

Preclinical Safety Evaluation Using Nonrodent Species: An Industry/Welfare Project to Minimize Dog Use

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Abstract

This review of the dog, the primary nonrodent species used in toxicology, and its use in the safety evaluation of pharmaceuticals, provides data on the number used in particular projects in an effort to establish a baseline from which some minimization can be measured. Opportunities for reduction and replacement, as identified by a European Industry/Welfare Steering Group, are discussed. The three distinct areas of potential approaches to minimize dog use are categorized as industrial cooperation/data sharing, achieving best practice in study design, and assessing the need for a particular study. The Steering Group prioritized the approaches based on the impact on the number of animals used, the impact on the welfare of the remaining animals, the potential for industry's acceptance of the scientific approach, the potential for regulators' acceptance of the validated approach, and the time/cost of evaluation or implementation. Examples of each category are presented, and the work needed to facilitate industry/regulatory change is discussed.

Key Words: alternatives; animal use; dogs; laboratory animal sciences; toxicity tests

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Introduction

Registration of pharmaceuticals for human use generally requires the inclusion of a nonrodent species in the safety assessment process (CPMP/ICH 1997). The dog is the most frequently used species and is commonly considered by toxicologists as the "default nonrodent." The criteria used to select the most appropriate nonrodent species are not discussed in this article but should be based on scientific justification, ethical perspectives, technical considerations, and regulatory acceptability.

The value of the dog (and primate) in the development of new pharmaceuticals has been reviewed over many years, and a recent International Life Sciences Institute study (Olson et al. 2000) has demonstrated that dog studies are considerably more predictive of human toxicity than rodent studies. However, other reviews have concluded that despite current constraints on dog use (EC 1986), it is possible to achieve a reduction without compromising human safety (Broadhead et al. 2000).

A Steering Group representing 10 European pharmaceutical companies and two animal welfare organizations was established in 2000 with the aim of recommending and, where possible, putting into practice scientifically valid and feasible approaches to minimize dog use. In achieving this aim, human safety would not be compromised, and the replacement of dog by other nonrodents would not be acceptable. The Steering Group, which is nearing the end of its first phase of work, has identified potential approaches to minimize dog use and has prioritized them for further analysis. Dialogue has now been established with US companies, and it is hoped that other organizations will participate in the venture after critical review of this publication. If successful, these approaches may well be appropriate for other nonrodent species such as the primate and minipig.

Use of the Dog in Toxicology

Before considering any reduction in dog use, it is important to know where in the development process and in what numbers dogs are used. It should then be possible to focus effort on those studies that deploy the most animals and those procedures that cause the most pain, distress, or lasting harm.

Study Types

For the purpose of this review, only studies included in the nonclinical toxicology summary are considered, with the exception of safety pharmacology, which is now part of the pharmacological summary (CPMP/ICH 2000). Animals used for discovery purposes and pharmacokinetics are excluded. In the majority of cases, studies are conducted according to good laboratory practice (GLP¹) standards. However, the increasing tendency to conduct toxicology earlier in the program (e.g., safety pharmacology or maximum tolerated dose) can require repeated studies during the development process to satisfy GLP. It is important to avoid this practice by using “fit for purpose” study designs, which are acceptable to regulators.

Safety Pharmacology

The area of safety pharmacology includes studies performed during the early development of a new chemical entity and usually before a first clinical dose. These studies include in vitro cardiovascular assessment using dog tissues/organs (e.g. Purkinje fibre/Langendorff preparation for action potential duration/QT interval evaluation); anesthetized nonrecovery studies to assess hemodynamics, electrocardiogram (ECG¹) and respiratory and renal parameters; and telemetry in surgically prepared, conscious dogs to assess cardiovascular system and ECG.

Maximum Tolerated Dose (MTD¹)/Dose Range Finding (DRF¹) Studies

Using MTD/DRF studies, it is possible to identify target organ toxicity and relate findings to exposure. Studies of varied design, either single or multiple doses, may elicit dose-limiting signs of toxicity. These studies are performed to allow selection of dose levels for regulatory studies.

Single Dose (Acute Toxicity)

Guidelines of the International Conference on Harmonisation (CPMP/ICH 2000) require two mammalian species for acute toxicology. In some territories (e.g., Japan), it is still customary for the regulatory authority to expect data in a nonrodent species.

