is intended to evaluate the safety ("tolerance" in clinical subjects), pharmacokinetics, and general biological effects of a new drug and is conducted in normal volunteers (almost always males).

Successful completion of Phase I testing allows, with the approval of the FDA, progression into Phase II clinical testing. Here, selected patients are enrolled to evaluate therapeutic efficacy, dose ranging, and more details about the pharmacokinetics and metabolism. Longer-term systemic toxicity studies must be in conformity with the guidelines that are presented in the next section. Once a sufficient understanding of the actions, therapeutic dose response, and potential risk-to-benefit ratio of the drug is in hand (once again, with FDA approval), trials move into Phase III testing.

Phase III tests are large, long, and expensive. They are conducted using large samples of selected patients and are intended to produce proof of safety and efficacy of the drug. Two studies providing statistically significant proof of the claimed therapeutic benefit must be provided. All the resulting data from preclinical and clinical animal studies are organized in a specified format in the form of an NDA, which is submitted to the FDA.

By the time that Phase III testing is completed, some additional preclinical safety tests must also generally be in hand. These include the three separate reproductive and developmental toxicity studies (Segments I and III in the rat, and Segment II in the rat and rabbit) and carcinogenicity studies in both rats and mice (unless the period of therapeutic usage is intended to be very short). Some assessment of genetic toxicity will also be expected.

The ultimate product of the pharmaceutical toxicologist will thus generally be the toxicology summaries of the IND and NDA (or PLA). For medical devices, the equivalents are the IDE and Product Development Notification (PDN). Data required to support each of these documents is specified in a series of guidelines, as discussed later.

Acceptance of these applications is contingent not only upon adherence to guidelines and good science, but also to GLPs.

37.5 TESTING GUIDELINES

37.5.1 Toxicity Testing: Traditional Pharmaceuticals

Although the 1938 Act required safety assessment studies, no consistent guidelines were available [11]. Guidelines were first proposed in 1949 and published in the *Food, Drug and Cosmetic Law Journal* that year [12]. Following several revisions, these guidelines were issued as *The Appraisal Handbook* in 1959. While never formally called a guideline, it set the standard for preclinical toxicity test design for several years. The current basic guidelines for testing required for safety assessment in support of the phases of clinical development of drugs were first outlined by Goldenthal [13, 14] and later incorporated into a 1971 FDA publication entitled *FDA Introduction to Total Drug Quality* [15].

37.5.2 General or Systematic Toxicity Assessment

Table 37.4 presents an overview of the current FDA toxicity testing guidelines for human drugs. Table 37.5 presents the parallel ICH guidelines [16], which are now largely supplanting the FDA guidelines. They are misleading in their apparent simplicity, however. First, each of the systemic toxicity studies in these guidelines must be designed and executed in a satisfactory manner. Sufficient animals must be used to have confidence in finding and characterizing any adverse drug actions that may be present. In practice, as the duration of the study increases, small doses are administered and larger numbers of animals must be employed per group. These two features—dosage level and group size—are critical to study designs. Table 37.6 presents general guidance on the number of animals to be used in systemic studies.

Note that for novel chemical structures, the FDA may still require 12 month nonrodent toxicity studies.

The protocols discussed thus far have focused on general or systemic toxicity assessment. The agency and, indeed, the lay public have a special set of concerns with reproductive toxicity, fetal/embryo toxicity, and developmental toxicity (also called *teratogenicity*). Collectively, these concerns often go by the acronyms *DART* (developmental and reproductive toxicity) or *RTF* (reproduction, teratogenicity, fertility). Segment II studies are more designed to detect developmental toxicity. Only pregnant females are dosed during the critical period of organogenesis. Generally, the first protocol DART test (exclusive of range-finding studies) is a Segment I study of rats in fertility and general reproductive performance. This is generally done while the drug is in Phase II clinical trials. Alternatively, many companies are now performing the Segment II teratology study in rats before the Segment I study because the former is less time and resource intensive. One or both should be completed before including women of child-bearing potential in clinical trials. The FDA requires teratogenicity testing in two species—a rodent (rat or mouse) and the rabbit. The use of the rabbit was instituted as a result of the finding that thalidomide was a positive teratogen in the rabbit but not in the rat. On occasion, when a test

TABLE 37.4 Synopsis of General Guidelines for Animal Toxicity Studies for Drugs

Category	Duration of Human Administration	Clinical Phase	Subacute or Chronic Toxicity	Special Studies
Oral or parenteral	Several days	I, II, III, NDA	Two species; 2 weeks	For parenterally administered drugs
	Up to 2 weeks	IIIIII, NDA	Two species; 4 weeksTwo species; up to 4 weeks Two species; up to 3 months	
	Up to 3 months	I, IIIIINDA	Two species; 4 weeksTwo species; 3 months Two species; up to 6 months	Compatibility with blood where applicable
	Six months to unlimited	I, IIIIINDA	Two species; 3 months Two species; 6 months or longer Two species; 9 months (nonrodent) and 6 months (rodent) +Two rodent species for CA; 18 months (mouse) May be replaced with an allowable transgenic mouse study; 24 months (rat)	
Inhalation (general anesthetics)		I, II, III, NDA	Four species; 5 days (3 h/day)	
Dermal	Single application	I	One species; single 24 hour exposure followed by 2 week observation	Sensitization
	Single or short-term application	II	One species; 20 day repeated exposure (intact and abraded skin)	
	Short-term application	III	As above	
	Unlimited application	NDA	As above, but intact skin study extended up to 6 months	
Ophthalmic	Single application	I		Eye irritation tests with graded doses
	Multiple applications	I, II, IIINDA	One species; 3 weeks daily applications, as in clinical use One species; duration commensurate with period of drug administration	
Vaginal or rectal	Single application Multiple applications	II, II, III, NDA	Two species; duration and number of applications determined by proposed use	Local and systematic toxicity after vaginal or rectal application in two species
Drug combinations	(4)	III, III, NDA	Two species; up to 3 months	Lethality by appropriate route, compared to components run concurrently in one species

TABLE 37.5 Duration of Repeated Dose Toxicity Studies to Support Clinical Trials and Marketing[a]

Duration of Clinical Trials	Minimum Duration of Repeated Dose Toxicity Studies[b]		Duration of Clinical Trials	Minimum Duration of Repeated Dose Toxicity Studies[c]	
	Rodents	Nonrodents		Rodents	Nonrodents
Single dose	2 weeks[d]	2 weeks	Up to 2 weeks	1 month	1 month
Up to 2 weeks	2 weeks[d]	2 weeks	Up to 1 month	3 months	3 months
Up to 1 month	1 month	1 month	Up to 3 months	6 months	3 months
Up to 6 months	6 months	6 months[b]	>3 months	6 months	Chronic[d]
>6 months	6 months	Chronic[b]			

[a]In Japan, if there are no Phase II clinical trials of equivalent duration to the planned Phase III trials, conduct of longer duration toxicity studies is recommended.[b]Data from 6 months of administration in nonrodents should be available before the initiation of clinical trials longer than 3 months. Alternatively, if applicable, data from a 9 month nonrodent study should be available before the treatment duration exceeds that which is supported by the available toxicity studies.
[c]The table also reflects the marketing recommendations in the three regions except that a chronic nonrodent study is recommended for clinical use >1 month.
[d]In the United States, as an alternative to 2 week studies, single-dose toxicity studies with extended examinations can support single-dose human trials.

TABLE 37.6 Numbers of Animals per Dosage Group in Systemic Toxicity Studies

Study Duration (per sex)	Rodents (per sex)	Nonrodents
2–4 weeks	5	3
13 weeks	20[a]	6
26 weeks	30	8
Chronic	50	10
Carcinogenicity	60[b]	Applies only to contraceptives
Bioassays		Applies only to contraceptives

[a]Starting with 13 week studies, one should consider adding animals (particularly to the high dose) to allow evaluation of reversal of effects.
[b]In recent years there have been decreasing levels of survival in rats on 2 year studies. What is required is that at least 20–25 animals/sex/group survive at the end of the study. Accordingly, practice is beginning to use 70 or 75 animals per sex, per group.

article is not compatible with the rabbit, teratogenicity data in the mouse may be substituted. There are also some specific classes of therapeutics (e.g., the quinalone antibioticsexample) where Segment II studies in primates are effectively required prior to product approval. Both should be completed before entering Phase III clinical trials. The most complicated of the DART protocols—Segment III—is generally commenced during Phase III trials and should be part of the NDA. There are differences in the different national guidelines (as discussed later with international considerations) regarding the conduct of these studies. The large multinational drug companies try to design their protocols to be in compliance with as many of the

guidelines as possible to avoid duplication of testing while allowing the broadest possible approval and marketing of therapeutics.

37.5.3 Genetic Toxicity Assessment

Genetic toxicity testing generally focuses on the potential of a new drug to cause mutations (in single-cell systems) or other forms of genetic damage. The tests, generally short in duration, often rely on *in vitro* systems and generally have a single endpoint of effect (point mutations, chromosomal damage, etc.). It is of interest that the FDA has no standard or statutory requirement for genetic toxicity testing but generally expects to see at least some such tests performed and will ask for them if the issue is not addressed. If one performs such a study, any data collected, of course, must be sent to the FDA as part of any INDA, PLA, or NDA. These studies have yet to gain favor with the FDA (or other national regulatory agencies) as substitutes for *in vivo* carcinogenicity testing. However, even with completed negative carcinogenicity tests, at least some genetic toxicity assays are generally required. Generally, pharmaceuticals in the United States are evaluated for mutagenic potential (e.g., the Ames' Assay) or for chromosomal damage (e.g., the *In Vivo* Mouse Micronucleus Test). In general, in the United States, pharmaceutical companies apply genetic toxicity testing in the following fashion:

- *As a Screen.* An agent that is positive in one or more genetic toxicity tests may be more likely than one that is negative to be carcinogenic and therefore may not warrant further development.
- *As an Adjunct.* An agent that is negative in carcinogenicity testing in two species and also negative in a genetic toxicity battery is more likely than not to be noncarcinogenic in human beings.
- *To Provide Mechanistic Insight.* For example, if an agent is negative in a wide range of genetic toxicity screens but still produces tumors in animals, then one could hypothesize that an epigenetic mechanism was involved.

While not officially required, the FDA does have the authority to request, on a case-by-case basis, specific tests it feels may be necessary to address a point of concern. A genetic toxicity test could be part of such a request. In general, therefore, companies deal with genetic toxicity (after "screening") on a case-by-case basis, dictated by good science. If more than a single administration is intended, common practice is to perform the tests prior to submitting an IND.

37.5.4 Toxicity Testing: Biotechnology Products

As mentioned, the regulation of traditional pharmaceuticals (small molecules such as aspirin or digitalis) and biologicals (proteins such as vaccines and antitoxins derived from animal sources) have very different histories. See Section 37.2.5. Until 1972, the NIH (or its forerunning agency, the Hygienic Laboratory of the Department of the Treasury) was charged with the responsibilities of administering the Virus Act of 1902. With the passage of the Food and Drug Laws of 1906, 1938, and 1962, there was recurring debate about whether these laws applied or should apply

to biologicals. This debate was resolved when the authority for the regulation of biologics was transferred to the FDA's new Bureau of Biologics (now the CBER) in 1972. Since then, there appears to have been little difference in the matter of regulation for biologics and pharmaceuticals. The FDA essentially regulates biologics as described under the 1902 act, but then uses the rule-making authority granted under the Food and Drug Act to "fill in the gaps."

The Bureau of Biologics was once a relatively "sleepy" agency, primarily concerned with the regulation of human blood products and vaccines used for mass immunization programs. The authors of the 1902 law could hardly have foreseen the explosion in biotechnology that occurred in the 1980s. New technology created a welter of new biological products, such as recombinant-DNA-produced proteins (e.g., tissue plasminogen activator), biological response modifiers (cytokinins and colony-stimulating factors), monoclonal antibodies, antisense oligonucleotides, and self-directed vaccines (raising an immune response to self-proteins such as gastrin for therapeutic reasons). The new products raised a variety of new questions on the appropriateness of traditional methods of evaluating drug toxicity that generated several points-to-consider documents [17–19]. For the sake of brevity, this discussion focuses on the recombinant DNA proteins. Some safety issues have been raised over the years [20]:

- The appropriateness of testing a human-specific peptide hormone in nonhuman species.
- The potential that the peptide could break down due to nonspecific metabolism, resulting in products that had no therapeutic value or even a toxic fragment.
- The potential sequelae to an immune response (formation of neutralizing antibodies, provoking an autoimmune or a hypersensitivity response), and pathology due to immune precipitation and so on.
- The presence of contamination with oncogenic virus DNA (depending on whether a bacterial or mammalian system was used on the synthesizing agent) or endotoxins.
- The difficulty interpreting the scientific relevance of response to supraphysiological systemic doses of potent biological response modifiers.

