Reduction Strategies in Animal Research: A Review of Scientific Approaches at the Intra-experimental, Supra-experimental and Extra-experimental Levels

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Summary — When discussing animal use and considering alternatives to animals in biomedical research and testing, the number of animals required gets to the root of the matter on ethics and justification. In this paper, some reduction strategies are reviewed. Many articles and reports on reduction of animal use focus mostly on the experimental level, but other approaches are also possible. Reduction at the intra-experimental level probably offers the greatest scope for reduction, as the design and statistical analysis of individual experiments can often be improved. Supra-experimental reduction aims to reduce the number of animals by a change in the setting in which a series of experiments take place — for example, by improved education and training, reduction of breeding surpluses, critical analysis of test specifications, and re-use of animals. At the extra-experimental level, reduction is a spin-off of other developments, rather than the direct goal. Through improved research or production strategies, aimed at better quality, consistency and safety, reduction in the number of animals used can be substantial. A revised definition of reduction is proposed, which does not include the level of information needed, as in some cases reduction in the number of animals resulting in less information or data, is still acceptable.

Key words: *alternative, animal, approach, reduction, research, strategy.*

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Introduction

As a result of many centuries of animal use for scientific purposes, different philosophies and several social movements have affected modern-day thinking about our treatment of animals. In 1959, Russell and Burch described humane techniques for the first time, and introduced the concept of alternatives as a means to diminish or remove inhumanity in our treatment of laboratory animals. In their book, *The Principles of Humane Experimental Technique* (1) they discussed several strategies, which have become well known as the Three Rs: *Replacement*, *Refinement* and *Reduction*. *Replacement* alternatives make the use of sentient beings for research superfluous; however, some replacement methods still require animal tissues or cells. *Refinement* methods are aimed at improving procedures, methods and the environment in such a way that animal suffering, pain and stress are reduced. *Reduction* approaches are means of lowering *"the number of animals used to obtain information of a given amount and precision"*. Discussions on the number of animals used in research and testing get to the root of the matter on ethics and the justification of animal use.

Although, the Three Rs concept is not explicitly mentioned in European law regulating the use of animals in scientific research, its principles are inte-grated into Article 7 of European Union (EU) *Directive 86/609/EEC* (2). According to Article 19 in the Directive, no more than the minimum number of animals should be used to ensure scientific and statistical validity in an animal experiment. In the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (3), Article 11 regulates the re-use of animals. Thus, both the *Directive* and the *Convention* make the Three Rs a legal requirement that European member countries should comply with.

In 2001, a project financed by the European Commission was launched that had as its main objective a re-examination of the conceptual, scientific and ethical elements of the Three Rs in the light of experience and developments during the last few decades. We participated in this study and focused on aspects of reduction. This paper will review some reduction strategies or approaches and current definitions, as well as offering a modified definition of reduction. Papers on replacement and refinement will be published separately by the other partners in the EU project.

Definitions of Reduction

Since the concept of the Three Rs was first developed in the 1950s, in 1978, Smyth (4) defined reduc-

tion as *"All procedures which can…reduce the number of animals required…in meeting the essential needs of man and other animals"*. Reduction is now generally viewed as *"the absolute minimum that is required to meet the experimental objectives"* (5). Russell and Burch (1) considered reduction of great importance as *"…of all modes of progress, it is one most obviously, immediately, and universally advantageous in terms of efficiency."* Efficiency might be the reason that reduction has become the key reference for monitoring progress in the implementation of alternatives in biomedical research. The UK Medical Research Council (6) defines reduction by stating that *"The number of animals used in an experiment must be the minimum sufficient to create adequate statistical power to answer the question posed"*. Festing *et al.* (7*)* said that *"Reduction means ways of obtaining comparable levels of information from the use of fewer experimental animals, or of obtaining more information from a given number of animals, so that fewer animals are needed to complete a given research project"*.

Most definitions focus on reduction at the experimental or research project level. It will be argued that reduction strategies could also be indirect, by modifying the research approach or by changing factors not directly related to scientific procedures themselves.

Reduction Approaches

Reduction approaches at different levels could have similar effects or complementary effects. In order to differentiate between these approaches, we would like to introduce a different concept of reduction at three levels.

1. *Intra-experimental Reduction*, which focuses on the reduction of the number of animals used within individual experiments.

