Netherlands perspective on 3R

Netherlands perspective on 3R alternatives to Animal Experiments

Replacing, Reducing, and Refining

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While gaining insight into the mechanisms in human health processes and environmental impacts has led to increasing use of animal experiments, opposition to animal experiments has also continued to increase. Yet up until comparatively recently, animal experiments have been the most effective means of assessing impacts on human health and safety, even though these methods have their limitations.

3R alternatives

In the last few decades, the debate has focused on whether it is possible to develop scientifically validated alternatives to animal experiments. A new generation of alternative methods has been developed which in many cases give better insight into mechanisms in human health processes and of environmental impacts. Collectively referred to as 3R methods or alternatives, these methods are designed to replace, reduce, and refine animal experiments. They include replacing animal experiments by using human stem cells and tissue cultures and by new innovative technologies such as '-omics' and systems biology, and by making a paradigm shift to develop research strategies focusing on 3R alternatives. These methods also include reducing and refining animal experiments by using innovative techniques, which require fewer animals to collect more data in a multidisciplinary research environment. The debate has now shifted to how to strengthen and speed up the process of further developing and implementing 3R alternatives.

Netherlands study

To this end, ZonMw, the Netherlands organisation for health research and development, with funding from the Netherlands Government commissioned the Netherlands Knowledge Centre on Alternatives to Animal Use (NKCA) to identify priority research areas for the development, implementation, and acceptance of 3R methods. This study was carried out by NKCA in 2010-2011 with the aim of supporting ZonMw and other organisations funding research in the Netherlands in programming research effectively to advance the application of 3R alternatives to animal experiments in both fundamental and applied research.

Legislation

The Netherlands has long been in the forefront in safeguarding the welfare of laboratory animals and in restricting the use of animal experiments. In 1977, the Law on Animal Experiments was enacted which prohibits animal experiments unless there are no alternative methods. Strict regulations apply to researchers and research organisations are required to obtain prior approval for animal experiments. Such experiments must be carried out under strictly controlled conditions and on as small a scale as possible. Since 1977, there has been a 60% reduction in animal experiments in the Netherlands.

Based on strong research tradition in developing 3R methods, the Netherlands has played a leading role in the debate in Europe. In fact, the standard established in the Netherlands is now being taken up to become the standard for all EU Member States under the EU Directive on Animal Experiments (2010/63/EU). This directive, which aims to protect animals used for scientific purposes, will give a significant stimulus to further implementation of 3R alternatives and will lead to harmonisation of national legislation on the use of animal experiments. While there are initiatives in other countries, international acceptance and implementation of alternatives to animal experiments is lagging behind progress made in the Netherlands.

Prioritising criteria

Within the scope of current research areas in the Netherlands, there are promising areas to stimulate 3R alternatives to animal experiments. To this end, criteria and a framework are needed to assess ongoing and new research in terms of relevance for the application of 3R alternatives. Formulating such a prioritising scheme was a key task of the Study on Alternatives to Animal Experiments, and was carried out by an expert committee comprising representatives of stakeholders in government, research, industry, and civil society.

The objective was to establish those areas that provide the greatest opportunity for 3R methods directed to reducing animal experiments and discomfort to laboratory animals as much as possible. These criteria are to some extent pragmatic and incorporate ethical, scientific, and political and practical considerations that are not necessarily given equal weight.

While derived for the situation in the Netherlands, these prioritising criteria can be applied in prioritising research activities for government authorities, researchers/scientists, companies, and NGOs/social stakeholders and to link optimally to international developments.

The expert committee devised a set of criteria that have been grouped as follows:

- Scale of the problem
- Chance of success
- Impact of further development of 3R methods.

Scale of the problem

This criterion puts extra weight on ethical considerations by giving preference to research areas where large numbers of animals are used and species that are evolutionary more complex. For instance, neurophysical research places greater pressure on the use of neurophysiologically more complex animals, particularly non-human primates. Preference was also given to research areas where there are indications of increased discomfort to laboratory animals.

Chance of success

In considering the chance of success, weight was given to scientific and practical aspects, with priority to those research areas considered to have the greatest chance of success in replacing, reducing or refining animal experiments. These areas include those where 3R alternatives have been incorporated in research planning, and areas where demonstrable progress has been made in implementing 3R methods in ongoing research. Another consideration was the potential to introduce 3R methods in the short term.