The intervening last few years have shown that some of these concerns were more relevant than others [21]. The "toxic peptide fragment" concern, for example, has been shown to be without merit. The presence of potentially oncogenic virus DNA and endotoxins is a quality assurance concern and is not truly a toxicological problem. Regardless of the type of synthetic pathway, all proteins must be synthesized in compliance with Good Manufacturing Practices. Products must be as pure as possible, not only free of rDNA but also free of other types of cell debris (endotoxin). Batch-to-batch consistency with regard to molecular structure must also be demonstrated using appropriate methods (e.g., amino acid). The regulatory thinking and experience over the last fifteen years has come together in the document *S6 Preclincial Safety Evaluation of Biotechnology-Derived Pharmaceuticals* prepared by the International Conference on Harmonization. The FDA (both the Center for Drug Evaluation and Research, and the Center for Biologics Evaluation

and Research jointly) has published the document as a *Guidance for Industry* [5, 6]. The document intended to provide basic guidance for the preclinical evaluation of biotechnology-derived products, including proteins and peptides, produced by cell culture using rDNA technology, but did not cover antibiotics, allergenic extracts, heparin, vitamins, cellular drug products, vaccines, or other products regulated as biologics. Items covered are summarized as follows [22]:

Test Article Specifications. In general, the product that is used in the definitive pharmacology and toxicology studies should be comparable to the product proposed for the initial clinical studies.

Animal Species/Model Selection. Safety evaluation should include the use of relevant species, in which the test article is pharmacologically active due, for example, to the expression of the appropriate receptor molecule. These can be screened with *in vitro* receptor binding assays. Safety evaluation should normally include two appropriate species, if possible and/or feasible. The potential utility of gene knockout and/or transgenic animals in safety assessment is discussed.

Group Size. No specific numbers are given, but it does state that a small sample size may lead to failure to observe toxic events.

Administration. The route and frequency should be as close as possible to that proposed for clinical use. Other routes can be used when scientifically warranted.

Immunogenicity. It has also been clearly demonstrated in the testing of rDNA protein products that animals will develop antibodies to foreign proteins. This response has been shown to neutralize (rapidly remove from circulation) the protein, but no pathological conditions have been shown to occur as a sequelae to the immune response. Bear in mind, however, that interleukins have powerful effects on immune response, but these are due to their physiological activity and not to an antigen–antibody response. The first has to do with "neutralizing antibodies." That is, is the immune response so great that the test article is being removed from circulation as fast as it is being added? If this is the case, does long-term testing of such a chemical make sense? In many cases, it does not. The safety testing of any large molecule should include the appropriate assays for determining whether the test system has developed a neutralizing antibody response. Depending on the species, route of administration, intended therapeutic use, and development of neutralizing antibodies (which generally takes about 2 weeks), it is rare for a toxicity test on an rDNA protein to be longer than 4 weeks duration. However, if the course of therapy in humans is to be longer than 2 weeks, formation of neutralizing antibodies must be demonstrated or longer-term testing performed. The second antigen–antibody formation concern is that a hypersensitivity response will be elicited. Traditional preclinical safety assays are generally adequate to guard against this if they are 2 weeks or longer in duration and the relevant endpoints are evaluated.

Safety Pharmacology. It is important to investigate the potential for unwanted pharmacological activity in appropriate animal models and to incorporate monitoring for these activities in the toxicity studies.

Exposure Assessment. Single- and multiple-dose pharmacokinetics, toxicokinetics, and tissue distribution studies in relevant species are useful. Proteins are not given orally; demonstrating absorption and mass balance is not typically a primary consideration. Rather, this segment of the test should be designed to determine half-life (and other appropriate pharmacokinetic descriptor parameters), the plasma concentration associated with biological effects, and potential changes due to the development of neutralizing antibodies.

Reproductive Performance and Developmental Toxicity Studies. These will be dictated by the product, clinical indication, and intended patient population.

Genotoxicity Studies. The S6 document states that the battery of genotoxicity studies routinely conducted for traditional pharmaceuticals are not appropriate for biotechnology-derived pharmaceuticals. In contrast to small molecules, genotoxicity testing with a battery of *in vitro* and *in vivo* techniques of protein molecules has not become common U.S. industry practice. Such tests are not formally required by the FDA but, if performed, have to be reported. They are required by European and Japanese regulatory authorities. This has sparked a debate as to whether or not genotoxicity testing is necessary or appropriate for rDNA protein molecules. It is the author's opinion that such testing is, scientifically, of little value. First, large protein molecules will not easily penetrate the cell wall of bacteria or yeast, and (depending on size, charge, lipophilicity, etc.) penetration across the plasma lemma of mammalian cells will be highly variable. Second, if one considers the well-established mechanism(s) of genotoxicity of small molecules, it is difficult to conceive of how a protein can act in the same fashion. For example, proteins will not be metabolized to be electrophilic active intermediates that will crosslink guanine residues. In general, therefore, genotoxicity testing with rDNA proteins is a waste of resources. It is conceivable, however, that some proteins, because of their biological mechanism of action, may stimulate the proliferation of transformed cells. For example, it is a feasible hypothesis that a colony-stimulating factor could stimulate the proliferation of leukemic cells (it should be emphasized that this is a hypothetical situation, presented here for illustrative purposes). Again, this is a question of a specific pharmacological property, and such considerations should be tested on a case-by-case basis.

Carcinogenicity Studies. These are generally inappropriate for biotechnology-derived pharmaceuticals; however, some products may have the potential to support or induce proliferation of transformed cells . . . possibly leading to neoplasia. When this concern is present, further studies in relevant animal models may be needed.

These items are covered in greater detail in the S6 guidance document and in a review by Ryffel [23].

So, given the above discussion, what should the toxicology testing package of a typical rDNA protein resemble? Based on the products that have successfully wound their way through the regulatory process, the following generalizations can be drawn.

- The safety tests look remarkably similar to those for traditional tests. Most have been done on three species: the rat, the dog, or the monkey. The big difference

has to do with the length of the test. It is rare for a safety test on a protein to be more than 13 weeks long.

- The dosing regimens can be quite variable and at times very technique intensive. These chemicals are almost always administered by a parenteral route—normally intravenously or subcutaneously. Dosing regimens have run the range from once every 2 weeks for an antihormone "vaccine" to continuous infusion for a short-lived protein.
- As reviewed by Ryffel [24], most side effects in humans of a rDNA therapy may be predicted by data from experimental toxicology studies, but there are exceptions. IL-6, for example, induced a sustained increase in blood platelets and acute phase proteins, with no increase in body temperature. In human trials, however, there were increases in temperature.
- The S6 document also mentions monoclonal antibody products. Indeed, many of the considerations for rDNA products are also applicable to monoclonal antibodies (including hybridized antibodies). With monoclonal antibodies, there is the additional concern of crossreactivity with nontarget molecules.

As mentioned, the rapid development in the biotechnology industry has created some confusion as to what arm of the FDA is responsible for such products. In October 1992, the two major reviewing groups, CBER and CDER, reached a series of agreements to explain and organize the FDA's position on products that did not easily fall into its traditional classification schemes. CDER will continue to have responsibility for traditional chemically synthesized molecules as well as those purified from mineral or plant sources (except allergenics), antibiotics, hormones (including insulin and growth hormone), most fungal or bacterial products (disaccharidase inhibitors), and most products from animal or solid human tissue sources. CBER will have responsibility for products subject to licensure (BLA), including all vaccines, human blood or blood-derived products (as well as drugs used for blood banking and transfusion), immunoglobulin products, products containing intact cells, fungi, viruses, proteins produced by cell culture or transgenic animals, and synthetic allergenic products. This situation was further simplified by the introduction of the concept of *well-characterized biologics*. When introduced during the debate on FDA reform in 1996, the proposed section of S-1447 stated that "biological products that the secretary determines to be well-characterized shall be regulated solely under the Federal Food, Drug and Cosmetic Act." Under this concept, highly purified, well-characterized therapeutic rDNA proteins would be regulated by CDER, regardless of therapeutic target [25].

37.5.5 Toxicity/Safety Testing: Cellular and Gene Therapy Products

Human clinical trials of cellular and gene therapies involve administration to patients of materials considered investigational biological, drug, or device products. Somatic cell therapy refers to the administration to humans of autologous, allogenic, or xenogenic cells, which have been manipulated or processed *ex vivo*. Gene therapy refers to the introduction into the human body of genes or cells containing genes foreign to the body for the purposes of prevention, treatment, diagnosing, or curing disease.

Sponsors of cellular or gene therapy clinical trials must file an Investigational New Drug Application (INDA) or in certain cases an Investigational Device Exemption (IDE) with the FDA before initiation of studies in humans. It is the responsibility of the Center of Biologics Evaluation and Research (CBER) to review the application and determine if the submitted data and the investigational product meet applicable standards. The critical parameters of identity, purity, potency, stability, consistency, safety, and efficacy relevant to biological products are also relevant to cellular and gene therapy products.

In 1991, the FDA first published *Points to Consider* in *Human Somatic Cell and Gene Therapy* [18]. At this time virtually all gene therapies were retroviral and were prepared as *ex vivo* somatic cell therapies. This was subsequently reviewed by Kessler et al. [26]. While the data for certain categories of information such as the data regarding the molecular biology were defined in previous guidance documents relating to recombinant DNA products, the standards for preclinical and clinical development were less well defined. Over the past five years, the field has advanced to include not only new vectors but also novel routes of administration. The *Points to Consider in Human Somatic Cell Therapy and Gene Therapy* [27] has thus been recently amended to reflect both the advancements in product development and more importantly the accumulation of safety information over the past five years.

FDA regulations state that the sponsor must submit, in the IND, adequate information about pharmacological and toxicological studies of the drug including laboratory animals or *in vitro* studies on the basis of which the sponsor has considered that it is reasonably safe to conduct the proposed clinical investigation. For cellular and gene therapies, designing and conducting relevant preclinical safety testing has been a challenge to both the FDA and the sponsor. For genes delivered using viral vectors, the safety of the vector system per se must be considered and evaluated.

The preclinical knowledge base is initially developed by designing studies to answer fundamental questions. The development of this knowledge base is generally applicable to most pharmaceuticals, as well as biopharmaceuticals, and includes data to support (1) the relationship of the dose to the biological activity, (2) the relationship of the dose to the toxicity, (3) the effect of route and/or schedule on activity or toxicity, and (4) identification of the potential risks for subsequent clinical studies. These questions are considered in the context of indication and/or disease state. In addition, there are often unique concerns relative to the specific category or product class.

For cellular therapies, safety concerns may include development of a database from studies specifically designed to answer questions relating to growth factor dependence, tumorigenicity, local and systemic toxicity, and effects on host immune responses including immune activation and altered susceptibility to disease. For viral-mediated gene therapies, specific questions may relate to the potential for overexpression of the transduced gene, transduction of normal cells/tissues, genetic transfer to germ cells and subsequent alterations to the genome, recombination/rescue with endogenous virus, reconstitutions of replication competence, potential for insertional mutagenesis/malignant transformation, altered susceptibility to disease, and/or potential risk(s) to the environment.

To date, cellular and gene therapy products submitted to the FDA have included clinical studies indicated for bone marrow marking, cancer, cystic fibrosis, AIDS, and

inborn errors of metabolism and infectious diseases. Of the current active INDs, approximately 78% have been sponsored by individual investigators or academic institutions and 22% have also been industry sponsored. In addition to the variety of clinical indications the cell types have also been varied. Examples include tumor infiltrating lymphocytes (TILs) and lymphocyte activated killer (LAK) cells, selected cells from bone marrow, and peripheral blood lymphocytes (e.g., stem cells, myoblasts, tumor cells), and encapsulated cells (e.g., islet cells and adrenal chromaffin cells).

