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The interplay between replacement, reduction and refinement: considerations where the Three Rs interact

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Abstract

Russell and Burch's Three Rs principle of replacement, reduction and refinement offers a useful concept for the scientific and ethical evaluation of the use of animals in scientific procedures. Replacement, reduction and refinement are often considered separately, but when applied, one of the Three Rs may have a positive or negative effect on one or both of the other Rs. This paper explores the interplay between the Three Rs and provides examples where the Three Rs have a positive interaction and where they are in conflict with each other. For example, all Three Rs positively interact in the use of cell cultures, but validation studies of replacement techniques may initially increase the numbers of animals used; therefore replacement and reduction are in conflict. Several models of cost-benefit analyses, used by animal ethics committees to justify or reject animal experimentation, contain elements such as quality and significance of the research, the credibility of the research group and the discomfort caused to the animals. Although these models consider the Three Rs, each R is considered independently of the others. Consequently, moral dilemmas may arise when reviewing proposals in which the Three Rs conflict. Currently there is no legal guidance relating to the prioritisation of the Three Rs, but guidance is required to facilitate their use. For example, does a significant reduction in animal numbers justify increased individual suffering? Moral justifications deserve more attention when considering the Three Rs in general, and when considering the application of one or more Rs to a procedure, to a protocol, or to the wider research programme.

Keywords: *alternatives*, *animal welfare*, *interplay*, *reduction*, *refinement*, *replacement*

Introduction

Russell and Burch's Three Rs principle of replacement, reduction and refinement (Russell & Burch 1959, reprinted 1992) is a critical concept in the ethical evaluation and justification of animal research. Although, the concept is not explicitly mentioned in European law regulating the use of animals in scientific research, its principles are integrated into Article 7, paragraphs 2–4, of the Council Directive 86/609/EEC; therefore, the application of the Three Rs to the use of animals in research is a legal requirement in Europe.

> "Article 7:
>
> Paragraph 2. An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available.
>
> Paragraph 3. When an experiment has to be performed, the choice of species shall be carefully considered and, where necessary, explained to the authority. In a choice between experiments, those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results shall be selected. Experiments on animals taken from the wild may not be carried out unless experiments on other animals would not suffice for the aims of the experiment.
>
> Paragraph 4. All experiments shall be designed to avoid distress and unnecessary pain and suffering to the experimental animals."

Although research programmes may include replacement techniques and protocols, the use of reduced animal numbers and implementation of refinement techniques, which reduce animal stress and promote animal welfare, the Three Rs concept is not intended to describe the impact on those research techniques and protocols, but the impact on the animals being used in the experiments. However, there may be conflicts between the benefits to the animals and advantages to the research management. This conflict is most likely with refinement. For example, a refinement technique might be to satisfy the appetitive need of an animal by rewarding with food on a regular basis, thereby reducing stress and enhancing well-being, but this may conflict with the research management because restricting food is often used to make animals work. The nature of reduction and replacement means that a conflict between the impact on the animals and on the research management

should not necessarily be present, except possibly in the validation stages. Finally, some Three Rs methods, which have a positive impact on animals, may be more costly and are therefore not to be considered as equivalent or superior to methods that were previously used by the research management. Throughout this paper, we refer to the impact of the Three Rs on the animals.

In many circumstances the Three Rs act in synergy to reduce harm associated with animal experiments. However, there are also circumstances in which the application of one of the Three Rs would have a negative effect on the experiment with respect to one or both of the other Rs. The interplay between the Three Rs can therefore have a considerable effect on the Rs applied to each experiment. Figure 1 highlights some examples, which demonstrate this interplay, and should be read in conjunction with the text.

