

Does regulation drive, manage or monitor change?

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Abstract

Regulatory frameworks for the use of animals for experimental and other scientific purposes ideally should provide a sufficiently flexible approach to anticipate, promote and make provision for technical progress in science and welfare; and inform and reflect the evolution of informed societal and political thinking. The focus needs to be on what must be achieved and why, and less on how it is to be achieved.

Unnecessarily stringent, dated and inflexible regulatory frameworks have the potential to delay progress with the 3Rs and compromise scientific and technical progress. Such approaches should be avoided.

The UK experience is that efficient and effective regulation is best achieved by evidence-based policies; processes and practices based on clearly-stated, sound general principles that, on a case-by-case basis, properly balance animal welfare and the legitimate needs of science and industry; coupled to the ability to introduce policy and operational changes without the need for further changes in primary legislation.

A range of case-studies reviews how some of the developments of the last 20 years have been accommodated in UK practice without changes to the UK legislation, and explores what the major drivers of, and obstacles to, change have been. Particular consideration is given to whether the regulatory system led to and required change, or simply adapted to reflect changes already being implemented for other reasons; and what progress has been made by means other than legislation and regulation.

Principles of good regulatory practice are set out, as are some of the lessons learned and pit-falls to be avoided. Consideration is given as to how these are reflected in the processes and outputs of the European regulatory framework.

Keywords: alternatives, regulatory testing, reduction, refinement, replacement

Introduction

Technical progress, representing scientific advances and our improving understanding of animal welfare, and changes in informed public opinion and society's views on the ethics of animal use, inevitably impact on the use of animals for experimental and other scientific purposes. Although change is a continuous process, it is occasionally punctuated by developments that demonstrably impact on animal care and use.

This paper reflects upon some real examples, and poses the question of whether regulation of animal research in the UK drove or managed those changes, or whether the regulator of animal research simply monitored and documented the changes taking place.

Background

In the United Kingdom the Animals (Scientific Procedures) Act 1986 (Anon., 1986a) regulates the

use of animals for experimental and other scientific purposes, seeking to balance the welfare of protected animals against the legitimate needs of science and industry. The Act does not allow animal use to be authorised when the scientific objectives can be obtained by a scientifically satisfactory, and reasonably and practicably available, non-animal alternative. The term "scientifically satisfactory" has been deemed to mean scientifically validated, and "reasonably and practicably available" to mean that the technology is available and there are agreed protocols for its use.

The scope of, and definitions within, the 1986 Act (and the ability to quickly change a range of technical annexes) have provided a surprisingly flexible framework, enabling the regulatory system to deal with a range of developments that could not have been foreseen when the legislation was drafted. For the last 20 years it has made provision for impartial, efficient and effective regulation in the UK without

further primary legislation being required to make provision for technical progress with consequential delays to research programmes, and animal welfare has not been compromised whilst such changes were made.

The general European regulatory framework, as set out in Directive 86/609/EEC (Anon., 1986b), has not proved quite so flexible and adaptable and is currently being revised.

Case Study 1: New technologies: Genetically altered animals

The UK legislation pre-dates scientists' ability to produce specific genetically altered, or cloned, animals.

Nevertheless their production and use has been incorporated seamlessly into the UK regulatory system, and they now account for of the order of one third of the animals used for licensed research and testing in the UK.

The UK legislation protects "vertebrates other than man", subjected to "regulated procedures" performed for experimental or other scientific purposes which may cause those animals pain, suffering, distress or lasting harm. Protection is afforded to certain immature forms, and extends to earlier interventions that might result in the birth or hatching of any protected animal that might experience adverse effects as a result. Thus the UK system has oversight of the *de novo* production of new genetically altered lines, the subsequent breeding of those lines, and the use of such animals for research or testing.

In this case the regulatory system did not drive or channel the changes that have taking place, but it has adapted in order to manage and monitor the resulting changes in animal production and use.

The EU Directive on the other hand, has not proved equally accommodating, and that is one of reasons why it is argued that the Directive should now be amended.

Case Study 2: Replacement: The production of monoclonal antibodies by the ascites method

Köhler and Milstein pioneered the production of monoclonal antibodies (Köhler & Milstein, 1975). Animals, typically mice, are immunised, and lymphocytes producing a specific class of antibody to a specific epitope of the antigen are isolated: these cells are then fused with tumour cells to immortalise the cell-line, and propagated. The specific (monoclonal) antibody they produce is collected and purified. The propagation and harvesting step has previously been done both in cell culture and by harvesting ascites fluid after the immortalised cells are injected intra-peritoneally into animals (again typically mice).

Over the years a number of reviews of the potential

to use *in vitro* methods for the propagation and harvest stage have identified a range arguments in favour of each of these methods, noting the continuous improvement that was being made with *in vitro* systems (see, for example, Anon., 1999).

In 1997 the UK Home Office again reviewed the available evidence and arguments, and concluded that it was increasingly difficult to justify the use of the ascites method – mindful of the legal prohibition on the authorisation of animal use when a suitable non-animal alternative was available.

A brief stakeholder consultation elicited no arguments in favour of continuing to use the ascites method when *in vitro* methods would suffice. Although some expressed a preference for materials produced by the ascites method, the Department made it clear that continued animal use was conditional upon scientific necessity, not preference.

Accordingly in November 1997 the policy was established of only authorising the use of the ascites method when good faith attempts at *in vitro* production had demonstrably failed (the conditions required for the successful *in vitro* propagation of the cells in question can vary between different cell lines).

Although *in vivo* production continues to be the mainstay of production in some parts of the world, no animals have been used for this purpose in the UK since 2001.

