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Harmonising the definition of refinement

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Abstract

Russell and Burch's Three Rs (replacement, reduction and refinement) remain the cornerstone for principles guiding humane experimental research. However, the concept of refinement has evolved considerably since its first inception and there have been numerous interpretations, some of which are regressive from the original definition. In this paper we examine the interpretations of refinement, and propose a harmonised progressive definition that is in line with changes in animal ethics and animal welfare science. Our definition should be applied to all aspects of refinement: those related to housing, husbandry and care, techniques used in scientific procedures, periprocedural care, health and welfare monitoring, and experimental design. We argue not only that the concept should include the avoidance or minimisation of adverse effects experienced at any time during the life of an animal destined for use in a laboratory, but also that it should be applied to the founder animals. Furthermore, we take a proactive stance and argue that refinement should include enhancing well-being through environmental enrichment. The acceptance and application of this new definition by legislative authorities and in guidelines would represent a significant step forward for animal welfare.

Keywords: *animal welfare, housing and husbandry, laboratory animals, refinement, scientific procedures, Three Rs*

Introduction

The principles behind the Three Rs (replacement, reduction and refinement) for animal experimentation (Russell & Burch 1959, reprinted 1992) are now widely accepted, and are fundamental to the philosophy underlying the guidelines and legislation that regulate animal experimentation in several countries in Western Europe (Balls *et al* 1995). Although the concept of the Three Rs is neither mentioned nor defined in the Council Directive 86/608/EEC *on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes*, the spirit of the Three Rs is clearly included in Article 7, paragraphs 7.2–7.4 (86/609/EEC). Similarly, some national, European and US legislation incorporates the requirement that consideration be given to the Three Rs (Balls *et al* 1995).

The Three Rs undoubtedly provide a systematic framework to advance humane experimental technique. Their value lies in their practical and straightforward approach, which has captured most of the key ethical issues in animal-based research. The origins of the Three Rs, and a comprehensive history of the events and thinking behind them, have been reviewed by Balls *et al* (1995). What is clear is that the concept of refinement has evolved from its original meaning and there is some confusion as to its current interpretation. It is our intention in this paper to focus on refinement, and to update its definition in light of current practice and changing attitudes toward the ethical and scientific importance of good animal welfare. Although Russell and Burch (1959, reprinted 1992) did not provide a definition of refinement as such, our need for a working definition has led to a plethora of new statements summarising the concept (see Table 1 for examples of the interpretation of refinement). Some of these statements regress from Russell and Burch's (1959, reprinted 1992) intended meaning, and refer only to the minimisation of suffering during experimental procedures. One statement refers to using animals to greater effect, and another to controlling extraneous variables that may increase data variability. Our aim is to justify and harmonise an updated definition, and to encourage those involved in animal experimentation to adopt this definition. This should lead to a wider appreciation of the full scope of refinement, and a wider application of appropriate techniques.

Within the context of animals used and bred for research purposes in laboratories, and including founder animals, the definition of refinement that we propose is: "any approach which avoids or minimises the actual or potential

pain, distress and other adverse effects experienced at any time during the life of the animals involved, and which enhances their well-being". We readily acknowledge that the definition we propose is not entirely original, but consolidates the work of others who have redefined and updated the concept. Below we justify and explain the rationale behind this definition.

Although there is a complex interplay between the Three Rs (de Boo *et al* 2005, pp 327–332, this issue), in theory, refinement starts when we cannot use replacement techniques, and every device of theory and practice has been used to reduce to a minimum the number of animals used in experiments. In a paper entitled *The increase of humanity in experimentation: replacement, reduction and refinement* that William Russell (1957) read at the UFAW symposium on Humane Technique in the Laboratory, he described refinement as "any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used" (cited in Russell & Burch 1959, reprinted 1992 p 64). In 1959 (reprinted 1992), Russell and Burch removed the ambiguity of what was meant by procedures (ie scientific procedures, or a broader interpretation of the term). In their seminal book *The Principles of Humane Experimental Technique*, refinement is described as "simply to reduce to an absolute minimum the amount of stress imposed on those animals that are still used" (Russell & Burch 1959, reprinted 1992 p 134). By this description it would appear that refinement includes what happens to the animal outside the scientific procedure(s), in addition to the direct consequence of the procedure(s). Although Russell and Burch do not explicitly state that refinement can apply to all stages of an animal's life, from birth to death (ie including breeding, weaning, acquisition, transport, housing, and the fate of the animals at the end of the protocols etc), they do, in the pages of their book, describe both the 'direct inhumanity' of the procedures themselves and the 'contingent inhumanity', which includes the negative effects of animal housing and husbandry on welfare (Russell & Burch 1959, reprinted 1992 p 54). That refinement should be applied to all aspects of the life of an animal destined for the laboratory has been expounded by others (eg Richmond 2000; Smith & Jennings 2003), and is now implicit in certain definitions and their associated explanations (Smaje *et al* 1998; FRAME). We also include this aspect in the harmonised definition that we propose in this paper.

