

An Approach to Minimise Dog Use in Regulatory Toxicology: Production of a Best Practice Guide to Study Design

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Summary — The primary non-rodent species used in toxicology is the dog. It is generally agreed that, for ethical reasons, dog use should be reduced to the minimum consistent with maintaining the scientific quality of toxicology studies and ensuring human safety. Dog use in toxicology has been discussed widely, both from a scientific and ethical viewpoint, and there appears to be real potential for achieving significant reductions in the number of dogs used in pharmaceutical safety testing. An industry animal welfare initiative commenced in 2000, with the aim of evaluating and, where possible, putting into practice, scientifically valid approaches to minimise dog use in regulatory toxicology without increasing the use of other non-rodent species, such as non-human primates or minipigs. The study's Steering Group categorised potential reduction approaches into three distinct areas, one of which is the production of a best practice guide on aspects of study design, including: group sizes, use of control animals, single sex studies and design of maximum tolerated dose (MTD) studies. Information on current practice and experience within the pharmaceutical industry is now being analysed, and additional input is invited.

Key words: dogs, pharmaceutical safety testing, reduction.

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Introduction

The use of a non-rodent species in the safety assessment process is required by most regulatory authorities for the registration of pharmaceuticals for human use (1–3). Selection of the most appropriate non-rodent species should be based on scientific justification, ethical perspectives, technical considerations and regulatory acceptability. However, the dog is the most frequently used species and is commonly considered by toxicologists as the “default non-rodent” (4).

The value of dog studies in the development of new pharmaceuticals has been under review for many years. Most recently, the International Life Sciences Institute has published the results of an analysis, showing that a relatively high proportion of human toxicities found in clinical trials were predictable from close examination of the dog studies (5). Concordance of animal and human toxicity was found to be greater for the dog than for rodents. However, close examination of the ways in which dog studies are conducted and used has

shown that there is potential for reducing dog use without compromising human safety (6).

In 2000, the *Industry/Animal Welfare Initiative to Evaluate Approaches to Reduce Dog Use in Preclinical Toxicology* was established, with the aim of recommending and, where possible, putting into practice scientifically valid and feasible approaches to minimise dog use. It was explicitly stated that, in achieving this aim, human safety would not be compromised, and that the replacement of the dog by other non-rodents would not be acceptable. The Steering Group, representing 12 European pharmaceutical companies and two animal welfare organisations, has identified many potential approaches to minimising dog use and has prioritised them for further analysis. Some of these approaches are currently being examined in detail. Dialogue has been established with US companies, and it is hoped that other organisations will participate in the venture. If successful, these approaches may well be appropriate for other non-rodent species, such as the non-human primate and minipig.

The Use of Dogs in Pharmaceutical Safety Testing

The types of study undertaken using dogs, and the numbers used in each of them, were analysed to help direct effort toward those studies that employ the most animals and those procedures that cause the most pain, suffering, distress or lasting harm. For the purpose of this analysis, only studies included in the non-clinical toxicology summary were considered, with the exception of safety pharmacology, which is now part of the pharmacological summary (7). Animals used for discovery purposes and pharmacokinetics were excluded.

The main studies involving dogs in safety assessment are as follows.

Safety pharmacology

This includes studies performed during the early development of a new chemical entity and usually before a first clinical dose:

- *in vitro* cardiovascular assessment using dog tissues/organs (e.g. the Purkinje fibre/Langendorff preparation for action potential duration/QT interval evaluation);
- anaesthetised non-recovery studies to assess haemodynamics, electrocardiogram (ECG) and respiratory and renal parameters; and
- telemetry in surgically prepared, conscious dogs to assess cardiovascular system and ECG.

Maximum Tolerated Dose (MTD)/Dose Range Finding (DRF) studies

These studies are performed primarily to allow selection of dose levels for regulatory studies, although, other information such as target organ toxicity can also be obtained.

Single dose (acute toxicity) studies

Guidelines of the International Conference on Harmonisation (ICH) require two mammalian species for acute toxicology (1) — in some territories (e.g. Japan), it is still customary for the regulatory authority to expect data from a non-rodent species.

Repeated dose studies

The pivotal studies in a regulatory package are repeated dose studies. Durations of 14 days–1 month, 3–6 months, and 9–12 months are generally used, but

some of these studies may be omitted, depending on the clinical programme (duration of human studies) or the therapeutic indication. Study designs are generally very standardised, as prescribed by regulatory bodies.