Cellular Therapies Since 1984 the CBER has reviewed close to 300 somatic cell therapy protocols. Examples of the specific categories include manipulation, selection, mobilization, tumor vaccines, and other.

Manipulation—autologous, allogenic, or xenogenic cells that have been expanded, propagated, manipulated, or had their biological characteristics altered *ex vivo* (e.g., TIL or LAK cells; islet cells housed in a membrane).

Selection—products designed for positive or negative selection if autologous or allogenic cells are intended for therapy (e.g., purging of tumor from bone marrow, selection of CD34+ cells).

Mobilization—in vivo mobilization of autologous stem cells intended for transplantation.

Tumor vaccines—autologous or allogenic tumor cells that are administered as vaccine (e.g., tumor cell lines, tumor cell lysates, primary explant). This group also includes autologous antigen presenting cells pulsed with tumor-specific peptides or tumor cell lysates.

Other—autologous, allogenic, and xenogenic cells that do not specifically fit the above; this group includes cellular therapies such as extracorporeal liver assist devices.

Gene Therapies The types of vectors that have been used, or proposed, for gene transduction include retrovirus, adenovirus, adeno-associated viruses, other viruses (e.g., herpes, vaccinia), and plasmid DNA. Methods for gene introduction include *ex vivo* replacement, drug delivery, and marker studies and others and *in vivo*, viral vectors, plasmid vectors, and vector producer cells.

Ex Vivo

Replacement—cells transduced with a vector expressing a normal gene in order to correct or replace the function of a defective gene.

Drug delivery—cells transduced with a vector expressing a gene encoding a therapeutic molecule that can be novel or native to the host.

Marker studies—cells (e.g., bone marrow, stem cells) transduced with a vector expressing a marker or reporter gene used to distinguish it from other similar host tissues.

Other—products that do not specifically fit the above (e.g., tumor vaccines in which cells are cultured or transduced *ex vivo* with a vector).

In Vivo

Viral vectors—the direct administration of a viral vector (e.g., retrovirus, adenovirus, adeno-associated virus, herpes, vaccinia) to patients.

Plasmid vectors—the direct administration of plasmid vectors with or without other vehicles (e.g., lipids) to patients.

Vector producer cells—the direct administration of retroviral vector producer cells (e.g., murine cells producing HTK vector) to patients.

Preclinical Safety Evaluation The goal of preclinical safety evaluation includes the recommendation of an initial safe starting dose and safe dose-escalation scheme in humans, identification of potential target organ(s) of toxicity, identification of appropriate parameters for clinical monitoring, and identification of "at risk" patient population(s). Therefore, when feasible, toxicity studies should be performed in relevant species to assess a dose-limiting toxicity. General considerations in study design include selection of the model (e.g., species, alternative model, animal model, or disease), dose (e.g., route, frequency, and duration), and study endpoint (e.g., activity and/or toxicity).

The approach to preclinical safety evaluation of biotechnology-derived products, including novel cellular and gene therapies, has been referred to as the *case-by case* approach. This approach is science-based, data-driven, and flexible. The major distinction from past practices from traditional pharmaceuticals is that the focus is directed at asking specific questions across various product categories. Additionally, there is a consistent reevaluation of the knowledge base to reassess real or theoretical safety concerns and hence reevaluation of the need to answer the same questions across all product categories. In some cases there may even be conditions that may not need specific toxicity studies: for example, when there is a strong efficacy model that is rationally designed to answer specific questions and/or there is previous human experience with a similar product with respect to dose and regimen.

Basic Principles for Preclinical Safety Evaluation of Cellular and Gene Therapies (Biotechnology-Derived Products in General)

- Use of product in animal studies that is comparable or the same as the product proposed for clinical trial(s).
- Adherence to basic principles of GLP to ensure quality of the study including a detailed protocol prepared prospectively.
- Use of the same or similar route and method of administration as proposed for clinical trials (whenever possible).
- Determination of appropriate doses delivered based on preliminary activity obtained from both *in vitro* and *in vivo* studies (i.e., finding a dose likely to be effective and not dangerous, a no observed adverse effect level, and a dose-causing dose-limiting toxicity).

- Selection of one or more species sensitive to the endpoint being measured (e.g., infections or pathologic sequelae) and/or biological activity or receptor binding.
- Consideration of animal model(s) of disease as better to assess the contribution of changes in physiologic or underlying physiology to safety and efficacy.
- Determination of effect on host immune response.
- Localization/distribution studies—evaluation of target tissue, normal surrounding tissue, and distal tissue sites and any alteration in normal or expected distribution, and local reactogenicity.

Additional Considerations for Cellular Therapies

- Evaluation of cytopathogenicity.
- Evaluation of signs of cell transformation/growth factor dependence—effect on animal cells, normal human cells, and cells prone to transform easily.
- Determination of alteration in cell phenotype, altered cell products, and/or function.
- Tumorigenicity.

Additional Considerations for Gene Therapies

- Determination of phenotype/activation state of effector cells.
- Determination of vector/transgene toxicity.
- Determination of potential transfer to germ line.
- *In vitro* challenge studies—evaluation of recombination or complementation, potential for "rescue" for subsequent infection with wild-type virus.
- Determination of persistence of cells/vector.
- Determination of potential for insertional mutagenesis (malignant transformation).
- Determination of environmental spread (e.g., viral shedding).

37.6 TOXICITY TESTING: SPECIAL CASES

On paper, the general case guidelines for the evaluation of the safety of drugs are relatively straightforward and well understood. However, there are also a number of special case situations under which either special rules apply or some additional requirements are relevant. The more common of these are summarized next.

37.6.1 Oral Contraceptives

Oral contraceptives are subject to special testing requirements. These have recently been modified so that in addition to those preclinical safety tests generally required, the following are also required [28]:

- A 3 year carcinogenicity study in beagles (this is a 1987 modification in practice from earlier FDA requirements and the 1974 publication).
- A rat reproductive (Segment I) study including a demonstration of return to fertility.

37.6.2 Life-Threatening Diseases (Compassionate Use)

Drugs to treat life-threatening diseases are not strictly held to the sequence of testing requirements because the potential benefit on any effective therapy in these situations is so high [29]. In the early 1990s, this situation applied to AIDS-associated diseases and cancer. The development of more effective HIV therapies (protease inhibitors) has now made cancer therapy more the focus of these considerations. Although the requirements for safety testing prior to initial human trials is unchanged, subsequent requirements are flexible and subject to negotiation and close consultation with the FDA's Division of Oncology (within CDER) [29, 30]. The more recent thinking on anticancer agents has been reviewed by DeGeorge et al. [31]. The preclinical studies that will be required to support clinical trials and marketing of new anticancer agents will depend on the mechanism of action and the target clinical population. Toxicity studies in animals will be required to support initial clinical trials. These studies have multiple goals: (1) determine a starting dose for clinical trials, (2) identify target organ toxicity and assess recovery, and (3) assist in the design of clinical dosing regimens.

The studies should generally confirm to the protocols recommended by the National Cancer Institute, as discussed by Greishaber [32]. In general, it can be assumed that most antineoplastic cytotoxic agents will be highly toxic. Two studies are essential to support initial clinical trials (IND phase) in patients with advanced disease. These are studies of 5–14 days in length, but with longer recovery periods. A study in rodents is required that identifies those doses that produce either life-threatening or non-life-threatening toxicity. Using the information from this first study, a second study in nonrodents (generally the dog) is conducted to determine if the tolerable dose in rodents produces life-threatening toxicity. Doses are compared on a mg/m^2 basis. The starting dose in initial clinical trails is generally one-tenth of that required to produce severe toxicity in rodents (STD10) or one-tenth the highest dose in nonrodents that does not cause severe irreversible toxicity. While not required, information on pharmacokinetic parameters, especially data comparing the plasma concentration associated with toxicity in both species, is very highly regarded. Special attention is paid to organs with high cell-division rates—bone marrow, testes, lymphoid tissue, and GI tract. As these agents are almost always given intravenously, special attention needs to be given relatively early in development to intravenous irritation and blood compatibility study. Subsequent studies to support the New Drug Application will be highly tailored, depending on the following:

- Therapeutic indication and mechanism of action.
- The results of the initial clinical trials.
- The nature of the toxicity.
- Proposed clinical regimen.

Even at the NDA stage, toxicity studies with more than 28 days of dosing are rarely required. While not required for the IND, assessment of genotoxicity and developmental toxicity will need to be addressed. For genotoxicity, it will be important to establish the ratio between cytotoxicity and mutagenicity. *In vivo* models (e.g., the mouse micronucleus test) can be particularly important in demonstrating the lack of genotoxicity at otherwise subtoxic doses. For developmental toxicity, ICH stage C–D studies (traditionally known as Segment II studies for teratogenicity in rat and rabbits) will also be necessary.

The emphasis of this discussion has been on purely cytotoxic neoplastic agents. Additional considerations must be given to cytotoxic agents that are administered under special circumstances: those that are photoactivated, delivered as liposomal emulsions, or delivered as antibody conjugates. These types of agents will require additional studies. For example, a liposomal agent will need to be compared to the free agent and a blank liposomal preparation. There are also studies that may be required for a particular class of agents. For example, anthracyclines are known to be cardiotoxic, so comparison of a new anthracycline agent to previously marketed anthracyclines will be expected.

In addition to antineoplastic, cytotoxic agents, there are cancer therapeutic or preventative drugs that are intended to be given on a chronic basis. This includes chemopreventatives, hormonal agents, and immunomodulators. The toxicity assessment studies on these will more closely resemble those of more traditional pharmaceutical agents. Chronic toxicity, carcinogenicity, and full developmental toxicity (ICH A–B, C–D, E–F) assessments will be required. For a more complete review, the reader is referred to DeGeorge et al. [31].

37.6.3 Optical Isomers

The FDA (and other regulatory agencies, as reviewed by Daniels et al. [33]) has become increasingly concerned with the safety of stereoisomeric or chiral drugs. Stereoisomers are molecules that are identical to one another in terms of atomic formula and covalent bonding, but differ in the three-dimensional projections of the atoms. Within this class are those molecules that are nonsuperimposable mirror images of one another. These are called enantiomers (normally designated as *R*- or *S*-). Enantiometric pairs of a molecule have identical physical and chemical characteristics except for the rotation of polarized light. Drugs have generally been mixtures of optical isomers (enantiomers), because of the difficulties in separating the isomers. It has become apparent in recent years, however, that these different isomers may have different degrees of both desirable therapeutic and undesirable toxicologic effects. Technology has also improved, to the extent that it is now possible to perform chiral-specific syntheses, separations, and/or analyses. It is now highly desirable from a regulatory [29, 30, 34, 35] basis to develop a single isomer unless all isomers have equivalent pharmacological and toxicologic activity. The FDA has divided enantiometric mixtures into the following categories:

- Both isomers have similar pharmacologic activity, which could be identical, or they could differ in the degree of efficacy.
- One isomer is pharmacologically active, while the other is inactive.
- Each isomer has completely different activity.

During preclinical assessment of an enantiometric mixture, it may be important to determine to which of these three classes it belongs. The pharmacological and toxicological properties of the individual isomers should be characterized. The pharmacokinetic profile of each isomer should be characterized in animal models with regard to disposition and interconversion. It is not at all unusual for each enantiomer to have a completely different pharmacokinetic behavior.

If the test article is an enantiomer isolated from a mixture that is already well characterized (e.g., already on the market), then appropriate bridging guides need to be performed which compare the toxicity of the isomer to that of the racemic mixture. The most common approach would be to conduct a subchronic (3 month) and a Sement II type teratology study with an appropriate "positive" control group that received the racemate. In most instances, no additional studies would be required if the enantiomer and the racemate did not differ in toxicity profile. If, on the other hand, differences are identified, then the reasons for this difference need to be investigated and the potential implications for human subjects need to be considered.