2. *Supra-experimental Reduction*, which aims to reduce the number of animals by changing the setting in which a series of experiments takes place, and which is independent of the individual scientific procedure.

3. *Extra-experimental Reduction*, which means reduction by means of developments that are not directly related to the animal procedures.

Intra-experimental Reduction

Intra-experimental reduction is the reduction approach which is most frequently referred to. In the landmark publication, *Alternatives to Animal Experiments*, by Smyth (4), only one paragraph is devoted to using fewer animals, and the examples given focus on the intra-experimental planning of experimental design and on reducing variation. The *Report of the FRAME Reduction Committee* (8) also mainly deals with controlling variation and statistical analysis. Indeed, these are very direct and powerful ways of reducing the numbers of animals needed. The main advantage of the intra-experimental reduction approach is its transparency: a smaller number of animals needed per experiment is clearly demonstrable, which makes it ideal for convincing policy makers about the need for a reduction strategy.

Experimental design

The designing of a research programme (research strategy) and of individual experiments should start with well-defined objectives, in which different stages in the study, and aspects such as time or effect in the study, are separated (9). The better the objectives are defined, the more likely that a reduction in the number of animals required can be achieved. There is no universal rule for calculating the exact number of animals necessary in experiments, but Festing (10) describes two ways of deciding how large the experiment needs to be: a "power" calculation, which is the preferred method, and the "resource equation". The power calculation is based on the calculation of sample sizes with a certain "power", which means that the observed effect is indeed due to the treatment and not just a coincidence. Van Wilgenburg *et al.* (11) developed an interactive computer-assisted learning program to guide students through the necessary steps when designing animal experiments and estimating optimal sample sizes. The program allows for more flexibility in designing experiments than when fixed formulae are used. The second method for calculating sample size is Mead's "resource equation" (12), which employs degrees of freedom, and incorporates treatment effect, block effect and error degrees of freedom. Reduction can be achieved by comparing different treatments to the same control group, combined in one experiment. Separate treatments compared to control groups require more animals than when they are combined. This also has the added advantage that the treatments can be directly compared with each other, which cannot be done in separate experiments.

Koudandé *et al.* (13) have developed methods for calculating the mean and variance of the number of animals with the desired genotype in each backcross generation for a marker-assisted introgression experiment. The methods can be used to design an experiment to determine the number of founder animals required, given the number of animals required at the completion of the backcross process and *vice versa*.

Multifactorial designs and randomised block designs

Festing (9) describes methods of increasing experimental efficiency that can be achieved by multifactorial designs and "blocking" treatments, instead of the most commonly-used single-factor design. A randomised block design corrects for differences such as time and space effects, by arranging animals in homogeneous groups in such a way that only variation within, but not between, groups contributes to experimental error (9). A factorial design blocks out certain effects, such as sex and strain, by using homogeneous groups. A higher efficiency might be expected in some designs where the total numbers of animals are kept the same, but more groups of different sexes and different strains are used than in single factor experiments. Moreover, several experiments can be performed simultaneously and interactions between factors can be detected (9). Many experiments are still poorly designed, use elementary statistics (9), and have low efficiency due to bad animal or data management, such as when data from one strain are extrapolated to the species as a whole. Bad animal or data management can lead to higher variation between individuals, and can thus increase the need for more animals.

Precision

In 1958, Cox (see 14) described five components of a good experiment, of which one, *precision*, relates directly to potential reduction in the number of animals needed. A high precision detects small biological effects which are not obscured by effects from the background variation. Increasing the precision of an animal model used in a study can lead to a reduction in the number of animals. Student's *t*-test is often used to look at the treatment effect and the background variation (14). In some cases, greater precision can be achieved by increasing treatment differences, although in toxicological testing, this may result in unacceptably high dose levels, if the treatment dose exceeds the maximum effective dose. Higher precision can also be achieved by using more-sensitive animals. An example is the use of animal models in carcinogenicity studies, which often lack precision due to the high background incidence of age-related tumours and the low sensitivity of the rat strain used, although (male) rats tend to be more sensitive than mice.