Juvenile toxicity

Juvenile toxicity studies are a relatively recent requirement of the US Food and Drug Administration to allow clinical paediatric treatment. The design of these studies is similar to those of repeated dose studies, but the age of the animals is pre-weaning.

Investigational studies

Investigations are project-specific and aimed at resolving safety issues arising from any of the other studies. Where possible, these are 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 safety data very early in the drug discovery process. Work is usually in a rodent, but occasionally, the dog is used on a project-specific basis.

Numbers of Dogs Used

Obtaining accurate information on the number of dogs used in different types of study is difficult, but it is necessary, both for targeting reduction strategies on major uses and for measuring the success of minimisation strategies once they are put into practice. Some countries publish sufficiently detailed annual statistics of animal use to be of use in this regard. For example, the data from the UK Home Office permit a partial analysis of dog use in Great Britain, as shown in Table 1. However, the value of these data is limited for a number of reasons. For example, drug development projects are managed globally, and particular types of study may be conducted in different countries. Additionally, the UK data relate primarily to procedures, rather than to the number of animals involved, and some repeated use of animals occurs. Finally, the classification of studies is too broad to allow a detailed analysis of study types.

A better method of monitoring dog use is to assess the number of animals used per drug development project. Data from ten recently submitted new drug applications from four organisations have been analysed to assess the value of such an

Table 1: Dog use in safety testing of pharmaceuticals in Great Britain: from Home Office Statistics, 2001 (8)

Type of test	Number of procedures ^a
Acute non-lethal	261
Subacute limit setting	471
Subacute toxicity	1808
Subchronic and chronic	1304
Toxicokinetics	542
Other toxicology	705
Total	5091

^aDogs may be subjected to more than one procedure, and the number of individual dogs used for each type of procedure is not given. However, the total number of dogs used for pharmaceutical safety testing was 4401.

approach. There was a large inter-project variation in the number of dogs used (150–290 per project), which was related to the therapeutic indication and the number of routes of administration used.

The percentages of animals used for each type of study are shown in Table 2. Clearly, the majority of dogs are used in repeat dose studies, although it should be recognised that this baseline might underestimate the number of dogs used in preliminary studies, because a significant number of projects end before longer term studies are conducted, and not all preliminary studies are included in submissions.

Potential Approaches to Minimising Dog Use

The Steering Group reviewed current study designs and working practices, and identified a plethora of potential opportunities to reduce dog use. To focus its effort, the Group prioritised them according to the potential impact on the number of animals used, the impact on the welfare of the remaining

Table 2: Analysis of dog use in drug development projects: data provided by pharmaceutical companies

Type of test	Percentage of dogs used
Safety pharmacology	4.6
Single dose studies	1.6
MTD/DRF studies	8.4
Repeat dose studies:	
14 days–1 month	28.0
3 months	4.5
6 months	24.1
9–12 months	18.1
Investigations	11.0

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.

After prioritisation, the opportunities were categorised into three areas: achieving best practice in study design, industrial cooperation/data sharing, and assessing the need for a particular study. For each of the categories, the Steering Group eliminated opportunities of low priority. These are still worthy of consideration in due course, but will not be discussed here.

Achieving best practice in study design

Approaches considered worthy of further analysis were: optimisation of the number of dogs per dose group, the use of single sex studies, rationalisation of recovery (off dose) groups, elimination/reduction of control groups (e.g. in MTD studies), and elimination of conventional acute toxicity testing.

An analysis of dose-group sizes from 12 European pharmaceutical companies is shown in Table 3. The majority of companies were using group sizes consistent with regulatory guidelines, but there may be the opportunity to harmonise. Sharing of best prac-

Table 3: Analysis of dose-group sizes used by 12 European pharmaceutical companies in repeat dose studies on dogs

Study duration (months)		1	3	6	9–12
		No. of dogs/sex/group			
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

tice may also result in rationalising the use of recovery animals (i.e. one should still ask whether they are needed in all treatment groups, and where controls are needed).

There appears to be little scope for reduction in dog use by conducting single sex studies. Most companies surveyed used a single sex in exploratory and specific pharmacokinetic studies, but only 2/9 used a single sex in regulatory studies. Even when a compound was intended for use only in one gender, there was reluctance to test in only one sex, because there was always a possibility that the compound might eventually be used in both genders. An additional consideration was that pooled data from both sexes were often used to constitute an adequate group size and that, to be acceptable, tests in a single sex might have to use the same number of dogs as two-sex studies.