Impact of further 3R developments

In selecting priority research areas, attention was given to potential spin-offs in relation to investment in 3R methods. In this respect, preference was given to areas where a radical change in research strategy offers potential to increase research relevance and also an effective route for eliminating animal experiments. Thus, priority was given to research areas using innovative techniques such as '-omics', stem cell culturing and physicochemical methods. These techniques have wider application in more research areas and come closer to human patho-physiology.

Consideration was given to the impact in relation to the effort required in developing alternative research methods, thus to areas in which 3R developments generate greater spin-offs in relation to the cost. Such spin-offs, however, also depend on knowledge and infrastructure and can contribute to strengthening other criteria in the prioritising scheme.

In terms of impact, preference is also given to research areas in which the Netherlands has some international standing and where development and implementation of 3R methods would contribute to innovation and progress in the international arena. In this respect, preference was given to research areas with spin-off, for instance, for regulatory research.

Priority areas for 3R alternatives

Application of the prioritising criteria led to identification of four priority research areas in the Netherlands where application of 3R alternatives is likely to have the greatest chance of success in the foreseeable future. The list of research areas and their groupings are based on an analysis of research areas using animal experiments in the Netherlands. This list is published by the Food and Consumer Product Safety Authority (VWA)[1], responsible for monitoring research organisations involved in animal experiments.

The priority areas identified are:
- Fundamental research on cancer and other diseases
- Fundamental research on the development of pharmaceuticals
- Quality control of pharmaceuticals and medicines including vaccines and serums
- Risk assessment of chemical substances.

Fundamental research on cancer and other diseases

- **Do less animal breeding with better techniques**
- **Structure research to use stem cells and tissue cultures in a tiered approach**
- **Use and improve on developments in the '-omics' and biomarkers**

Cancer research is becoming increasingly more complex but still much research depends on animal experiments. However, use of laboratory animals is decreasing in the initial research phases of collecting factual evidence, while use is increasing in the last phases in confirming the existence and progression of a cancer. Thus, in terms of alternatives for animal experiments, replacement will have impact on the initial research phases while the focus in the latter phases will be on reducing and refining animal experiments. However, in developing these techniques, knowledge and research from different disciplines needs to be integrated and space created for further innovation. Knowledge from fundamental research needs to be shared with knowledge from applied research and clinics in developing equipment and technology for use in biomedical research as in translational research.

Within this framework, more attention will need to be given to extracting more information from each animal experiment by means of more extensive use of analytical techniques such as non-invasive measuring techniques. However, efficient use of advanced techniques and equipment places increasing demand on closer cooperation between research institutes, compounded by practical considerations of sharing expenditure especially in the current climate of budget cuts.

Where animal experiments are used, there is a need to develop techniques that refine them and that bring humane endpoints in relation to scientific endpoints. In addition, efforts are needed to develop real-time monitoring techniques and non-invasive measuring techniques.

Cell culturing techniques

The most significant replacement for animal experiments in recent years has been the development of cell culturing techniques, which offer numerous advantages over animal experiments. In some cases, tissues and organs from embryonic stem cells offer better predictive value for humans, with the advantage of better standardisation and extrapolation of findings to humans than in animal experiments. In addition, cell culturing techniques have advantages in terms of availability of material and the storage of different types of cells. While the use of human stem cells is highly regulated internationally, used in combination with biomarkers offers good potential for reducing animal experiments.

Furthermore, there is a need for better alignment of stem cell research, for example by setting up national coordination and registration points for tissue banks would

[1] Annual Report on laboratory animal and animal experiment statistics

improve the quality and availability and thus improve the research.

'-omics'

Another technique in investigating cellular processes is genomics. In cancer research, for instance, '-omics' are used to map cellular processes and to study cellular mechanisms. However, some experts consider that '-omics' research may lead to an increase in animal experiments and in the animal species used. Others suggest that more knowledge about mechanisms could ultimately lead to replacing, reducing, and refining animal experiments.

-Omics offer opportunities to refine animal experiments for use in, for example, developing early biomarkers for screening for higher expression of cancer genes. Biomarkers offer the potential to use micro-dosing thus reducing discomfort to laboratory animals. Comparative genomics can be used in selecting animal models for specific research as screening method in tiered testing approach and as an easy and sensitive biomarker. While '-omics' are not a replacement for animal experiments, used in combination with *in vitro* methods enhances these methods.