In this case the regulator did drive the optimisation and uptake of a replacement alternative, and managed and monitored the increased use of *in vitro* methods and the phasing out of the *in vivo* method.

However, as a cautionary note, it is not possible to say with absolute confidence that monoclonal antibodies produced elsewhere in the world by the ascites method are not exported to the UK.

Case Study 3: Reduction: Marine biotoxin testing

A number of shellfish species can accumulate and concentrate a range of marine biotoxins that can pose a health risk to consumers. These include a range of paralytic shellfish toxins.

Historically the mainstay of safety testing programmes has been a mouse bioassay (see, for example, Yasumoto et al, 1978), with groups of three mice being injected with specific extracts of shellfish matrix: the standard test method is deemed to have given a positive result (requiring action be taken to protect consumer safety) if two or more of the mice die as a result.

A review of test results, looking at the prevalence and nature of positive tests, indicated that typically the same outcome was observed in all three mice, and that an outcome where two mice die and one lives was not common.

This led to the insight that animal use could be

reduced by almost one third by running the initial test using only two mice (if both live the sample MUST have been 'negative'; if both die the sample MUST be deemed to be positive – in either case the result in a third mouse would not alter the regulatory decision). Only if one mouse lived and one mouse died was the result in a third animal necessary to properly evaluate the sample.

Working with the relevant UK national competent authority and the test labs this "reduced" test method was introduced, in my view without any compromise to consumer safety. It has not however been accepted at the European level as being an appropriate, equivalent or better, and more refined method – although the rationale for it not compromising consumer safety seems indisputable.

In this case the regulator played a key part in the development, promotion and use of the more refined method.

This is an also area where progress with *in vitro* alternatives are expected to supersede the need to use an *in vivo* method.

Case Study 4: Refinement: Murine local lymph node assay

The potential of chemicals and products to produce skin sensitisation is an important toxicological endpoint for protecting human safety, with the development and expression of skin sensitisation being the end product of two different, but related, biological events. The clinical condition becomes manifest when, having been sensitised by exposure to a foreign material (the induction phase), a specific immune response to subsequent exposure produces a range of adverse effects produced by the specific immunological response in the sensitised subject (the elicitation phase).

Until recently the *in vivo* test methods use to evaluate the potential of test materials to cause skin sensitisation was based on tests using guinea pigs (see, for example, Magnusson & Kligman, 1970) which required deliberately trying to induce sensitisation, and then eliciting the adverse clinical effects produced when a sensitised subject again encountered the test material.

The realisation that the key toxicological endpoint was the immunisation stage, and not the subsequent demonstration of the adverse effects seen with subsequent exposure, prompted work to develop more refined test methods where the toxicological hazard could be confirmed on the basis that the subject had become sensitised – that is, that the immune system had been stimulated and programmed to produce an adverse clinical response if the same test material was subsequently encountered.

This is the basis for the murine local lymph node assay (Kimber & Basketter, 1997): the test material

is applied to the mouse ear, potential skin sensitizers cause proliferation of the local auricular lymph nodes, and if the stimulation index reaches a critical threshold value, it can be assumed that the test material is a potential skin sensitiser.

This offers three refinements over the earlier guinea pig test systems: a lower species is used, fewer animals may be required, and the test material can be evaluated without the need for the animals to experience the adverse effects that result when a sensitised subject is re-exposed to the test material. In addition to reducing the negative welfare states associated with the Guinea pig tests, it also reduces the time taken to undertake the test and evaluate the results.

The UK legislation requires that where animal use can still be justified, the most refined methods consistent with attaining the scientific objectives must be used. Accordingly, once the murine local lymph node assay had been scientifically validated and a protocol for regulatory use was available, The Home Office reviewed its policies and the relevant licence authorities.

Through consultation with UK regulators and licensees it was agreed that the local lymph node assay was in many cases well-suited to the regulatory testing and decision making, providing the Guinea pig test might still be considered on a case-by-case basis where exceptionally the mouse assay might not be scientifically suitable. The licence authorities, and laboratory practice, were promptly amended.

The impact of this change is reflected in the annual statistical reports of animal use in Great Britain: in 2000 20,000 procedures involving Guinea pigs and 3,000 involving mice were used for skin sensitisation studies for regulatory purposes – in 2006 2,640 procedures involving mice (and four in "other rodents") were used for the same purpose.

Whilst these figures confirm the Guinea pig test has effectively been phased out in the UK, they pose the question of whether the Guinea pig testing has ceased or alternatively is now undertaken in other jurisdictions.

In this case the regulatory framework, the regulators and the scientific community worked actively to ensure the prompt uptake of the more refined method.

Lessons learned

The current UK legislation has, by setting out sound general principles and dealing with requests for animal use on a case-by-case basis, proved sufficiently flexible to incorporate technical progress.

It is important to note that in each case the 3R benefits were dependent on technical progress, rather than being driven by targets or ideology, and that it needs to be recognised that provision must always be

made for special cases which will not fit any revised framework.

In each case there were clear scientific benefits in changing – the alternative methods being both more efficient and overcoming some of the technical limitation of the earlier methods.

Peer pressure was also important – any reluctance to be the first the change counts for little when compared by the need not to be the last to adopt improved methods.

The case studies also illustrate the need for scientists and regulators to accept that change is necessary to adapt to technical progress, and that inclusive processes that involve communication and consultation are necessary to bring about the required changes through non-adversarial processes.

The main unresolved concern must nevertheless be that, in some instances, the use of the previous animal-based methods may have been displaced to other jurisdictions rather than stopping.

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