Alternative methods in animal experimentation: evaluating scientific, ethical and social issues in the 3Rs context

FINAL REPORT AND RECOMMENDATIONS
D8
PART A

1. Title of the project and project website

Anim.Al.See (Animal alternatives : Scientific/Ethical Evaluation)

Official title in the contract:

“Alternative methods in animal experimentation: evaluating scientific, ethical and social issues in the 3Rs context.”

Web-Site: www.inemm.cnr.it/animalsee.html

1st edition (June 2004): Project presentation. Work packages, List of participants, C.V. of participants, questionnaire, Pert diagram, Dissemination (list of publications), Links, Events.

2nd edition (September 2004): Brussels Conference program, Updating of the previous information, Up-dating of the dissemination activities, Executive summary of the project.

3rd edition (December 2004): Brussels Conference minutes, List of participants to the Conference, Power point presentations of the 4 invited speakers.

2. Contract number:

QLG6/QLAM (CT-2001-00028)

3. Official contractual period:

Start: 01-01-02 End: 30-12-04

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7. Abstract

The use of animals in biomedical research remains a question of topical interest both in science and in other sectors. The current project aims to address and evaluate existing alternative methods for animal experimentation and their wider cultural implications from different perspectives. The objective is to assess if Russell and Burch's 3Rs model is still valid and applicable today. This evaluation is the outcome of the interplay between scientific and philosophical analyses and comprises accountability of the scientific results, animal welfare, compliance with existing norms, the social context, and ethical acceptability. These aspects were initially analysed to define the key concepts. These were then tested in case studies on each “R”: Replacement, Reduction, Refinement. As final step the conceptual, scientific and ethical assumptions underlying the 3 Rs were re-examined, in view of the current cultural context and recent scientific progress.

The final objective of the project was to update conceptual landmarks in order to promote the awareness of the complexity of the issue, of the diversity of roles and responsibilities and of possible improvements in ethical and legislative activities.

8. Conclusions, results

The main conclusions emphasise the need to:

- Acknowledge that the ethical dimension to animal experiments is equally as important as the scientific dimension.
- Clarify concepts underpinning current terminology concerning alternatives and animal experiments, methods, procedures etc., in recognition of the role played by semantics in this debate.
- Adopt a narrow and a wider definition of alternatives, related to the socio-political context.
- Reinforce the “3Rs” model as a sound scientific approach to improve animal experimentation.
- Update definitions of the principles of Replacement, Reduction and Refinement.
- Promote the application of alternatives procedures to animal experiments, according to this new framework of reference.
9. Project related publications and relevant dissemination activities:


experiments: closing the gap between ethical and scientific issues. Toxicology and Applied Pharmacology 197, 245.

**Conference presentations**


Pollo, S. Animal Welfare and Refinement, Days of bioethics, International Conference organised by The Croatian Philosophical Society, Mali Lošinj (Croatia), June 13th-16th 2004


**Other dissemination**

PART B

Background/Rationale/Context

The motivation behind the original proposal submitted to the Quality of Life Programme (5th Framework Programme of RTD) was that the issue of animal experimentation and possible alternatives has not been sufficiently investigated from the theoretical and ethical points of view. Theoretical and ethical aspects deserve as much study and elaboration as practical and scientific aspects in the evaluation of possible alternatives and they should be strictly linked to the scientific advancement in the field. The absence of this kind of theoretical research may underlie the stalemate in the debate between the proponents and opponents of animal use in scientific procedures.

A. The need for a more advanced ethical inquiry

In western societies animals are increasingly regarded as having intrinsic value. This recognition is the starting point for the debate on the moral justification of the use of animals in scientific procedures. The debate concerns: a) the justification of the use of animals; b) the value of animals and of the suffering they experience; c) the human-animal bond and the responsibility of human beings; d) the inclusion of these issues in a model for the ethical evaluation of scientific procedures using animals. Moreover other changes in the cultural context, extensively examined in this project, should also be considered. These include:

1) Criticisms of science that have surfaced in recent years, not only among public, but also among experts and even scientists (no longer accepted as authoritative, often controversial viewpoints, insufficient transparency, overtly technological and market bound etc).
2) The change in the current mode of thinking about the discontinuity between animals and humans in favour of a more holistic view, in an endeavour to reconcile the human species with the natural environment within a less anthropocentric worldview.

B. Bridging the two cultures

C.P. Snow's diagnosis that modern society is split into "two cultures", humanities on the one side and science, on the other, still holds after 40 years. Science can no longer be considered as the only vehicle of progress, but must be accompanied by the humanities and their investigative approach to the question of progress in civilisation. Technological advancement needs to be combined with the principles of ethical analysis that are grounded in the humanities (sociology, history, psychology, philosophy).

C. Breaking down the language barrier

Criticisms of science have also regarded the language it uses to communicate. Scientific language is considered increasingly incomprehensible as it struggles to communicate new concepts and theories emerging from the advancement of
knowledge. This issue has been tackled on a number of fronts in order to try and overcome the breakdown in communication between science and the public. It is also increasingly admitted that the analysis of theoretical concepts and assumptions and a revision of the current language is indispensable to progress in specific fields of science.

These aspects have all been crucial in analysing the issue of animal experiments and possible alternatives, beginning with the fundamental book by Russell and Burch.

**D. Russell and Burch’s 3Rs model and legislation**

‘The Principles of Humane Experimental Technique’, by the British scientists Russell and Burch, was published in 1959. The book seriously challenged the scientific community to improve the treatment of animals in scientific procedures. The authors introduced the concept of the 3Rs - reducing, replacing and refining animal use in experiments. More precisely,

‘Replacement means the substitution for conscious living higher animals of non-sentient material.
Reduction means reduction in the numbers of animals used to obtain information of a given amount and precision.
Refinement means any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used.’

This book marked a starting point in attempts, by scientists, to improve the treatment of animals used in the laboratory and introduced the central problem of determining what is humane in any experimental procedure on animals. Slowly the concept of the 3Rs trickled down among the scientific community throughout the following decade. The late 1960s were particularly important, which saw the spread of a critical cultural and political upheaval in the Western world that demanded more democratisation and equality, and was particularly suspicious of the established institutions, including science. Regarding the reception of the 3Rs this development was important for two reasons.

First, the political nature of the critical climate in Western societies paved the way for the introduction of the concept of ‘alternatives’ in the debate. Principles of democracy, equality and justice were pursued. The notion of ‘alternative’ became a token for this critical attitude towards established institutions including science and was introduced as a summary notion for the 3Rs.

Second, the general moral emphasis on justice and welfare helped shape the idea that animals, sentient beings like humans, were morally valuable. Their plight regarding their use in research as a tool and material for experimentation according to many, could no longer be ignored by governments and society. Animal ethics as a branch of applied ethics was born and animal liberation became an important movement in society.

The concept of the 3Rs, and Replacement-alternatives, constituted an acceptable alternative between the needs of science and radical, abolitionist views on the use of animals. Hence, throughout the 1980s and 1990s various initiatives were taken to promote the concept of the 3Rs’ principle.

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1 Page 64. We will refer to the Special Edition of 1992, published by UFAW, South Mimms, England
First a number of legal actions were taken either motivated by or promoting the 3Rs principle.

(2) ‘The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Procedures’.

In 1991 the European Centre for the Validation of Alternative Methods (ECVAM) was established, with a brief that explicitly mentions the promotion of the 3Rs. The USA already had seen the establishment of a similar centre, specifically seeking alternatives for testing, CAAT (Johns Hopkins Center for Alternatives to Animal Testing). In a number of countries National Centres were installed to further the objectives of the 3Rs principle.

Nationally as well as on the European level, budgets were made available by governments for research into and dissemination of alternative methods. Industry and NGOs sometimes joined in with financial backing.

The scientific community showed its commitment to the 3Rs principle in a number of ways including specialized journals, congresses, work groups, etc.

Since some years EC funded research is subject to ethical review by an independent, trans-national panel, which takes account of alternatives and animal welfare. The EC has also extensively supported the development of alternative methods and research on animal welfare (see ref. 6).

