Editorial

Scientific Issues Raised by the Use of Dogs as Laboratory Animals

Michael Balls

Preclinical toxicity tests in dogs cannot be used to predict that a new drug will not have unacceptable adverse effects in humans, so they should not be a regulatory requirement

The use of dogs as laboratory animals in the UK is controlled by the *Animals (Scientific Procedures) Act 1986* (*ASPA*),[1] as amended in 2012 to comply with *Directive 2010/63/EU*.[2]

Article 38 of the Directive requires that the evaluation of each proposed project involving protected animals must include: *A harm–benefit analysis of the programme of work, to assess whether the harm that would be caused to protected animals in terms of suffering, pain and distresss is justified by the expected outome, taking into account ethical considerations and the expected benefit to human beings, animals or the environment.*

Such a requirement has been part of the *ASPA* since it came into force in 1987,[3] where these words were used: *In determining whether and on what terms to grant a project licence the Secretary of State shall weigh the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the programme to be specified in the licence.*

One advantage of the Directive is that a retrospective analysis of *actual* harm and *actual* benefit will now be required for each project.

It can be argued that the bias in the evaluation has overwhelmingly been in favour of the benefit side of the equation, and that the mere mention of possible progress in relation to a disease, such as cancer or Parkinson's disease, or of the need for safe new drugs for human use, has been, and still is, sufficient for the programme of work to be allowed to proceed, regardless of the cost to the animals to be used.

How Many Dogs Are Used?

Approximately 80,000 dogs are used as laboratory animals each year in the USA and Europe. Some of them are used in basic medical research, such as that on cardiovascular disease and sleep disorders, but the vast majority of them are used in compliance with regulations related to the efficacy of drugs or the safety of drugs and other chemical products.

In Great Britain in 2014, 4,107 procedures involved dogs,[4] of which 3,434 (84%) were for regulatory purposes; 3,420 of these procedures were concerned with "toxicity and other safety including pharmacology", and 2,903 (85%) of those procedures were in compliance with "legislation on medicinal products for human use", all of them "to satisfy EU requirements".

Why Are Dogs Used?

The main reason for tests on dogs is that dogs are not rodents. Data from rodents alone have been found not to be sufficiently relevant or reliable, so data from a second, non-rodent, mammalian species, usually a dog or a non-human primate (NHP), are required by the regulations related to the safety testing of drugs and other products. This requirement has been in place since the thalidomide tragedy, although, ironically, dogs are not susceptible to the teratogenic effects of thalidomide.

Dogs tend to be used as the default non-rodent species, as they are much easier to breed, house and use than are NHPs, such as macaques. Nevertheless most of the scientific and welfare concerns raised by the use of dogs would also apply to the use of NHPs, and often, to the use of rodents as well.

General Criticisms of the Use of Animals as Models

There have always been strong, scientific grounds for considering the reliance of so much of bio-

medical research and testing on animals as models of humans to be unwise to the point of foolishness. This is because:

1. The development of models is dependent on having sufficient understanding of what is to be modelled: we usually don't have sufficient understanding.
2. It also requires sufficient understanding of the models to be used: we never have that either. Rodents, dogs and NHPs have not evolved to be models of *Homo sapiens*. Fundamental differences result from their adaptation to their different lifestyles and from their different evolutionary pedigrees.
3. The models should be simple and without uncontrollable variables: they never are.
4. Modifying the models, e.g. by selective breeding or genetic manipulation, will make them even more complicated and untypical of their species: the result will inevitably be an increase in uncontrollable variables and greater uncertainties.
5. The human being to be modelled doesn't actually exist: inherited differences in humans result in an infinite diversity of susceptibilities and responses, further complicated by a variety of different lifestyle factors, other diseases, and exposure to many other factors.
6. The diseases being modelled don't exist either: there is no such thing as Alzheimer's disease or Parkinson's disease: these names refer to collections of symptoms in complex, multi-factorial conditions, which should not be regarded as single diseases. For example, there are at least 100 forms of dementia: finding relevant and usable animal models for them would be impossible. Similarly, the answer to the question of the "safety" of a drug or other chemical is never straightforward.