Replacement, reduction and refinement

Replacement

Russell and Burch defined the term 'replacement' as "any scientific method employing non-sentient material which may, in the history of experimentation, replace methods which use conscious living vertebrates" (Russell & Burch 1959, reprinted 1992). Examples of replacement techniques that were described by Russell and Burch included plants, micro-organisms, endoparasites and non-living physical and chemical systems (Russell & Burch 1959, reprinted 1992). Current replacement methods also include the use of human volunteers, (3D) models and *in silico* methods, including virtual reality systems. Russell and Burch also introduced the concept of *absolute* and *relative* replacement (Russell & Burch 1959, reprinted 1992). The term *absolute* replacement describes replacement methods in which animals are not required at all at any stage, whilst *relative* replacement describes those methods of replacement in which animals of lower neurophysiological sentience or animal tissues are used.

Reduction

Russell and Burch defined the term 'reduction' as a "reduction in the number of animals used to obtain information of a given amount and precision" (Russell & Burch 1959, reprinted 1992). For example, pilot trials can be used to determine the magnitude of effects of an experimental manipulation, the ease with which these effects can be identified and the degree of extraneous experimental variation. This information can be used to accurately calculate the number of animals required to obtain scientifically relevant results. Appropriate experimental design (eg sample size and power, variation and precision) and the correct use of statistics are also crucial to reducing the number of animals required (eg Festing & Altman 2002). Examples of retrospective analyses show that the number of animals needed could be reduced in certain types of research, for example, in vaccine potency assays (Hendriksen *et al* 1987). Furthermore, international harmonisation of protocols and standardised requirements for regulatory tests should, in theory, ensure that the same experiments are not repeated in different countries; therefore reducing the number of animals used in these tests (van Cauteren 1996).

Refinement

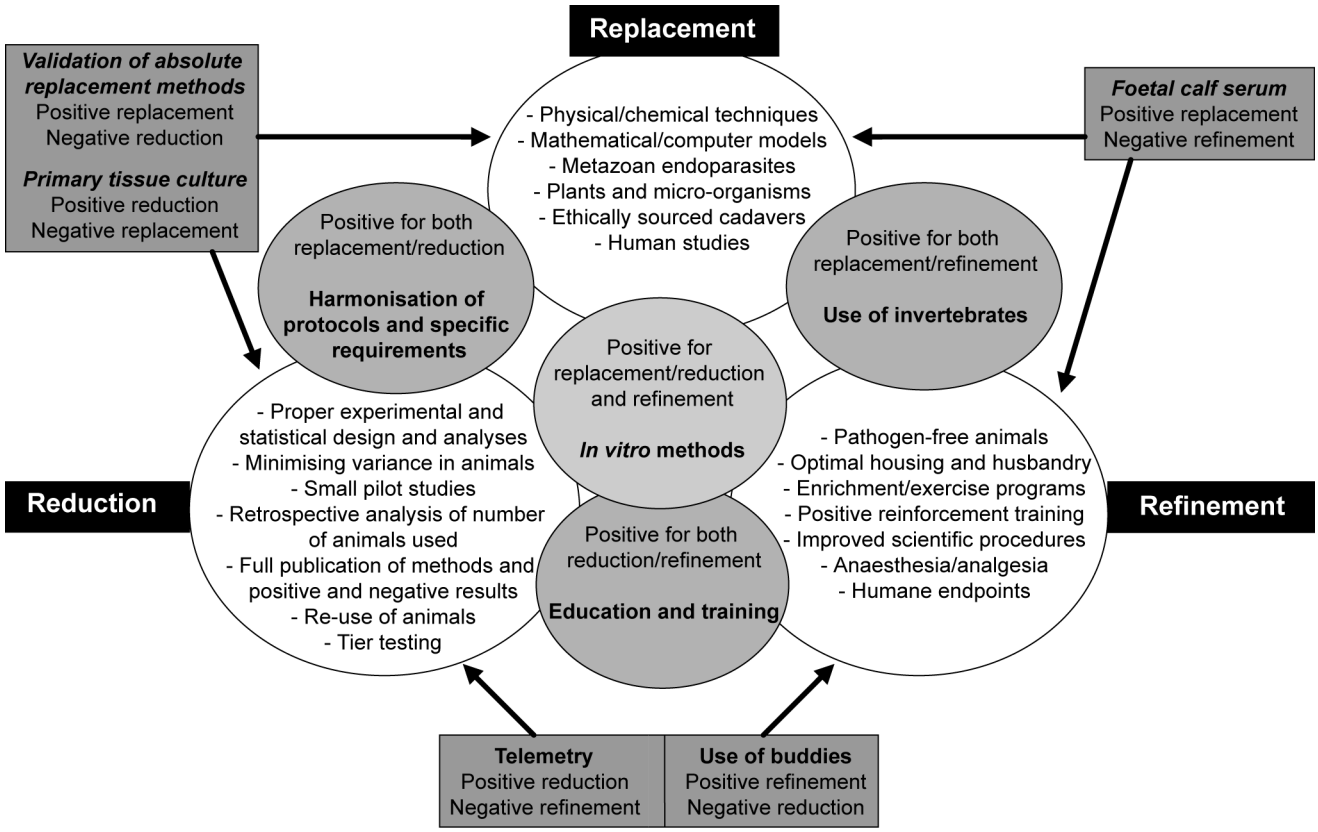
Russell and Burch defined the term 'refinement' as "simply to reduce to an absolute minimum the amount of stress imposed on those animals that are still used" (Russell & Burch 1959, reprinted 1992). Since 1959, the concept of refinement has been redefined many times, resulting in confusion and misunderstanding over the scope of the concept. In the interests of harmonisation, Buchanan-Smith *et al* (2005, pp 379-384, this issue) have recently produced a definition of refinement, which encompasses many of the ideas expressed by other authors (eg Balls *et al* 1995; Richmond 1998; Smaje *et al* 1998; Smith & Jennings 2003), while constructing a broadened concept of refinement. Buchanan-Smith *et al* (2005) define refinement as "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". The use of positive reinforcement training is a good example of refinement; animals are given the opportunity to cooperate with scientific procedures, reducing the need for restraint and other adverse practices, while being rewarded (Scott 1990; Reinhardt *et al* 1995; Laule 1999; European Commission 2002; Prescott & Buchanan-Smith 2003).