A system of Animal Welfare Committees has been installed in various EU countries. One of their main tasks is to monitor and apply the 3Rs principle to scientific procedures.

The 3Rs have steadily become better and more widely known, although much still has to be done to ensure their wider implementation. However, the general context regarding their acceptability and application has also become increasingly more complex than it was in the 1950’s. The discussion of issues such as unnecessary use or reasonable alternatives and their interpretation according to the law is essential for a critical, workable and consensual approach to the 3Rs principle.

Objectives:
According to the previous analysis, the force of the arguments in favour of the 3Rs, that derives from a strictly science-based approach, should not be forgotten. However, there are many conceptual philosophical and ethical issue involved in elaborating the 3Rs, that force one to go beyond the scientific context and these arguments. The main objective of the project has been to re-examine the conceptual, scientific and ethical rationales underlying the 3Rs in view of recent experiences. Scientists and philosophers from three European countries, Italy, United Kingdom and the Netherlands, participated in this project. The opinion was that since 1959 many developments and changes had occurred, both in science and in society, as well as in the interface between science and the wider society. It was thought that many of these changes, for instance concerning the moral perception of animals in society, are of consequence for the meaning, the purport and the application of the 3Rs concept. However, these consequences have not been the focus of systematic study integrating scientific, philosophical, ethical and policy perspectives.
The project Animal.Al.See has been done by an interdisciplinary group. Scientists from several fields and philosophers worked closely together and discussed a variety of issues. Participants in the project and invited experts represented a broad range of EU countries. This made it possible to place more specifically scientific and general questions within the legal, political and cultural contexts of each country and compare them.

The objective was to combine more general discussion on the 3Rs with a detailed study of some representative cases on each 3R. Concerning Replacement animal experiments for testing *cosmetic products* was selected, for Reduction *vaccine potency testing* and for Refinement various aspects of the use of *non-human primates* were examined and discussed. Each case was investigated with a view to establishing what factors contributed to or deterred the acceptance or non-acceptance of Replacement, Reduction and Refinement alternatives to existing practices.

The general aim of the project was to combine science, philosophy and politics and provoke informed discussion in the scientific community and the political arena. The objective was to obtain a challenging and realistic list of proposals and recommendations, based on thorough, integrated scientific and philosophical inquiry, finalised to:

- Define and clarify the influence of each factor in evaluating possible alternatives to animal experimentation;
- Identify the different roles and responsibilities as well as interaction among those involved;
- Help promote awareness among the scientific community, the general public and regulators, regarding the complex interaction between the different players;
- Provide recommendations for bio-ethical committees to help them evaluate and decide on possible alternatives on the use of animals as experimental models in selected research projects;
- Help decision makers improve existing legislation.

**Methodology**

This project proposed to tackle the 3R model and each 3R singly, by analysing their ethical and scientific aspects and implications in the light of the novelties generated in science. By doing so the intention was to assess the validity of the current conceptual framework underlying the model and identify possible improvements. Given these aims, the project largely pursued philosophical and sociological methods of analysis, particularly with regard to defining the various aspects of the evaluation process, pinpointing new criteria and definitive conceptual landmarks. Moreover, as the evaluation also concerned the scientific validity and feasibility of procedures to replace animal testing, reduction methods and measurement of animal well-being, competence and analysis in the relative scientific areas were involved. The multidisciplinary aptitudes of the participants ensured that the difficulties posed by the use of such a variety of methodologies were overcome.
The project was developed in three phases.

1. **Phase one**
   Each partner obtained an updated background of current practices in the 3Rs as alternatives to animal experiments. The preliminary step was to define key concepts of the different aspects involved.
   A questionnaire (in electronic format) was then formulated assisted by an expert in social surveys in an endeavour to enquire into the conceptual background. The mailing list was carefully drawn up in order to cover the main experts in the different fields: scientists, regulatory boards, industry, consumers, animal associations and ethicists but, at the same time, targeted to avoid dispersion. Priority was given to organisations such as: ECVAM (European Centre for Validation of Alternative Methods) and its scientific committee; representatives of EU Commissions (i.e. DG Research, DG Environment, DG Consumers); OECD; representatives of Government bodies (i.e. Home Office, Ministry of Health); representatives of industry (i.e. COLIPA; UNILEVER, SANOFI); steering boards of scientific societies in the field (i.e. ESTIV, ASAB, EFB); main protectionist organisations (i.e. EUROGROUP; UFAW), members of ethical boards.
   The responses were then analysed and the results compared to the definition of the key concepts on which the questionnaire had been formulated. (A paper has been published on this specific aspect.)
   At the same time, during the last six months of phase one, the WP’s began the preparatory work to undertake specific case studies regarding Replacement, Reduction and Refinement, reviewing and selecting the relevant literature, in preparation for phase two.

2. **Phase two**
   Three different interdisciplinary groups challenged the outcomes of the first phase against case studies chosen as relevant for each “R”. Thus the extent to which the framework, developed during phase one, reflected the reality of the problems raised by practical approaches to alternatives to animal experiments, was verified.
   Two case-studies were analysed as being relevant to the concept **Refinement**: experimental protocols involving non-human primates; housing of non-human primates in biomedical research. In both cases, a philosopher analysed the appropriateness of the criteria used to define the ethical aspects of the research when these criteria are applied to the welfare of non-human primates in biomedical laboratories, whereas primatologists focussed on the possibility of applying non-invasive procedures in experimental protocols and environmental enrichment in housing conditions.
   Regarding **Reduction**, the “single-dose” test alternatives to the multi-dilution test in vaccine quality control and the case of telemetry were analysed. These examples are of interest for various reasons: traditional testing uses large numbers of animals and a high level of distress is involved, the tests are foreseen within a regulatory framework. Telemetry is interesting due to its recent widespread diffusion, as a possible means of reducing the numbers of animals involved, but it can conflict with refinement. The WP was composed of bio-statisticians, legislative authorities, toxicologists, vaccinologists and philosophers, ensuring a multidisciplinary analysis of the interplay
and relative importance of all the aspects involved. Particular attention was paid to the terminology used by the different parties. 

The case study on *Replacement* dealt with issues such as in vitro methods as alternatives in cosmetic testing. This is a high impact area on public opinion and has produced a series of amendments to the legislation, mostly concerning postponing the European ban on animal testing for cosmetics. Moreover, many in vitro tests have been included in the legislation or have been validated by the ECVAM/ESAC, and are close to regulatory acceptance. An evaluation has been performed of the interplay between the different aspects underlying these tests and their development, and how they comply with the existing conceptual framework. The relative importance of each aspect has been assessed in relation to the practicability and feasibility of eventual innovations, as well as the ethical issues involved in the so-called alternatives.

On this issue, beside the partners in charge of it, representatives of different stakeholders have provided contributions and comments. A specific report has been prepared for each R, containing a proposed redefinition of Russell and Burch’s original proposals.

3. *Phase three*

Phase three proposes a more updated version of the “3Rs” model, reflecting the outcomes of the case studies performed and the current cultural context. In this phase the Bioethics Institute of the University of Utrecht played a key role. In collaboration with the Coordinator and the WP leaders, the findings from the case-studies on each 3R were integrated and pooled as far as possible in order to gain an overview of the issue. A general framework of reference (social, cultural, etc.) regarding this endeavour has been included.

As a first step, a unified report was prepared by merging and blending the three independent reports with respect to the general frame of reference selected to update the concepts contained in the 3R model. A draft of possible recommendations has been elaborated. A short executive summary of the unified report and the drafted recommendations have been submitted to selected experts invited to the final conference, scheduled shortly before the termination of the project, in order to obtain the opinion of representatives of the different stakeholders. The outcome of the conference has been integrated in the final edition of the project report.

**Results:**

The results are contained under different headings emphasising the main areas pinpointed by the study and relevant proposals.