37.6.4 Special Populations: Pediatric and Geriatric Claims

Relatively few drugs marketed in the United States (approximately 20%) have pediatric dosing information available. Clinical trials had rarely been done specifically on pediatric patients. Traditionally, dosing regimens for children have been derived empirically by extrapolating on the basis of body weight or surface area. This approach assumes that the pediatric patient is a young adult, which simply may not be the case. There are many examples of how adults and children differ qualitatively in metabolic and/or pharmacodynamic responses to pharmaceutical agents. In their review, Schacter and DeSantis [36] state that "the benefit of having appropriate usage information in the product label is that health care practitioners are given the information necessary to administer drugs and biologics in a manner that maximizes safety, minimizes unexpected adverse events, and optimizes treatment efficacy. Without specific knowledge of potential drug effects, children may be placed at risk. In addition, [in] the absence of appropriate proscribing information, drugs and biologics that represent new therapeutic advances may not be administered to the pediatric population in a timely manner." In response to the need for pediatric information, the FDA had developed a pediatric plan. This two-phase plan called first for the development of pediatric information on marketed drugs. The second phase focused on new drugs. The implementation of the plan was to be coordinated by the Pediatric Subcommittee of the Medical Policy Coordinating Committee of CDER. The Pediatric Use Labeling Rule was a direct result of phase 1 in 1994 [7]. Phase 2 resulted in 1997 from a proposed rule entitled *Pediatric Patients: Regulations Requiring Manufacturers to Assess the Safety and Effectiveness of New Drugs and Biologics*. Soon after this rule was proposed, the FDA Modernization Act of 1997 was passed. FDAMA contained provisions that specifically addressed the needs and requirements for the development of drugs for the pediatric population.

The FDAMA bill essentially codified and expanded several regulatory actions initiated by the FDA during the 1990s. Among the incentives offered by the bill, companies would be given an additional six months of patent protection for performing pediatric studies (clinical trials) on already approved products. In fact, the

FDA was mandated by FDAMA to develop a list of over 500 drugs for which additional information would produce benefits for pediatric patients. The FDA is supposed to provide a written request for pediatric studies to the manufacturers [37].

In response to the pediatric initiatives, the FDA has published policies and guidelines and conducted a variety of meetings. CDER has established a web site (http// www.fda.gov/cder/pediatric), which lists three pages of such information. Interestingly, the focus has been on clinical trials, and almost no attention has been given to the preclinical toxicology studies that may be necessary to support such trials. There are three pages of documents on the pediatric web site. None appear to address the issue of appropriate testing. This is a situation that is just now being addressed and is in a great deal of flux.

In the absence of any guidelines from the FDA for testing drugs in young or "pediatric" animals, one must fall back on the maxim of designing a program that makes the most scientific sense. As a guide, the FDA designated levels of postnatal human development and the approximate equivalent ages (in the author's considered opinion) in various animal models are given in Table 37.7. The table is somewhat inaccurate, however, because of differences in the stages of development at birth. A rat is born quite underdeveloped when compared to a human being. A one-day old rat is not equivalent to a one-day old full-term human infant. A four-day old rat would be more appropriate. In terms of development, the pig may be the best model of those listed; however, one should bear in mind that different organs have different developmental schedules in different species.

Table 37.7 can be used as a rough guide in designing toxicity assessment experiments in developing animals. In designing the treatment period, one needs to consider not only the dose and the proposed course of clinical treatment, but also the proposed age of the patient, and whether or not an equivalent dosing period in the selected animal model covers more than one developmental stage. For example, if the proposed patient population is human infants, initiating a toxicity study of the new pharmaceutical agent in 3-day-old rats is not appropriate. Furthermore, if the proposed course of treatment in adult children is 2 weeks, it is unlikely that this would cross over into a different developmental stage. A 2-week treatment initiated in puppies, however, might easily span two developmental stages. Thus, in designing an experiment in young animals, one must carefully consider the length of the treatment period, balancing the developmental age of the animal model and the proposed length of clinical treatment. Where appropriate (infant animals), one needs to also assess changes in standard developmental landmarks, (e.g., eye opening, pinae eruption, external genitalia development) as well as the more standard

TABLE 37.7 Comparison of Postnatal Development Stages

Stage	Human	Rat	Dog	Pig
Neonate	Birth to 1 month	Birth to 1 week	Birth to 3 weeks	Birth to 2 weeks
Infant	1 month to 2 years	1–3 weeks	3–6 weeks	2–4 weeks
Child	2–12 years	3–9 weeks	6 weeks to 5 months	4 weeks to 4 months
Adolescent	12–16 years	9–13 weeks	5–9 months	4–7 months
Adult	Over 16 years	Over 13 weeks	Over 9 months	Over 7 months

indicators of target organ toxicity. The need for maintaining the experimental animals past the dosing period, perhaps into sexual maturity, to assess recovery or delayed effects needs also to be carefully considered.

To summarize, the current status of assessment of toxicity in postnatal mammals, in response to the pediatric initiatives covered in FDAMA, is an extremely fluid situation. One needs to carefully consider a variety of factors in designing the study and should discuss proposed testing programs with the appropriate office at the CDER.

Drugs intended for use in the elderly, like those intended for the very young, may also have special requirements for safety evaluation, but geriatric issues were not addressed in the FDAMA of 1997. The FDA has published a separate guidance document for geriatric labeling [7]. As was the case with pediatric guidance, this document does not address preclinical testing. With the elderly, the toxicological concerns are quite different from the developmental concerns associated with pediatric patients. With the elderly, one must be concerned with the possible interactions between the test article and compromised organ function. The FDA had previously issued a guidance for examining the clinical safety of new pharmaceutical agents in patients with compromised renal and/or hepatic function [38]. The equivalent ICH guideline (S5A) was issued in 1994. Whether this type of emphasis will require toxicity testing in animal models with specifically induced organ insufficiency remains to be seen. In the interim, we must realize that there is tacit evaluation of test article-related toxicity in geriatric rodents for those agents that undergo 2 year carcinogenicity testing. As the graying of America continues, labeling for geriatric use may become more of an issue in the future.

37.6.5 Orphan Drugs

The development of sophisticated technologies, coupled with the rigors and time required for clinical and preclinical testing, has made pharmaceutical development very expensive. In order to recoup such expenses, pharmaceutical companies have tended to focus on therapeutic agents with large potential markets. Treatment for rare but life-threatening diseases have been "orphaned" as a result. An orphan product is defined as one targeted at a disease that affects 200,000 or fewer individuals in the United States. Alternatively, the therapy may be targeted for more than 200,000 but the developer would have no hope of recovering the initial investment without exclusivity. The Orphan Drug Act of 1983 was passed in an attempt to address this state of affairs. Currently applicable regulations were put in place in 1992 [39]. In 1994, there was an attempt in Congress to amend the Act, but it failed to be passed into law. The current regulations are administered by the Office of Orphan Product Development (OPD). The Act offers the following incentives to encourage the development of products to treat rare diseases:

- Seven years exclusive market following the approval of a product for an orphan disease.
- Written protocol assistance from the FDA.
- Tax credits for up to 50% of qualified clinical research expenses.
- Available grant to support pivotal clinical trials.

As reviewed by Haffner [40], other developed countries have similar regulations.

The ODA did not change the requirements of testing drug products. The nonclinical testing programs are similar to those used for more conventional products. They will undergo the same FDA review process. A major difference, however, is the involvement of the OPD. A sponsor must request OPD review. Once OPD determines that a drug meets the criteria for orphan drug status, it will work with the sponsor to provide the assistance required under the Act. The ODA does not review a product for approval. The IND/NDA process is still handled by the appropriate reviewing division (e.g., Cardiovascular) for formal review. The Act does not waive the necessity for submission of an IND and for the responsibility of toxicological assessment. As always, in cases where there is ambiguity, a sponsor may be well served to request a pre-IND meeting at the appropriate division to discuss the acceptability of a toxicology assessment plan.

37.6.6 Botanical Drug Products

There is an old saying, "What goes around, comes around," and so it is with botanicals. At the beginning of the 20th century, most marketed pharmaceutical agents were botanical in origin. For example, aspirin was first isolated from willow bark. These led the way in the middle part of the century, for reasons having to do with patentability, manufacturing costs, standardization, selectivity, and potency. The dawning of the 21st century has seen a grass-roots return to botanical preparations (also sold as herbals or dietary supplements). These preparations are being marketed to the lay public as "natural" supplements to the nasty synthetic chemicals now proscribed as pharmaceutical products. In 1994, the Dietary Supplement Health and Education Act was passed, which permitted the marketing of dietary supplements (including botanicals) with limited submissions to the FDA [33]. If a producer makes a claim that an herbal preparation is beneficial to a specific part of the body (e.g., enhanced memory), then it may be marketed after a 75 day period of FDA review but without formal approval. On the other hand, if any curative properties are claimed, then the botanical will be regulated as a drug and producers will be required to follow the IND/NDA process. In 1997 and 1998 combined, some 26 INDs were filed for botanical products [41].

The weakness in the current regulation has to do with its ambiguity. The line between a beneficial claim and a curative claim is sometimes difficult to draw. What is the difference, for example, between an agent that enhances memory and one that prevents memory loss? Given the number of products and claims hitting the shelves every day, this situation will probably demand increased regulatory scrutiny in the future.

37.7 INTERNATIONAL PHARMACEUTICAL REGULATION AND REGISTRATION

37.7.1 International Conference on Harmonization

The International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use was established to make the

drug-regulatory process more efficient in the United States, Europe, and Japan. The U.S. involvement grew out of the fact that the United States is party to the General Agreement on Tariffs and Trade, which included the Agreement on Technical Barriers to Trade, negotiated in the 1970s, to encourage reduction of nontariff barriers to trade [42]. The main purpose of the ICH is, through harmonization, to make new medicines available to patients with a minimum of delay. More recently, the need to harmonize regulation has been driven, according to the ICH, by the escalation of the cost of R&D. The regulatory systems in all countries have the same fundamental concerns about safety, efficacy, and quality, yet sponsors had to repeat many time-consuming and expensive technical tests to meet country-specific requirements. Secondarily, there was a legitimate concern over the unnecessary use of animals. Conference participants include representatives from the drug-regulatory bodies and research-based pharmaceutical industrial organizations of three regions: the European Union, the United States, and Japan, where over 90% of the world's pharmaceutical industries are located. Representation is summarized in Table 37.8. The biennial conference has met four times, beginning in 1991, rotating between sites in the United States, Europe, and Japan.

The ICH meets its objectives by issuing guidelines for the manufacturing, development, and testing of new pharmaceutical agents that are acceptable to all three major parties. For each new guideline, the ICH Steering Committee establishes an expert working group with representation from each of the six major participatory ICH bodies. So far, the ICH has proposed or adopted over 40 safety, efficacy, and quality guidelines (listed in Table 37.9) for use by the drug-regulatory agencies in the United States, Europe, and Japan. The guidelines are organized under broad categories: the "E" series having to do with clinical trials, the "Q" series having to do with quality (including chemical manufacturing and control as well as traditional GLP issues), and the "S" series having to do with safety. Guidelines may be obtained from the ICH secretariat, c/o of IFPMA, 30 rue de St.-Jean, PO Box 9, 1211 Geneva 18, Switzerland, or may be downloaded from a web site set up by Ms. Nancy McClure (http://www.mcclurenet.com/index.html). They are also published in the *Federal Register*. The guidelines of the "S" series will have the most impact on

TABLE 37.8 ICH Representation

Country/Region	Regulatory[a]	Industry[a]
European Union	European Commission (2)	European Federation of Pharmaceutical Industries Associations (2)
Japan	Ministry of Health, Labor and Welfare (2)	Japanese Pharmaceutical Manufacturers Association (2)
United States	Food and Drug Administration (2)	Pharmaceutical Research and Manufacturers of America (2)
Observing organizations	World Health Organization, European Free Trade Area, Canadian Health Protection Branch	International Federation of Pharmaceutical Manufacturers Associations (2): also provides the secretariat

[a]( ) = number of representatives on the ICH Steering Committee.