There are numerous methods by which all Three Rs can be applied to minimise the impact of the use of animals in scientific procedures; some of these methods are listed in Figure 1 under the headings replacement, reduction and refinement in the larger circles.

Positive interaction between the Three Rs

There are many instances in which the use of one of the Three Rs can have a positive impact on one or both the other two Rs. Examples of positive interactions between the Three Rs are shown in the smaller circles in Figure 1. For example, the introduction of species-specific education and training programs for staff is likely to improve the care of animals, improve the detection of welfare problems, and therefore the rate at which these problems are resolved, and aid the detection of experimental effects (Hau 1999; Bayne 2002; Chang & Hart 2002; European Commission 2002). This may result in a reduction in stress-related variation and an improvement in the recording of results, which, in turn, can result in a reduction of the number of animals required (Brockway *et al* 1993). The substitution of animals of higher neurophysiological sensitivity with invertebrates or metazoan parasites is clearly a positive replacement strategy, but the use of animals with a lower capacity for suffering must also be considered a refinement because the experience of harm may also be reduced. The international harmonisation of protocols and legally required safety tests represents an important strategy for reducing the number of animal experiments that must be carried out in individual countries, but it is also a means by

Figure 1



A ven diagram showing examples of the interplay between the Three Rs; see the text for an explanation.

which we can ensure that obsolete invasive animal studies can be replaced by the most advanced techniques using insentient material. Similarly, the replacement of tests on live animals with the testing of substances on cells in culture not only reduces the number of animals used but also minimises the harm experienced by those animals that are used, and is a clear example of a case in which all Three Rs converge for the benefit of animal welfare.

Conflicts between the Three Rs

In contrast with the positive interactions described above, there are also circumstances in which the application of one of the Three Rs may have a negative influence with respect to one or both of the other two Rs. When these conflicts arise, researchers and ethical review committees are left to evaluate the impact of one R on the other and must make difficult decisions concerning the prioritisation of each individual R. Examples of these conflicts are given in the boxes in Figure 1.