**Results of the theoretical analysis:**

The definition of alternatives according to the “3R’s” model, *Reduction, Refinement and Replacement* is widely agreed upon by experts in the field, but it is not widely understood nor applied, so that disagreement is likely to continue. It is important to clarify terms and principles and thus focus is given to three important notions: scientific procedure, animals and alternatives, used as a summary notion for the 3Rs.
Although the European Directive uses and defines ‘experiment’ as a central term, it is generally felt that this reference is too limited. The word does not describe some uses to which animals are put, which are clearly not experimental. Scientific procedure may be used to replace ‘experiment’.

The Technical Expert Working Group for the Revision of Directive 86/609/EEC, Subgroup on ‘Scope’, has proposed the following definition of ‘scientific procedure’:

‘A combination of one or more technical acts carried out on an animal for an experimental or other scientific purpose and which may cause that animal pain, suffering, distress or lasting harm’.

Mentioned as examples of ‘technical acts’ are: injection, laparatomy and withholding of food and water. The examples suggest that ‘technical acts’ are limited to the experiment or procedure stricto sensu. In other sections of this document, however, mention is made of other aspects of the life of an experimental animal, such as breeding and housing. Here the approach seems to be broader. It is the opinion of our group (that) for the application of the 3R principles in a wide sense, and regarding the responsibilities of institutions such as Animal Welfare Committees, not to limit the application of the 3Rs only to the experiment itself. Removing the word ‘technical’ might overcome this problem as it may be held that all acts and the conditions of laboratory animals are pertinent to the experiment or other scientific purposes. The following definition is suggested:

‘A combination of one or more acts carried out on an animal for an experimental or other scientific purpose, and which may cause that animal pain, suffering, distress or lasting harm’

The mention of ‘pain, suffering etc.’ shows that the definition is framed with the intention of denoting morally relevant acts. The interpolation of ‘may’ (‘risk of..) leaves the definition sufficiently open to cover all scientific procedures with animals, if we accept that the captivity of laboratory animals implies harm.

The scope of the 3Rs: sentiency as a criterion of admission to qualify for moral consideration

Vertebrate animals are perceived by most people as sentient beings, which qualify for moral treatment, on account of their capacity to suffer from pain and distress. The fact that many alternatives, which are proposed, involve micro-organisms, indicates that the extension of the notion of animals, covered by the Directive, and the protection which is one of the purposes of the Directive, is limited to sentient beings. So, the beings considered in scientific procedures, are:

‘sentient, non-human vertebrates, and other species of animals that have interests and capacities that are comparable to those of vertebrates.

Additional questions concern the position of larval, embryonic and foetal forms of vertebrates. Little scientific information about the sentience of animals at these stages
is available. Provisions should be made to include vertebrates at these stages, in the future, as our knowledge in this area increases.

**The definition of ‘alternatives’**

Many countries have legal regulations that make the use of ‘alternatives’ to animal experiments mandatory, if this alternative is available. However, several problems arise regarding the use of the word “alternatives”.

First, the concept is often limited to the Replacement-concept, often interpreted as the *total* replacement of animal testing. This ignores the concepts of relative Replacement (the substitution of animals with higher grades of sensitivity with those with low neuro-physiological sensitivity), Reduction (a procedure in which a small number of animals are used) and Refinement options.

Second, ‘alternative’ is often stated as such, leaving open the question ‘alternative to what?’ It might be understood (1) as a specified alternative to a proposed *singular* animal experiment, (2) as an alternative to a particular *method, procedure or condition* of animal experimentation, (3) as an alternative strategy or *approach* to the scientific or technical question that motivates the experiment in the first place.

Third, ‘alternative’ is often understood with reference to an *established* practice or method. It is tinged with a ‘political’ meaning that, according to one’s stance, is positively or negatively valued. This prejudices an open and reasonable discussion about the evaluation of proposals and arguments.

Considering these misunderstandings, for the sake of clarity it is recommended:

1) That Replacement, Refinement and Reduction, is referred to Replacement-alternatives etc., and not to ‘alternatives’ as such, or
2) To use the term as an adjective: alternative method, alternative approach or to specify as fully as possible: ‘an alternative approach to the problem that motivates the animal experiment, is to look for other ways of dealing with the problem, for instance considering a different policy’.

However, the concept of alternatives continues to be used to summarise the 3R’s model and an acceptable definition is urged. The definition proposed by the project members is as follows:

> ‘An alternative to an animal experiment is any procedure – i.e., any method or technique, proposal or approach - that is meant to replace a particular science-based procedure that may harm the interests of animals, to reduce the numbers of animals required, or to refine the procedure in such a way that the welfare of the animals in the procedure itself or in its context, is optimised.

This definition covers the 3Rs and includes all the relevant elements that are eligible for further elaboration and discussion.

**A narrow and a broad conception of alternatives**

Essential to this definition is the notion of a procedure, which is kept deliberately open. It is not specified as a ‘scientific procedure’ so as not to exclude other (non-scientific, not un-scientific!) uses of animals in experiments. A procedure is any formally planned human action (a technique, a practice, a project, a policy) that is meant to lead to a result that conforms to previously specified requirements. The most
common procedures in the context of animal experiments are scientific procedures or methods intended to provide an answer to a scientific question and that conform to specific scientific requirements. This does not exclude other possibilities, such as different scientific approaches to a set of problems, or policies that offer a different solution to the issue requiring the use of animals in the first case.\(^3\)

For reasons of clarity, it is helpful to distinguish between a broad and a narrow interpretation of this definition. A narrow interpretation specifies the procedure as a ‘scientific procedure’. It interprets ‘alternative’ in the strict sense as a proposed scientific-based procedure which, relative to an existing, accepted standard or consensual procedure, achieves one or more of the 3Rs. The standards to which the procedure is supposed to conform, or ‘the aim’ of the original procedure, are not disputed. The alternative in this narrow sense has to be argued for on scientific grounds, as a condition sine qua non.

A broad interpretation considers the motivating context behind animal experiments, as well as the scientific or other background that have an influence to play regarding the experiment. This introduces a broader range of options that might be called ‘alternatives’. Accordingly, the scientific question may be redefined, the use of animal experiments questioned, different solutions to the experimental procedures proposed. Moral aspects are also to be (re)considered regarding the relationship between non-human animals and human beings.

What is important to realise is that the term alternative in this context serves the welfare of animals. Apart from this element, the definition of alternatives does not reflect more fundamental ‘ethical’ or ‘political’ normative instances in the debate on animal experiments, such as which scientific projects where animals suffer are worthwhile.

**Replacement, Reduction and Refinement**

In light of this analysis a number of new definitions of the 3Rs are proposed. The 3Rs are clearly represented in the definition of alternatives. A few remarks on each R are called for. Concerning the definition of absolute 'Replacement', any reference to non-sentient material (that figures prominently in the original description by Russell and Burch) has been removed. The reason for this is that by introducing this reference, the definition would be limited to scientific procedures and to scientific experiments as singular acts. This would foreclose the discussion on 'broader' conceptions of alternatives.

Reduction is also defined with reference to procedures in a wide sense. In some cases, such as the creation of transgenic laboratory animals, and the use of 'buddy animals' as a Refinement-alternative to isolated housing of laboratory animals, it is important to take into consideration not only the animals actually used but all the animals involved. Only in this way can the application and evaluation of Reduction-strategies be based on adequate information.

Regarding Refinement, a reference to 'optimising' animal welfare was added. Given experimental conditions and the focus on experiment, Refinement is often confined to the relief of pain and suffering during the experiment. But it is important to apply the Refinement principle to the complete life-course of laboratory animals, and respect animals as valuable sentient beings in their own right.

\(^3\) For an elaboration of these options see part 2, chapter 4, the discussion of Replacement.
Absolute Replacement refers to any approach in scientific research, product testing, and other technical procedures in which no animals are used.

(The ex-vivo aspects of the cell cultures - serum provision and primary cell cultures - are thought of as falling under Reduction and Refinement practices.)

Reduction refers to any approach in scientific research, product testing or education that leads, directly or indirectly, to a decrease in the number of animals used.

Refinement refers to any approach which avoids or minimises the actual or potential pain, distress and other adverse effects suffered at any time during the life of the animals involved, and which enhances their well-being.

