Replacement, Reduction and Refinement in Biomedical Research with Particular Emphasis to the –omics Technologies

Coenraad F.M. Hendriksen

Summary
Although no exact figures are available, it is estimated that more than 100 million laboratory animals are used each year for experimental and other scientific purposes world wide. Even though animal research remains indispensable for progress in medical research, consensus is spreading over the need for replacement, reduction and refinement of animal use, the so-called 3R’s Principle of Russell & Burch. Apart from moral considerations, there are also scientific and economical reasons for the increased interest in 3Rs and these will be discussed. The 3R’s Principle includes a diversity of new methods and research strategies, some of which have become a routine practice in laboratories. A particular interesting approach is now coming from the area of molecular biology. Progress in –omics technologies enable the expression of large numbers of individual genes and proteins to be monitored simultaneously. This makes the technology an interesting tool in drug development and the hazard and safety assessment of chemicals. It is also believed that –omics might have potential to reduce and refine the use of laboratory animals. This paper will review these potentials as well as the obstacles that might limit the implementation of –omics technologies in research and testing.

Introduction
People seldom realise how closely everyday life is intertwined with the use of laboratory animals. Diagnostic testing, drug development, immunoprophylaxis, or food safety: knowledge in all these fields has partly been acquired through research on animals. As a consequence, large numbers of laboratory animals have been and are still being used in biomedical research. Detailed figures on animal use are reported every three years by the Member States of the European Union. The statistics of the European Union show that 10.7 million animals were required for scientific purposes in 2002. It is estimated that world wide figures account to over 100 million animals per year. Figure 1 and Figure 2 shows a breakdown of the EU statistics with regard to scientific purposes and animal species being used in 2002. (European Commission, 2005).

Figure 3 shows trends in animal use in three European Countries that have statistics already since long. From this figure it can be seen that a period of substantial reduction (1978 – 2003) is followed by a slight increase, probably due to the popularity of genetically modified animals that became available in the nineties.
Although many consider animal experimentation and testing as vital for progress in biomedical research, there also is a strong social, political and scientific pressure to Replace, Reduce and/or Refine the use of animals, the reasons that are given below.

- Replacement contains the replacement of vertebrates with non-vertebrate organisms, tissue cultures or non-biological material.
- Reduction covers the minimisation of the number of animals needed to obtain a valid result, e.g. by advanced experimental design and statistical analysis or by using in vitro pre-screening tests.
- Refinement deals with minimising pain and suffering for the animal’s involved and maximising welfare (de Boo et al., 2005), e.g. by environmental enrichment, use of analgesics or application of humane endpoints.
- The principle of the 3Rs was first described by Russell and Burch in 1959 in their fascinating book ‘The Principles of Humane Experimental Technique’ (Russell & Burch, 1959). The elegance of the principle is that it allows a combination of scientific progress and improvement of ethical principles in research and testing. The principle has found widespread acceptance, both in the animal welfare community and in scientific circles and has become the leading principle in the existing laws, regulations and guidelines on animal experimentation, such as the EU law on animal experimentation or the testing guidelines of WHO.

**Reasons to Replace, Reduce and Refine Animal Use**

The next paragraph will discuss the reasons for an increased interest in the 3R’s principle.

- Ethical reasons. The use of laboratory animals in biomedical research is a controversial issue. In many European countries and in the US, animal experiments are heavily criticised. Results of an enquiry about animal experimentation, held in the UK in (Aldhous et al., 1999) showed that a substantial part of the individuals interviewed disagreed with using animals when no details of the research purpose were given. If research details were provided, responses become more divers. Most people agreed when animals are needed for highly relevant medical problems, such as the development of cytostatic drugs in case of children’s cancer. On the other hand, most people disagreed with animal use when the purposes are trivial, such as testing of cosmetic products. The enquiry also showed that acceptance rates decrease if the specific research is involved with severe pain and suffering. Most European countries and the US now require applications for animal research to be approved by animal ethics committees. These committees balance the potential social and scientific benefits of the proposed experiment against the costs for the animals in terms of pain and suffering. Furthermore, some European countries now assign an intrinsic value to animals, meaning that the individual animal should be protected for the sake of the animal itself, rather than for human ethical values.
Economical reasons. Costs of animal experimentation increase. Performing animal studies according to Good Laboratory Practice (GLP) principles require the use of Specific Pathogen Free (SPF) animals, qualified personnel, housing in facilities with standardised environmental conditions, barrier systems, air filtering, etc. Therefore, housing costs generally account to almost 40% of total experimental costs. Expenses will further increase in Europe when the new housing requirements of the Council of Europe, allowing fewer animals per square meter, will come into force.

