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# TOXICOLOGY`

NEWSLETTERS

*"Traditional animal testing is expensive, time-consuming, uses a lot of animals & from a scientific perspective. The results do not necessarily translate to humans"*

*-Dr.Christopher P. Austin*

**October 2019**

## Interactive Meet on Insights in Toxicology - 2019

### Pathology for Toxicologists

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# Limitations of Animal Toxicity Data for Human Safety

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## A. Introduction

Evaluation of the toxicity of chemicals in laboratory animals has been a cornerstone of human safety evaluation. *Preclinical toxicology studies helps to establish a presumably safe starting dose for first-in-human studies and define potential toxicities and their reversibility.* Experimentation with animals makes it possible to learn a great deal about the toxic potential of drugs and other chemicals. Explicitly defined investigations in laboratory animals are prescribed by EPA, FDA, EMA and other regulatory authorities for approval of pesticides and drugs. Animals can be utilized for short, intermediate, and chronic exposure studies through which scientists can characterize the spectrum of adverse effects of a compound over a wide range of doses, dosage regimens, and exposure durations. Often, the toxicologist initially will administer high doses and evaluate a broad spectrum of parameters in order to identify target organs. Focused dose-response studies employing a limited number of sensitive indices of injury can then be performed. Ideally, dosage routes and regimens will be designed to mimic actual human exposure situations. The use of laboratory animals as toxicology research subjects is advantageous for several reasons. Most rodent species are relatively inexpensive and easily maintained. Large numbers of rodents can be assessed over a wide range of doses, increasing the likelihood of detecting adverse events. Several biochemical, cellular, and physiological endpoints that can be examined only in human biopsy samples or at autopsy can be evaluated in animals. *In addition, considerable background information often is available on commonly used strains of mice, rats, and dogs, including their genetic makeup, their abilities to metabolize xenobiotics, and their responses to other compounds.* Groups of uniform animals can be administered measured doses of chemical(s) under defined and carefully controlled conditions, circumstances under which adverse effects to specific chemical exposure can be attributed with greater certainty. Human populations, in contrast, are much more genetically diverse, with endogenous and exogenous factors (e.g., diet, stress, health, age, personal habits, use of drugs, exposures to other chemicals) that may not be recognized or controllable. In addition, the degree and duration of an individual's exposure to the chemical of interest are often unclear in epidemiological studies and case histories.

As of now, animal data are essential for the safety assessment of new drugs, particularly when initiating clinical trials where little or no human data are available. However, the duration of toxicity studies required to support repeated dosing in humans should take into consideration the relationship between the duration of repeated dosing and new toxicity findings in animals, the reliability

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of the results of animal studies at predicting potential safety in clinical use, and the relationship of the time-scale in different animal species to man.

*It is proposed that four weeks of repeated dosing in animals is enough to support studies in humans up to four weeks. Following this, 3-month repeated dose toxicity studies should be recommended as the minimum duration to support repeated dosing of longer than four weeks in all phases of clinical investigation.*

Long-term dosing in humans, such as that over six months, can only be supported by human data which are accumulated by the stepwise process of clinical investigation.

## B. Historic Precedence of Preclinical Studies in Drug Development

The need for careful testing of new drugs in animal models before study in humans has been recognized by physicians since the First World War. Now, first human studies on new drugs are subject to detailed government guidelines, which are being reinforced through the wide-ranging *Clinical Trials Directive*. However, despite their long history and widespread application, these guidelines are empirical and have been formulated with a paucity of critical scientific evidence. Here, described are a review of the principles and the available, albeit limited, evidence that supports the design and conduct of preclinical studies in a way that permits effective and safe first-dose studies of potential new medicines in humans.

a. *The aim of a first study of a new drug in humans is to explore in a safe and ethical manner the dose and exposure range that is well-tolerated, and, if possible, to identify any dose-limiting adverse events*. Achievement of these aims represents a major leap from the laboratory bench to humans. It requires a substantial body of information characterizing the test substance, which can only be derived from animal studies.

b. Despite these activities being regulated by many national and international guidelines, the approach to preclinical studies remains largely empirical. There is a paucity of evidence about the performance of the widely employed preclinical tests in the prediction of toxicity in humans. There is a need for much better performance data in this area.

