The objective of this manuscript is to provide a brief overview of the pharmaceutical industry, role of a toxicologist, testing requirements for pharmaceuticals and detailed information on the reproductive toxicology testing of human and veterinary drugs.

Introduction

The unprecedented medical progress we have made over the past few decades is nothing short of remarkable. Much of this progress, past and future, is due to the discovery and development of new medicines for both humans and animals.

Today, prescription drugs play a leading role in health care. Innovative medicines available today can ease the symptoms of arthritis, help reduce the risk of osteoporosis and breast cancer, make life easier for people with congestive heart failure, generally, prevent the disability that was once considered a normal part of the aging process. Animals have been the beneficiaries of many of the advances made in human medicine as the new medicines developed for humans have many of the same beneficial properties in animals. Medicines have turned many diseases that were once virtual death warrants into treatable conditions. And we can’t stop now.

The prospects for better health at all stages of life have never been brighter. Scientists now have greater knowledge of how disease works and the high tech tools needed to design more effective medicines. Pharmaceutical research companies are constantly increasing their commitment to R&D; in 2001 companies will invest more than $26 billion to discover and develop new medicines.

The knowledge needed to discover and develop new medicines does not come cheap. Discovering, developing, testing, and gaining regulatory approval for new medicines is expensive, time consuming, and risky.

- Of every 5,000 medicines tested, on average, only 5 are tested in clinical trials and only 1 of those is approved for patient use. Revenues from successful medicines must cover the costs of the “dry holes.” (Fig. 1)
- The average cost of bringing one new medicine to market is $500 million.
- It takes an average of 12-15 years to discover and develop a new medicine. Most of that time is spent testing the drug to make sure it is safe.
- On average, only 3 of every 10 prescription drugs available to treat Americans generate revenues that meet or exceed average R&D costs. (Fig. 2)
Although the cost of developing drugs is soaring, the time that companies have to recoup their investment is shrinking due to stepped-up competition from competitor drugs and generics. (Fig. 3)

Figure 1. Compound Success Rates by Development Stage

Figure 2. Success rate of new drugs recovering R&D expenditures.
Figure 3. Marketing exclusivity of breakthrough drugs from 1965 to 1999

**Regulatory Drug Safety**

Regulatory drug safety for human drugs is generally divided into two separate groups—preclinical and clinical. The focus of this presentation will be on preclinical safety assessment of human pharmaceuticals and requirements for reproductive toxicology assessment of veterinary drugs.

Preclinical safety assessment plays a critical role in the drug development process. Indeed, this step is often considered to be rate-limiting. Subsequent to chemical synthesis of a novel, pharmacologically active therapeutic candidate, extensive toxicity testing is initiated. The objective of this testing is to provide a comprehensive and systematic evaluation of the toxic potential of the novel chemical entity based on the best available technology.

The purpose of this testing is three-fold: to provide a valid risk assessment for man prior to the initiation of clinical trials; to fulfill rigid domestic and international regulatory requirements; and to avoid liability in protecting the company from potential litigation.

General toxicologic considerations in the course of evaluation of novel therapeutic candidates include development of an understanding of the dose-response relationships for toxicity (need to establish a no-effect level (NOEL) and a maximally tolerated dose (MTD)), identification of important target organs, determination of the cause of toxicity (e.g. exaggerated pharmacology versus a unique, unidentified toxic mechanism), definition of species and sex-
related differences in toxic potential, determination of reversibility, and elucidation of the mechanism of toxicity.

The process of safety evaluation of therapeutic candidates must employ the best scientific approach possible. In today’s world it is becoming increasingly difficult to identify breakthrough (“blockbuster” > $1 billion in sales) therapeutics and therefore, the toxicologist must understand clearly the animal toxicity, spending a rigorous effort in defining it relationships to potential human toxicity. In the past, a less proactive approach resulted in more therapeutic candidates being terminated at early stages in the process due to undefined or poorly understood animal toxicology. A strong competitive advantage can be provided by mechanistic studies aimed at understanding differences in the biology of the toxicologic response in animal versus man. This information can be used to support the argument that a toxic response in animals may not be relevant for man. Indeed, there are numerous examples of species specific toxicity and/or toxicity that occurs only at enormous dose/exposure exaggerations in animals making the finding less important to human safety based on different biology or lower targeted dose/exposure(s) in man. Using this approach, it is possible to provide a rationale defense for further development of a given therapeutic candidate.

General Considerations in Regulatory Drug Safety

1. **Dose-response relationships** It is important that clear toxic effects are demonstrated in animal safety studies. While toxicologists are often questioned about the large dose employed in toxicology studies, it is a highly conservative approach which unfortunately, can produce artifact in the test system (e.g. effects which are non-physiological). This requirement is expected to allow for the large heterogeneity of response anticipated in the highly diverse human population. Lower doses typically include a mild-moderately toxic level and a “clean dose” or no effect level. The low dose is typically on the order of 5-10 times the anticipated human clinical dose.