In conclusion, the definitions of 'procedures', 'animals' and 'alternatives' have made it clear that the 3Rs model is strongly based on a moral principle, the guiding principles being the welfare of sentient animals. A comprehensive conception of 'procedure' is urged, that does not ignore other possibilities and requirements for improving the plight of laboratory animals. A relatively formal and open definition of 'alternatives' was opted for, so as to make it possible to broaden, where needed, the discussion on alternatives to embrace other perspectives other than those of established scientific paradigms.

There is also a need to reconsider the conceptual, political and moral content of the 3Rs, in light of a changed cultural context. These changes concern how animals are viewed, moral implications, the status of animals, the role of experiments and scientific perspectives and practices.

The moral status of animals reconsidered
There are several arguments in favour of reconsidering and strengthening the moral status of animals for experimentation. Described below are a number of the scientific, philosophical and ethical arguments of relevance.

Scientific arguments
Scientific arguments are the result of developments in several fields. It has been shown that the significance of findings in animal experiments is more sensitive to the complex differences between animals and human beings than is often assumed. Moreover the reliability of animals as models is also sensitive to how they are treated within and outside the experimental situation. Animal laboratory science, but especially animal welfare studies, have contributed to greater moral sensitivity to the predicament of animals. Consequent to showing how complex the responses of animals to experimental situations and to conditions in general are, respect for the complexity and uniqueness of animals has increased. The idea of animals as machines has been shown to be wide off the mark regarding their true nature.

Both laboratory animal science and applied ethology draw on evolutionary theory. From this point of view the continuity more than the discontinuity between animals and human beings has been emphasised. This continuity thesis is also an important argument in favour of the moral status of animals. The tension between the idea that
animals are used to model human beings, when considering physiological and biomedical processes, and that this assumed analogy is denied any significance when it comes to considering the animal’s moral worth, has been pointed out. This is particularly relevant in view of the fact that the moral prevalence of human beings over animals has been traditionally justified with reference to the sort of properties that animals are increasingly shown to possess. Ignoring this evidence is hard to defend.  

Philosophical arguments

The philosophical arguments that inform the emerging image we have of animals to a certain extent rely on scientific findings. The idea of animals as beings that react more or less automatically and passively to stimuli with reflexes - unconditioned and conditioned - has been shown to be, in many respects, an artefact of psychological learning paradigms. On the contrary, the working group on Refinement have shown that animal functioning is marked by agency and self-development rather than by passivity. Animals have been shown not only to feel pain, but also to experience stress, fear, anxiety, depression, boredom, panic, etc. Although their capacities to cope with these experiences are not to be underestimated and testify for their flexibility and active attitude towards the environment, there are limits to these capacities. If ignored, these limits lead to extreme negative states of distress and eventually to death.

Ethical arguments

It is now a widely acknowledged principle that human action, both individual as well as collective, that harms animals’ well-being have to be justified by providing reasons that are seen to prevail over the moral status of animals. Essentially animal ethics maintains that they are beings with interests of their own and a value in their own right and that, without good reason, are not subject to that of human beings. Four considerations that have been defended as morally relevant in the case of animals are:

1. The most generally acknowledged consideration is animal welfare. Animals are recognized as sentient beings, capable of a range of experiences of a positive or negative kind. Moral principles of doing no harm and beneficence therefore should be applied to actions on animals.

2. The killing of animals is sometimes regarded as a moral issue in itself, not reducible to the short-duration negative experience of fear that the animal might have immediately before being slaughtered, as it is commonly treated in ethical reviews of experiments. Human beings have a conception of animal life as a complete and flourishing ‘whole’, as a series of experiences, lived by an identifiable being. It seems that a violent and untimely death goes against this conception and should be devalued.

3. A third consideration is respect for the integrity of animals, presumably grounded in the moral feelings of human beings that there is a limit to the use and malleability of living beings by humans.

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4 One could say that this analogy also favours animal studies and is against in-vitro-methods. A possible rebuttal is that the moral status supervenes on general properties such as sentiency or cognitive ability, while the functionality of biological modelling often relies on specific and subtle properties and mechanisms, which may differ considerably, even within a species. So at the very least the selection of animals should be based on specific similarities argued for, not assumed. For an elaborate discussion of this analogy, see La Folette and Shanks (1995).
4. A fourth consideration is the ‘naturalness’ of animals. This consideration might not seem directly relevant, as only a small number of scientific procedures concern animals in the wild. But the ‘natural’ state of the animal, for many people, remains a normative reference point for what we may and may not do to animals, and, when it comes to ‘optimisation’, of what we must do to care for them.

It seems that the practice of animal experimentation has largely ignored most of these issues. Acknowledging the relevance of these considerations would have large implications for the justifiability of animal experiments, while the moral agenda of experimentation is already over laden. But it is evident that interpretation of the 3Rs will be influenced by positions taken on these issues.

Reconsidering the role of animal experiments
Science is an organized and holistic practice of a theoretical as well as a practical nature. The experiment is an important element in this practice. But, it is a test for the reliability of a claim to knowledge, which is embedded and relative. The content and relevance of the experiment is derived from a much larger network of assumptions, conditions and standards. Not only the historical and theoretical, but also the practical and institutional embeddedness of animal experiments should be recognized in thinking about the 3Rs. Limiting the 3Rs to a single experiment or type of experiment, although it may certainly contribute to Reduction and Refinement, would be a too narrow approach to the issue of alternatives from a moral point of view, and ignore the nature of much scientific design and scientific valuation of experiments.

We may develop this reconsideration of the experiment from within three perspectives: that of science, that of the life of the experimental animal, and that of society.

1. The scientific perspective: the 3Rs and the four Ps
We may distinguish between four levels, at which the applicability of the 3Rs can be considered.

The first level is that of the Particular concrete acts that the animals are subjected to. This is the most obvious way of applying the 3Rs, covering for instance (under Refinement) the application of anaesthesia and humane end-points.

The second level is that of the Proposal, described in a scientific protocol. This proposal contains a coherent description of the particular concrete acts with respect to their contribution to a clearly defined purpose, such as the acquisition of information needed to draw scientifically sound conclusions. The Proposal contains much more information about the objectives and conditions of the experiment. Sometimes it covers a series of experiments. The contextualisation makes it possible to look for additional possibilities to apply the 3Rs.

This same process of contextualisation can be carried out at a higher level of abstraction by concentrating on the level of the Project that the Proposal is part of. While Proposals are reviewed most of the time by Animal Welfare Committees, the judgment of Projects is often reserved for scientific boards in a university or institution, or for the management of a company. But Animal Welfare Committees may be in a position to advise these boards on ethical and animal welfare issues related to the 3Rs.
The fourth P refers to the P of Research Programmes or Policies such as the requirements for testing procedures. These are even more comprehensive levels of organising scientific (and economic) activities, which may offer again possibilities for applying the 3Rs and for suggesting alternatives.

The distinction between these four levels is important for two reasons. First, it enables a much more realistic and pointed evaluation of the scientific aspects of the animal experiment. Second, it suggests new prospects for applying the Three Rs by taking into account the broader range of time within which animals are bred, housed and used and animal experiments are designed and conducted.

2. The perspective of animal life: protection of the 3Rs from first to last breath

Animals, used for experiments, live a life that originates in, and is almost completely dominated by this purpose of experimentation with the harmful consequences that come with it. Therefore, the application of the 3Rs should not be limited to the actual use of the animals in the experiment, but should extend to the various phases and aspects of the life of the animal as a whole, such as breeding, feeding, housing, preparing and killing of the animal. The issues of housing, preparation for experiments and killing are already taken into account in applying the 3Rs by many Governments, Animal Welfare Committees and License holders. What would be desirable and that requires considerable effort would be international agreements on a set of standards and regulations supported by the various actors to apply the 3s.