Conflicts between replacement and reduction

The development of *absolute* and *relative* replacement techniques clearly has a positive impact on the ability of researchers to implement replacement, thereby increasing the range of studies that can be carried out without the use of animals, or using animals of lower neurophysiological sensitivity. However, in validation studies of replacement techniques, a comparison of the proposed new technique with the conventional *in vivo* technique is required, therefore having a negative impact on reduction. Validation is also required for the development, and consequent acceptance, of many reduction and refinement techniques, but need not have a negative impact upon reduction. However, developing non-animal (replacement) methods, which need to be compared with the *in vivo* model, often leads to an increase in the numbers of animals used in the short-term, which seems to be paradoxical.

Previously, we mentioned the difference between *absolute* and *relative* replacement, which Russell and Burch (1959, reprinted 1992) used to distinguish between methods that fully replace the need for sentient beings and methods in which sentient beings are still necessary. Using primary tissue from animals as a *relative* replacement method could be considered to have a positive effect on reduction. Fewer live animals are needed and the animals that are used for their primary tissue are killed prior to the collection of the tissue, and will therefore not be subjected to experimental conditions. The use of cell lines is another replacement method in which animals are used initially; cell lines are then immortalised making further use of animals obsolete. Therefore, the number of animals used as a source of primary tissue is higher than those required for immortalised cell lines and therefore decreases the positive impact of reduction.

Conflicts between replacement and refinement

It seems difficult to conceive of a conflict between a replacement method and a refinement method; however, the use of foetal calf serum is one such method that has drawn the attention of animal welfare scientists. Foetal bovine serum (FBS) is commonly used in *in vitro* methods, but the collection of blood causes suffering to the foetus (Jochems *et al* 2002). In the workshop 'Towards Better *In Vitro* Methods: The Replacement of Fetal Bovine Serum' (van der Valk *et al* 2004), a 'Foetal Calf Slaughter Welfare Protocol' was proposed to minimise suffering during collection. Furthermore, although the use of serum-free media is currently limited to a small number of cells, serum-free media formulations for primary cell cultures and cell lines are being published in an increasing number. The benefits of serum-free culturing include chemically defined and controlled culture conditions, reduced variability in qualitative and quantitative culture medium composition, reduced risk of microbial contamination and advantages in the isolation of cell culture products (downstream processing) (Gstraunthaler 2003). Therefore, because of batch-to-batch variability and ethical concerns, it would be better if FBS was not used anymore in routine testing. However, the effects of serum-free media on test results need to be carefully assessed, because serum-free media components may interact with the substance of study at the cellular level. No clear examples of techniques that have a positive impact upon refinement and a negative impact upon replacement could be identified.

Conflicts between reduction and refinement

The use of fully implanted telemetry devices enable researchers to collect data remotely from undisturbed animals, refining a previously intrusive method of data collection, for example, taking regular blood samples or using permanent catheters (Brockway *et al* 1993; Einstein *et al* 2000). From this point of view, the technique of using fully implanted telemetry devices might be considered to be a useful method of refinement: data are obtained by remote sampling so stress-related variation is considerably reduced. Because the data are of better quality (Brockway *et al* 1993; Schnell & Gerber 1997) it may be possible to reduce the number of animals needed to obtain the required information (Brockway *et al* 1993); the quantity of data that may be obtained from a single experiment is greatly increased; and, provided these data are used in an efficient way, fewer animal experiments may need to be performed. Therefore, telemetry can constitute an effective reduction method. However, implantation of the telemetry device requires extensive surgery and may be associated with considerable post-operative pain. Furthermore, the size of the transmitter is such that it may cause significant physiological stress, especially in small animals (Einstein *et al* 2000; Gerber *et al* 2002; Morton *et al* 2003). Consequently, telemetry can have a very negative impact on refinement. From this example it is clear that the interplay between the Three Rs can be extremely complex, involving more than straightforward positive or negative interactions, but sometimes a combination of both.