Variation

Controlling variation can be a very effective way of reducing the number of animals required, because the resulting increase of experimental power might allow a reduction in sample size (8). Background variation can be reduced by having equal numbers in each group, and by increasing the sample size (14). Using uniform animals, such as genetically homogeneous (isogenic) strains of animals may contribute to reducing variation. Hendriksen *et al.* (15) showed that the number of animals could be reduced by 30%, without any effect on the range of the confidence intervals, when F1 hybrid animals were used instead of the outbred animals commonly used in diphtheria and tetanus vaccine potency testing. However, in some cases, there might be a need to use outbred animals, such as in behavioural studies. A reduction in variation can also be achieved by using specified pathogen-free (SPF) animals, both in the breeding phase and in their later life. Since clinical and sub-clinical infections might affect the outcome of an experiment, thereby increasing variation, SPF animals may be more robust than "conventional" animals, so fewer animals are needed to account for losses during the experiment (16). Whether SPF animals are really robust can be questioned, since they may show less variation as a result of disease under SPF conditions, but not outside SPF laboratories. Animals with disease might be more variable, so more animals would be needed to achieve a given level of statistical precision. The refinement of procedures, experimental techniques and husbandry practices, may result in less-stressed animals, which may also reduce variation. Another important area of variation is measurement error. When taking measurements, human errors and equipment aberrations can make experimental animals appear to be much more variable than they really are, leading to the need for increased sample sizes. Measurement errors need to be identified and solved; for example, when doing behavioural research, intra-observer and inter-observer reliability errors may occur, which can be reduced by proper training. The use of improved apparatus, repeated measurements, and standardised protocols, can also reduce measurement errors.

Statistical analysis

Statistical analysis is of crucial importance for drawing correct conclusions about the outcome of an animal study. However, according to the FRAME Reduction Committee (8), *"…surveys of published papers as well as more anecdotal information suggest that more than half of the published papers in biomedical research have statistical mistakes, many seem to use excessive numbers of animals, and a proportion are poorly designed"*. It will be clear that this both interferes with the quality of research and causes wastage of animals. Several papers have been published that discuss the correct statistical analysis of test data (see 17). In principle,

the method of analysis should be closely linked to the experimental design and to the type of data to be produced (7), for example, randomised block designs must be analysed by a two-way analysis of variance (ANOVA). Scientists do not generally know these matters. For that reason, additional training of researchers in statistics has been recommended (18). Alternatively, statisticians could be involved directly in the design of an experiment.

Number of animals in experiments depending on practical factors

Statistical input in the design of a study is necessary, in order to find a balance between the reduction approach and the minimum number of animals required to demonstrate significance for the experimental parameter. In other words, using too many animals is unethical, but the same is true for using too few animals, as experiments may fail and have to be repeated with another group of animals. In the testing of biologicals, very small numbers of large animals are sometimes used, and occasionally, so few that no statistical analysis is possible. The scientific validity of such experiments is questionable. They might be justifiable as part of a series of experiments to assess safety, whereas as individual experiments they would not be acceptable. There are situations where a small group size can be justified, such as when only descriptive information rather than statistical significance is required, or in pilot studies. However, there is a tendency to increase or decrease the numbers of animals used on the basis of scientifically-irrelevant issues, such as availability or cost, giving the impression that the total body weight of animals is more important than statistical consideration. A typical example of such an inconsistency is the regulatory requirement for the Target Animal Safety Test (TAST) in the quality control of veterinary vaccines (19). In a TAST where chickens are used, the number of animals often exceeds 10, while in a TAST using horses, only two animals are specified. Apart from its non-specific character, the relevance of the TAST can be questioned when using such a limited number of animals (19). It is argued that reduction should always be placed in the context of the test reliability required. This means that either the number of animals should be increased to a level that allows significance or the test should not be performed at all.

Other intra-experimental reduction strategies

Other examples of intra-experimental strategies that can achieve a reduction in animal use, but which are not restricted to the design of the experiment, include the following.

Pre-screening

Using replacement methods in part of the entire study can result in a significant reduction in the number of animals used. Potential new pharmaceutical agents can be pre-screened by using computer simulation programmes and by *in vitro* methods. These methods, which are already widely used by the pharmaceutical industry, have the potential to replace and reduce the number of animals needed, at least in the initial phases of testing and drug development.

Pilot studies

A pilot study is recommended where no information is available about the size and variability of the response. Pilot studies can also provide information about critical stages in the experiment. The data from a pilot study can be used to assist in the design of the main study, to determine the number of animals needed and to avoid using too many or too few animals. In some cases, the results of pilot studies can also be used in the main study.