Specific Criticisms Related to New Drugs

There is increasing recognition of the failure of data from animal models to provide sufficiently relevant and reliable guidance on likely toxicity ahead of the entry of new drugs into human trials. Drug attrition has increased significantly over recent decades.[5] About 95% of all the drugs that survive the regulatory preclinical testing, fail in clinical trials, mostly due to unforeseen toxicities, and half of those that succeed may be subsequently withdrawn or have to be re-labelled, due to lack of efficacy or adverse drug reactions not detected in animal tests. Adverse drug reactions are a major cause of premature death in developed countries. One recent study showed that 63% of human adverse drug reactions had no counterparts in animals, and that less than 20% had a positive equivalent in animal studies.[6]

With specific regard to the dog, the most extensive study prior to our own recent study,[7] concluded that 92% of dog toxicity studies did not provide relevant information in addition to that provided by the rat, and that the other 8% did not result in the immediate withdrawal of drugs from development.[8] There is a scientific reason for this: among several notable species differences which confound the extrapolation of data from dogs to humans, there are major differences between humans and dogs in, for example, the intestinal absorption of drugs, and in the most important enzymes involved in drug metabolism, so the unchallengeable conclusion must be that the dog cannot be a good metabolic model for man. Thus, the continuing and significant extent of the use of dogs in the preclinical testing intended for humans, cannot be considered to be either necessary or ethically acceptable.

In addition, the problem of inter-species differences is likely to be amplified by intra-species differences, since, just as there is no such thing as a standard human being, there is no such thing as a standard dog (or, for that matter, a standard macaque, or rat, or mouse or rabbit).

The Use of the Instem Data

Until recently, it had been very difficult to conduct a satisfactory analysis of the value, or lack of value, of animal studies in predicting human toxicity or safety, mainly because it was not possible to gain access to detailed data about the use of laboratory animals in preclinical drug testing, since these data were the property of drug companies and were not made available for public scrutiny.

However, we were fortunate in being able to obtain data on the effects of more than 3,000 drugs in humans, dogs, NHPs, rats, mice and rabbits. This information had been independently collected and classified by Instem Scientific Limited, i.e. without any bias imposed by my collaborators, Jarrod Bailey and Michelle Thew, and me.

Our analysis of the data was based on the calculation of likelihood ratios (LRs), as used in evaluating the performance of diagnostic tests. The sensitivity (true positive rate) and specificity (true negative rate) of the data provided by a test are used to determine whether a test result usefully changes the judgement and/or prediction that a certain condition (such as a disease or a toxic effect) is or is not likely.

Two versions of the LR exist, one for positive test results (the positive LR, or PLR), and one for negative results (the negative LR, or NLR). A likelihood ratio much greater than 1.0 indicates that the result is associated with the condition, but tests

where the likelihood ratios are close to 1.0 have little practical significance, as the post-test judgement of the likelihood of the occurrence of a condition or of the non-occurrence of the condition is not very different from the pre-test judgement.

The conviction on which our study was based is that the value of a test must depend on both its ability to predict positive effects (e.g. overt toxicity) *and* its ability to predict the absence of effects (e.g. no toxicity), not on the prediction of positive effects alone.

In three articles,[7,9,10] we have compared dog–human data, then rat–human, mouse–human and rabbit–human data, and finally, NHP–human, NHP–dog, NHP–rat, NHP–mouse, dog–rat, dog–mouse and rat–mouse data. The results of the LR analysis showed that both the PLR and the NLR were so close to 1.0 that they added no useful information to what was already known about the drug. The data showed that tests in one species are not reliably and consistently able to provide significant evidential weight with regard to toxicity or lack of toxicity for any other species.

The presence of toxicity in these species can sometimes add meaningful evidential weight concerning human risk, but the positive LRs are extremely inconsistent, varying by over two orders of magnitude for different classes of compounds and their effects. Of particular concern is the clear indication that the absence of toxicity in the animal models provides little or virtually no evidential weight that adverse drug reactions will also be absent in humans. This greatly weakens the scientific case for the routine preclinical testing in laboratory animals of new drugs intended for human patients.

The Need for Immediate Action

The results of our analysis have important implications for the use of preclinical testing in animal models, and indicate that relevant and reliable human-focused alternative methods are urgently required.

