The Role of Individuals in Progressing the Three Rs in Regulatory Testing

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Summary

Where regulatory testing regimes require the use of animals, attitudes and approaches of individuals at all levels within the system can significantly alter whether or not replacement, reduction, and refinement (Three Rs) strategies are developed and fully utilized. Within the UK, progress in the Three Rs in testing shellfish for marine biotoxins has been driven by a number of key individuals working within laboratories and Government departments and agencies. Advances have been made by teamwork and good communication within and between these parties. Flexibility is essential, particularly on the part of regulators. The driver for testing regimes should primarily be the need for valid, robust, and reliable results, in conjunction with consideration of how these can be achieved using the fewest animals and techniques that cause the least harm, rather than relying on blind adherence to regulations. Regulations should be changed if they do not allow methods to be developed and improved.

Keywords: marine biotoxins, Three Rs, weight of evidence based approach

1 Introduction

European safety monitoring programs to detect marine biotoxins in shellfish harvested for human consumption have relied heavily on the use of Mouse Bioassays (MBAs) because, until recently, these have been the only method acceptable under food hygiene legislation (EC, 2005). The tests are among the most severe performed on animals. Until revision in 2011, these regulations were highly prescriptive, specifying the number of animals to be used per sample, the duration of the protocols, and death as the endpoint. The requirements of European legislation regulating the use of animals for experiments, including the Animals (Scientific Procedures) Act 1986 (HMSO, 1986) in the UK, are in direct conflict, requiring that alternatives that do not entail the use of animals must be used if reasonably and practicably available, and, where animals must be used, that the fewest possible animals must be used in the most refined protocols, including consideration of anesthesia and analgesia where possible, in order to give satisfactory results.

Those working under the two legislations at one UK laboratory believed that by refining the methodology there was scope to permit the achievement of safe food product AND minimal cost to the fewest animals, and that the case could withstand weight of evidence scrutiny by the regulators to allow its formal use. That this belief was not shared by all individuals at all locations could have been due to a variety of reasons, including lack of skills, experience, and knowledge of the procedures being undertaken, differing ethical opinions or levels of concern for animal welfare, and different levels of motivation and drive for change, and/or perceptions of risk associated with change.

2 UK framework and responsibilities of individuals: potential to influence progress on implementing 3Rs

The Animals (Scientific Procedures) Act specifies certain key individuals who have specified roles and responsibilities relating to the use of animals in research (Tab. 1). Significant progress in refining and reducing the numbers of animals used in the shellfish monitoring program was made at the laboratory where the persons in each of these roles had the correct combination of experience, knowledge, and drive and were also prepared to seek help and work with others where specific skills that were not contained within the group were recognized. At a local level, cooperation was needed among animal technicians, the veterinary staff caring for the animals, and the scientists involved in running the monitoring program in order to identify the specific welfare issues for the animals and develop strategies to ameliorate these.

– *Persons responsible for designing experiments* in the UK (Project Licence Holders) have a legal requirement to confirm, prior to the use of animals in any protocol, that no alternative method is available that does not entail the use of animals and would produce satisfactory results. If no reasonable and practically available alternative exists, then methods used must be the most refined and use the fewest numbers of animals. A number of potential refinements were considered for shellfish test protocols, including changing the nature of the injectate to make it less acidic, shortening the duration of the assays, the use of anesthesia, and the development of clinical endpoints.

Tab. 1: Responsibilities of Key Personnel working in the UK under the Animals (Scientific Procedures) Act