3. The perspectives of society, policy and politics

The main point of this perspective is that the research objectives of many animal experiments are strongly linked to societal problems and choices. These research objectives usually uncritically assume standards of risk-avoidance that are the result of social negotiations, take for granted wants and preferences of the public, and follow without discussion the pressure for innovation that is characteristic of markets etc. In many cases the consequences in terms of harm to the animal’s interests are not known, let alone transparent. Consequently the ultimate justification for experiments is not critically examined. Replacement-alternatives may be limited to finding scientifically validated alternatives and take the purposes of the experiment for granted. However, at a political level such an attitude is becoming increasingly difficult.

Practices and perspectives in science re-considered

Practices and perspectives in science have considerably changed since the 1950s. Scientific aims and practices are no longer totally uncontested within society. This change may imply a breakdown in the modern contract between science and society and urge that it be renewed. Changes are both due to internal and external pressures. Internal causes involve increasing theoretical, technological and practical complexity and how sophisticated science has become as a practice. External causes include the financial, institutional and societal conditions underlying science.

Regarding internal causes three main issues are involved.

The first issue concerns opinions on animal experimentation and regards:

(1) contextualisation - the specific background conditions necessary to ensure reliable animal experiments,
(2) **representation** - the extent to which animals and how they are prepared constitute a reliable basis for the acquisition of generalized knowledge,

(3) **extrapolation** - the extent to which animal experiments are relevant to the human condition.

These aspects are more compelling than was previously the case. There is a growing sense that as we acquire deeper and more complex biological and biomedical knowledge, we need to be more cautious when relying on animal experiments for conclusions. Science is moving away from a mechanistic to a more dynamic view of biological entities that places greater emphasis on their genetic and evolutionary specificity and the relation between organisms and their surroundings. This may have major implications for the future of animal experimentation, especially regarding applications. The arguments and trends involved should be examined carefully and should not be regarded as conclusive even though they are crucial in the debate on the future use of animals in science.

The second issue concerns the fact that technology enables scientists to invent new ways of conducting research and assembling information. This influences the role and relevance of animal experiments as well as how they are conducted. Sometimes, this leads to a reduction in the number of animal experiments as, for instance, new techniques of imaging make it possible to monitor physiological processes in more detail and better model of the dynamics of life, without having to kill an animal. However, new techniques may also jeopardise the 3Rs, as it is clear from the case of telemetry, a technical procedure in which electronic recording devices are surgically implanted in animals. The number of animals involved are fewer, as the information obtained is much more reliable and direct, but it also causes more suffering as surgery is needed. An important aspect regarding the opportunities offered by new technologies is that it often elicits discussion about the trustworthiness, applicability and relevance of new techniques compared to old. Particularly influential in this respect are molecular biology, genetics and many affiliated new sciences, such as ‘informational genetics’. The technological possibilities of creating transgenic and knock-out laboratory animals, as models for fundamental and applied research, has caused a revolution in recent years. The consequences for animal experimentation, the 3Rs and ethics are difficult to evaluate at this stage.

A third issue regards theories in the history and philosophy of science. The critical work of influential thinkers (Popper, Lakatos, Kuhn, Latour) has weakened the positivistic picture of science and superseded the idea of ‘Pure Science’ as a representation of the ‘Eternal Truth’. Science has its own image of reality, facts and experiments, outcomes, interpretations and knowledge advancement. These views are not new. Science has a long history of (self-)reflection culminating, in the 20th century, in disciplines such as the sociology of science and the psychology of science. But the relativising view of science has now become more common and it impacts on the general public’s belief and trust in science.

The views of these philosophers, especially those of Latour, herald the external causes of change. These are twofold. First, scientific practice has become vastly more complex. The financial, social and institutional contexts for practicing science have changed. For instance, investment from corporate business is greater, there is more policy steering on the political level and much more attention from the media and the
public. So the societal climate, with which science is confronted, is more pressing and influential, both institutionally and in respect of relations with the public.

Second, science as a practice, as well as specific scientific results and activities, are much more debated and controversial. Many meta-studies have put into perspective the achievements of science and made society much more aware of the drawbacks and side-effects of scientific and technological progress.

It is our opinion that these changes might well contribute to a situation where science is more integrated in a democratic society. Because science meets with criticism from outside, it is bound to be more self-critical and to deliver more value for money, support and other facilitating conditions that flow from society to science. Sciences which use animal experimentation will be in a better position to stand the test of the ethical acceptability of its activities. The challenge is to accommodate the demand for moral justification, public accountability and transparency without jeopardizing scientific freedom, creativity and progress.

Re-considering society

The changes described so far are in various ways related to the perspective that turns on the deep-rooted and complex changes that have affected western societies in the past 40 years. These changes have also influenced the conceptualisation of the 3Rs. Reduction, for instance, takes serious issue with the complex institutional frameworks of society and formulates strategies that commit actors to an effort to reduce numbers of animals on the extra- and supra-experimental level. Replacement is analysed from the perspective of the changing views on science, on societal needs and on the relevance of animal models.

From a moral point of view, the upshot of these changes is that there is a need for a more ‘inclusive’ and encompassing approach of the 3Rs. This approach must take the idea of caring for laboratory animals seriously and that in the broadest sense. At the same time, the implementation of the 3Rs needs to take account of the many factual, practical, institutional and moral consequences that concrete proposals for implementation imply.

From a political and institutional point of view, these changes mean that the central decision making process on the national level has to reckon with the broader international and global institutional context and with the fact that various stakeholders, on the societal level, have an essential role to play in reaching workable solutions.

For the objectives of this project, changes in the European area are the most important, so mention will only be made of developments on this level, particularly those that will, in all likelihood, have a large impact on the issue of animal experimentation. We will start with referring to four important institutional and political developments.

First, on the political level, authority has to a considerable extent shifted from national governments to Europe. This has created a need for harmonization of regulations and standards between member states, a process that offers chances and challenges as well as obstacles for a renewed effort to achieve the 3Rs.

Second, national political authority has been weakened, not only by processes of globalization, especially economic globalization, but also by the ‘sub-political’
powers\(^5\) of economic and technological forces and organizations that influence to a large extent the lives of people and the plight of animals. The shape these influences will take is as difficult to foretell as it is to control by political authorities.

Third, the influence of the media on the political debate and on policy, and the involvement of the public have considerably strengthened and have made it much more difficult to pursue policies on the basis of exclusively ‘rational’, informed arguments.

A fourth factor is the influence of Non-Governmental Organizations (NGOs) and pressure groups, such as the Animal Protection Movement. Animal Welfare Committees that now exist in many countries, witness the attempts of the authorities to accommodate a more critical attitude on the part of the public to animal experiments. Their success will be mainly determined by the trust people have in their procedural transparency, how moral judgments are motivated and the impartiality of its members.

There are signs that western societies are heading for a period of transition concerning social and cultural values. The pattern of values - technological comfort, economic prosperity, material welfare, public health and safety, individual freedom - that has motivated the growth of the modern nation state has proved to have its limitations and drawbacks. Western societies, rightly proud of their freedom, individualism, the longevity of its members and the technological achievements of its industry, seem however out of balance with the environment and nature and with society and morality as a shared enterprise when it comes to issues of safety, justice and meaning. Environmental catastrophes, terrorism, the concern about risks, a generalized feeling of uncertainty about the future may all be symptoms of this process of transition. It is probable that these changes in the appreciation of current values will also be reflected in opinions on animal experimentation.

There is also a growing awareness that policies should be open to alternative ways of solving problems than by investing in science and technology... As social and political conflicts and dilemmas dig deeper into the key values of societies and technocratic solutions are running out, the call for more political discussion as well as authentic responsibility and leadership from politics and politicians becomes louder.

The general picture, emerging from these analyses, can be summarised in three conclusions:

1. The moral and legal position of vertebrate animals (or animals with similar sentiency) as subjects of experiment has been recognized and strengthened in many countries. There is more sensitivity regarding the suffering of animals, more knowledge of the capacities and conditions for welfare of animals and more concern that the use of animals is warranted and justified by strong reasons and realistic expectations. The implication for the 3Rs model of this more comprehensive view is that the search for Replacement, Reduction and Refinement-strategies and applications should not be limited to the single experiment and the condition of the

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\(^5\) The notion of ‘sub-politics’ has been coined by Ulrich Beck, a German sociologist. It means that the powers that determine the future shape of society are no longer to be found in the Parliament and the public, democratically controlled and formal authority of Governments, but must be located in the decision-making processes that take place in laboratories, directory rooms of large firms or strategic discussion of NGO’s which make use of the influence of the media to reach their objectives.
animal in the experiment, but should be approached in a more inclusive way by taking into account the complete path of life and death of laboratory animals (breeding, birth, housing, feeding, using, killing).