Scientific reasons. Animal experiments are dealing with a number of shortcomings. Studies are difficult to standardise. It is well known that experimental procedures, such as intraperitoneal injection (Walvoort, 1991), are prone to large variation. In addition, many factors such as handling of the animals may influence the experimental outcome. Standardisation problems might also occur when different strains of animals are used. For instance, it has been well documented that testing a batch of vaccine in different mouse strains, even when using a reference preparation, might result in different estimated batch potencies (Hardegree et al., 1972). Next, animal models generally provide little mechanistic information. Due to the complexity of the system biological processes are difficult to monitor and consequently models often behave like black-boxes. Causality between treatment and effect can be shown, but why there is causality often remains unclear. Probably, the biggest limitation of results obtained in an animal experiment is that these are difficult to extrapolate to the human situation. A human being is not a big rat. The results of a carcinogenicity study in rats are relevant for rats, but whether this is also true for humane beings remains an open question. These problems are clearly illustrated in the recent phase 1 tragedy in the UK, when 6 volunteers were injected with a therapeutic monoclonal antibody that was previously being tested in rodents and non-human primates without showing adverse effects.

3R's Alternatives
Without claiming that 3R's alternatives offer an overall solution for the problems specified above, there is a widespread believes that these methods include a number of advantages. 3R's methods, particularly when replacing animal use, are generally more cost- and time effective, require less expensive facilities and allow us to study biological processes in more detail. Replacement alternatives most frequently used include tissue cultures, invertebrate organisms, in silico models such as computer modelling and physicochemical- and immunochemical methods. A replacement alternative that has saved substantial numbers of animals is the use of continuous cell lines for the production of viral vaccines. The traditional procedure was to culture the virus in primary cells obtained from NHPs or rabbits or even in vivo culturing (culturing in the brain of suckling mice) as is the case for the rabies vaccine. Other striking replacement alternatives are the LAL (using the extract — Limulus Amebocyte Lysate — from blood cells of the horseshoe crab) test for pyrogenicity testing instead of using rabbits, the in vitro methods instead of ascites induction in mice for production of monoclonal antibodies and the high-throughput in vitro systems for drug characterisation and evaluation.
However, although many replacement alternatives are now common practice in the laboratories, also these methods are facing their limitations. Some are inherent to the system being used. Thus, it will never be possible to simulate systemic processes such as blood pressure, CNS involvement or hormonal regulation in relative simple cell culture systems. Furthermore, also in vitro systems lack full understanding of mechanistic pathways. Finally, extrapolation still is a big hurdle, even in case human cells are used for culturing. Dose levels, distribution, uptake and effects of metabolic processes in cell cultures differ from real life situation, making these models very valuable for pre-screenings purposes, but of limited value for final conclusions. This makes that animal studies are given a different position in research strategies: from research model to confirmation model (the ‘proof-of-the pudding’).

This different position might be supported by new and highly innovative scientific developments from the area of stem cell research and molecular biology. The latter one will be discussed in more detail.