TABLE 37.9 International Conference on Harmonization Guidelines

Reference	Guideline	Date Issued
E1	The Extent of Population Exposure to Assess Clinical Safety	Oct 94
E2A	Clinical Safety Data Management: Definitions and Standards for Expedited Reporting	Oct 94
E2B(M)	Maintenance of the Clinical Safety Data Management including: Data Elements for Transmission of Individual Case Safety Reports	Nov 00
E2B(R)	Revision of the E2B(M) ICH Guideline on Clinical Safety Data Management Data Elements for Transmission of Individual Case Safety Reports	May 05
E2C	Clinical Safety Data Management: Periodic Safety Update Reports for Marketed Drugs	Nov 96
E2D	Post-Approval Safety Data Management: Definitions and Standards for Expedited Reporting	Nov 03
E2E	Pharmacovigilance Planning	Nov 04
E3	Structure and Content of Clinical Study Reports	Nov 95
E4	Dose Response Information to Support Drug Registration	Mar 94
E5	Ethnic Factors in the Acceptability of Foreign Clinical Data	Feb 98
E6	Good Clinical Practice: Consolidated Guideline; Notice of Availability	May 96
E6A	GCP Addendum on Investigator's Brochure	Mar 95
E6B	GCP: Addendum on Essential Documents for the Conduct of a Clinical Trial	Oct 94
E7	Studies in Support of Special Populations: Geriatrics	Jun 93
E8	Guidance on General Considerations for Clinical Trials; Notice	July 97
E9	Draft Guideline on Statistical Principles for Clinical Trials; Notice of Availability	Feb 98
E10	Choice of Control Group and Related Issues in Clinical Trials	July 00
E11	Clinical Investigation of Medicinal Products in the Pediatric Population	July 00
E12A	Principles for Clinical Evaluation of New Antihypertensive Drugs	Mar 00
E14	Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs	May 05
M2	Electronic Transmission of Individual Case Safety Reports Message Specification	Nov 00
M3	Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals	July 97
M4	Organization of the Common Technical Document	Oct 05
M5	Data Elements and Standards for Drug Dictionaries	May 05
Q1A(R)	Stability Testing of New Drug Substances and Products (2nd Revision)	May 03
Q1B	Photostability Testing of New Drug Substances and Products	Nov 96
Q1C	Stability Testing for New Dosage Forms	Nov 96
Q1D	Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Drug Products	Feb 02
Q1E	Evaluation of Stability Data	Feb 03
Q1F	Stability Data Package for Registration Applications in Climatic Zones III and IV	Feb 03
Q2A	Validation of Analytical Procedures: Definitions and Terminology	Oct 94
Q2B	Validation of Analytical Procedures: Methodology	Nov 96
Q3A(R)	Impurities in New Drug Substances Revised Guideline	Feb 02

TABLE 37.9 *Continued*

Reference	Guideline	Date Issued
Q3B(R)	Impurities in New Drug Products Revised Guideline	Feb 03
Q3C	Impurities: Guideline for Residual Solvents	July 97
Q3C(M)	Impurities: Guideline for Residual Solvents (Maintenance)	Sept 02
Q5A	Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin	Mar 97
Q5B	Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products	Nov 95
Q5C	Q5C: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products	Nov 95
Q5D	Q5D: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products	July 97
Q5E	Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process	Nov 04
Q6A	Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances	Oct 99
Q6B	Specifications: Test Procedures and Acceptance Criteria for Biotechnological/ Biological Products	May 99
Q7A	Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients	Nov 00
Q8	Pharmaceutical Development	Nov 05
Q9	Quality Risk Management	Nov 05
S1A	Guideline on the Need for Carcinogenicity Studies of Pharmaceuticals	Nov 95
S1B	Draft Guideline on Testing for Carcinogenicity of Pharmaceuticals	July 97
S1C	Dose Selection for Carcinogenicity Studies of Pharmaceuticals	Oct 94
S1C(R)	Guidance on Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addendum on a Limit Dose and Related Notes; Availability; Notice	July 97
S2A	Genotoxicity: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals	Jul 95
S2B	Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals; Availability; Notice	July 97
S3A	Toxicokinetics: Guidance on the Assessment of Systemic Exposure in Toxicity Studies	Oct 94
S3B	Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies	Oct 94
S4	Single Dose Toxicity Testing; Revised Guidance	Aug 96
S4A	Duration of Chronic Toxicity Testing in Animals (Rodent and Non-Rodent Toxicity Testing)	Sept 98
S5A	Detection of Toxicity to Reproduction for Medicinal Products	Jun 93
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	Nov 95
S6A	Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals; Availability	Nov 97
S7A	Safety Pharmacology Studies for Human Pharmaceuticals	Nov 00
S7B	The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals	May 05
S8	Immunotoxicology Studies for Human Pharmaceuticals	Sept 05

toxicologists. The biggest changes having to do with toxicological assessment are summarized next.

Carcinogenicity Studies Carcinogenicity studies are covered in Guidelines S1A, S1B, and S1C. The guidelines are almost more philosophical than they are technical. In comparison to the EPA guidelines, for example, the ICH guidelines contain little in the way of concrete study criteria (e.g., the number of animals, the necessity for clinical chemistry). There is discussion on when carcinogenicity studies should be done, whether two species are more appropriate than one, and how to set dosages on the basis of human clinical PK data. The following major changes are being wrought by these guidelines:

- Only one 2 year carcinogenicity study should generally be required. Ideally, the species chosen should be the one most like humans in terms of metabolic transformations of the test article.
- The traditional second long-term carcinogenicity study can be replaced by a shorter-term alternative model. In practical terms, this guideline is beginning to result in sponsors conducting a 2 year study in the rat and a 6 month study in an alternative mouse model, such as the P53 or the TG.AC genetically manipulated mouse strains.
- In the absence of target organ toxicity with which to set the high dose at the maximally tolerated dose, the high dose can be set at the dose that produces an area under the curve (AUC). This is 25-fold higher than that obtained in human subjects.

Chronic Toxicity Traditionally, chronic toxicity of new pharmaceuticals in the United States was assessed in studies of 1 year duration in both the rodent and the nonrodent species of choice. The European view was that studies of 6 months are generally sufficient. The resulting guideline (S4A) was a compromise. Studies of 6 months duration were recommended for the rodent, as rodents would also be examined in 2 year studies. For the nonrodent (dog, nonhuman primate, and pig) studies of 9 months duration were recommended.

Developmental and Reproductive Toxicity This was an area in which there was considerable international disagreement and the area in which the ICH has promulgated the most technically detailed guidelines (S5A and S5B). Some of the major changes include the following:

- The traditional Segment I, II, and III nomenclature has been replaced with different nomenclature, as summarized in Table 37.10.
- The dosing period of the pregnant animals during studies on embryonic development (traditional Segment II studies) has been standardized.
- New guidelines for fertility assessment (traditional Segment I) studies have shortened the premating dosing schedule (e.g., in male rats from 10 weeks to 4 weeks). There has been an increased interest in assessment of spermatogenesis and sperm function.

TABLE 37.10 Comparison of Traditional and ICH Guidelines for Reproductive and Developmental Toxicology

Traditional Protocol	Stages Covered	ICH Protocol	Dosing Regimen
Segment I (rats)	A. Premating to conception B. Conception to implantation	Fertility and early embryonic development, including implantation	Males: 4 weeks premating, mating (1–3 weeks), plus 3 weeks postmating Females: 2 weeks premating, mating through day 7 of gestation
Segment II (rabbits)	C. Implantation to closure of hard palate D. Closure of hard palate to the end of pregnancy	Embryo–fetal development	Female rabbits: day 6 to day 20 of pregnancy

Study Title	Termination	Endpoints: In-life	Endpoints: Postmortem
Fertility and early embryonic development, including implantation	Females: day 13–15 of pregnancy Males: day after completion of dosing	Clinical signs and mortality Body weights and feed in-take Vaginal cytology	Macroscopic exam + histological exam of gross lesions Collection of reproductive organs for possible histology Quantitation of corpora lutea and implantation sites Seminology (count, motility, and morphology)
Embryo–fetal development		Clinical signs and mortality Body weights and changes Feed in-take	Macroscopic exam + histological exam of gross lesions Quantitation of corpora lutea and implantation sites Fetal body weights Fetal abnormalities
Pre- and postnatal development, including maternal function		Clinical signs and mortality Body weights and changes Feed in-take Duration of pregnancy Parturition	Macroscopic exam + histological exam of gross lesions Implantation Abnormalities (including terata) Live/dead offspring at birth Pre- and postweaning survival and growth (F_1) Physical development (F_1) Sensory functions and reflexes (F_1) Behavior (F_1)

- The new guidelines allow for a combination of studies in which the endpoints typically assessed in the traditional Segment II and Segment III studies are now examined under a single protocol.

For a more complete review of the various study designs, the reader is refereed to the review by Manson [43].

While they were not quite as sweeping in approach as the aforementioned guidelines, a toxicologist working in pharmaceutical safety assessment should become familiar with all the other ICH guidelines in the S series.

In an interesting recent article, Ohno [44] discussed not the harmonization of nonclinical guidelines, but the need to harmonize the timing of nonclinical tests in relation to the conduct of clinical trials. For example, there are regional differences in the inclusion of women of childbearing potential in clinical trials. In the United States, including women in such trials is becoming more important, and therefore evaluation of embryo–fetal development will occur earlier in the drug development process than in Japan. Whether or not such timing or staging of nonclinical tests becomes part of an ICH guideline in the near future remains to be established.

37.7.2 Other International Considerations

The United States is the single largest pharmaceutical market in the world. But the rest of the world (particularly but not limited to the second and third largest markets, Japan and the European Union) represents in aggregate a much larger market, so no one develops a new pharmaceutical for marketing just in the United States. The effort at harmonization (exemplified by the International Conference on Harmonization, or ICH) has significantly reduced differences in requirements for these other countries, but certainly not obliterated them. Although a detailed understanding of their regulatory schemes is beyond this chapter, the bare bones and differences in toxicology requirements are not.

European Union The standard European Union toxicology and pharmacologic data requirements for a pharmaceutical include the following

- Single-dose toxicity
- Repeat-dose toxicity (subacute and chronic trials)
- Reproduction studies (fertility and general reproductive performance, embryotoxicity, and peri/postnatal toxicity)
- Mutagenic potential (*in vitro* and *in vivo*)
- Carcinogenicity
- Pharmacodynamics
  Effects related to proposed drug indication
  General pharmacodynamics
  Drug interactions
- Pharmacokinetics
  Single dose
  Repeat dose

Distribution in normal and pregnant animals
Biotransformation
- Local tissue tolerance
- Environmental toxicity

In general, the registration process in the European Union allows one to apply to either an overall medicines authority or to an individual national authority. Either of these steps is supposed to lead to mutual recognition by all the individual members.

Japan In Japan, the Ministry of Health, Labor and Welfare is the national regulatory body for new drugs [45].

The standard LD_{50} test is no longer a regulatory requirement for new medicines in the United States, the European Union, or Japan. The Japanese guidelines were the first to be amended in accordance with this agreement, with the revised guidelines becoming effective in August 1993. The Japanese may still anticipate that single-dose (acute) toxicity studies should be conducted in at least two species, one rodent and one nonrodent (the rabbit is not accepted as a nonrodent). Both males and females should be included from at least one of the species selected: if the rodent, then a minimum of 5 per sex; if the nonrodent, at least 2 per sex. In nonrodents, both the oral and parenteral routes should be used, and normally the clinical route of administration should be employed. In nonrodents, only the intended route of administration need be employed; if the intended route of administration in humans is intravenous, then use of this route in both species is acceptable. An appropriate number of doses should be employed to obtain a complete toxicity profile and to establish any dose–response relationship. The severity, onset, progression, and reversibility of toxicity should be studied during a 14 day follow-up period, with all animals being necropsied. When macroscopic changes are noted, the tissue must be subjected to histological examination.

Chronic and subchronic toxicity studies are conducted to define the dose level, when given repeatedly, that causes toxicity, and the dose level that does not lead to toxic findings. In Japan, such studies are referred to as repeated-dose toxicity studies. As with single-dose studies, at least two animal species should be used, one rodent and one nonrodent (rabbit not acceptable). In rodent studies, each group should consist of at least 10 males and 10 females; in nonrodent species, 3 of each sex are deemed adequate. Where interim examinations are planned, however, the numbers of animals employed should be increased accordingly. The planned route of administration in human subjects is normally explored. The duration of the study will be dictated by the planned duration of clinical use (Table 37.11).