2. **Identification of target organ(s) and biomarkers of toxicity** Another reason to “push the doses” in toxicology studies is to produce target organ toxicity that may be subtle or beneath the sensitivity of toxicologic/pathologic parameters of measurement (e.g. clinical pathology, histopathology) at lower doses. This exercise is aimed at identifying possible target sites of toxicity in man. Since each organ system has hallmark signs that can be evaluated for specific target organ toxicity, it is possible to identify target organs, often without sacrificing the animal. Biomarkers can include the standard array of test which are employed in most toxicity studies, but special emphasis should be given to tests that could reflect the expected pharmacologic activity of the test agent. Relevant biomarkers that are identified in toxicity studies are often then employed in human clinical trials and in this way the toxicologist provides a tool for the clinical monitor.

3. **Exaggerated pharmacology versus unique toxic action** As pharmaceutical candidates become more and more specific for their biologic target, the notion of exaggerated pharmacology or “mechanism based toxicity” has become popular. Exaggerated pharmacology refers to toxicity, which results from an exaggeration of
the desired pharmacologic response. For example, the lowering of blood pressure with a potent antihypertensive in a normal animal to levels, which limit organ perfusion, will result in toxicity. The toxicologist is generally comfortable with toxicity results based on exaggerated pharmacology since the mechanism is known and the dose can be titrated. Of greater concern is toxicity, albeit well-characterized, of unknown cause. In this case it becomes far more difficult to extrapolate the response from animals to man.

4. Species-, age-, sex-related differences in toxicity and reversibility It is critically important to establish the species-specific nature of toxicity to a therapeutic candidate. Many agents are toxic in a single or limited number of species and understanding of the species restricted nature of the toxicity can be important in estimating the potential human response. The converse is also true. For example, certain species are often resistant to the expected human pharmacological response and therefore would not be predictive of a response associated with exaggerated pharmacology. This is often true in biotechnology drug development where recombinant human proteins do not act in lower species. Hepatocarcinogenicity in response to certain hypolipidemics in rodents appears to be rodent specific phenomenon. However, in the past, a poor understanding of the mechanism did not allow the toxicologist to argue the irrelevance of this response for man. Such a response would result in termination of the development program often in very late stages of development after large expenditures were incurred. This resulted in a loss of money, time, and opportunity. The age and sex of animals in toxicology studies are also important factors – given the differences in organ function with age and hormonal status with sex, there are many examples of differences in sensitivity to toxic response that may be relevant to humans. Standard toxicity programs include an analysis of these possible interactions. The reversibility of toxicity is also critical. That is, the potential irreversible injury to a biological system should be clearly evaluated in any toxicology program as demonstration of such an effect will demand a more conservative approach to the eventual use of a given therapeutic candidate, including a careful risk/benefit analysis.

Summary

Regulatory drug safety studies are critically important in the drug development process. These highly-regulated studies are conducted according to rigid regulatory guidelines, and based on the tremendous level of competition in the industry, the studies are often conducted under internal pressure to trim timelines and move reports through the system as quickly as possible. These studies are not simply “box-checking” exercises to fulfill regulatory requirements. Rather, these studies must result in a clear understanding of the potential relevance of animal toxicity to man. It is possible and indeed not uncommon, that such an understanding will result ultimately in a marketed product as opposed to a lost opportunity.
Reproductive Toxicology Testing

Human Pharmaceuticals

There is considerable overlap in the methodology that could be used to test chemicals and medicinal products for potential reproductive toxicity. As the first step to using a wider methodology for efficient testing, the International Conference on Harmonization (ICH; adopted by the USA, EU and Japan) drafted guidelines to consolidate a strategy based on study designs currently used for testing of medicinal products worldwide; it encouraged the full assessment of the safety of a chemical on the development of the offspring. It is perceived that tests in which animals are treated during defined stages of reproduction better reflect human exposure to medicinal products and allow more specific identification of stages at risk. While this approach may be useful for medicines, long term exposure to low doses does occur and may be represented better by a one or two generation study approach.

The actual testing strategy should be determined by:
- anticipated drug use especially in relation to reproduction
- the form of the substance and route(s) of administration intended for humans
- making use of any existing data on toxicity, pharmaco-dynamics, kinetics, and similarity to other compounds in structure/activity

The aim of reproduction toxicity studies is to reveal any effect of one or more active substance(s) on mammalian reproduction. For this purpose both the investigation and interpretation of the results should be related to all other pharmacological and toxicological data available to determine whether potential reproductive risks to humans are greater, lesser or equal to those posed by other toxicological manifestations. Further, repeated dose toxicity studies, can provide important information regarding potential effects on reproduction, particularly male fertility. To extrapolate the results to humans (assess the relevance), data on likely human exposures, comparative kinetics, and mechanisms of reproductive toxicity may be helpful.

The combination of studies selected should allow exposure to mature adults and all stages of development from conception to sexual maturity. To allow detection of immediate and latent effects of exposure, observations should be continued through one complete life cycle, i.e. from conception in one generation through conception in the following generation. For convenience of testing this integrated sequence can be subdivided into the following stages.