Despite this, the interpretation of refinement is very limited in the Animals (Scientific Procedures) Act (1986), which is often considered to be the strongest legislation protecting animals used in scientific research, and is therefore discussed in more depth here. In the guidance on the operation of the Animals (Scientific Procedures) Act (1986), the Home Office states that "regulated procedures can only be authorised and performed if there are no scientifically suitable alternatives that *replace* animal use, *reduce* the number of animals needed or *refine* the procedures used to cause less suffering — these are known as the Three Rs" (Section 2.3). Similarly, as recently as 2003, in a review of cost–benefit assessment in the use of animals in research, the Animal Procedures Committee (APC 2003 p 8) has interpreted Russell and Burch's definition as "refinement of experimental procedures to reduce the incidence and severity of procedures on those animals that are still used following application of the previous two Rs". Nevertheless, this APC report later documents a wide range of factors that should be included in the assessment of costs to animals, in addition to those described by the Home Office. The Home Office limits the description of refinement to the "nature and extent of all the likely adverse effects on the animals due to all interventions *from the time that the animals are issued from stock, until they are discharged from the control of the Act*" (our emphasis, APC 2003 p 38). However, the APC (2003 p 40) recommends that the assessment of costs should include "contingent harms such as those caused in animal breeding" and "factors other than those associated with the actual scientific procedures" such as capture, confinement, transport systems and general handling. The Home Office already considers the fate of the animals at the end of the protocols (eg method of killing, continued use, re-use, release into the wild, re-homing); therefore, although not explicitly incorporated into the Home Office's definition of refinement, there are already measures in place to include wider aspects of the welfare of animals destined for the laboratory in a cost–benefit analysis, and hence refinements should apply to all aspects of the animal's life, from birth to death. The refinement of contingent harms not only applies to the housing, husbandry and care of the animals destined for laboratories, but also should be applied to the housing, husbandry and care of the breeding animals (the founders). Although this may be less practicable if the founders are at another location, it is an important goal as there is also potential for pain, distress and other adverse effects associated with, for example, the capture of primates from the wild for breeding purposes (Prescott & Jennings 2004), or the generation of genetically modified mice (Robinson *et al* 2003). Therefore, we argue that refinement should also be applied to founders and their welfare should be included in any cost–benefit analyses.

Russell and Burch's (1959, reprinted 1992) description of refinement includes "...reduce to an absolute minimum the amount of *stress*..." (our emphasis). Although Russell and Burch (1959, reprinted 1992) dedicated a chapter of their book to negative welfare states, including pain, stress and distress, they chose to use the term 'stress' in this sentence. The definition of terms used to describe negative welfare states is still open to much debate, as is their assessment (Flecknell 1994), but referring only to stress is limiting. The majority of statements on refinement refer to pain, distress, and suffering (see Table 1), although the Animal Welfare Institute includes discomfort, fear and stress in its definition. We choose to use the phrase "pain, distress and other adverse effects experienced" to cover all negative welfare states. These include states such as boredom, anxiety and fear, which are probably the most common adverse effects of captivity (Morton 1997). Furthermore,

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Table 1 Examples of definitions/descriptions of, and mission statements referring to, refinement.