New developments in Molecular Biology
Genetically modified animals

Recent developments in molecular biology have opened the door to many new applications in biomedical research. The ‘construction’ of genetically modified animals is probably the most striking example in terms of laboratory animal use. By insertion of new genes (quite often human resulting in ‘humanised’ animals) or by knocking out existing genes studies can be performed towards the role of these genes in specific biological pathways. Nowadays, thousands of transgenic (Tg) animal strains are available and these models have offered interesting tools to clarify disease processes and improve drug development. However, the use of Tg animals is also linked with several inherent problems, both moral and scientific. Apart from the increase in the use of laboratory animals that has been a spin-off of the popularity of Tg strains; genetic modification has also resulted in an increased number of animal strains with intrinsic health problems, such as early-life tumour development or birth defects. Another issue for moral concern is the large number of animals bred that are genetically unsuited for the experiment. Breeding surplus often accounts to over 50% of the offspring. Next, the question frequently asked is whether a mouse with one inserted humane gene offers a good model for studying human diseases. Biological phenomenons are often based on multi-gene interactions. Replacing one gene might result in compensation mechanisms by the other genes. Replacing multiple genes in the genome is not an option as this will result in genetic instability. Therefore many believe that Tg animals will remain useful but limited ‘tools’ within the laboratory.

-omics technologies

One of the most promising scientific developments in biomedical research is the –omics technologies. The –omics, in fact, include a number of approaches for which many definitions exist. In general, it covers a broad research area aimed at the identification and characterisation of the genes of living organisms as well as their products, such as proteins and metabolites,
and studying their functionality by using specific in vitro techniques such as DNA micro-array and gel-electrophoresis. Progress in this field now enables the expression of large numbers of individual genes (up to 40,000), proteins and metabolites to be monitored simultaneously. The promise is reflected in the large financial budgets that are made available word-wide as well as in the –omics centres that have been established in many countries. Based on the scientific techniques used, -omics technologies can refer to mRNA profiles produced in a cell (transcriptomics), cell protein profiles and characteristics (proteomics) or metabolomics (metabolite profiles in biological samples such as urine, saliva and blood plasma). An important supporting tool in –omics is the area of bioinformatics. This discipline should allow efficient data storage and subsequent analysis for detecting subtle relationships between gene-activity, mRNA, proteins, metabolites on one hand and (patho-) physiological processes on the other hand (Davidov et al., 2003).

The –omics have created a stream of opportunities, such as efficient drug development (pharmacogenomics) (Walgren et al. 2004), the development of new prophylactic and therapeutic vaccines (immunogenomics) and better hazard and safety assessment of chemicals (toxicogenomics) (Tenant, 2002).

-omics and the 3Rs
Until now little attention has been given to the way –omics can contribute to humane research without or with limited numbers of animals (Jenkins et al., 2002). There may be many although predictions about their impact are yet hard to be made. The promise is linked to one of the inherent advantages of –omics technologies; to increase our understanding of the fundamental nature of biological processes. The 3R spin off when using –omics technologies can be summarised as follows:

Refinement of animal experiments can be attained through a more rational choice of the best animal model to be used and the acquisition of more and more specific information and the application of early pre-clinical endpoints in animal studies, thus limiting pain and distress for the animals. Furthermore, application of metabolomics would enable the use of less invasive procedures e.g. by testing urine instead of blood. Reduction of laboratory animal use can be achieved through a better general set-up of animal experiments and a rational and science-based selection of the animal species/strain to be used. In addition, the use of –omics will result in more information out of fewer animals. The potential of –omics to Replace animal experiments as such are still limited but might increase in the future. For now, -omics is a technology that complements traditional research models (animal as well as in vitro). However, in future It might improve existing, but imperfect in vitro models to make these more relevant to the in vivo situation, allow the use of invertebrate organisms that, based on comparative genetics, have shown to be a good model for the research question, or allow the use of low doses, e.g. in pharmacology and toxicology, for use in human volunteers.
Conclusions
Although 3R’s alternatives have demonstrated their value in biomedical research, there still is scepticism in the scientific and regulatory community. The reason might be the ‘high fidelity fallacy’ (Russell & Burch, 1959), which means that because animals are more like us than cells in culture, more value is given to results of animal studies than to data of in vitro studies. Tedious validation studies are therefore often required to demonstrate unequivocally that the 3R alternative is at least as good as the classical animal model. You might wonder how many animal models would have been accepted if these had been validated likewise. The -omics technologies will contribute to better understand biological processes. This might improve the relevance of the 3R’s models and, consequently, might speed up the validation process and ultimately the acceptance of the 3R methods. It might help if some case studies be performed as a proof of concept.

References