Retrospective analyses

Fixed numbers of laboratory animals are frequently used in routine tests, particularly if these tests are based on regulations. No mechanism exists to regularly review these numbers, and as a result, the number of animals actually used might exceed the number that is required for the statistical validity of a test. However, it is anticipated that modifications should be made in the performance of routine tests that would ultimately lead to reductions in the numbers of animals required, for example, as a result of an increase in expertise, further standardisation of procedures and/or less variation among the animals. Using retrospective analyses of test data yields information about test variance, which can then be used to calculate the number of animals needed. Through such retrospective analyses, Hendriksen *et al*. (15) and Knight & Roberts (20) showed that, within tetanus potency testing routinely performed in their institutes, a reduction of about 25% in the number of animals used would be possible, with only a minor effect on the range of the confidence intervals.

Supra-experimental Reduction

Supra-experimental reduction strategies focus on changing the conditions and settings in which animal experiments are performed. This approach is independent of the experimental research context.

Education and training

Since many experimental designs use too many animals, scientists should be trained in experimental design and statistics, so that they realise that fewer animals are appropriate in particular circumstances.

Education also includes learning about performing a literature search before the design of an experiment. A proper literature study might avert improper study design and avoid the unnecessary duplication of testing. Several centres such as the Animal Welfare Information Center (AWIC, http://www.nal.usda.gov/awic.alternatives/alternat.htm) provide useful information on literature searching and alternatives. Policy Guideline Number 12 in the Animal Care Resource Guide, issued in June 2000 (21), was developed to support the US *Animal Welfare Act*, and requires principal investigators in animal research to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals used, and to provide a written narrative description of the methods and sources used to determine that alternatives were not available. All the Three Rs must be addressed, and not just absolute replacement methods. Policy Guideline 12 should also be implemented in European countries. Another idea is to increase accountability for the use of animals by stimulating PhD students to include a paragraph in their dissertations on the justification of animal use, and a formal indication of why alternative methods were not employed.

A further aspect of education and training concerns improving the competence of those performing experiments, in the handling and use of laboratory animals. Experienced researchers and caretakers cause less stress to animals and this can lead to a reduction in test variability. The Federation of European Laboratory Animal Science Associations (FELASA) has identified four categories of professionals that should receive education and training (22): a) those taking care of animals; b) those carrying out animal experiments; c) those responsible for directing animal experiments; and d) laboratory animal science specialists. Education and training are now mandatory in some EU countries. In The Netherlands, for example, only scientists with an academic degree in one of the biomedical disciplines and who have followed a 3-week course in laboratory animal science are permitted to perform animal experiments.

Ethical review

Proposals to carry out animal experiments are assessed by ethical review committees in most countries. One of the aims of the ethical review process is to ensure that the essential minimum number of animals is used, and that alternative procedures requiring fewer animals have been considered. To guarantee that experiments are scientifically sound in terms of design and statistical analysis, it has been recommended by Balls *et al.* (18) that a "named statistician" should be appointed to all animal ethics committees, analogous to named veterinarians. Although official statistics are not available, it might be expected that the establishment of animal ethics committees has generally resulted in the use of a lower number of animals per protocol.

Reduction of breeding surplus

Laboratory animals are mostly purchased from commercial breeders, or are obtained from an institute's own breeding facilities. In order to adequately provide for the demand of researchers, breeders tend to have animals in stock. When the animals are not sold, they are considered as surplus to requirements and killed. The number of surplus animals varies according to the circumstances, but for rodents, the surplus may exceed 10% of the total number of animals bred. Data on such surpluses are not included in annual reports on animal use. Striving for zero surplus animals is not a realistic option, as the precise numbers of animals required cannot be predicted beforehand. However, finding a better balance between supply and demand might reduce the surplus percentage to a more acceptable level. Sharing information on the availability of laboratory animals in specific databases could be a solution to the problem of breeding too many animals. Furthermore, the number of discarded animals is largely accounted for by using animals from a single sex, mostly males. When both males and females are used, for example, in an appropriate factorial experimental design, more information about possible effects on sex can be collected and this would also reduce breeding surplus (M. Festing, personal communication, 2004).