Definitions/description	Reference
Any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used.	Russell (1957, cited in Russell & Burch 1959, reprinted 1992 p 64)
Simply to reduce to an absolute minimum the amount of stress imposed on those animals that are still used.	Russell & Burch (1959, reprinted 1992 p 134)
Refinement alternatives encompass those methods which alleviate or minimise potential pain and distress, and which enhance animal wellbeing.	Balls *et al* (1995 p 848)
Refinement incorporates all measures taken to avoid, minimize, recognize and alleviate pain, suffering, distress or lasting harm — or to otherwise improve the welfare and well-being of the experimental subjects.	Richmond (1998 p 27)
Refined methods in animal research are those which alleviate or minimise the pain, distress or other adverse effects suffered by the animals involved, and/or enhance animal well-being.	Smaje *et al* (1998 p 137)
Refinement alternatives: methods which alleviate or minimise potential pain, suffering and distress, and which enhance animal well-being.	Hansen (2002 p 2)
Refinement of experimental procedures to reduce the incidence and severity of procedures on those animals that are still used following application of the previous two Rs.	Animals Procedures Committee (2003 p 8)
Refinement of husbandry and procedures, so as to cause less suffering and improve animal welfare.	Smith & Jennings (2003 p 49)
The refinement of animal experimentation — using the minimum number of animals and using them to a greater effect.	Wright (2003)
Refine the way experiments are carried out, to make sure animals suffer as little as possible.	Research Defence Society
Refine the tests to ensure the most comfortable and humane conditions possible.	Foundation for Biomedical Research
The term refinement signifies the modification of any procedures that operate from the time a laboratory animal is born until its death, so as to minimise the pain and distress experienced by the animal and enhance its well-being.	Fund for the Replacement of Animals in Medical Experiments (FRAME)
Refinement is the attempt to enhance animal welfare and control extraneous variables which may increase research data variability.	Animal Welfare Institute
Refinement of housing, handling and experimental procedures to reduce discomfort, pain, fear, stress and suffering.	Database on Refinement of Housing and Handling Conditions and Environmental Enrichment for Laboratory Animals
Refinement refers to methods which alleviate or minimise potential pain, suffering or distress, and which enhance animal welfare, for those animals which still have to be used.	National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)
Any approach which avoids or minimises the actual or potential pain, distress and other adverse effects experienced at any time during the life of the animals involved, and which enhances their wellbeing.	This paper

we add an original element to the definition: given that the effect of a procedure may not be known precisely when the experiment is designed, we include the expression "*actual or potential* pain, distress and other adverse effects experienced", as refinements that aim to reduce potentially adverse effects should be considered to be refinements even if the animal does not actually experience the adverse effects. For example, using positive reinforcement training to reduce the adverse effects associated with restraint is still a refinement, even if certain individuals did not experience adverse effects when they were restrained.

Russell and Burch (1959, reprinted 1992) used the phrase "*...reduce to an absolute minimum* the amount of stress..." (our emphasis). Other authors have phrased this as "alleviate or minimise" (eg Balls *et al* 1995; Smaje *et al* 1998; Hansen 2002), or include "recognition" (Richmond 1998). We consider that including "alleviate" is unnecessary because, by minimising adverse effects, one is including the alleviation of such effects. We consider recognition to be a necessary step, but not a refinement in itself. However, we argue that techniques which avoid pain, distress or other adverse effects should be included in the definition. Rather

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than focussing on the minimisation of adverse effects through improved use of, for example, analgesics and anaesthetics, our definition encompasses refined techniques that replace traditional techniques that may cause pain, distress or other adverse effects. For example, it is known that restraint has adverse effects on the welfare of primates, but positive reinforcement training avoids the needs for restraint in some situations (McKinley *et al* 2003; Reinhardt 2003). Furthermore, Russell and Burch (1959, reprinted 1992) focus on two aspects of refinement — that of "generally superimposed procedures" and "choice of procedures". For the former they give examples of well-known refinement techniques such as anaesthesia. For the latter, concerning choice of procedures — and they include choice of species under this umbrella — they acclaim procedures that are rapid, elegant and simple, and emphasise the importance of careful formulation of questions. Therefore, refinement of the experimental design has always been integral to the original concept of refinement (Russell & Burch 1959, reprinted 1992). Our definition uses the word "approach" (as opposed to technique, method, procedure etc), to be broad enough to include all aspects of refinement, including refinement to the experimental design.