2. Animal experiments are embedded as central elements in practices of science and regulation. These practices are dynamic and complex, internally as well as in respect of external conditions and causes. This fact of embeddedness requires on the one hand a broader analysis and discussions of the reasons, value and importance of animal experiments, on the other hand it offers a broader range of possibilities to apply the 3Rs, not only on the level of single experiments but also on other levels and phases, not only on the basis of scientific arguments but also on the basis of other considerations.

3. Animal experiments are human actions, sometimes of a crucial kind, that fit into a wider pattern of a society that thrives on scientific, technological and economic dynamics that constantly enforce legal, moral and political changes. The case of risk-management is an outstanding example to illustrate this wider context. Traditionally, scientific knowledge and results, but also trust and confidence in science, has been a firm basis for policy and progress. But this situation has changed for reasons described above. We now witness a much more intricate and complex pattern of reasons, values and considerations that influence public and political decision making. Recognition of the fact that multiple and plural normative frameworks (scientific, economic, legal, political, cultural and ethical) have to be taken into consideration and balanced, is important to find a way of taking on the many challenges that the principle behind to, and the model of the 3Rs harbours.

The presentation of the general backgrounds of the studies we conducted in this project, has produced many general suggestions, reasons and ideas to re-conceptualise and broaden the ideas that informed the launching of the 3Rs model. In the following we will make a summary of the process of converting these suggestions into specific proposals and solutions by reviewing the 3Rs separately and inquiring into the possibilities of re-conceptualisation, reinterpretation and wider application of each of the Rs.

Let it be clear from the outset that the structure of each of the 3Rs is quite different, in many respects. For instance, the call for Replacement is meant, ideally, to eliminate animal experiments or specific categories of experiments, while Refinement aims at improving the predicament of the animal subjects, given that the experiment currently being carried out, claims that this improvement is either neutral with regard to the scientific validity of experiments, or even optimising. Problems, arguments, suggestions for application, stakeholders and obstacles for implementation will likewise be different for the discussion of each of the 3Rs.

**Results of the case-studies:**

**Replacement: the cosmetic case**

The work on Replacement starts from the regulatory history leading to the ban of animal experiment in the field of cosmetic testing, and from the practical and theoretical points present in the argument pros and con concerning the ban. This issue
was chosen because is the one in which many non-animal “alternative” tests have already been accepted by regulation, and others have been validated. However, most Replacement-tests available for regulatory purposes, in the case of cosmetic products, are adopted not as real Replacement alternatives, but as steps in a procedure. Given this uncompleted scientific process, there is a need for arguments of a more principled nature to defend the ban as such. What arguments could justify this regulatory choice, and to what extent are these arguments tenable so as to offer reasonable grounds to decide in favour of a complete ban? We envisage at least three distinct approaches to these issues.

1. The first approach relates to a debate between those who hold that there could be some risks in the production and use of cosmetic products, and those who argue that there are no risks or that risks are not unavoidable. This first approach, then, justifies the ban on marketing cosmetic products/ingredients tested on animals on the supposition that these tests are unnecessary: they are either superfluous, because there is not a question of risk to human health, or they are unnecessary from a broader point of view: that of the replaceable, trivial character of the needs they attend to. In this respect the concepts of innocuousness nature of those products, their luxury nature, of dispensability of innovation have been criticised. Although these arguments are not tenable in a general sense, it can be defended that these kinds of products, and given the magnitude and nature of the risks involved, confront us with the possibility - and the responsibility - to consider collective or individual choices based on different values, e.g. choices that put the right to seek profit above some moral values, held by large segments of the population, or some serious interests of humans above fundamental interests of non-human vertebrates. The discussion has led away from categorical assertions concerning the harmlessness of substances or the vanity of some people, and toward the recognition that a reasonable balance between animal suffering and the kind of benefits for humans, involved in the production and use of cosmetics, ought to be a subject for further research and consideration.

2. A second approach, that comes as a natural second step - if we accept that there could be risks for human health involved in non-tested ingredients of cosmetics - is to search for scientifically validated alternative methods to minimise these risks, to develop alternative methods that can be considered as reliable as the traditional in vivo ones. In this approach the existing paradigms of scientific efficacy and safety testing are accepted. In this section the in vitro methods, mainly cell culture are discussed concerning their ethical (ex-vivo procedures) and practical (long term and systemic exposure) shortcoming. Additional consideration concerns safety evaluation and the use of human subjects. We conclude with regard to the scientific defence of the ban on animal testing for cosmetics, that some clear advantages are counterbalanced by a number of problems with the suggested alternatives that remain unsolved and may well require a long time to solve. This does not imply that these alternatives ought not to be used, but that the scientific arguments in favour of them should be more coherent and well-grounded. In view of the possible progress to be made in this area, it also remains true that more involvement of the scientific community at large in this kind of research and more adequately funded research will considerably strengthen the success and impact of this approach.

3. A third approach is to let the ethical concerns about the use of animals prevail, over the actual available knowledge and tests, and accept those risks (not predictable with the current non-animal studies) to human consumers of cosmetics as a cost for
upholding the respect for animals. This position is usually not defended, but bringing it out, might illuminate some of the reasons behind the request of this type of ban. In the light of these analyses, it appears that the ban of cosmetic testing should be addressed more on an ethical ground than a scientific one, therefore the concept of alternative appears to be more linked to the ethical and sociopolitical framework than to the scientific one. It is difficult to say whether this approach can be easily implemented. To make this approach work within existing economic, legal and social structures, is obviously difficult, as it is clear that cosmetic companies involved in the development and marketing of new substances are entitled to reliable and transparent regulations. Moreover, the possible risk of exposure for employees, as well as the problem of the toxic waste elimination, has to be taken into account too (Hartung et al. 2003).

**Reduction**

In the case of **Reduction**, an inventory has been carried out of legislation regarding statistics of animal use in Europe, with special reference to the United Kingdom, the Netherlands and Switzerland, and various available Reduction strategies have been described. One case study concerned the historical analysis of the evolution from the multi-dose to the single-dose procedure as an alternative in the potency testing of toxoid vaccines. The second case concerned telemetry, because it is a technique increasingly used and is relevant to the investigation of the conflicts, which may arise between Reduction and Refinement. In this case, while feasibility of Reduction seems not to be a restrictive factor from the point of view of correct experimental planning, the conflict seems to be in relation to the increased suffering to which the animals that are still used are subjected to. Again, ethical assessment of costs and benefits to animals and the value of research results play an important role.

An important outcome has been the identification of three different levels of reduction: intra-experimental (Reduction of numbers of animals within individual experiments/tests); supra-experimental (Reduction of number of animals by modifying experiment/test related aspects); extra-experimental (Reduction of number of animals by introduction of strategic research decisions or reduction as a result of spin-off developments not directly related to research). On this basis the new definition of Reduction has been proposed.