Conversely, it has been shown that the presence of a conspecific (or 'buddy') can significantly reduce stress associated with procedures (Hennessy 1984; Smith *et al* 1998) and therefore may be considered a refinement. However, although the conspecific animal will not be subjected to the test itself, it may be exposed to many stressful aspects of the study, including handling and separation from a larger social group. Therefore, the number of animals exposed to stress as a result of the procedure, albeit of a lesser severity, is doubled, having a negative impact on reduction.

Cost-benefit analyses of animal experimentation

Before exploring possible prioritisation guidelines for situations where the Three Rs interact, we will briefly discuss the general cost-benefit analyses used in Ethical Review Committees (or Animal Ethics Committees) to justify or reject proposals for research involving animals.

In written narratives or research proposals submitted to the Ethic Review Committee, the level of suffering, or discomfort, is often indicated in categories. For example, in the Dutch system, minor, moderate and severe discomfort is balanced against three levels of significance for society and science: minor, moderate and great significance. Examples of these three categories of discomfort are an injection (minor), individual housing (moderate), and prolonged pain (severe). Research projects with minor significance are generally rejected; projects that cause severe discomfort to the animal, but only have minor significance, are also rejected. On one hand, the quality, aims and significance of the animal experiment and the credentials of the research group are assessed and, on the other hand, the discomfort for the animals (de Cock Buning & Theune 1994). There is a set of questions for members of the Ethical Review Committee to judge each of the following: the quality of the research, the discomfort caused to the animal or animals, the significance of the proposed study and the credibility of the research group. Aside from the three categories of discomfort, the duration of discomfort, in days, is also taken into account, as well as housing conditions, physical health and the possible inhibition of species-specific behaviour. Questions are also asked regarding possible replacement techniques, including whether adequate sources, such as journals and databases, have been consulted for alternative techniques and, if alternative techniques do exist, what the reviewer thinks of the justifications for not using them. Questions relating to reduction only focus on the technical aspects of the research, such as whether it is possible to reduce the number of animals, whether the proposed research is repeating existing research, or whether closely related research has been carried out or is being carried out elsewhere, and if so, whether collaboration exists. Dolan (1999) compares the British and Dutch review systems in detail, and summarises other systems developed to decide ethical acceptability (eg those from the Universities of Nottingham and Utrecht). Several other attempts have been made to provide a set of decision rules for justifying or rejecting animal experimentation, for example, Bateson's

initial 'square', and later 'decision cube', which take into account quality of research, certainty of human benefit and animal suffering (Bateson 1986).

Prioritisation of replacement, reduction and refinement

The problem with the existing ethical review models, or decision trees, is that although replacement, reduction and refinement are considered, they are considered independently of each other and not in conjunction. In general, the different cost-benefit analysis models guide the Ethical Review Committees in their justification of animal experimentation, but when one or more Rs are conflicting, there is likely to be a dilemma. The Ethical Review Committees generally use 'commonsense' consensus decisions when they are faced with dilemmas, which may result in the ethics of principles dominating the ethics of consequence. The ethics of principles often reflect a utilitarian approach, which tend to maximise happiness for the greatest number. Ethics of consequence tend to focus on the result of an action (or absence of an action). Where the Three Rs are in conflict, the need for improved for and against arguments becomes apparent and the ethics of consequence should be taken into account.

Although each procedure, protocol and research programme requires a different approach when the Three Rs are considered, because of positive and negative interactions between the Three Rs, some guidance or prioritisation is needed. As the Three Rs are not specifically mentioned in the Council Directive 86/609/EEC, there is no indication of how the concepts of replacement, reduction and refinement should be prioritised, although in some national guidelines refinement is given priority over reduction (eg the Home Office 2000; Anon 2004). The reasoning that follows from this is that the experience of an individual animal is paramount to the number of animals, and that additional suffering for the individual in exchange for a reduced total number of animals is not acceptable. However, when a significant reduction in the number of animals can be achieved by minor additional discomfort or distress, perhaps reduction could be more acceptable. Although balancing the number of animals versus individual suffering remains rather subjective, it is extremely important that the moral dilemmas between the Three Rs are debated, and guidance is given on how to prioritise reduction and refinement. We hope that the 7th Framework Programme, funded by the European Commission (2005), and their reviews of primates and genetically modified animals in research, will pay more attention to ethical analyses of proposals and the Three Rs and the interactions between them.