Re-evaluation of laboratory animal specifications

Experimental protocols often specify the characteristics of the animals to be used, in order to limit test variability. For example, specifications for sex, age and/or body weight are laid down in test guidelines for regulatory purposes. In toxicity testing, for instance, male animals are preferred, as they have no sexual cycle. However, the narrower these specifications are, the fewer animals from litters will be suitable, and more surplus animals will be produced. Reduction in the number of animals can therefore be achieved by critically analysing test specifications. For example, it might be more effective to use an age range in a test specification, instead of body weight. This is already partly achieved, as inbred strains are usually supplied by age, whereas outbred stocks are sold by weight (M. Festing, personal communication, 2004).

The re-use of animals and longitudinal studies

The re-use of animals for research can be defined as the sequential use of the same animal for unrelated animal experiments (23), and can be seen as an approach to reducing the overall number of animals required. However, there might be both scientific and ethical reasons for discouraging this practice. Re-use is sometimes accompanied by a lack of standardisation of animals that have been used before, as the effects of previous treatments may not always be clear. This might lead to the need for larger numbers of animals in the second study (23). On the other hand, animals that have been used as control animals in a previous study, in which they were not exposed to factors that might have lasting effects, can be considered for use in a subsequent experiment. The re-use of animals that have been trained to co-operate in routine laboratory procedures might be less stressful overall than when new individuals have to be recruited and trained. However, re-use must be avoided if it results in the substantial accumulation of pain and suffering. This moral principle is regulated in legislation in both the US *Animal Welfare Act* (24) and in the EU *Directive 86/609/EEC*.

Several procedures allow for following animals over time, where animals could act as their own control. This makes the use of parallel control groups without treatment, as well as interim kills, unnecessary. Examples of these techniques are the instrumentation of an animal to provide easy access for sampling (for example, with permanent vascular cannulae), the real-time measurement of physiological functions (for example, with telemetric devices) and the extra-corporal monitoring of pathophysiological processes (for example, by biophotonic imaging). However, since the animals used might be subjected to additional pain and suffering, these techniques may have negative effects in terms of animal welfare.

Extra-experimental Reduction

Extra-experimental reduction refers to developments that are not related to animal experiments, but which, as a spin-off, result in a reduction in the use of laboratory animals. Safety, high quality and efficiency are key concepts in these developments.

Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP)

Good Laboratory Practice (GLP) consists of administrative procedures that cover the organisation of test facilities and the conditions under which studies are carried out. The purpose of GLP is to ensure the generation of high quality and reliable data related to regulatory testing. Good Manufacturing Practice (GMP) is used by pharmaceutical companies, and incorporates regulations that ensure the quality, safety and efficacy of the medicines or biologicals produced. Most countries have issued regulations on GLP and GMP, as well as implementing a compliance-monitoring programme through laboratory inspections and data audits. GLP and GMP contribute to reduction, because high quality and reliable data limit the frequency of doubtful results and the need for re-testing; GLP guarantees consistency in testing, and protocols are specified in standard operating procedures (SOPs), based on best practice. Lastly, GLP and GMP permit the international harmonisation of test procedures and contribute to the mutual acceptance of data.

Harmonisation of guidelines

Substantial numbers of animals are used for regulatory purposes. Regulations are issued by national and international regulatory bodies, such as the European Commission, the US Food and Drug Administration (FDA) and the US Environmental Protection Agency (EPA). The regulations and underlying test programmes/specifications may differ between regulatory bodies. As a result, companies that want to launch their products in several countries must comply with a variety of test requirements. Quite often, this results in additional or duplicate animal tests. Lack of harmonisation is disadvantageous to globally-active companies, particularly because of barriers to trade, but also because of the additional and unnecessary use of animals. Several activities have been initiated to harmonise the various types of regulations. The International Conference on Harmonisation (ICH) is a co-operation between regulatory authorities in Europe, Japan and the USA and pharmaceutical industry, with the aim of achieving greater harmonisation in the interpretation and application of requirements for product registration and reducing or obviating the need to duplicate testing. The Pharmacopoeia Discussion Group is an initiative with a similar aim and represents the same groups. Unfortunately, harmonisation activities are very tedious and tend not to progress very well. Another approach is to develop agreements on the mutual recognition and acceptance of test data.

New research and testing strategies

In some areas of biomedical research, the emphasis on the use of animal models is closely linked to the research strategy chosen. A change in strategy might lead to a different appraisal of the role of animal models and, consequently, to reductions in the

numbers of animals used, for example, in vaccine quality control and in toxicological testing.