Title	Responsibilities
Certificate Holder (of the Certificate of Designation)	Maintenance of management systems that prevent the performance of unauthorized procedures. Appoints and accountable to the regulator for the performance and conduct of the Named Persons (NVS and NACWO), including ensuring they are properly trained and resourced in order to function effectively. Required to implement and maintain a LERP.
Named Veterinary Surgeon (NVS)	Responsible for the health and welfare of all animals on the designated premises. Advises licensees on best practice and how specific procedures might be refined.
Named Animal Care and Welfare Officer (NACWO)/Day to Day Care Person	Responsible for the day-to-day husbandry care of the animals.
Members of Local Ethical Review Process (LERP)	Provision of independent ethical advice to certificate holder with respect to proposed and on-going work involving the use of animals. Supports and advises the Named Persons and licensees regarding animal welfare and ethical issues arising from the work and promotion of the widest possible application of the Three Rs
Personal Licence Holder	Primary responsibility for the welfare of the animals that they have performed procedures on. Required to ensure analgesia and anesthesia are used where possible and that protocols used are the most refined to achieve the scientific purpose.
Project Licence Holder	Ensures proper design and conduct of program of work, including on-going application of the Three Rs, training, supervision and competence of personal licensees and compliance with licence authorities granted.
Animals (Scientific Procedures) Inspector	Advises Secretary of State whether and on what terms certificates of designation, and personal and project licences should be granted. Inspection of personnel and premises to ensure compliance with licence authorities and that the Three Rs are being implemented.

– *The Named Veterinary Surgeon (NVS)* has a responsibility to ensure the health and welfare of all animals within a scientific establishment. They are required to advise on ways that adverse effects of tests may be ameliorated. The program of work, particularly investigation of the use of anesthesia and clinical endpoints to replace lethality, required significant input from the veterinarian, who became heavily involved in the day-to-day program of testing to develop the suggested refinements. This involvement continued in order to check that the criteria and techniques were being applied correctly and to allow analysis of the results and the submission of grant applications to further develop the work.
– *The animal technicians/those investigators (PIs) performing procedures on animals* (Personal Licence Holders) are required to ensure the welfare of all animals under their care. To develop refined protocols, a great deal of additional work was required from the technicians in terms of observation and data collection, over and above the minimal legal requirement. They also provided the historical data that was the basis of the reduction in animal numbers per sample.
– *The Local Ethical Review Process (LERP)* in the UK is a statutory requirement under a condition placed on the licence (certificate) that provides authorization of a facility. It comprises at least the veterinarian, an experienced animal technician, a senior scientist who is involved in developing plans of work, a PI, and a layperson. The membership of a statistician is recommended. The LERP is required to promote and develop the uptake of the Three Rs, review the welfare cost to the animals of proposed work against the likely benefits, and ensure that staff are kept up to date with best practice. Development of refined MBA protocols required approval by the LERP to ensure that the work would be of satisfactory scientific standard and to realize the potential welfare benefits. The LERP supported the principles being applied and provided advice on initial experimental design via an experienced biomedical statistician. Applying the endpoints identified by the NVS and PIs subsequently needed further input from the scientists and the statistician to ensure continuing validity of approach. Similarly, the expertise of all those involved in the process ensured robust analysis of historical data that resulted in confidence that fewer animals could be used per sample.
– *The Animals (Scientific Procedures) Inspectorate (ASPI)* are required to advise the Secretary of State on application for licences and certificates under the Animals (Scientific Procedures) Act (ASPA), to visit the establishment performing experiments on animals to ensure compliance with licences, and to report where there is non-compliance. ASPI must ensure that applications and the on-going program of work apply the Three Rs. Regular inspection of the marine biotoxin program by the local ASPI and his interaction with the various key personnel helped drive forward the process for change. The inspector promoted the need for full consideration of the three Rs. His knowledge and advice on how problems had been dealt with in similar fields of work, advice to the LERP on scope for further enquiry, and engagement of the food hygiene regulator with respect to the concerns of those performing the MBAs and the requirements of ASPA was essential.
– *The UK Food Hygiene Regulator* (FSA) had a critical role to play in allowing changes to the biotoxin monitoring program. The overly restrictive wording of the European food hygiene regulations made advancing Three Rs strategies much more

difficult than would have been the case had there been in-built flexibility allowing tests to be used based on whether they were valid and performed to a particular standard. At this time, the FSA took a similar position to that of US and Canadian regulators, i.e., alternatives needed further validation and therefore replacement of MBAs was not possible. This was in contrast to the position taken by the equivalent regulators in Germany and New Zealand. These differences may have related to differences within national legislations, views on validation criteria and/or levels of risk aversion. Differences in the experience, knowledge, and flexibility of individuals within FSA meant that views on the robustness and reliability of the proposed modified protocols were supported at the branch local to the laboratory that had produced the data but not elsewhere.