*c. The available limited, retrospective evidence indicates that the conventional approach using experimental pharmacology alongside toxicity studies of one month's duration reasonably predicts adverse events in the first human studies. The conventional methods identify more than 90% of toxicities that can be detected in animals.*

d. If toxicity studies are shorter than one month, there is a risk of certain organ toxicities being overlooked. However, single studies seem to have the capacity to detect many of the most important potential adverse effects.

e. *Data obtained from dog studies are frequently better predictors than data from rodent experiments.*

*f. Although uncommon, serious idiosyncratic drug reactions involving skin, liver, and hemopoiesis - which conventional animal studies usually fail to predict - are major problems in drug development.*

## C. Limitations of Animal Studies

Much of the uncertainty about the relevance for humans of animal studies stems from three factors: *1) sensitivity, 2) reproducibility and 3) predictivity.* For example, researchers from Amgen attempted to repeat cancer treatment studies using mice. They succeeded in only six out of 53 cases, after repeated attempts. Similar results were obtained by researchers from Bayer. All these factors are inherently tied to the central drawbacks of animal studies, which is that it is a living model system that is being used to extrapolate results from. *Animal model systems have high intrinsic variability, differences within species and are also mechanistically not completely understood. Study design can influence all three factors.*

In retrospect, the concordance of human toxicity that was observed in any animal species is roughly 71%. *Rodent studies using rats or mice predicted only 43% of human toxicity, and non-rodents such as dogs only 63%.* The advent of recombinant technologies in the early 1980s has introduced protein drugs (biotherapeutics) to the arsenal of available therapeutics. These highly complex proteins are also very species-specific and are usually immunogenic in animals. Therefore, animal data for these types of products are also not likely to yield informative data. Animal studies with low predictive value might hamper the development of innovative pharmaceutical drugs because during development of drugs may be wrongly identified as being too toxic and never reach the patient. A false sense of safety can be given when drugs were considered non-toxic in animal studies and serious adverse effects can occur after a drug has been granted a marketing authorization. *For instance, in 2004 Vioxx (Rofecoxib), a COX-2 inhibitor was withdrawn from the market after analysis of clinical trial data revealed that there was an increased risk of adverse cardiovascular effects. During non-clinical studies with Rofecoxib, such adverse effects were not observed.*

In short, convincing data concerning the predictive value of animal studies in the development of pharmaceutics is lacking. The main reason for this is that existing databases with relevant non-clinical animal data and clinical data are confidential. submissions on file in regulatory agencies, neither of which are easily available for research.

## D. Comparison to Short Term Effects to Humans

Findings in animal toxicology studies generally are applicable to humans, although, responses of laboratory animals and humans to chemicals may differ qualitatively and/or quantitatively. The most definitive study to date of interspecies concordance involved an International Life Sciences Institute-sponsored review of data supplied by 12 pharmaceutical companies. The database consisted of toxicity findings from preclinical (i.e., experimental animal) and clinical (i.e., human) studies of 150 compounds in 15 therapeutic classes. Interspecies concordance of toxicity was said to exist if generally severe effects on the same organ occurred in humans and in laboratory animals. *There*

*was an overall interspecies concordance for 61 percent of the compounds. Rodents alone were predictive of human toxicities for 43 percent of the agents, while nonrodents (primarily dogs) alone were predictive for 63 percent. In another comparative investigation, 43 percent of the clinical toxicities of 64 marketed drugs in Japan were not predicted from animal experiments.* The poorest concordance in all surveys was for cutaneous hypersensitivity and endocrine and hepatic functions. Obviously, animal studies cannot reveal subjective effects such as headache, myalgias, dizziness, nausea, or mental disturbances. Many of the cases apparently involved idiosyncratic reactions that occurred with a very low incidence in patient populations, a phenomenon that reflects the pronounced influence of exogenous and endogenous factors on interindividual responses.

### E. Comparison for Chronic and Carcinogenicity Effects to Humans

A considerable amount of information has been published on interspecies similarities and differences in susceptibility of chemical carcinogenesis. *All human carcinogens that have been adequately tested in animals have produced cancer in at least one animal model.* However, an evaluation of the National Toxicology Program (NTP) cancer bioassay data for 400 chemicals revealed that only 23 percent of the carcinogenic compounds produced tumors in both mice and rats. Some carcinogens, such as vinyl chloride, produce tumors in humans and in both sexes of other species tested. Conversely, many other carcinogens appear to be sex, strain and/or species-specific. Unleaded gasoline-induced kidney toxicity and cancer, for example, are limited to male rats, which is attributed to binding of gasoline to $\propto_{2u}$-globulin, a male rat-specific protein. The protein is hypothesized to accumulate to toxic levels in kidney cells and thereby induce sustained cellular proliferation, with its attendant cancer risk factors. It also is hypothesized that oxidative moieties produced by peroxisomal enzymes and modification of cell signaling by activation of Peroxisome Proliferator-Activated Receptor-α (PPAR) can elicit liver cancer. A variety of compounds, including drugs such as ciprofibrate and nafenopin and solvents such as trichloroethylene and perchloroethylene, markedly induce hepatic peroxisomes and produce hepatic tumors in mice and/or rats. Studies of humans taking clofibrate and gemfibrozil, however, reveal little peroxisome proliferation and no increased incidence of liver cancer. Pharmacodynamic differences (i.e., disparities in receptor numbers and affinities) appear to account for this phenomenon.