Repeat Dose

The pivotal studies in a regulatory package are repeat dose studies and durations of 14 days/1 mo, 3 mo/6 mo, and 9

mo/12 mo are generally used. In some cases, some of these studies may be omitted depending on the clinical program (duration of human studies) or the therapeutic indication (6-mo sufficiency for some territories/indications). Study designs are generally well prescribed and include extensive in-life monitoring (e.g., ECG, haematology, clinical chemistry, and ophthalmology) and detailed histopathology.

Juvenile Toxicity

Juvenile toxicity studies comprise a relatively recent requirement of the US Food and Drug Administration to allow clinical pediatric treatment. The design of these studies is similar to those of repeat dose studies; however, the age of the animal at commencement of study is preweaning whereas adult animals are used in conventional studies.

Investigations

Investigations are project specific and aimed at resolving issues arising from any of the studies. Where possible, these studies are usually performed in vitro or in rodents, but occasionally there may be the need for a study in the dog.

Discovery Support

Toxicologists working with discovery teams may generate data (identification and optimization of chemical series) very early in the drug discovery process. Work is usually in a rodent, but occasionally the dog is used on a project-specific basis.

Number of Dogs Used

Generating data on the number of dogs used in toxicology is a difficult but essential task if we are to have a measure of success with any minimization initiative. Many countries publish annual statistics. One such example is the data from the UK Home Office shown in Table 1 (Home Office 2000).

Although the data described above permit trend analysis in the UK, there are limitations when assessing dog use per toxicology project because such projects are managed globally and some particular types of study may be conducted overseas. Additionally, the data reflect procedures and not the number of animals, and some repeated use of animals does occur. Finally, the classification of studies is too broad to allow a detailed analysis of study types.

One other method of monitoring dog use is to assess the number of animals used per project, a measure that truly reflects the efficiency of the safety evaluation process. Data from 10 recently submitted new drug applications from four organizations have been analyzed to assess the value of such

¹Abbreviations used in this presentation: ECG, electrocardiogram; GLP, good laboratory practice; MTD, maximum tolerated dose.

Table 1 Annual dog use in the United Kingdom for 1999

Type of test	No. of procedures
Acute nonlethal	105
Subacute limit setting	797
Subacute toxicity	1513
Subchronic and chronic	1101
Toxicokinetics	356
Other toxicology	817
Total	4689

an approach. A large interproject variation in the number of dogs used (150-290) was seen, which was related to the therapeutic indication and the number of potential routes of administration. The percentages of animals used for each type of study are illustrated in Figure 1.

With the addition of further data sets, a baseline could be established from which future dog use could be monitored. However, it should be recognized that this baseline might underestimate the number of dogs used in preliminary studies because (1) a significant number of projects end before longer term studies are conducted, and (2) not all preliminary studies are included in submissions.

Potential Approaches to Minimize Dog Use

The Steering Group's review of study designs and working practices identified a plethora of potential opportunities to minimize dog use. To focus its effort, the Group prioritized them according to the impact on the number of animals used, the impact on the welfare of the remaining animals, the potential for industry's acceptance of the scientific approach, the potential for regulators' acceptance of the vali-

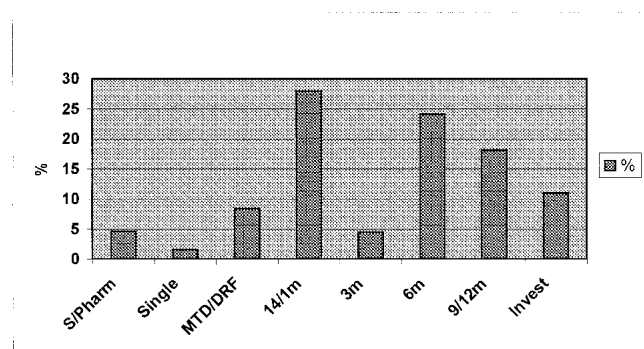


Figure 1 Analysis of dog use per research project. S/Pharm, safety pharmacology; MTD/DRF, maximum tolerated dose/dose range finding study.

dated approach, and the time/cost of evaluation or implementation.

After prioritization, the opportunities were categorized into three areas: (1) industrial cooperation/data sharing, (2) achieving best practice in study design, and (3) assessing the need for a particular study. For each of the categories, the Steering Group eliminated opportunities of low priority but that were still worthy of consideration in due course. The opportunities they eliminated and those they selected for further analysis are presented below. Several of those chosen for further analysis are described in more detail to illustrate what may be achieved.