Although it has been argued that the welfare of animals can be compromised through the denial of what is pleasurable (Richmond 2002 p S66), or through boredom (Wemelsfelder 1994), we believe that refinement should include techniques that are specifically designed to enhance an animal's well-being (eg Balls *et al* 1995; Smaje *et al* 1998; Hansen 2002; FRAME; NC3Rs); this additional clause is included in many definitions of refinement, but in far from all (see Table 1). We are using the term 'well-being' to relate to both the physical health of the animal and to its psychological well-being. Improving well-being is directly associated with minimising poor welfare and therefore we use "and" rather than "and/or" in our harmonised definition (following Balls *et al* 1995; Hansen 2002; Smith & Jennings 2003; FRAME; NC3Rs). However, refinement should also be proactive in enhancing well-being. Environmental enrichment should be implicit in the interpretation and implementation of refinement. The aim should be not only to avoid or minimise adverse effects, but also to maximise well-being. This means that we must take a proactive approach in promoting the positive elements of welfare, such as companionship, comfort, and security. For example, one may be able to refine the diet of animals, not simply so that they receive a nutritionally balanced diet, but so that the diet is also satisfying for the animals — preferably in terms of both its appetitive nature and the way the animals have to forage and process the food (Lindburg 1998; Johnson & Patterson-Kane 2003). Furthermore, the enhancement of well-being has potential benefits to the science; 'happy' and healthy animals increase the validity and accuracy of scientific results (Poole 1997; Bohannon 2002; Garner & Mason 2002). Providing animals with a species-adequate social and physical environment that enhances well-being may allow them to cope with stress more effectively (eg Fraser & Broom 1990; Kingston & Hoffman-Goetz 1996; Smith *et al* 1998; Bassett *et al* 2003) and possibly modulates their experiences of pain or distress (Gentle & Corr 1995; Gentle & Tilston 1999).

Establishments should take all reasonable steps to refine animal use for ethical and scientific reasons; however, we are acutely aware of the restrictions of the laboratory environment, such as the limitations of space. Animals are kept in laboratories specifically so that experimentation can take place; therefore, refinements must be consistent with the scientific objectives. There has been considerable debate about how, and whether, refinement affects experimental results, and this has been used as an argument for not implementing refinement. However, there are many refinement techniques, such as habituation, desensitisation, training and improved post-operative care, which can be used without compromising the scientific protocol (eg Chilcott *et al* 2001; Bassett *et al* 2003).

There are also a number of examples in which refinements are shown to enhance the science (eg Reinhardt *et al* 1995; Schnell & Gerber 1997; Scott *et al* 2003). Many traditional husbandry, caging and handling practices are sources of poor welfare and stress (eg see Draper & Bernstein 1963; Lidfors 1997; Novak 2003; Reinhardt 2003), and it is well established that stress can have a disruptive effect on the physiology and behaviour of mammals (eg Rivier & Rivest 1991; see Terlouw *et al* 1997). One of the prominent physiological changes in response to a stressor is increased activity in the hypothalamic–pituitary–adrenal axis and concurrent increases in circulating glucocorticoids, in addition to increased activity in the sympathetic nervous system (see Terlouw *et al* 1997). Other responses to stress include hypertension, immune deficiencies and increased disease susceptibility (Bahr *et al* 2000). The welfare of stressed animals is compromised and studies using these animals, whose behaviour, physiology and immunology are abnormal, may reach unreliable conclusions (Poole 1997; Neigh *et al* 2005). We must also be aware that although in many cases refinement enhances science, certain refinements may interfere with the scientific objectives. For example, changes to routine protocols may introduce novel confounding variables, which would prevent comparisons with previous data collected using the original technique (Smaje *et al* 1998). These refinements should not be dismissed; instead any possible negative effects of a refinement on the scientific objectives should be balanced against the positive nature of the refinement, for both science and the animals (Morton 1995; Poole 1997).

Our proposed definition includes all aspects of refinement; for example, housing, husbandry and care, techniques used in scientific procedures, periprocedural care, health and welfare monitoring, and experimental design. It is acknowledged that for refinement to be successful, appropriate measures of welfare states need to be developed and validated (Flecknell 1994). The prevailing focus on poor welfare has led to numerous measures of compromised welfare states, including behavioural, physiological and biochemical. More research should concentrate on the recognition, assessment and validation of good welfare.

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Improved assessment and validation of welfare is just one of the ways to advance refinement. Other advances include: increasing the awareness of the scientific and ethical obligation to refine all aspects of animal use; making information about refinement more readily accessible; and clearly delineating the responsibilities for implementing refinement techniques (Smaje *et al* 1998).

Conclusions and animal welfare implications

There have been numerous interpretations of Russell and Burch's (1959, reprinted 1992) refinement since its first inception. On closer analysis of the wider sense proposed by Russell and Burch (1959, reprinted 1992), some of these interpretations are regressive. By demonstrating the wide range of interpretations, the need for harmonisation of an updated and progressive definition becomes evident. Our proposed definition is in line with new developments in animal ethics and animal welfare science, and not only covers the essence of the original definition, but also clarifies and adds substantially to it. The acceptance and application of this new definition by legislative authorities, and its promotion in guidelines and mission statements, would represent a significant step forward for animal welfare.

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