### F. Impact of Pharmacokinetics (PK)

Variances in pharmacokinetics are often responsible for pronounced interspecies differences in susceptibility to toxic agents. The term "pharmacokinetics" encompasses systemic absorption, distribution, metabolism, and elimination. Many chemicals undergo metabolic activation (i.e., are metabolized to toxic metabolites). Others are detoxified through metabolism. *Aflatoxin $B_1$, one of the*

*most potent hepatocarcinogens known, is metabolically activated by cytochrome P450s and subsequently detoxified by conjugation with glutathione.* Mice have been found to be much more resistant to aflatoxin $B_1$-induced liver cancer than rats. This disparity has been attributed to very efficient conjugation of the major reactive metabolite by mice.

Interspecies extrapolations based on body surface area and comparative metabolism studies with primary hepatocytes of mice, rats, and humans indicate that the susceptibility of humans to several compounds resembles that of rats. Tamoxifen is a non-steroidal antiestrogen that is used to treat pre- and postmenopausal women with breast cancer. It is a full estrogen in mice, a partial estrogen/ antiestrogen in rats and humans, and an antiestrogen in chicks. Tamoxifen is metabolically activated to a DNA-binding metabolite by a combination of Phase I and II metabolism. Biotransformation of tamoxifen is qualitatively similar in rats and humans, but the amounts of reactive metabolites and DNA adducts formed in the human liver are much lower than those formed in rats. *Knowledge of qualitative and quantitative species differences in the metabolism of a xenobiotic allows the selection of the animal strain and species that is most like the human.*

### G. Use of Body Surface Area in Comparative Extrapolation

There are a number of quantitative methods for extrapolation of animal toxicity data to humans. The standard uncertainty factor default approach is frequently used because of a paucity of data. *Linear extrapolations based on body weight equivalence often are inaccurate unless species-specific conversion factors are applied, while allometric scaling on the basis of Body Surface Area (BSA) is more accurate.* The primary impetus for the application of BSA-based dose extrapolation across species derives from cross-species comparisons of the sensitivity to small-molecule anticancer agents and other small-molecule drugs, for which poor predictivity of human sensitivity was obtained with direct extrapolation of mg/kg dose levels and for which much better predictivity was obtained when doses were converted to BSA ($mg/m^2$).

In general, doses of anticancer drugs lethal to 10 percent of rodents and maximally tolerated doses (MTDs) in nonrodents correlate with MTDs in human patients, when the doses are normalized to the surface area of each species. Normalization of body weight to the 2/3 or 3/4 power results in accurate predictions of body surface area, since both size (weight) and form (height), are considered. *FDA (2002) describes the use of standard species-specific factors that allow conversion of animal doses in mg/kg to animal doses in $mg/m^3$ and human doses in mg/kg.* The use of PK and metabolism data, when available for each species of interest, facilitate the most reliable interspecies conversions.

*FDA (2002) has published a guidance document that describes a strategy recommended for deriving safe starting doses of therapeutic agents for clinical*

*trials with healthy research participants*. The first step in the process involves the identification of NOAELs (no observed adverse effect levels) from animal toxicity studies. The NOAEL for the most appropriate species is selected, regardless of whether this species is the most sensitive. The selection is based on information available on relative bioavailability, metabolic profile, molecular biology, physiology, and reactions to similar compounds. Humans and marmosets, for example, have constitutive levels of hepatic CYP1A2, a P450 isozyme that activates heterocyclic amines to reactive metabolites. Cynomolgus monkeys lack constitutive CYP1A2. Marmosets are thus a more suitable animal model for heterocyclic amines than cynomolgus monkeys. *For drugs, the most appropriate animal NOAEL is converted to the human equivalent dose (HED) by the body surface area normalization process described by FDA (2002). Finally, the HED is divided by a safety factor to yield the maximum recommended starting dose.*

Pharmacokinetics-based conversions provide the most reliable means of extrapolating from one species to another. Such approaches require PK data for each species of interest. Optimally, animal blood and target organ time-course data and metabolic information will be available for a range of doses, including those within which toxicity occurs. Human metabolic and blood-level data for low doses also would be necessary. Blood time-course data alone allow comparison of areas under blood concentration versus time curves (AUCs) for test animals and humans. The so-called HED approach has been used successfully for several chemicals including, among others, methylene chloride, acrylic acid, and chlorpyrifos.

### H. Use of Reference Dose (RfDs)

An evaluation of susceptibilities to industrial and agricultural chemicals has provided some additional information on the reliability of animal toxicology findings. *Comparison of human data-based reference doses (RfDs) for 22 chemicals in EPA's Integrated Risk Information System (IRIS) database with RfDs calculated from animal data in IRIS using standard uncertainty factors.* Seven of the 22 compounds were pesticides, for which cholinesterase inhibition was measured in intentionally dosed research participants. The interspecies concordance rate was approximately 40 percent. The human-based RfDs were lower than the animal-based values for 7 (32 percent) of the 22 chemicals. The human values were more than three times lower for five of these seven compounds, leading the authors to conclude that exposure limits based upon animal data may not be protective of public health.