Industrial Cooperation/Data Sharing

Eliminated approaches include control value databases and compound class databases. One approach that is worthy of further analysis is the vehicle effect database.

A vehicle database would contain qualitative and quantitative findings of all vehicles, excipients, solvents, and preservatives used in the preparation of dosing formulations and would be "owned" by the industry. Although repetition of studies is rare, there are occasions when vehicles are being used either for the first time or by a different route of administration. Data may not be in the public domain, and sharing of toxicity profiles would avoid the need for investigation/MTD/DRF studies to precede regulatory studies. The Group recommended development of a global, informal, datasharing process, which exists in the UK.

Achieving Best Practice in Study Design

Approaches that have been eliminated include sharing controls from parallel studies, using a wider age range of animals, reusing animals, and applying power analysis statistics to experimental design. Approaches worthy of further analysis are those that use single sex studies, use the optimum number of dogs/dose group, rationalize recovery (off dose) groups, eliminate/reduce control groups (e.g., in MTD studies), and eliminate conventional acute toxicity testing. Analysis of dose group sizes from 12 European pharmaceutical companies is shown in Table 2.

Although data revealed that the majority of companies were using group sizes consistent with regulatory guidelines, there may be the opportunity to harmonize. Sharing of best practice may also result in rationalizing the use of recovery animals (i.e., one should still ask whether they are needed in all treatment groups and where controls are needed).

An additional example of the need to share best practice is in the design of the MTD study. An appropriately designed study could eliminate the need for an acute toxicity test when regulation requires it in a nonrodent. Inclusion of the following parameters in dose ranging studies has been shown to be successful: an adequate washout period, toxicokinetics, histopathology of major organs and macroscopic

Table 2 Analysis of 12 European pharmaceutical companies: Dose group sizes

Duration of study	No. of dogs/sex/group			
	1 m	3 m	6 m	9/12 m
Main study				
Norm	3	3	4	4
Range	2-6	3-6	3-6	3-6
Recovery				
Norm	2	2	2	2
Range	0-2	0-2	0-2	0-2

abnormalities after 14 days of observation, a characterization/impurity profile of the compound, and conducting the study “in accordance with GLP.”

Assessing the Need for a Particular Study

One approach that has been eliminated is that of replacing the Purkinje fiber model (e.g., another species, myocyte culture). An approach worthy of further analysis is that of replacing terminal 3 mo/6 mo studies.

Advance assessment of the need for particular studies, if successful, would have a significant effect on animal numbers. After the 1-mo study, the aim would be to conduct a single study of 9 mo/12 mo duration, which would provide interim data at 3 and/or 6 mo to allow progression of clinical trials. Necropsies would not be performed at these time points, and the study would rely on biomarkers of toxicity, as in clinical trials.

Currently, it may not be possible to achieve this aim; however, as technology develops, we must be in a position to capitalize on it. To do so, it is necessary to identify toxicities that occur after 1 mo but before 9/12 mo and to assess the potential to detect each case by other means. A database, not unlike that of the International Life Sciences Institute project, would be established to gather such information; and over the same period, a number of the new technologies would be assessed for their ability to detect effects in long-term ongoing studies (e.g., the utility of metabonomics and genomics in the dog—although most work is focused on the rat). Generation of additional data would also be required to assess how many times an early-

initiated study may be aborted because group sizes for the long-term studies are larger than those for the 3-mo studies. Of course, on the positive side, the power of such a single study with increased group size would be increased—an issue frequently raised by regulators such as the US Food and Drug Administration.

The Way Forward

The Steering Group advocates a dual approach to reduce dog numbers in safety evaluation projects without compromising human safety and without replacing the dog by another nonrodent species. The approach consists of “Quick Wins” and longer term projects. There are benefits of such an approach inasmuch as formulation of a Best Practice Guide in study design (a Quick Win approach) would demonstrate that animal welfare groups and industry can work together to produce perhaps a modest reduction in animal use. In the longer term, it will be necessary to influence project managers, regulatory affairs staff within companies, and clinicians to accept nonstandard data sets and not to rely on a box-ticking approach to assure themselves of the safety of new medicines. The stage would then be set to introduce a new testing strategy that would significantly reduce dog use.

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