At least three different dose groups should be included, with the goals of demonstrating an overtly toxic dose and a no-effect dose, and establishing any dose–response relationship. The establishment of a nontoxic dose within the framework of these studies is more rigorously adhered to in Japan than elsewhere in the world. All surviving animals should also be necropsied, either at the completion of the study or during its extension recovery period, to assess reversal of toxicity and the possible appearance of delayed toxicity. Full histological examination is mandated on all nonrodent animals used in a chronic toxicity study; at a minimum, the highest-

TABLE 37.11 Determination of Duration of Studies

Duration of Dosing in Toxicity Study	Duration of Human Exposure
2 weeks	Single dose or repeated dosage not exceeding 12 days
1 month	Single dose or repeated dosage not exceeding 4 weeks
3 months	Repeated dosing exceeding 1 week and to a maximum of 12 weeks
6 months (rodent)	Repeated dosing exceeding 13 weeks and to a maximum of lifetime
9/12 months[a] (nonrodent)	Repeated dosing exceeding 6 months or where this is deemed to be appropriate

[a]Where carcinogenicity studies are to be conducted, the Koseisho had agreed to forego chronic dosage beyond 6 months.

Source: New Drugs Division Notification No. 43, June 1992.

dose and control groups of rodents must be submitted to a full histological examination.

While the value of repeated-dose testing beyond 6 months has been questioned [46], such testing is a regulatory requirement for a number of agencies, including the U.S. FDA and the Koseisho. In Japan, repeated-dose testing for 12 months is required only for new medicines expected to be administered to humans for periods in excess of 6 months [47]. At the First International Conference on Harmonization held in Brussels, the consensus was that 12 month toxicity studies in rodents could be reduced to 6 months where carcinogenicity studies are required. While not yet adopted in the Japanese guidelines, 6 month repeated-dose toxicity studies have been accepted by the agencies of all three regions. Japan—like the European Union—accepts a 6 month duration if accompanied by a carcinogenicity study. The United States still requires a 9 month nonrodent study.

With regard to reproductive toxicology, as a consequence of the first ICH, the United States, European Union, and Japan agreed to recommend mutual recognition of their respective current guidelines. A tripartite, harmonized guideline on reproductive toxicology has achieved ICH Step 4 status and should be incorporated into the local regulations of all three regions soon [48]. This agreement represents a very significant achievement that should eliminate many obstacles to drug registration.

Preclinical Male Fertility Studies Before conducting a single-dose male volunteer study in Japan, it is usually necessary to have completed a preclinical male fertility study (Segment I) that has an in-life phase of 10 or more weeks (i.e., 10 weeks of dosing, plus follow-up). Although government guidelines do not require this study to be completed before Phase I trials begin, the responsible Institutional Review Board or the investigator usually imposes this condition. Japanese regulatory authorities are aware that the Segment I male fertility study is of poor predictive value. The rat, which is used in this study, produces a marked excess of sperm. Many scientists therefore believe that the test is less sensitive than the evaluation of testicular weight and histology that constitute part of the routine toxicology assessment.

Female Reproductive Studies Before entering a female into a clinical study, it is necessary to have completed the entire reproductive toxicology program, which consists of the following studies:

- Segment 1: Fertility studies in the rat or mouse species used in the Segment 2 program.
- Segment 2: Teratology studies in the rat or mouse, and the rabbit.
- Segment 3: Late gestation and lactation studies in a species used in the Segment 2 studies.

Such studies usually take approximately 2 years. Although the U.S. regulations state the need for completion of Segments 1 and 2, and the demonstration of efficacy in male patients, where appropriate, before entering females into a clinical program, the current trend in the United States is toward relaxation of the requirements to encourage investigation of the drug both earlier and in a larger number of females during product development. Growing pressure for the earlier inclusion of women in drug testing may encourage selection of this issue as a future ICH topic. The trend in the United States and the European Union toward including women earlier in the critical program has not yet been embraced in Japan, however.

The three tests required in Japan for genotoxicity evaluation are a bacterial gene mutation test, *in vitro* cytogenetics, and *in vivo* tests for genetic damage. The Japanese regulations state these tests to be the minimum requirement and encourage additional tests. Currently, Japanese guidelines do not require a mammalian cell gene mutation assay. Harmonization will likely be achieved by the Koseisho recommending all four tests, which will match requirements in the United States and European Union. At present, this topic is at Step 1 in the ICH harmonization process. The mutagenicity studies should be completed before the commencement of Phase II clinical studies.

Guidelines presented at the second ICH are likely to alter the preclinical requirements for registration in Japan; they cover toxicokinetics and when to conduct repeated-dose tissue distribution studies. The former document may improve the ability of animal toxicology studies to predict possible adverse events in humans; currently, there are no toxicokinetic requirements in Japan, and their relevance is questioned by many there. Although there is general agreement on the registration requirement for single-dose tissue distribution studies, implementation of the repeated-dose study requirement has been inconsistent across the three ICH parties.

37.7.3 Safety Pharmacology

Japan was the first major country to require extensive pharmacological profiling on all new pharmaceutical agents as part of the safety assessment profile. Prior to commencement of initial clinical studies, the drug's pharmacology must be characterized in animal models. In the United States and Europe, these studies have been collectively called safety pharmacology studies. For a good general review of the issues surrounding safety pharmacology the reader is referred to Hite [1]. The Japanese guidelines for such characterizations were published in 1991 (New Drugs Division Notification No. 4, January 1991). They include the following:

- Effects on general activity and behavior.
- Effects on the central nervous system.
- Effects on the autonomic nervous system and smooth muscle.
- Effects on the respiratory and cardiovascular systems.
- Effects on the digestive system.
- Effects on water and electrolyte metabolism.
- Other important pharmacological effects.

In the United States, pharmacological studies in demonstration of efficacy have always been required, but specific safety pharmacological studies have never been required. Special situational or mechanistic data would be requested on a case-by-case basis. This is a situation that is changing. In the United States the activities of the Safety Pharmacology Discussion Group, for example, have helped bring attention to the utility and issues surrounding safety pharmacology data. In 1999 and 2000, the major toxicological and pharmacological societal meetings had symposia on safety pharmacological testing. Many major U.S. pharmaceutical companies are in the process of implementing programs in safety pharmacology. The issue has been taken up by the ICH and the draft guideline is currently at the initial stages of review. This initial draft (Guideline S7) includes core tests in the assessment of CNS, cardiovascular, and respiratory function. Studies will be expected to be performed under GLP guidelines.

37.8 CONCLUSION

In summary, we have touched upon the regulations that currently control the types of preclinical toxicity testing done on potential human pharmaceuticals. This chapter has reviewed the history, the law, the regulations themselves, the guidelines, and common practices employed to meet regulatory standards.

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38

DATA ANALYSIS

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Contents

38.1 Introduction and Scope
38.2 Systemic Concentration–Time Profiles and Urinary Excretion
38.2.1 Single Intravenous Dose
38.2.2 Multiple Intravenous Doses
38.2.3 Single Oral Dose
38.2.4 Other Routes of Administration
38.3 Pharmacokinetic Analysis
38.3.1 Example 1: Intravenous Dosing
38.3.2 Example 2: Oral Dosing
38.4 Implications of Pharmacokinetics on Drug Discovery and Development
References

38.1 INTRODUCTION AND SCOPE

Pharmacokinetic analysis of systemic (or blood or plasma) concentration–time data, or amount excreted in urine over various time intervals, represents a critical activity in the preclinical development of drug candidates. This chapter describes the common methods for analysis of such data in support of drug development efforts, with an emphasis on nonclinical drug discovery and development. Theoretical principles underlying pharmacokinetic data analysis are presented elsewhere in other chapters of this volume.

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Concentration–time or urinary excretion data are typically analyzed to generate estimates of bioavailability, clearance, apparent volume of distribution, and fraction of dose converted to a specific metabolite. These parameters are vital for determination of appropriate dose(s), administration route(s), and dosing regimens in preclinical efficacy and toxicology studies.

Noncompartmental or compartmental methods are generally employed to perform these analyses [1, 2]. At their core, noncompartmental methods, based on statistical moment theory, require estimation of the area under the concentration–time curve. Compartmental methods assume that the body is composed of a series of compartments, even though such compartments have no physiologic relevance, with first-order (concentration-independent) transfer or elimination rate constants between compartments. A one-compartment model, for instance, depicts the body as a single, homogeneous unit, with first-order elimination.

Many modern software applications are available for rapid, reliable, and pharmacokinetic analyses by noncompartmental or compartmental methods (e.g., WinNonlin®, Kinetica®, PKAnalyst®, NCOMP) [3].

This chapter discusses noncompartmental methods of pharmacokinetic analysis with primary emphasis on data analysis following intravenous and oral dosing. This chapter is meant to provide an introductory discussion on pharmacokinetic data analysis with a few "hands-on" examples. A comprehensive discussion of all current and emerging pharmacokinetic data analysis techniques is documented in several excellent books [1–5].

38.2 SYSTEMIC CONCENTRATION–TIME PROFILES AND URINARY EXCRETION

38.2.1 Single Intravenous Dose

Following a single intravenous bolus administration, the test article (or drug) is assumed to instantaneously mix within the components of the circulating blood (red and white cells, plasma water, and plasma proteins). Concentration of drug in blood is then gradually diluted through distribution to tissues and organs. Concurrently, the drug may be metabolized or degraded by circulating enzymes (such as esterases) or by metabolic enzymes in the liver (or lungs) or may be excreted unchanged in urine through the kidneys. This complex combination of distribution, degradation, metabolism, and excretion results in a gradual decline in blood or systemic concentrations of drug over time. Whereas individual processes may not be generally discernible, their combination is generally evident as a monoexponential decline in systemic concentrations of drug over time. In instances when one or more processes are relatively slow, a multiexponential decline in systemic concentrations over time is evident. In any event, the apparent terminal monoexponential portion of concentration–time profile is assumed to represent elimination of drug from systemic circulation. A schematic of monoand multiexponential systemic concentration–time profiles following intravenous administration of a drug is depicted in Fig. 38.1.

The complex interplay between distribution and elimination processes observed in systemic circulation following an intravenous dose is also reflected in urine. As the systemic concentration of drug declines, the corresponding amount of unchanged

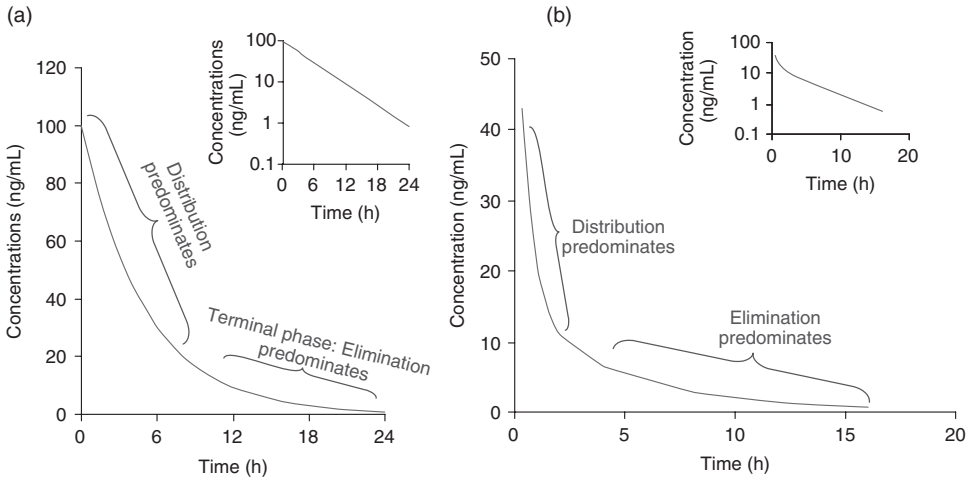


FIGURE 38.1 Schematic of (a) monoexponential and (b) multiexponential concentration–time curves following a single intravenous dose of a drug. (Inset: Semilog concentration–time plot.)