Action is urgently needed, since it is scientifically and ethically unacceptable that dogs (and other laboratory animals) should continue to suffer in unreliable tests, and that patients should continue to suffer as a result of the misinformation provided by such tests.

It is therefore a matter of great concern, and something of a mystery, that the UK Government has overruled a decision of the East Riding Council, and the advice of the Planning Inspectorate, by approving an application by B&K Universal to establish a new beagle breeding unit at Grimston, in Yorkshire.[11] The matter is still *sub judice*, since a High Court judge has given Cruelty Free International leave to bring a judicial review against the Home Office over the Government's decision.[12]

There is still time for common sense to prevail, but in any case, the focus should be on the development, validation and strategic use of human-based, human-oriented tests employing modern *in vitro* and *in silico* methods, and procedures based on molecular biology and clinical experience, including well-designed and relatively-safe human studies.[13]

The application of the laws and regulation on the protection of laboratory animals is the duty of all concerned, including governments, politicians, regulators, industries, scientists, and organisations such as the National Centre for the Three Rs (NC3Rs).

This should include the diversion of human and other resources away from expensive, unnecessary and unreliable animal studies, in favour of fund-starved research on replacement alternative methods and the more economic and more productive use of the investment by the pharmaceutical industry in new drug discovery and development, for the benefit of humans and animals alike.

Professor Michael Balls
c/o FRAME
Russell & Burch House
96–98 North Sherwood Street
Nottingham NG1 4EE
UK
E-mail: michael.balls@btopenworld.com

References

[1] Anon. (2013). *Consolidated version of the* Animals (Scientific Procedures) Act 1986*, prepared from the* Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012*, to include the changes that were effected by those Regulations on 1st January 2013,* 40pp. London, UK: Home Office. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/308593/ConsolidatedASPA1Jan2013.pdf

[2] Anon. (2010). *Directive 2010/63/EU* of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* **L276**, 20.10.2010, 33–79.

[3] Anon. (1986). *Animals (Scientific Procedures) Act 1986*, 36pp. London, UK: HMSO.

[4] Home Office (2015). *Annual Statistics of Scientific Procedures on Living Animals Great Britain 2014.* HC 511, 62pp. London, UK: Her Majesty's Stationery Office. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/469508/spanimals14.pdf

[5] Harding, A. (2004). More compounds failing phase I. FDA chief warns that high drug attrition rate is pushing up the cost of drug development. *The Scientist*, 6 August 2004. Available at: http://www.the-scientist.com/?articles.view/articleNo/23003/title/More-

compounds-failing-Phase-I/

[6] van Meer, P.J., Kooijman, M., Gispen-de Wied, C.C., Moors, E.H. & Schellekens, H. (2012). The ability of animal studies to detect serious post marketing adverse events is limited. *Regulatory Toxicology & Pharmacology* **64**, 345–349.

[7] Bailey, J., Thew, M. & Balls, M. (2013). An analysis of the use of dogs in predicting human toxicology and drug safety. *ATLA* **41**, 335–350.

[8] Broadhead, C.L., Jennings, M. & Combes R. (1999). *A Critical Evaluation of the Use of Drugs in the Regulatory Testing of Pharmaceuticals*, 106pp. Nottingham, UK: FRAME.

[9] Bailey, J., Thew, M. & Balls, M. (2014). An analysis of the use of animal models in predicting human toxicology and drug safety. *ATLA* **42**, 181–199.

[10] Bailey, J., Thew, M. & Balls, M. (2015). Predicting human drug toxicity and safety via animal tests: Can *any* one species predict drug toxicity in any other, and do monkeys help? *ATLA* **43**, 393–403.

[11] Anon. (2015). Grimston beagle breeding farm approved by government. *BBC News*, 17 July 2015. Available at: http://www.bbc.co.uk/news/uk-england-humber-33538574

[12] Anon. (2015). Activists given leave for judicial review over beagles. *Yorkshire Post*, 5 November 2015. Available at: http://www.yorkshirepost.co.uk/news/activists-given-leave-for-judicial-review-over-beagles-1-7555691

[13] Combes, R.D. & Balls, M. (2011). Integrated strategies for toxicity employing new and existing technologies. *ATLA* **39**, 213–225.