When retrospectively analysing the role of various stakeholders in the development of the single-dose approach, first it has to be noticed that there has been little attention of the public as a whole and animal welfare organizations particularly for the issue of Reduction, notwithstanding the fact that (for instance) tetanus vaccine potency testing both requires significant numbers of animals and involves significant pain and distress. This might be because the purpose of this use of animals, safe vaccination of children, is not contested such as is the case with cosmetic testing. Another reason might be that vaccine potency testing is not criticized because of its relevance (scientific validity), which was a major reason for challenging the LD50 test. The underlying discussion was science-based and could only be carried forward after new developments in vaccine production and quality assurance had taken place. Vaccine manufacturers particularly fuelled criticism on the parallel-line assay as the positive outcome would be advantageous for their goals: producing safe and potent vaccines in the most efficient way and at lowest possible costs. The input of the scientist was more or less an outcome of the position manufacturers took in this case.
Refinement

The working group on *Refinement* has focused its interest on the use of non-human primates in laboratory experimentation. Due to their closeness to human beings they represent a good case study for the conceptual redefinition of the scientific and ethical issues of animal welfare related to the concept of Refinement. Besides the concerns on the scientific value of a particular experimental protocol, the use of primates in experimental research raises a series of ethical issues. One of the questions relates to the moral acceptability of the use of animals, such as primates. Primates are a special case amongst laboratory-housed animals because they have complex cognitive capacities and social lives. Also, primates have no genetic adaptations to laboratory conditions and may also have a greater capacity for suffering than some other laboratory-housed animals. The core of a new concept of animal welfare is the idea that not only the welfare of human beings consists in the opportunity to develop themselves, but that of non-human animals as well. Such an idea is grounded both on scientific and philosophical premises. From the philosophical point of view, the idea of self-development seems more able to meet the requirement of an individualistic and naturalistic approach to ethics. The welfare of an animal is based both on his/her species-specific needs and his/her individual ‘character’ and nature. The question is how many of these needs do we allow to be compromised by experimental procedures? This question has to be taken into account seriously in our daily practice with experimental animals. Generally speaking, it can be said that the welfare of individual animals can be enhanced by improving degrees of behavioural freedom. Actually, the opportunity of making choices is the fulcrum of the process of self-development. From a practical point of view that means that the individual must be given the opportunity to choose, for example, whether and how to use a particular environmental enrichment. According to the concept of “degrees of behavioural freedom”, it is possible to increase, by means of Refinement techniques, the level of control that the captive animal has of its own life and of the environment. A dynamic and gradual approach to the improvement of the animal’s quality of life in captivity can also help to disentangle the apparent contradiction between the idea of “self-development” and the life behind bars.

As a starting point the legislation in housing and husbandry practices across the main primate users in Europe (UK, France and Germany) and the number of primates used was compared, and thorough review of Refinement practices, and welfare indices for the most commonly used primates was made. Provision of adequate useable space for captive animals is essential for good welfare but, because of its expenses, determining minimum cage sizes for legislative purposes it is highly contentious. Cage design (i.e. size and furnishings) affects the behavior, and its size determines whether there is sufficient room for appropriate environmental enrichment, and whether animals can be housed in socially harmonious groups. For animals used in laboratory, the environment can be an additional source of suffering and distress. If they can be better housed and cared for - to reduce the overall impact of experiments upon them - then we are obliged to do so for ethical and scientific reasons. Practically, all minimum standards are based on weight of primate (e.g. Council of Europe; European Community; Home Office Code of Practice-Scientific Procedures; Institute of Laboratory Animal resources; Royal Society/UFAW Guidelines). No single factor, such as body weight or size, is sufficient to determine minimum cage size. Instead, a suite of characteristics should be used that include morphometric, physiological, ecological, postural, locomotor, social and behavioural characteristics. Ideally one
should also take into account the number, age, sex and individual histories of the primates.

**Final results:**

**General Conclusions**

1. The cultural and institutional changes of the past decades urge a rethinking of the 3R’s model. Especially the increasing recognition of the value of animals, the critical appraisal of science and technology and the dynamics of European and global change need to be taken into account.

2. In defining the essential terms of the debate around the 3Rs, due account should be given to the fact that the model is meant to contribute to the general improvement of the welfare condition of animals. The definitions should be kept as clear and simple as possible.

3. A distinction between a narrow, science-based conception of ‘alternatives’ which accepts the purposes of scientific and regulatory purposes, and a broad conception of ‘alternatives’ which takes the underlying motives, approaches and contexts of scientific procedures, into critical account, is desirable from the point of view of public debate and policy.

4. 3R’s model implementation should reckon with the fact that adoption of any R-alternative is influenced by various kinds of normative constraints (scientific, economic, legal, political, cultural and ethical) which sometimes point in the same direction, and sometimes conflict and need to be balanced.

5. Science is not always able to answer to the ethically and politically motivated request to develop Replacement-alternatives and to guarantee safety without using animals. The relative certainty of risk assessment and of scientific knowledge in general, should be made better known and more account should be taken of it in discussing the use of animal experiments and alternatives.

6. The development of Reduction-alternatives (at least in the case study on vaccine potency testing) has been largely a matter of science and economics, without much attention from external stakeholders such as animal protection organisations and regulatory institutions.

7. Animal welfare, the central concept in Refinement-policy, is a complex notion which, incorrectly, in discussing Refinement is often limited to alleviating negative experiences of animals. There is often ample scientific evidence as to which conditions contribute to the general ‘flourishing’ of animals of various species.

8. Application of the 3Rs principle not always points to an undivided option. Three types of problems can be indicated: 1) problems of planning and coordinating efforts and means; 2) conflicts between Reduction and Refinement-strategies; 3)
conflicts between Replacement and Refinement and other moral consideration regarding animals, such as killing or interference with integrity..

9. The 3Rs principle is an important, but not the only, moral principle relevant for decision making concerning science-based procedures. Ethical principles related to human beings, such as the no-harm principle and acceptable levels of risk exposition, should be taken into account as well. Therefore, integration of the arguments derived from the 3Rs principle into a wider ethical framework is needed.

10. The policy inspired by the 3Rs is best based on an intelligent mixture of regulation, stimulation and education, with responsibilities assigned to various agents, individual as well as institutional, according to their different levels of power.

Recommendations

Concerning terms and concepts

1. In view of the ambiguity of the notion of ‘alternatives’, often taken to be covering only Replacement-options, it is recommended that referral to the Three Rs principle in contexts of animal use in scientific procedures is promoted, in order to make the full range of applications of Replacement, Reduction and Refinement regarding animal experiments more widely known.

2. It is recommended to promote a distinction between a narrow interpretation and a broad interpretation of ‘R-alternatives’, especially ‘Replacement-alternatives’. The narrow interpretation specifies procedure as ‘scientific procedure’. All efforts are directed to substitute existing scientific procedures which use animals with alternatives procedures that conform to the requirements of the 3Rs principle, accepting the framework behind the procedures. The broad interpretation takes up the approaches, reasons and purposes that motivate scientific procedures, and also the background conditions of a scientific, cultural or social nature, and invites the ethical and political consideration of radically alternative approaches and policies. It also invites looking for completely new ways of viewing scientific or regulatory problems (eg different regulatory solutions or testing). Presumably, the reviews and evaluations with regard to these two interpretations call for distinct procedures and policies and assignment to different responsible agents.

Concerning moral foundations and general policy

3. The fact that in many cases and conditions a serious consideration and application of 3Rs opportunities leads to better science, i.e. more reliable and valuable results,
ought to be a spearhead of EU policy of furthering and disseminating the 3Rs model.\footnote{This issue has been extensively covered by Prof. Merel Ritskes in her lecture “The scientific challenge” at the October conference organized by the EU commission and the Project Coordinator.}

4. Sentiency and the capacity of vertebrate animals to enjoy welfare and to flourish should be acknowledged as the morally relevant properties that motivate the 3Rs principle. From this principle the general preference – ceteris paribus – for in-vitro procedures, the preference for sparing vertebrate animals over invertebrates and the preference for sparing vertebrates with higher neurophysiological capacities over those with lesser neurophysiological capacities (again ceteris paribus), can be derived - (even the preference for not using human beings over other vertebrate animals, at least in cases involving serious or fundamental interests, could be argued for from this moral premise, amongst others).

5. An inclusive approach to the 3Rs principle should be promoted in three ways:
   a. a broader range of reasons (than just ethical reasons), that may positively influence the decision on implementation of the Three Rs should be taken into account. 3Rs policy makes sense from the point of view of science, economics and politics as well.
   b. the complete life of the animal – not only the experimental episode – should be considered as the field of application for Refinement-alternatives
   c. not only the singular (type) of experiment, but also projects, programs, areas of research and policies should be evaluated on opportunities to apply the 3Rs principle, especially to search for Replacement alternatives in the broadest sense.