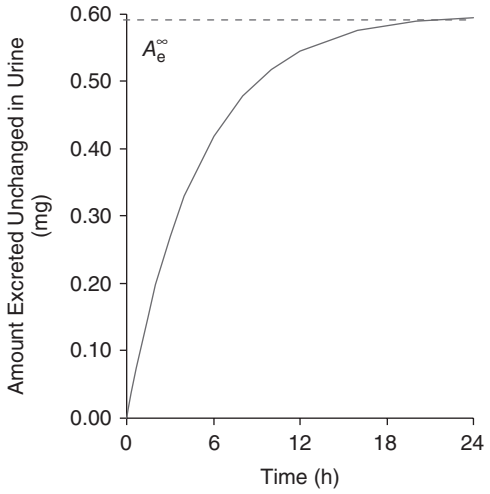


FIGURE 38.2 Schematic of urinary excretion–time profile following a single intravenous dose of a drug.

(unmetabolized) drug excreted in urine increases, reaching a maximum value referred to as the maximum amount of drug excreted unchanged in urine (A_e^∞). In cases when systemic concentration of an administered drug cannot be determined due to inaccessibility of appropriate sampling sites (such as in pediatric or infant patients), measurement of urinary excretion serves as a convenient tool for determination of corresponding pharmacokinetic parameters. A schematic of the urinary excretion–time profile of an intravenously administered drug is presented below in Fig. 38.2. This approach to pharmacokinetic characterization is utilized even for

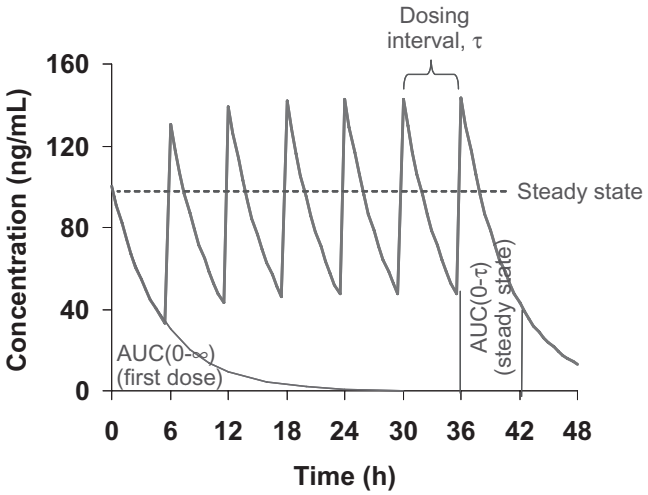


FIGURE 38.3 Schematic of systemic concentration–time profile following multiple intravenous administration of a drug.

agents that are fully metabolized and some of the metabolites excreted in urine. The reader is referred to the literature for further discussions [5].

38.2.2 Multiple Intravenous Doses

Multiple drug administration generally results in increasing systemic exposure when concentrations from prior doses are not completely eliminated, reaching "steady-state" levels (Fig. 38.3). Such an increase in systemic exposure is commonly referred to as *accumulation*.

If additional doses are administered following "almost complete" elimination of prior doses then accumulation is minimal. The implications of accumulation (or lack thereof) are discussed elsewhere in this book and in the literature [5].

38.2.3 Single Oral Dose

Following an oral (or other nonparenteral) route of administration, the drug must be released from its dosage form and be absorbed from the site of administration into the systemic circulation. Distribution, degradation, metabolism, and excretion processes occur in parallel with drug absorption. At least initially, absorption rate predominates over other processes, resulting in increasing systemic concentrations of drug with time. As the amount of dose remaining to be absorbed declines at the site of administration, the rate of absorption declines and systemic concentrations reach a peak before declining thereafter in mono- or multiexponential fashion with time, as described previously. A schematic of systemic concentration–time profile following oral (or nonparenteral) administration is presented in Fig. 38.4. As discussed earlier, urinary excretion–time profiles provide an additional window into the absorption, distribution, and elimination processes following oral administration of a drug.

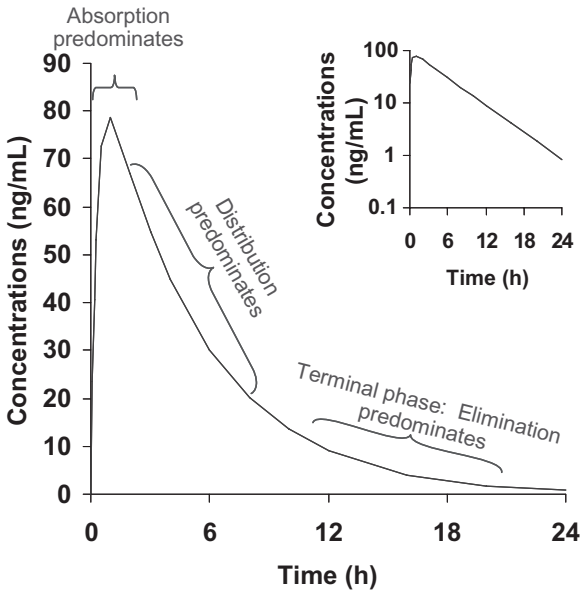


FIGURE 38.4 Schematic of monoexponential concentration–time curve following a single oral (or nonparenteral) dose of a drug. (Inset: Semilog concentration–time plot.)

38.2.4 Other Routes of Administration

The test article may be administered to animals and humans during drug development by a number of other routes, including subcutaneous, intraperitoneally, and transdermally. Such routes result in systemic concentration–time profiles that mimic those following oral dosing (Fig. 38.4). Each includes absorption of the test article from the site of administration to systemic circulation with concurrent distribution, metabolism, and elimination processes. In such cases, drug absorption depends on the amount of administered dose remaining at the administration site. As the amount of administered dose decreases with time, the absorption rate declines as well. Such an absorption process is referred to as *first-order absorption*.

In contrast to changing absorption rates over time, a *zero-order* drug administration maintains constant rate of absorption. Such constant absorption rate is generally achieved by a constant rate intravenous infusion or through osmotic pumps. Typically, drugs with narrow therapeutic window (i.e., low safety margins) are administered in this fashion. Systemic concentrations increase steadily, reaching "steady state" when the rate of drug "input" equals the rate of drug "output" (i.e., removal of drug from systemic circulation). Once the infusion is terminated (or drug delivery device, such as an osmotic pump, is removed), systemic concentrations decline in exponential manner, reflecting either the mono- or multiexponential characteristics described earlier. A schematic of systemic concentration–time profile following a constant rate infusion is presented in Fig. 38.5.

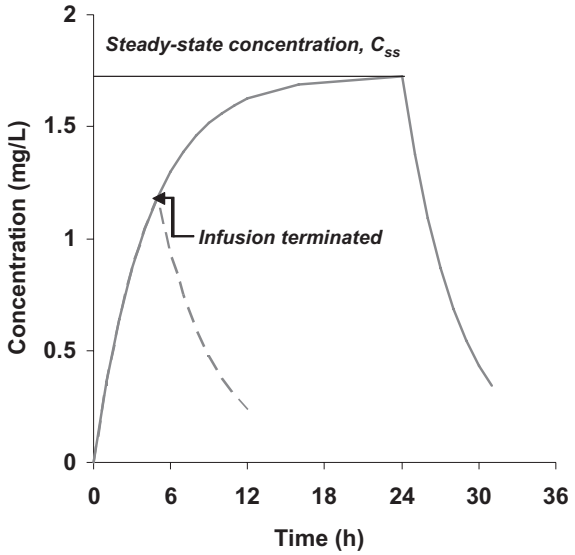


FIGURE 38.5 Schematic of systemic concentration–time profile following intravenous infusion of drug. Infusion was terminated (a) prior to achievement of steady state or (b) following steady state.

38.3 PHARMACOKINETIC ANALYSIS

Pharmacokinetic analysis of systemic concentration–time data is typically initiated by noncompartmental methods resulting, at least initially, in estimation of the apparent terminal elimination half-life ($t_{1/2}$) and area under the concentration–time curve (AUC) (systemic exposure). The terminal elimination rate constant is calculated from the slope of the terminal $\log_{10}$(concentration)–time data:

$$\text{Terminal elimination rate constant, } k_e = -Slope \times 2.303 \tag{38.1}$$

where, *Slope* is the slope of the terminal portion of $\log_{10}$(concentration)–time data

$$\text{Terminal elimination half-life, } t_{1/2} = \frac{0.693}{k_e} \tag{38.2}$$

AUC values are generally estimated using the trapezoidal rule, which is the sum of the area of all trapezoids between two sampling points. The area of the curve beyond the last sampling point is estimated using the ratio of the corresponding concentration value and the terminal elimination rate constant.

$$AUC(0\text{–}t_{\text{last}}) = \sum_{t=0}^{t_n} \frac{(C_{n-1} + C_n)}{2} \cdot (t_n - t_{n-1}) \tag{38.3}$$

$$AUC(0\text{–}\infty) = AUC(0\text{–}t_{\text{last}}) + AUC_{\text{terminal}} \tag{38.4}$$

where

$$AUC_{\text{terminal}} = \frac{C_{\text{last}}}{k_e} \tag{38.5}$$

38.3.1 Example 1: Intravenous Dosing

Following an intravenous dose of 1 mg of Drug X to human volunteers, blood samples were collected for 24 hours. Plasma concentration–time data are summarized in Table 38.1.

Elimination $t_{1/2}$ and AUC Values The terminal elimination rate constant (k_e) and terminal elimination half-life ($t_{1/2}$) may be estimated by examination of $\log_{10}$(concentration)–time profile to identify the terminal elimination phase and estimate the slope of the corresponding line (Eq. 38.1) (Table 38.2).

The application of Eqs. 38.3–38.5 for estimation of AUC values is exemplified in Table 38.3.

Clearance, Mean Residence Time, and Volume of Distribution Estimation of apparent terminal half-life ($t_{1/2}$) and AUC values is directly useful in estimation of other key pharmacokinetic parameters, such as clearance (CL), mean residence time (MRT), and volume of distribution (V_{ss}).

$$\text{Systemic clearance } (CL) = \frac{Dose_{\text{IV}}}{AUC(0-\infty)} \tag{38.6}$$

TABLE 38.1 Plasma Concentration–Time Profile Following a Single Intravenous Dose of 1 mg Drug X to Human Volunteers

Time (h)	Plasma Concentration (ng/mL)
0	100
0.0833	98.3
0.167	96.7
0.25	95.1
0.5	90.5
1	81.9
2	67.0
3	54.9
4	44.9
6	30.1
8	20.2
10	13.5
12	9.07
16	4.08
20	1.83
24	0.82

TABLE 38.2 Estimation of Apparent Terminal Elimination Rate Constant and Half-life Following a Single Intravenous Dose of 1 mg of Drug X to Human Volunteers

Time (h)	Plasma Concentration (ng/mL)	$\log_{10}$(Concentration)
0	100	
0.0833	98.3	
0.167	96.7	
0.25	95.1	
0.5	90.5	
1	81.9	
2	67.0	
3	54.9	
4	44.9	
6	30.1	
8	20.2	
10	13.5	
12	9.07	0.958
16	4.08	0.610
20	1.83	0.263
24	0.823	–0.0846
	Slope = –0.0869	
	k_e (Eq. 38.1) = $0.2\,h^{-1}$	
	$t_{1/2}$ (**Eq. 38.2**) = **3.46 h**	

TABLE 38.3 Estimation of Area Under Plasma Concentration–Time Curve (*AUC*) Following a Single Intravenous Dose of 1 mg Drug X to Human Volunteers

(1) Time (h)	(2) Plasma Concentration (ng/mL)	(3) $\frac{(C_{n-1}+C_n)}{2}$	(4) $(t_n - t_{n-1})$	(5) *AUC* Value of Each Trapezoid (Column 3 × Column 4)	(6) Cumulative *AUC* Values $[AUC(0–t_{last})]$
0	100	99.2	0.0833	8.26	8.26
0.0833	98.3	97.5	0.0833	8.13	16.4
0.167	96.7	95.9	0.0833	7.99	24.4
0.25	95.1	92.8	0.25	23.2	47.6
0.5	90.5	86.2	0.5	43.1	90.7
1	81.9	74.5	1	74.5	165
2	67.0	61.0	1	61.0	226
3	54.9	49.9	1	49.9	276
4	44.9	37.5	2	75.1	351
6	30.1	25.2	2	50.3	401
8	20.2	16.9	2	33.7	435
10	13.5	11.3	2	22.6	458
12	9.07	6.57	4	26.3	484
16	4.08	2.95	4	11.8	496
20	1.83	1.33	4	5.31	501
24	0.823				
				$AUC(0–t_{last})$ (Eq. 38.3) = 501 ng·h/mL	
				$AUC_{terminal}$ (Eq. 38.5) = 4.11 ng·h/mL	
				$AUC(0–\infty)$ (**Eq. 38.4**) = **505 ng·h/mL**	

$$\text{Mean residence time } (MRT) = \frac{AUMC}{AUC} \tag{38.7}$$

where $AUMC$ is the area under the first-moment time curve, calculated in similar fashion to AUC, using the following formula:

$$AUMC = \left\{\sum_{t=0}^{t_n} \frac{(t_{n-1} \cdot C_{n-1} + t_n \cdot C_n)}{2} \cdot (t_n - t_{n-1})\right\} + \left\{\frac{C_{\text{last}} \cdot t_{\text{last}}}{k_e} + \frac{C_{\text{last}}}{k_e^2}\right\} \tag{38.8}$$

The apparent volume of distribution at steady state (V_{ss}) is the product of CL and MRT after a single intravenous dose of a drug:

$$V_{ss} = CL \cdot MRT \tag{38.9}$$

For Drug X, clearance is calculated using Eq. 38.6:

$$CL = 1\,\text{mg}/(505\,\text{ng}\cdot\text{h/mL}) = 1979\,\text{mL/h}$$

Calculation of MRT requires estimation of $AUMC$ (Eq. 38.8) as illustrated in Table 38.4.

