



Triple check: the 3Rs and the evaluation of research protocols

1. The 3Rs an overview

Definition, History and Transformations of the 3Rs

The 3Rs - first described by W. Russell and R. Burch in the book 'The Principles of Humane Experimental Techniques' (1959) – are guiding principles for a more ethical use of animals in laboratory testing preserving, and often improving, the quality and reliability of the experimental data. The 3Rs are: 1) *Replacement* (use of non-animal over animal methods, whenever possible, to achieve the same scientific aims); 2) *Reduction* (using the lowest number of animals necessary to achieve reliable scientific results); 3) *Refinement* (use of methods that alleviate or minimize potential pain, suffering or distress, and enhance the wellbeing of the animals used).

Replacement can be intended then as the use of alternative and/or complementary methods that allows to replace and/or accompany some methods or techniques traditionally used. Reduction is achieved by means of appropriate experimental design and precise statistical assessment of the smallest sample size useful for a particular experimental protocol, Refinement must not only aim at minimizing – or preventing – pain and distress from experimental procedures, but also enhance the welfare of each animal throughout all of its life span, as much as possible and to as many animals as possible.

The 3Rs in the 2010/63/EU Directive

The 3Rs are the overarching principle of Directive 2010/63/EU on the protection of animals used for scientific (1), as patent in Article 4. Knowledge of the '3Rs' is required by the personnel taking part (at any level) in projects involving in vivo models (Annex 5), and any researcher presenting a project application to the national Competent Authority must explain in which way he/she will apply the 3Rs Principle through the different stages of his/her project (Annex 6).

The interaction among the 3Rs

The '3Rs' are not always independent one from another, and both negative and positive interactions can occur. (2, 3) For example, Refinement can minimize procedure-related stress – a confounding factor in animal experiments – and hence improve the signal/'noise' ratio, allowing a Reduction of the sample size necessary to identify meaningful differences between groups. A negative interaction can occur when animal numbers are increased to preserve or enhance animal welfare. This is the case of some experiments where a given substance must be administered in the water or food. This implies that the cage – rather than the animal – is the experimental unit, so choosing to house animals in pairs over single housing will increase animal numbers, but prevent much distress from social isolation.

Which 'R' to choose? There is no definitive rule to follow, and the decision has to be taken on a 'case-by-case' basis. However, it is our view that the degree of welfare of the animals involved should always be given particular consideration.

2. Project application and the '3Rs'

Replacement

In the application, the justification for the use of that particular species to generate that particular model should be provided, it should be explained why the research cannot be done by methods not involving animal experimentation.

Reduction

In a project application, a thorough description of the experimental design and the statistical test used to determine the sample size should be presented, through an appropriate power analysis. This is an area in which there is a special need to raise the level of awareness of the researchers' community. In many cases poor experimental design and statistics have been responsible for unreliable and unreproducible results (e.g. 4, 5, 6).

Refinement

In the case of Refinement, the applicants should consider whether they can improve both procedures and housing conditions of their experimental animals for all stages of an animal research project – that is, before, during and after procedures - including the breeding of animals with a deleterious phenotype.

A potential problem in the implementation of the '3Rs' Principle is the perception of the principle as something static, while the '3Rs' should rather be intended as dynamic and interconnected concepts, driven by new evidence and dramatic changes in accepted methodologies, but also little improvements and small progressive developments. These changes are affected by both technical/scientific advancements and progresses in the ethical sensibilities regarding animal research.

3. Education, Evaluation, Moral Progress

Education through evaluation: is it feasible?

The evaluators of a project application should be in the position to advise the applicant on how to improve the quality of the project, also through a correct understanding of the '3Rs'. This can be done in two ways 1) by setting up courses dedicated to researchers on how to provide the needed information, in compliance with legislation and other regulatory demands; 2) by providing specific and detailed feedback to applicants. In order to accomplish this, the competent authority and institutional animal welfare bodies should be given adequate resources. Such an evaluation process has the potential to make project submission a process of self-education on the 3Rs, for researchers.

The 3Rs and the public common-sense idea of 'alternatives'

It is advisable that evaluation principles could be somehow transparent and understandable by the general public. As a matter of fact, the common and lay understanding identifies 'alternatives' just as 'alternatives to animal experimentation', i.e. Replacement. However, the concept of 'alternatives', as originally proposed by David Smyth in 1978 (7), encompasses all the 3Rs, hence including Reduction alternatives and Refinement alternatives, crucial principles given the present irreplaceability of animal models in biomedical research. Transparency on the evaluative principles – i.e. how animal research is regulated and evaluated – can promote a better understanding of the scientific and ethical issues at hand in animal research and to a better informed and more sensible public debate.

The 3Rs: ethical and scientific progress

As a matter of fact, the 3R Principle is at present the most effective tool for dealing with the dilemma brought forth by the need for scientific and medical progress and the ethical relevance of animal welfare. If correctly understood and applied, the 3R Principle promotes both ethical and scientifically sound progress. Moreover, each of the 3Rs is driven forward by improvements in research techniques and new scientific and technological developments, and evaluation of research protocols should always take in consideration the multifaceted and dynamic nature of the 3Rs. More than just a legal requirement, evaluation of research protocols is part of a more general process of ethical transformation of human/animal relationships affecting all our society, where members of the scientific community can play a significant role, as regulator, animal caretakers, or animal users.

References

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