Animal welfare implications

Russell and Burch's concept (1959, reprinted 1992) of the Three Rs has provided researchers and ethicists with a framework by which the direct and contingent harms of animal research can be reduced and within which ethical evaluation can be logically practiced. The application of many replacement, reduction and refinement techniques will have a positive impact on the design of studies with respect to one or all of the Three Rs. However, the simplicity of the Three Rs framework breaks down when conflicts between the Three Rs arise. Without legal guidance on the degree of priority that should be given to each of the Three Rs under these circumstances, we must draw our own conclusions as to the relative impact of the harms and benefits of the implementation of such conflicting techniques, potentially having considerable animal welfare effects. This should be assessed on a case-to-case basis because it depends on the nature of the experiment and the animal suffering involved. Moral reasons also deserve more attention when considering the Three Rs in general, and when considering the application of one or more Rs to a procedure, to a protocol, or to the wider research programme. Despite the difficulties, the Three Rs remain an important concept for the scientific and ethical evaluation of the use of animals in scientific procedures. If, as can only be recommended, the Three Rs are to be incorporated into the revised Council Directive 86/609/EEC, guidance on the prioritisation of each of the Three Rs must be provided to facilitate their use.

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References

Anon 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition. http://www.nhmrc.gov.au/publications/_files/ea16.pdf (accessed 20 September 2005)

Balls M, Goldberg AM, Fentem JH, Broadhead CL, Burch RL, Festing MFW, Frazier JM, Hendriksen CFM, Jennings M, van der Kamp MDO, Morton DB, Rowan AN, Russell C, Russell WMS, Spielmann H, Stephens ML, Stokes WS, Straughan DW, Yager JD, Zurlo J and van Zutphen BFM 1995 The Three Rs: the way forward. *Alternatives to Laboratory Animals 23*: 838-866

Bateson P 1986 When to experiment on animals. *New Scientist 20 February*: 30-32

Bayne K 2002 Development of the human-research animal bond and its impact on animal well-being. *Institute of Laboratory Animal Research Journal 43*: 4-9

Brockway BP, Hassler CR and Hicks N 1993 *Minimizing Stress During Physiological Monitoring*. SCAW: Bethesda, Maryland, USA

Buchanan-Smith HM, Rennie AE, Vitale A, Pollo S, Prescott MJ and Morton DB 2005 Harmonising the definition of refinement. *Animal Welfare 14*: 379-384

Chang FT and Hart LA 2002 Human-animal bonds in the laboratory: how animal behavior affects the perspective of caregivers. *Institute for Laboratory Animal Research Journal 43*: 10-18

Council Directive 86/609/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. http://europa.eu.int/comm/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf (accessed 20 September 2005)

de Cock Buning T and Theune EP 1994 A comparison of three models for ethical evaluation of proposed animal experiments. *Animal Welfare 3*: 107-128

Dolan K 1999 *Ethics, Animals and Science*. Blackwell Science: Oxford, UK

Einstein R, Rowan C, Billing R and Lavidis N 2000 The use of telemetry to refine experimental technique. In: Balls M, van Zeller AM and Halder ME (eds) *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* pp 1187-1197. Elsevier: Oxford, UK

European Commission 2002 The Welfare of Non-Human Primates used in Research. Scientific Committee on Animal Health and Animal Welfare, European Commission http://europa.eu.int/comm/food/fs/sc/scah/out83_en.pdf (accessed 20 September 2005)

European Commission 2005 Future European Union Research Policy. http://europa.eu.int/comm/research/future/index_en.cfm (accessed 20 September 2005)

Festing MF and Altman DG 2002 Guidelines for the design and statistical analysis of experiments using laboratory animals. *Institute for Laboratory Animal Research Journal 43(4)*: 244-258

Gerber P, Schnell CR and Anzenberger G 2002 Behavioral and cardiophysiological responses of common marmosets (*Callithrix jacchus*) to social and environmental changes. *Primates 3*: 201-216