### I. Using Human Data to Develop Risk Values

*One of the criticisms of industry-sponsored human subject testing of toxicants is based on the perception that it is often motivated by an attempt to raise the acceptable exposure limit for the chemical.* When Reference Doses (RfDs) or Reference Concentrations (RfCs) are based upon no-effect levels from human

rather than animal data, an animal-to-human uncertainty factor (usually 10) is not required, which could conceivably result in a higher safe exposure limit. There has been little in the way of study of the effect of using human vs. animal data on the development of RfDs and RfCs to lend empirical support to this argument. *An analysis of comparing RfDs and RfCs derived from human data with toxicity values for the same chemicals based on animal data found that the use of human data did not always result in higher RfDs or RfCs.* In 36% of the comparisons, human-based RfDs or RfCs were lower than the corresponding animal-based toxicity values and were more than 3-fold lower in 23% of the comparisons. In 10 out of 43 possible comparisons (23%), insufficient experimental animal data are readily available, or data are inappropriate to estimate either RfDs or RfCs. Given the inherent ability of human data to reduce uncertainty regarding risks from human exposures, its use in conjunction with data gathered from experimental animals is a public health-protective policy that should be encouraged.

## J.Conclusions

The need for careful testing of new drugs in animal models before study in humans has been recognized by physicians since the First World War. Now, first human studies on new drugs are subject to detailed government guidelines, which are being reinforced through various regulations. Any drug product not previously authorized for marketing in the United States requires the submission of an Investigational New Drug application (IND). Although the IND submission is regulated by law (21CFR 312), there are several issues that are not covered in the law or U.S. Food and Drug Administration (FDA) guidance that are important for a successful IND submission. For oncology products, the International Conference
on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) S9 guidance is the most relevant. *The most difficult issues to solve in an IND are chemistry, manufacturing and control information, and pharmacology and toxicology.* In the United States, pivotal toxicological studies are done in two species: one rodent (i.e., rats) and one nonrodent (i.e., dogs). The safe starting dose is based on toxicological findings observed in the most sensitive species.

However, despite their long history and widespread use of two species toxicology studies, these guidelines are empirical and have been formulated with a paucity of critical scientific evidence. The current review of the principles and the available, albeit limited, evidence that supports the design and conduct of preclinical studies in a way that permits effective and safe first-dose studies of potential new medicines in humans can be summarized as follows:

1.*The aim of a first study of a new drug in humans is to explore in a safe and ethical manner the dose and exposure range that is well-tolerated, and, if possible, to identify any dose-limiting adverse events.* Achievement of these aims represents a major leap from the laboratory bench to humans. It requires a substantial body of information characterizing the test substance, which can only be derived from animal studies.

2.Despite these activities being regulated by many national and international guidelines, the approach to preclinical studies remains largely empirical. There is a paucity of evidence about the performance of the widely employed preclinical tests in the prediction of toxicity in humans. There is a need for much better performance data in this area.

3.The available limited, retrospective evidence indicates that the conventional approach using experimental pharmacology alongside toxicity studies of one month's duration reasonably predicts adverse events in the first human studies. *The conventional methods identify more than 90% of toxicities that can be detected in animals.*

4.If toxicity studies are shorter than one month, there is a risk of certain organ toxicities being overlooked. However, single studies seem to have the capacity to detect many of the most important potential adverse effects.

5.Data obtained from dog studies are frequently better predictors than data from rodent experiments.

6.Although uncommon, serious idiosyncratic drug reactions involving skin, liver, and hemopoiesis — which conventional animal studies usually fail to predict — are major problems in drug development.

7.For molecularly targeted agents (MTAs), it remains to be elucidated which animal models and toxicology parameters best predict a safe starting dose for first-in-human evaluation.

i. Despite their more selective mechanisms of action, MTAs can exhibit a wide spectrum of adverse effects. If the observed toxicities are off-target effects, they may not follow a clear dose-effect relationship as is seen with cytotoxic agents.

ii. *Therefore, traditional preclinical toxicology may be inadequate for determining a safe starting dose for first-in-human evaluation of MTAs*, and the design of appropriate preclinical toxicology studies for such agents is challenging.

8.Ultimately, the choice of animal species is likely to depend on the mechanism of action and structure of the novel agent under evaluation and the ability of the animal species to render and reflect the specific mechanism-based and off-target effects.

**9.**It is anticipated that over time, there will be substantial replacement of *in vivo* studies with *in vitro* studies, as more large and small molcules with the specified target are identified and developed for human use.

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