There appears to be more potential for reducing dog use in earlier studies, such as MTD/DRF, than in the later regulatory studies. A survey of MTD/DRF study designs currently used by member companies revealed a considerable variation in the number of dogs used. Fifteen study designs were submitted, using from 2 to 16 dogs. To a large extent, this variation was due to differences in the intended purpose of the studies, and consequently, the type of data they were designed to produce. The primary purpose of such studies is to define appropriate doses for the pivotal (repeat dose) studies, but in some cases, they were also used to support candidate selection or confirmation and to provide data on acute toxicity. The Steering Group discussed whether a single study design could be adopted that would fulfill the various objectives whilst using the minimum number of dogs. A basic design was proposed, consisting of two phases; a single dose, escalating dose phase using one male and one female up to the MTD, with or without wash-out between doses, followed by a repeat dose phase of five to seven days at the MTD, or lower, using the same two dogs plus one male and one female naïve dogs. Measurements and observations would include toxicokinetics, clinical pathology, ECG, blood pressure, clinical signs, tachyphylaxia, histopathology and target organ toxicity. This design, with modifications on a project-by-project basis, was considered to be acceptable for achieving the main purposes of MTD/DRF studies whilst reducing dog use as far as possible. Some of the parameters considered in assessing the proposed design are shown in Table 4. It was also considered that this design, provided that the study is performed according to Good Laboratory Practice (GLP) regulations, could eliminate the need for an acute toxicity test when regulation requires it in a non-rodent.

The proposals of the Steering Group will be written up in detail in the near future, as a guide to best practice.

Table 4: Assessment of a minimised MTD/DRF study design using an escalating dose phase followed by a repeat dose phase, using a total of four dogs

Parameter	Opinion
Minimisation of dog use	Good
Regulatory acceptability	We believe our recommendations will be acceptable for regulatory purposes
Utility for dose selection	As good as other study designs in current use
Risk of failure (incorrect determination of doses for pivotal studies)	Low
Utility for candidate selection	Good
Test material requirement	Low
Target organ identification	Satisfactory
Detection of tolerance/tachyphylaxia	Satisfactory
Differentiation of single and repeat dose effects	Satisfactory
Dosing up to MTD	Possible if MTD is carefully defined in terms of clinical signs
Inclusion of toxicokinetics	Possible but needs rapid analysis
Amenability to GLP	Possible but not always necessary
Definition of no-observed-effect level (NOEL)	Only possible with regard to clinical signs
Time requirement	Slightly slower than single/repeat dose studies in parallel

Industrial cooperation/data sharing

The value of sharing data of various types was considered, and a vehicle effect database was thought to have the most potential for eliminating unnecessary repetition.

A vehicle database would contain qualitative and quantitative findings for all vehicles, excipients, solvents and preservatives used in the preparation of dosing formulations, especially for parenteral administration, 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 or, in some cases, whole toxicology packages on particular vehicles or excipients. The Steering Group recommended development of a global, informal data sharing process, as exists already in the UK, and will progress this recommendation in the future.

Assessing the need for a particular study

An approach worthy of further development is that of replacing terminal 3- and/or 6-month studies. The proposal is that, following a 1-month study, a single study of 9–12 months duration would be conducted which would provide interim data at 3 and/or 6 months 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. Advance assessment of the need for particular studies, if successful, would have a significant effect on the numbers of dogs used in repeated dose studies.

Currently, it may not be possible to achieve this aim. However, as technology develops, we must be in a position to capitalise on it. To do so, it is necessary to identify toxicities that occur after 1 month but before 9–12 months and to develop other means of detecting these effects. 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, on-going studies. 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 longer-term studies are larger than those normally used for 3-month studies: if a 9–12 month study is eventually not required, animals could be wasted.

The Way Forward

The approach taken by this initiative is firstly to identify “quick wins”, and then, to undertake longer-term projects. There are benefits from such a dual approach. The formulation of a best practice guide in study design (a quick win approach) may

produce only a modest reduction in dog use, but it will demonstrate that animal welfare groups and industry can work together to develop ideas that are scientifically sound and that do not compromise human safety. This will increase the confidence of others, such as project managers, regulatory affairs staff within companies and clinicians, who will need to be persuaded to accept non-standard 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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