A. Premating to conception (adult male and female reproductive functions, development and maturation of gametes, mating behavior, and fertilization).
B. Conception to implantation (adult female reproductive functions, preimplantation development, implantation)
C. Implantation to closure of the hard palate (adult female reproductive functions, embryonic development, major organ formation)
D. Closure of the hard palate to the end of pregnancy (adult female reproductive functions, fetal development and growth, organ development and growth)
E. Birth to weaning (adult female reproductive functions, neonate adaptation to extrauterine life, preweaning development and growth)
Weaning to sexual maturity (postweaning development and growth, adaptation to independent life, attainment of full sexual function)

**Study Design**

**Selection of Animal Species**

Studies should be conducted in mammalian species. It is generally desirable to use the same species and strain as in other toxicological studies. Reasons for using rats as the predominate rodent species are practicality, comparability with other results obtained in this species and the large amount of background knowledge accumulated.

In embryotoxicity studies only, a second mammalian species traditionally has been required, the rabbit being the preferred choice as a “non-rodent”. The reasons for choosing the rabbit are similar to those outlined for the rat.

Where the rat and/or rabbit are not suitable an alternative specie(s) may be acceptable and should be considered on a case by case basis. Alternative species include mice, guinea pigs, domestic and/or mini pigs, ferrets, hamsters, dogs and non-human primates.

**Dosages**

Selection of dosages is one of the most critical issues in design of the reproductive toxicity study. The choice of the high dose should be based on data from all available studies. When sufficient information is not available preliminary studies are advisable. Some minimal toxicity is expected to induced in the high dose dams (i.e. reduced body weight, specific target organ toxicity, alterations in hematology or clinical chemistry, saturation of kinetics, or marked increase in embryo-fetal lethality in preliminary studies). Under most circumstances 1 g/kg/day should be an adequate limit dose.

**Number of Animals per Group**

The number of animals specified in the ICH guidelines are educated guesses governed by the maximum study size that can be managed without undue loss of overall study control. This is indicated by the fact that the more expensive the animal is to obtain or keep, the smaller the group size proposed. Ideally, at least the same group size should be required for all species and there is a case for using larger group sizes for less frequently used species such as primates.

For all but the rarest events (such as malformations, abortions, total litter loss), evaluation of between 16 to 20 litters for rodents and rabbits tends to provide a degree of consistency between studies.

**Design of Studies to Cover All Stages**

For most medicinal products the 3-study design will usually be adequate. Other strategies, combinations of studies and study designs could be as valid or more valid as the “most probable option” according to circumstances. The key factor is that, in total, they leave no gaps between the stages and allow direct or indirect evaluation of all stages of the reproductive process.
The most probable options can be equated to a combination of studies covering various segments or stages for effects on:

- Fertility and early embryonic development (Seg I)
- Embryo-fetal development (Seg II)
- Pre- and postnatal development including maternal function (Seg III)

The outlines of the study designs for each of these combinations is illustrated in Figure 4.

**Veterinary Products**

For veterinary products the need for reproductive toxicity studies depends on the desired label (i.e. if the labeling indicates that the drug in the target species is not intended for breeding animals or during pregnancy), a reproduction study is not required. Otherwise, the Center for Veterinary Medicine, the governing body within FDA for veterinary products, would require a reproduction study in the target species.

The reproductive toxicology study designs are similar for all target animal species. The studies are designed to evaluate the entire reproductive life cycle of the parental generation in addition to potential cumulative effects in the offspring, including any reproductive effects. The FDA has provided guidance in two separate documents that included guidance from CVM and a separate FDA document referred to as the “Redbook.” The websites for the documents are listed below:

http://www.fda.gov/cvm/guidance/guidance.html
http://www.cfsan.fda.gov/~redbook/red-toca.html

In general, the guidance recommends that reproductive studies should be conducted on both sexes to evaluate possible drug effects in the target species on fertility, ovulation and spermatogenesis, egg and sperm integrity, transportion, implantation and development of the zygote, parturition and the neonate, lactation, weaning, and care of the young, on delayed postnatal deviations and future breeding potential with special interest directed to the teratogenic, fetotoxic and mutagenic potential of the drug.

The studies can be divided into three segments as described above for human pharmaceuticals or be combined into a two-generation study as recommended by the “Redbook.” In a two-generation study which is most commonly used for agro-chemicals and industrial chemicals there is direct exposure of the F₀ generation, indirect (gestational and lactational) and direct exposure of the F₁ generation and indirect exposure of the F₂ generation. The high dose in these studies should be 3X the anticipated maximum therapeutic dose.


References available upon request.
Gestational Cycle of the Rat and Reproductive Toxicity Testing

- Ovulation/Fertilization
- Blastocyst Formation
- Implantation/Decidualization
- Embryo Transport
- Organogenesis
- Fetal Growth
- C-section
- Birth

Day 0 3 4 5 6 18 21 22

Segment I
Dosing through Day 7 of Gestation
Early Embryonic Development
• Fertility
• Fecundity

Segment II
Dosing Day 6 - 18 (Gestation)
Teratology/Developmental Toxicity
( also conducted in rabbits dosing Days 6-20)
• Malformations

Segment III
Dosing Day 6 (Gestation) - Day 21 Postpartum
Perinatal Development
• Fetal Viability
• Neonatal Viability
• Multi-generational Effects

Figure 4