Vaccine quality control is carried out within a regulatory framework, and guidelines for quality control tests are described by (inter)national regulatory bodies such as the FDA and US Department of Agriculture (USDA) for the USA, and the European Pharmacopoeia for the Member States of the Council of Europe. Each vaccine batch produced used to be regarded as a unique product, and each individual batch was tested for safety and potency. Extensive numbers of animals were used. However, vaccine batches are not as unique as was assumed. Vaccine manufacturers produce batches of tetanus vaccine originating from the same starting material (the seed lot), and perform a standardised production process. Consequently, the batches are not independent of each other. This observation has resulted in a new concept of quality control, which focuses on consistency in production rather than on the quality of an individual batch. Consistency testing emphasises in-process control, which is predominantly based on biochemical and physicochemical tests, rather than on final product testing, which requires animals. This change of concept will lead to a reduction in the number of animals used for this purpose.

New strategies have also been developed in toxicological testing. The aim of toxicity testing is to evaluate the risks of exposure to a chemical. Traditionally, the research strategy to assess risk characteristics consists of four consecutive phases: hazard characterisation, hazard assessment, exposure assessment, and finally, risk assessment. Consequently, the toxicological profile of each chemical is evaluated, even if it becomes clear on completion of the tests that the likelihood of exposure is so low that the minimal toxic dose will not be reached in real life. A more realistic approach, which would require less testing and therefore fewer animals, would be to first assess exposure levels. This is the reversed toxicology approach. Only in the case of potential exposure would hazard have to be assessed before assessing risk. Related and obvious examples in toxicity testing are the Fixed Dose procedure, the Acute Toxic Class method and the Up-and-Down procedure as alternatives to the classical LD50 test. The OECD has accepted these methods (25–27), which are less precise but yield sufficient data for the purpose of classifying chemicals in terms of their potential hazard. These procedures involve both reduction and refinement. Another example of a new research strategy in toxicology that will lead to a reduction in the use of animals, is to use a tailor-made testing programme for a particular chemical instead of the rigid testing scheme that is currently required. An opening to this approach has been given in the White Paper on a new EU Chemicals Policy (28), now known as the REACH system.

New production strategies

The use of animals in biomedical research and testing can be related to the production of (biological) products. A reduction in the number of animals might be achieved when production techniques are optimised or modified. Polyclonal antibodies (Pabs) are often used in diagnostic testing and in immunological studies. Traditionally, these products are produced by immunising rabbits or mice with the antigen, then bleeding the animals several weeks after the last immunisation. In a number of cases, particularly when antibodies are required toward highly preserved mammalian antigens, it might be favourable to obtain polyclonal antibodies from the egg yolk of an immunised chicken. Apart from the refinement aspect (no animals have to be bled), this approach also offers a reduction approach, as the total amount of Pabs collected from one chicken is equivalent to the use of about 10 rabbits (29). There are a few drawbacks to the use of chicken Pabs, such as its inability to activate complement (29) and the specific housing conditions required by these animals. Another example comes from hormone production. Traditionally, these products were obtained from animal material or from donated human tissue, but large differences could be expected between batches. Several of the hormones are now produced by rDNA technologies, resulting in standardised products. This allows for the use of *in vitro* methods for quality control, particularly for potency testing, and for less emphasis on safety testing. As part of the safety tests are based on animal models, the change from conventional to rDNA products has offered an opportunity to minimise the number of animals required.
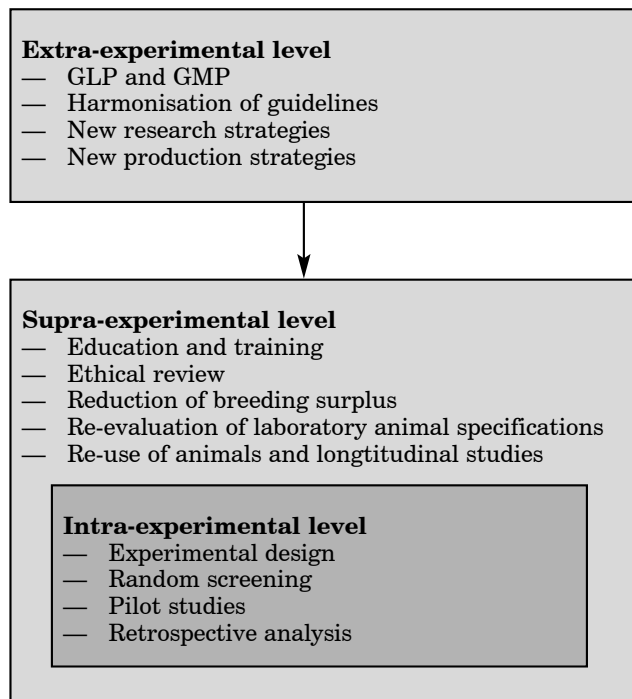
Conclusion