TABLE 38.4 Estimation of Area Under First-Moment Time Curve (*AUMC*)

(1) Time (h)	(2) Plasma Concentration (ng/mL)	(3) $t{\cdot}C$ (Column 1 × Column 2)	(4) $\frac{(t_{n-1}C_{n-1} + t_nC_n)}{2}$	(5) $(t_n - t_{n-1})$	(6) *AUMC* (Column 4 × Column 5)	(7) Cumulative *AUMC* Values
0	100	0	4.10	0.0833	0.341	0.341
0.0833	98.3	8.20	12.2	0.0833	1.01	1.35
0.167	96.7	16.1	20.0	0.083	1.66	3.02
0.25	95.1	23.8	34.5	0.250	8.63	11.6
0.5	90.5	45.2	63.6	1	31.8	43.4
1	81.9	81.9	108	1	108	151
2	67.0	134	149	1	149	301
3	54.9	165	172	1	172	473
4	44.9	180	180	2	360	833
6	30.1	181	171	2	342	1176
8	20.2	162	148	2	297	1472
10	13.5	135	122	2	244	1717
12	9.07	109	87.0	4	348	2065
16	4.08	65.2	50.9	4	204	2269
20	1.83	36.6	28.2	4	113	2381
24	0.823	19.8				
					$AUMC\ (0\text{–}t_{\text{last}}) = 2381$	
					$(C_{\text{last}} \cdot t_{\text{last}})/k_e = 98.7$	
					$C_{\text{last}}/k_e^2 = 20.6$	
					$AUMC(0\text{–}\infty)$ (**Eq. 38.8**)	
					= **2501 ng·h²/mL**	

Therefore, using Eq. 38.7, mean residence time (MRT) is calculated as

$$MRT = 2501/505 = 4.95\,\text{h}$$

The apparent volume of distribution at steady state, V_{ss}, is calculated using Eq. 38.9:

$$V_{ss} = CL \cdot MRT = (1979\,\text{mL/h}) \cdot 4.95\,\text{h} = 9797\,\text{mL}$$

Urinary Excretion Assume that urine samples were collected over various time intervals following a single intravenous dose of Drug X. The volume of urine collected over each interval was recorded and the corresponding concentration of unchanged Drug X was determined, as summarized in Table 38.5.

These apparent terminal elimination half-life ($t_{1/2}$) values as well as the fraction of dose excreted unchanged in urine (f_e) may be estimated by plotting the excretion rate (dA_e/dt) against the "midpoint" of sample collection (t_{midp}) (Fig. 38.6, Table 38.6). The theoretical basis for this derivation is beyond the scope of this chapter and is exhaustively discussed in the literature [2, 3, 5].

$$\log \frac{A_e}{dt} = \log(f_e \cdot Dose) - \frac{k_e t}{2.303} \tag{38.10}$$

where f_e, is the fraction of administered dose excreted unchanged in urine.

$$Slope = -k_e/2.303$$

$$t_{1/2} = 0.693/k_e = 3.46\,\text{h}$$

The intercept of the semilogarithmic plot provides an estimate of the fraction of administered dose excreted unchanged in urine at infinity ($f_e \cdot Dose$), 0.123 mg.

TABLE 38.5 Urinary Concentration of Unchanged Drug Following Single Intravenous Administration of 1 mg Drug X to Healthy Volunteers

Time Interval (h)	Urine Volume (mL)	Concentration in Urine (ng/mL)
0–0.5	100	571
0.5–1	74	698
1–2	88	1012
2–4	150	884
4–6	170	523
6–8	250	238
8–12	340	196
12–16	500	59.9
16–20	520	25.9
20–24	450	13.4

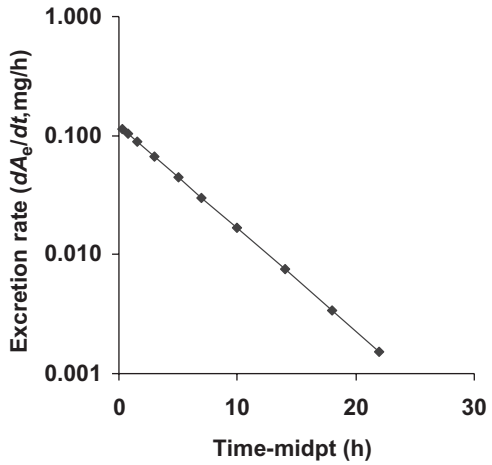


FIGURE 38.6 Semilogarithmic plot of urinary excretion rate versus t_{midpt}.

TABLE 38.6 Urinary Excretion Analysis Following Intravenous Administration of 1 mg Drug X to Healthy Volunteers

(1) Time Interval (h)	(2) Urine Volume (mL)	(3) Concentration in Urine (ng/mL)	(4) Amount Excreted in Urine (A_e, mg) (Column 2 × Column 3)	(5)[a] t_{midpta}	(6)[b] dA_e/dt
0–0.5	100	571	0.0571	0.25	0.114
0.5–1	74	698	0.0517	0.75	0.103
1–2	88	1012	0.0890	1.5	0.0890
2–4	150	884	0.133	3	0.0663
4–6	170	523	0.0889	5	0.0444
6–8	250	238	0.0596	7	0.0298
8–12	340	196	0.0667	10	0.0167
12–16	500	59.9	0.0300	14	0.00749
16–20	520	25.9	0.0135	18	0.00337
20–24	450	13.4	0.00605	22	0.00151

[a] t_{midpt} is the midpoint of the urine collection interval.
[b] dA_e/dt is calculated as $(dA_{e(n+1)}-dA_en)/(t_{n+1}-t_n)$.

Multiple Intravenous Dosing For a drug following *linear* (dose- or concentration-independent) *pharmacokinetics*, *AUC* values at steady state equal those following the first dose:

$$AUC(0-\tau)_{\text{steady state}} = AUC(0-\infty)_{\text{first dose}} \tag{38.11}$$

where τ is the dosing interval. $AUC(0-\tau)_{\text{sleady state}}$ is calculated using the trapezoidal rule as illustrated in Table 38.1.

TABLE 38.7 Estimation of Pharmacokinetic Parameters Following a Single 1 mg Oral Dose to Human Volunteers

(1) Time (h)	(2) Plasma Concentration (ng/mL)	(3) Log_{10} (Concentration)	(4) $\frac{(C_{n+1}+C_n)}{2}$	(5) $(t_{n+1}+t_n)$	(6) AUC Value of Each Trapezoid	(7) $t \cdot C$	(8) $(t_{n-1}C_{n-1}-t_nC_n)/2$	(9) $AUMC$ Values
0	0		1.29	0.0833	0.107	0	0.107	0.00896
0.0833	2.58		3.78	0.0833	0.315	0.215	0.523	0.0436
0.167	4.99		6.12	0.0833	0.510	0.832	1.321	0.110
0.25	7.24		10.2	0.25	2.55	1.81	4.19	1.05
0.5	13.1		17.4	0.5	8.68	6.56	14.1	7.04
1	21.6		25.6	1	25.6	21.6	40.3	40.3
2	29.5		30.1	1	30.1	59.1	75.6	75.6
3	30.7		29.7	1	29.7	92.0	103	103
4	28.7		25.3	2	50.6	115	123	246
6	21.9		18.7	2	37.4	131	128	255
8	15.5		13.1	2	26.1	124	115	230
10	10.6		8.91	2	17.8	106	96.3	193
12	7.20	0.857	5.23	4	20.9	86.4	69.2	277
16	3.26	0.513	2.36	4	9.44	52.1	40.7	163
20	1.46	0.166	1.06	4	4.25	29.3	22.5	90.2
24	0.658	–0.182				15.8		
	Slope = –0.0866			$AUC(0–t_{last})$= 264			$AUMC(0–t_{last})$ = 1682	
	$k_e = 0.199\,h^{-1}$			C_{last}/k_e = 3.30			$(C_{last} \cdot t_{last})/k_e$ = 79.2	
	$t_{1/2}$ = **3.48 h**			***AUC*(0–∞) = 267 ng·h/mL**			C_{last}/k_e^2 = 16.6	
				***F* = 52.9%**			***AUMC*(0–∞) = 1778 ng·h²/mL**	
							***MRT* = 0.150 h**	

TABLE 38.8 Implications of Pharmacokinetic Data Analyses on Drug Discovery and Development

Key Pharmacokinetic Information	Methodological Approach	Drug Discovery and Development Implication
Systemic exposure and peak systemic concentrations	Analysis of systemic concentration–time profile: determination of the area under the curve (AUC) and C_{max}	Potential correlation with drug response (efficacy and/or toxicity)
Rate of drug absorption	Analysis of systemic concentration–time profile: time to C_{max} (T_{max})	Choice of an appropriate route of administration and drug formulation
Systemically available fraction of administered dose	Bioavailability assessment	Choice of an appropriate route of administration and dose
Presystemic or first-pass elimination following oral dosing	Comparison of nonparenteral AUC, and other routes such as portal administration with AUC after intravenous administration	Factors affecting bioavailability
Drug persistence in the body	Apparent terminal elimination half-life ($t_{1/2}$)	Frequency of drug administration and designing "acceptable" dosing regimen
Drug accumulation following multiple dosing (parenteral or nonparenteral)	Analysis of systemic concentration–time profiles at steady state	Relationship of the drug levels to pharmacologically relevant drug levels (therapeutic window)
Route of drug clearance	Rate and extent of urinary excretion and extent of drug metabolism (including *in vitro* methodologies)	Factors affecting drug elimination; extent of intersubject variability in drug disposition; and drug–drug interactions

38.3.2 Example 2: Oral Dosing

The plasma concentration–time data following a single oral dose of 1 mg Drug X is summarized in Table 38.7. Apparent terminal elimination $t_{1/2}$, AUC values, CL, and MRT values are calculated as indicated in Eqs. 38.1–38.2. The corresponding calculations are included in Table 38.7. The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) are generally determined by observation. Therefore, in this instance,

$$C_{max} = 30.7\,\text{ng/mL}$$

$$T_{max} = 3\,\text{h}$$

Oral bioavailability is defined by the following equation:

$$F(\%) = \frac{AUC_{\text{oral}}/Dose_{\text{oral}}}{AUC_{\text{IV}}/Dose_{\text{IV}}} \tag{38.12}$$

38.4 IMPLICATIONS OF PHARMACOKINETICS ON DRUG DISCOVERY AND DEVELOPMENT

This chapter describes the typical data analysis methodologies used to characterize the pharmacokinetics of a drug following single or multiple intravenous or oral dosing using systemic (plasma) concentration–time and urinary excretion–time data. The implications of such analyses on drug discovery and development are summarized in Table 38.8.

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