Gstraunthaler G 2003 Alternatives to the use of fetal bovine serum: serum-free cell culture. *Alternativen zu Tierexperimenten 20*: 275-281

Hau J 1999 Humane endpoints and the importance of training. In: Hendriksen CFM and Morton DB (eds) *Humane Endpoints in Animals Experiments for Biomedical Research* pp 71-74. The Royal Society of Medicine Press: London, UK

Hendriksen CFM, van der Gun JW, Marsman FR and Kreeftenberg JG 1987 The effects of reductions in the numbers of animals used for the potency assay of the diphtheria and tetanus components of adsorbed vaccines by the methods of the European pharmacopoeia. *Journal of Biological Standardization 15(4)*: 353-362

Hennessy MB 1984 Presence of companion moderates arousal of monkeys with restricted social experience. *Physiology & Behaviour 33*: 693-698

Home Office 2000 Guidance on the Operation of the Animals (Scientific Procedures) Act, 1986. HMSO: London, UK

Jochems CEA, van der Valk JBF, Stafleu FR and Baumans V 2002 The use of fetal bovine serum: ethical or scientific problem? *ATLA 30*: 219-227

Laule G 1999 Training laboratory animals. In: Poole T (ed) *The UFAW Handbook on the Care and Management of Laboratory Animals* pp 21-27. Blackwell Science Ltd: Oxon, UK

Morton DB, Hawkins P, Bevan R, Heath K, Kirkwood J, Pearce P, Scott L, Whelan G and Webb A 2003 Refinements in telemetry procedures. *Laboratory Animals 37*: 261-299

Prescott MJ and Buchanan-Smith HM 2003 Training non-human primates using positive reinforcement techniques: Guest editor's introduction. *Journal of Applied Animal Welfare Science 6*:157-161

Reinhardt V, Liss C and Stevens C 1995 Restraint methods of laboratory non-human primates: a critical review. *Animal Welfare 4*: 221-238

Richmond J 1998 Criteria for humane endpoints. In: Hendriksen CFM and Morton DB (eds) *Proceedings of the International Conference* pp 26-32. *Zeist, The Netherlands*. The Royal Society of Medicine Press: London, UK

Russell WMS and Burch RL 1959 (reprinted 1992) *The Principles of Humane Experimental Technique*. Universities Federation for Animal Welfare: Wheathampstead, UK

Schnell CR and Gerber P 1997 Training and remote monitoring of cardiovascular parameters in non-human primates. *Primate Report 49*: 61-70

Scott L 1990 Training non-human primates: meeting their behavioural needs. In: UFAW (ed) *Animal Training: A Review and Commentary* pp 129-133. Universities Federation for Animal Welfare: Wheathampstead, UK

Smaje LH, Smith JA, Combes RD, Ewbank R, Gregory JE, Jennings M, Moore GJ and Morton DB 1998 Advancing refinement of laboratory animal use. *Laboratory Animals 32*: 137-142

Smith J and Jennings M 2003 *A Resource Book for Lay Members of Local Ethical Review Processes*. Royal Society for the Protection of Animals: Horsham, West Sussex, UK

Smith TE, McGreer-Whitworth B and French JA 1998 Close proximity of the heterosexual partner reduces the physiological and behavioral consequences of novel-cage housing in black tufted-ear marmosets (*Callithrix kuhli*). *Hormones and Behavior 34*: 211-222

van Cauteren H 1996 Implementation and impact of the ICH safety guidelines. In: D'Arcy PF and Harron DWG (eds) *Proceedings of the Third International Conference on Harmonisation* pp 213-220. Queens University Press: Belfast, UK

van der Valk J , Mellor D, Brands R, Fischer R, Gruber F, Gstraunthaler G, Hellebrekers L, Hyllner J, Jonker FH, Prieto P, Thalen M and Baumans V 2004 The humane collection of fetal bovine serum and possibilities for serum-free cell and tissue culture. *Toxicology In Vitro 18*: 1-12