– *Those without direct association to the legal requirements of the two pieces of legislation:* Resource commitment from laboratory managers was required throughout the process in order to move from the status quo, primarily in terms of staff time but also with respect to some consumables. Similarly, senior managers who were also committing time and money to developing alternative technologies required commitment to advance refinement and reduction as well.

3 Reproducibility and transferability

In the other laboratories, while alternative strategies were under investigation, there was reluctance from staff to make protocol changes, even with the provision of data from the first laboratory. This may have been due to insufficient breadth of knowledge, particularly with respect to how to develop and use humane endpoints. There was not enough local data for confidence in reducing the number of animals used per sample. Lack of background skills and experience can result from changes in laboratories, where samples are tested after contracts go out to tender. Differences in attitudes with respect to both the interpretation of the food hygiene legislation requirements and acceptance of change among individuals at local branches of the Competent Authority for Food Safety – and therefore the perception of risk derived – were unhelpful for transferability.

The issues were resolved by increasing local data and knowledge of staff at laboratories and within the FSA so that they had confidence in the changed protocols. When protocols were adopted they were found to be fully transferable.

4 Outcome

After a number of years, progress eventually was made in implementing refined and reduced strategies for the MBAs throughout the UK. Introduction of greater flexibility into the food hygiene regulations was helpful in allowing consideration of modified protocols. There was sufficient, consistent evidence for the replacement of the standard MBA with assays of reduced duration, using two rather than three mice, and, for one of the two assays, for the use of clinical endpoints that predicted death rather than a lethal endpoint. For the local FSA regulators to accept these refined approaches to testing for shellfish toxins there had been an extension of knowledge, the confidence that there was a sound evidence base for the revised protocols, and less risk aversion.

The ultimate goal of the Three Rs is to replace the use of animals, and this has now been achieved for around 98% or more of marine biotoxin testing in the UK (Morris et al., 2009; Turner et al., 2010). However, the value of interim Two Rs strategies is shown by considering that, based on an estimate of the number of animals used within Europe in marine biotoxins testing in the 15-year timeframe it has taken to introduce alternative methods, animal numbers could have been around a third lower and suffering reduced for more than 400,000 mice if modified protocols had been used.

5 Discussion

MBAs to detect marine biotoxins cause significant suffering to the animals used and are considered to have some serious scientific drawbacks (BfR, 2005; Hess et al., 2006; EFSA, 2008). This combination of factors has driven a need for change in how such testing is performed within the UK, despite the potential for conflict with poorly drafted food hygiene regulations.

Attitudes of those involved at all levels have been vital to successfully implement the Three Rs. Progress in refining protocols and in minimizing animal use was quicker at one laboratory than at others, despite uniform regulatory requirements. This demonstrates that much of the drive for change within regulatory testing comes from highly motivated individuals who are prepared to prioritize animal welfare, even though this may be difficult to do within the framework available.

Cross-disciplinary discussion and good communication has been essential at all levels in order to implement change. Trust has had to be developed and maintained. Individual personalities have had a significant impact on the speed of progress. Compromise by all parties has been necessary.

Improved harmonization is needed to create a level playing field. However, this is not easy to achieve where there are disagreements as to what constitutes a validated method. Similarly, harmonization to the middle ground is unlikely to be acceptable to those who believe they have "better" tests, and it may be equally unacceptable to those who believe that such change is "over the top" and unnecessary. This, along with inertia and poor drafting of legislation, remains a significant hurdle. Weight of evidence approaches need to be further considered by international regulators as a means of progressing the Three Rs.

6 Conclusions

Where new legislation that requires the use of animals is established, it is essential to engage all relevant stakeholders, especially those involved with the performance and regulation of

such testing, and to ensure sufficient flexibility and that the testing strategy is fit for the purpose and complies with all required legislation.

Development of separate methods at different laboratories may offer the most robust way of ensuring optimal methods in the long term, but collaboration on a single method initially should be given priority if significant animal suffering can be prevented by this approach.

Individuals can, and do, make a significant difference to ensuring the Three Rs are addressed in regulatory testing. If scientists and regulators do not challenge existing dogma, animals will continue to be subjected to unnecessary testing and suffering. Improved communication strategies between scientists and regulators need to be developed to enable rapid introduction of new Three Rs methodologies.

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