The various reduction strategies can be represented as in Figure 1. Ideally, when considering reduction alternatives, all research questions and proposals should focus on an integrated approach involving all three types of reduction.

A New Definition of Reduction

Earlier, we discussed definitions of reduction focusing on the intra-experimental approach. Although Smyth (4) defines reduction in more general terms, his definition includes some concepts that need further explanation. Probably the most comprehensive definition is that of Festing *et al.* (7): *"ways of obtaining comparable levels of information from the use of fewer experimental animals, or of obtaining more information from a given number of animals, so that fewer animals are needed to complete a given*

Figure 1: Reduction strategies or approaches at three levels

Extra-experimental level
— GLP and GMP
— Harmonisation of guidelines
— New research strategies
— New production strategies

Supra-experimental level
— Education and training
— Ethical review
— Reduction of breeding surplus
— Re-evaluation of laboratory animal specifications
— Re-use of animals and longtitudinal studies

Intra-experimental level
— Experimental design
— Random screening
— Pilot studies
— Retrospective analysis

research project". In this definition, reduction can only lead to comparable levels or higher amounts of information. However, we have given examples, particularly in extra-experimental approaches, in which reduction might even be possible and acceptable when limiting the amount of information obtainable from that given number of animals. Therefore, a new definition should not include the level of information needed, but only the condition that the reduced number of animals allows for meeting the scientific needs of the exercise. Hence, the following definition of reduction is suggested after a consensus among several experts (C.F.M. Hendriksen, J. de Boo, M.F.W. Festing, H.M. Buchanan-Smith, A.E. Rennie, F. Zucco, M. Ritskes-Hoitinga, K. Cüssler and A. Knight, personal communication, 2004) as:

"Any approach in scientific research, product testing or education that leads directly or indirectly to a decrease in the number of animals used while meeting the scientific requirements."

Acknowledgement

References

1. Russell, W.M.S. & Burch, R.L. (1959). *The Principles of Humane Experimental Technique*. 238pp. London, UK: Methuen.
2. Anon. (1986). European Directive of 24 November 1986 on the Approximation of Laws, Regulations and Administrative Provisions of the Member States Regarding the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Directive 86/609/EEC). *Official Journal of the European Communities* **L358**, 1–29.
3. Anon. (1986). *Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS 123)*, 51pp. Strassbourg, France: Council of Europe.
4. Smyth, D.H. (1978). *Alternatives to Animal Experiments*. 216pp. London, UK: Scolar Press.
5. Festing, M.F.W. & Lovell, D.P. (1996). Reducing the use of laboratory animals in toxicological research and testing by better experimental design. *Journal of the Royal Statistical Society* **58**, 127–140.
6. Anon. (1993). *Responsibility in The Use of Animals in Medical Research*. Guidance issued by the Medical Research Council, London.
7. Festing, M.F.W., Baumans, V., Combes, R.D., Halder, M., Hendriksen, C.F.M., Howard, B.R., Lovell, D.P., Moore, G.J., Overend, Ph. & Wilson, M.S. (1998). Reducing the use of laboratory animals in biomedical research: problems and possible solutions. *ATLA* **26**, 283–301.
8. Balls, M., Festing, M.F.W. & Vaughan, S., eds. (2004). Reducing the use of experimental animals where no replacement is yet available. *ATLA* **32**, Suppl. 2, 1–104.
9. Fry, D. (2004). Reduction by well-defined objectives. *ATLA* **32**, Suppl.1, 241–244.
10. Festing, M.F.W. (1997). Experimental design and husbandry. *Experimental Gerontology* **32**, 39–47.
11. Wilgenburg, H. van, Van Schaick Zillesen, P.G. & Krulichova, I. (2003). Sample power and ExpDesign: tools for improving design of animal experiments. *Laboratory Animals* **32**, 40–44.
12. Mead, R. (1988). *The Design of Experiments*. 634pp. Cambridge University Press, New York.
13. Koudandé, O.D., Thomson, P.C. & Van Arendonk, J.A.M. (1999). Model for population growth of laboratory animals subjected to marker-assisted introgression: how many animals do we need? *Heredity* **82**, 16–24.
14. Festing, M.F.W. (1992). The scope for improving the design of laboratory animal experiments. *Laboratory Animals* **26**, 256–267.
15. Hendriksen, C.F.M., Van der Gun, J.W., Marsman, F.R. & Kreeftenberg, J.G. (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**, 353–362.
16. Festing, M.F.W. (1995). Reduction in animal use 35 years after Russell & Burch's principles of humane experimental technique. *ATLA* **23**, 51–60.
17. Festing, M.F.W. & Altman, D.G. (2002). Guidelines for the design and statistical analysis of experiments