6. There is a need to promote positive welfare rather than just focus on the reduction of suffering. The notion of animal welfare as simply the ability of an animal to cope with the environment should be abandoned in favor of a richer one, which includes the need to actively improve conditions of welfare. The concept of self-development could be related to the possibility for an individual animal to choose if, and how, to exploit a particular enrichment or to choose among different enrichments.

Concerning EU Three R’s policy  [Legal and policy implications]

7. The promotion, review and application of 3Rs opportunities should become a central object of the remit of Animal Welfare Committees. The European Commission should promote a legal and uniform basis for AWCs in EU countries, especially with regard to public accountability and the composition of AWCs. The composition of AWCs – especially requirements concerning available expertise - should reflect the central place of the 3R’s principle in the remit. AWCs should also develop a policy concerning the broad conception of ‘alternatives’.
8. The EC should develop and issue guidelines concerning the training, education and competencies of people involved in scientific procedures using animals. The 3R’s principle, in the narrow as well as the broader sense - referring to an attitude of sensitivity and caring for the value of animals - should have a central place in these training programs.

9. Information about the 3Rs should be made more readily accessible, and clear lines of responsibility for implementing the 3Rs, each in conformance with formal and practical requirements, should be delineated. The uniform system (issued by the Commission in 1997) for collecting and sharing information on numbers and types of use of animals in science-based procedures should be promoted so as to get, from all MS, reliable data to develop and evaluate policies, especially with regard to reduction.

10. The EC should also encourage editors of books and journals to require from scientists an explanation of the use of animals from the 3Rs point of view. Sponsors, financiers and governments should be made aware of the 3Rs quality of research that involves animals, and should be encouraged to evaluate their support in relation to the 3Rs as well as in relation to the ratio between the numbers and ways of animal use and scientific value and output of research.

11. The EC should see to it that, in case of new policies and regulations which require new tests and procedures with animals, an assessment from the point of view of 3Rs consequences is carried out beforehand. The results of this assessment should be brought to the attention of decision makers.

12. Reduction strategies, not directly related to a test situation, should be on the political agenda to increase public interest and counteract the passivity of legislators. Intra-experimental (Reduction of numbers of animals within individual experiments/tests), supra-experimental (Reduction of number of animals by modifying experiment/test related aspects) and extra-experimental (Reduction of number of animals by introduction of strategic research decisions or reduction as a result of spin-off developments not directly related to research) strategies for reducing the number of animals used can and should be specified in legislation and applied to all situations involving animals used in research, testing and education, with corresponding directives concerning duties and responsibilities of agents.

13. Based on periodic re-evaluation studies of the number of animals needed, guidelines should be adjusted to require tests using the minimum number of animals needed to obtain relevant results.

14. To create an economic incentive for stakeholders to develop and implement the 3Rs, measures such as tax-deduction or earmarking of a fixed percentage of research and/or testing budget for the 3Rs should be considered. Other instruments of policy, challenging stakeholders to search for and invest in alternatives, should also be made the subject of discussion, such as

   a. the ‘budgeting’ of numbers of animals per license holder
b. the introduction of Animal Issue-audits and
c. the development of a Certification system (including an official authority
issuing Certificates for Companies and Institutions carrying out animal-
using scientific procedures)

15. A code of ethics for the use of animals in science, education and testing, with a
prominent place for rules regarding the implementation of the 3Rs should be
considered.

16. The dissemination and promotion of the updated 3Rs principle and the ethics of
animal experimentation in all countries of the European Community, but
especially in the New Members States and Candidate Member States, which are
speedily catching up with scientific developments, should be a matter of the
highest concern, as these countries might profit from experiences in other
countries.

**Concerning research priorities from a 3Rs point of view**

**[Future research need]**

17. More research should be concentrated on animal suffering and the assessment and
validation of good welfare, the results and recommendations of which should be
disseminated in a clear and accessible way. The common and proven knowledge
that already exist about welfare conditions of various animal species should be
integrated and applied to the treatment, in a wide sense, of laboratory animals.

18. Research regarding the foundations and the moral and cultural aspects of the
protection of experimental animals within the EU is strongly recommended. This
project should consider the following topics:

a. The relation between moral considerations that are based in the experience
of the animals themselves (‘What matters to the animal’) and moral
considerations that are based in the human-animal-relationship and the
conception of animals and connected emotions that ground human
responses to the treatment and being of animals

b. The relation between patho-centric moral considerations relating to the
suffering of animals, welfare-centred moral considerations relating to the
optimal conditions of living for animals and moral considerations that
transcend these frameworks and refer to notions of flourishing, naturalness
and integrity.

c. The differences between legal regulations and opinions in society in
different member states concerning animal protection as well as
concerning the grounds for animal protection

19. Retrospective as well as prospective research concerning the impact of methods of
genetic modification on the implementation of the 3R’s model is highly
recommended.
Final suggestions:

The theoretical approach and the practical methodology adopted in this project may be useful in approaching other areas in which science and society come to a conflict. The more important aspects that can be transferred to other issues are:

- Honest, exhaustive and interdisciplinary analysis of the issue;
- Analysis of theoretical concepts, assumptions of the framework of reference and of the languages used, is indispensable to progress in specific fields of science.
- Making scientific aspects more accessible, reducing technicalities and making explicit embedded concepts and values;
- The importance of a minimal philosophical background to understand the relevance of ethical evaluation in technological implementation.

Project related publications and relevant dissemination activities:

- 20 publications in peer reviewed journal
- 8 Papers submitted for publication
- 7 Papers in preparation
- 47 Conference presentation

Publications

2002


2003


2004


2005


**Papers submitted for publication**

Botti C., Stammati A., Zucco F. Banning the use of animals in cosmetic testing: philosophical considerations upon a European debate. *ATLA*


Boo J de, Rennie AE, Buchanan-Smith HM, Vorstenbosch JMG and Hendriksen CFM. The interplay between reduction and refinement: a case study on telemetry in rodents and primates. *Laboratory Animals*. 


**Papers in preparation**

Boo de, J. and Hendriksen, C.F.M. Trends in animal use in biomedical research in the Netherlands, the United Kingdom and Switzerland: legislation and statistics. *Lab Animals (Europe).*


Hendriksen, C.F.M. Reduction and the single-dose approach in potency testing of vaccines.


Pollo, S. L’antropomorfismo tra etica filosofica ed etologia, *Rivista di Filosofia?*


**Conference presentations**

**2002**

*The general poster of the project has been presented:*

by partner 1, at the Conference “The three Rs, state of the art: Research, Development and Implementation of Alternative Methods”, Brussels(July 9th-10th 2002);
by Partner 4, at the Fourth World Congress on Alternatives and Animal Use in the Life Sciences, New Orleans (USA), August 11-15, 2002.

Zucco F., Botti C., Stammati A.L., An European Project to evaluate scientific and ethical issues in the “3Rs” implementation, Poster presented at INVITOX 2002, Formia, Italy (October 16th-19th 2002), Book of Abstracts P8-02.

2003


Hendriksen C. (2003), An introduction on 3Rs in the production and quality control of biologicals. Conference on Alternatives to Animal Testing, Delhi (India), February 16 – 19.

Hendriksen C. (2003), Alternatives to Animal Use. AllChemE seminar for MEPs. Brussels, 7 May.


Stammati A.: Presentation of the Project at the 3rd ECOPA Workshop in Brussels, November 9th-10th 2002;


Vorstenbosch J, (2003) Some ideas on ‘Frames of reference’ in the interface between science and ethics, Presentation at the meeting of the Replacement Working Group, 1 December, Brussels, CNR.


**2004**

Boo (de) Jasmijn. The interplay between the Three Rs. MEGAT meeting on Alternatives to Animal Use, Linz, October 16-17, 2004


Pollo, S. Animal Welfare and Refinement, Days of bioethics, International Conference organised by The Croatian Philosophical Society, Mali Lošinj (Croatia), June 13th-16th 2004


Other dissemination