using laboratory animals. *ILAR Journal* **43**, 244–258.

18. Balls, M., Goldberg, A.M., Fentem, J.H., Broadhead, C.L., Burch, R.L., Festing, M.F.W., Frazier, J.M., Hendriksen, C.F., Jennings, M., van der Kamp, M.D., Morton, D.B., Rowan, A.N., Russell, C., Russell, W.M.S., Spielmann, H., Stephens, M.L., Stokes, W.S., Straughan, D.W., Yager, J.D., Zurlo, J. & Van Zutphen, B.F. (1995). The three Rs: the way forward: the report and recommendations of ECVAM Workshop 11. *ATLA* **23**, 838–866.

19. Pössnecker, A. & Cüssler, K. (1998). Evaluation of the relevance of the target animal safety test for the quality control of veterinary immunological medicinal products. *ALTEX* **1**, Suppl., 71–75.

20. Knight, P.A. & Roberts, P.A. (1987). An evaluation of some proposals for a reduction in the number of animals used for the potency testing of diphtheria and tetanus vaccines. *Journal of Biological Standardization* **15**, 165–175.

21. Anon. (2002). *Consideration of Alternatives to Painful/Distressful Procedures*. Animal Care Resource Guide Policy Number 12, 3pp. Riverdale, MD, USA: USDA/APHIS. Website http://www.aphis.usda.gov/ac/policy/policy12.pdf.

22. Nevalainen, T., Berge, E., Gallix, P., Jilge, B., Melloni, E., Thomann, P., Waynforth, B. & Van Zutphen, L.F.M. (1999). FELASA guidelines for education of specialists in laboratory animal science (Category D). *Laboratory Animals* **33**, 1–15.

23. Fentener van Vlissingen, J.M. (1999). The re-use of animals for research — a humane endpoint? In: *Humane Endpoints in Animal Experiments for Biomedical Research*. Hendriksen C.F.M. & Morton D.B. (eds.) 150pp. London, UK, The Royal Society of Medicine Press Ltd.

24. Anon. (1966). US *Animal Welfare Act*. Public Law 89-554 of 24 August 1966. United States Code, Title 7, Sections 2131–2159. Washington, DC, USA: US Congress.

25. OECD (2001). *OECD Guidelines for the Testing of Chemicals No. 420: Acute Oral Toxicity — Fixed Dose Procedure*. 14pp. Paris, France: Organization for Economic Cooperation and Development.

26. OECD (2001). *OECD Guidelines for the Testing of Chemicals No. 423: Acute Oral Toxicity — Acute Toxic Class Method*. 14pp. Paris, France: Organization for Economic Cooperation and Development.

27. OECD (2001). *OECD Guidelines for the Testing of Chemicals No. 425: Acute Oral Toxicity — Up-and-Down Procedure*. 26pp. Paris, France: Organization for Economic Cooperation and Development.

28. Anon. (2001). *White Paper on the Strategy for a Future Chemicals Policy (COM [2001]88 final)*. Brussels, Belgium: European Commission. Website http://europa.eu.int/comm/environment/chemicals/whitepaper.htm.

29. Schade, R., Calzado, E.G., Sarmiento, R., Chacana, P.A., Porankiewicz-Asplund, J. & Terzolo, H.R. (2005). Chicken egg yolk antibodies (Ig-Y-technology): a review of progress in production and use in research and human and veterinary medicine. *ATLA* **33**, 129–154.