Fundamental research on the development of pharmaceuticals

- **Use new 3R methods in developing neuropharmaceuticals and biological products**
- **Make closer links between medical and fundamental research in developing new pharmaceuticals**

Many experts consider that pharmaceuticals cannot be developed without the use of animals because of the need to protect the safety of patients. However, a favourable development is the reduction in animal experiments as the standard and their use mainly for complex issues in assessing the effects of a medicine on the whole body. This is a result of the range of alternatives now available including cell tissue culturing, computer modelling and physicochemical methods, and which are used to study aspects of a larger problem. With mounting pressure to eliminate animal experiments, it is anticipated that more technical innovations will be developed. It is essential that regulatory authorities and industry are involved in an early stage in this process.

Nevertheless, more 3R alternatives can be used than is now the case. While there is a tendency to continue using animal experiments, more creative and multidisciplinary cooperation can lead to replacing as well as to reducing and refining animal experiments. The real issue, however, is whether research results can justify ethically the use of animal experiments, which is not always weighed sufficiently in pharma/biomedical research. In this respect, more medical professionals have to be more closely involved in fundamental research on pharmaceuticals using laboratory animals.

A favourable basis for these developments is neuropharmaceuticals (clinical, pharma and experimental), an area in which scientists are increasingly questioning the value of animal experiments as a model for humans. For instance, in studies on medicines for depression and behaviour disturbances, animal experiments produce little useful information and under strict conditions there are direct overlaps with clinical studies in humans.

A new development is micro-dosing in which low doses are given so that no side-effects are expected and very sensitive measurement methods such as mass spectrometry are used. This technique gives data on the kinetics of the substance in humans. As many animal experiments do not give a good prediction of these processes, there is a demand for models that do not use laboratory animals (micro-dosing in combination with *in vitro methods)*

These opportunities for 3R alternatives in neuropharmaceuticals combine techniques such as genetics, neuro imaging and computational modelling. If no replacement methods are available, then the research design needs to be adjusted on ethical consideration to reduce and refine the proposed animal models. In the development phases of 3R methods, account needs to be taken of regulatory acceptance and implementation further down the track so that animal experiments are not necessary in the process of launching a product on the market. Also, medical professionals have to be involved in fundamental research on pharmaceuticals in answering questions regarding the benefits to the patient. This will contribute to a better flow of knowledge to applied research. Research agendas need to be coordinated internationally and government, scientists, and NGOs actively involved.

Risk assessment of chemical substances

- **applying 3R methods**
- **Use retrospective studies on combinations of 3R methods in Integrated Testing Strategies**

Risk assessment of chemical substances (innovative risk assessment) contains many initiatives to replace or reduce animal experiments. For instance, there is new legislation such as REACH, and the EU Cosmetic Directive restricting the use of animal experiments that will come into effect on 2013. Risk assessment of chemical substances is dealt with under various frameworks regarding product safety such as the USA Toxicology in the 21st century and the Netherlands ASAT Assuring Safety without Animal Testing. Furthermore, technological developments such as nanotechnology offer opportunities to replace or reduce animal experiments.

3R alternatives are used in scientific validation tests for skin irritation, photo toxicity, skin absorption, genotoxicity / mutagenicity and embryo toxicity. With the exception of embryo toxicity, these tests are part of regulatory procedures. However, it is difficult to completely replace tests for acute and chronic toxicity, sensitisation, carcinogenicity and reproduction toxicity, because one-on-one replacement is difficult because of the complex biological processes that come into play.

Strategies can be developed to reduce the number of animals used in experiments, for instance by using human data from epidemiological studies and health assessment monitoring in the workplace, and data obtained from earlier animal studies. This smart combination and interpretation of data on which assessments can be made is referred to as Integrated Testing Strategy. Such an example is tracing toxicological pathways in combination with mathematical models in physiological-based pharmacokinetics.

Acceptance and adoption of 3R alternatives requires more interaction between parties in the various research phases. Closer cooperation is needed in legal frameworks which are complex and international, with the organisations involved often having different interests, cultures, norms and values, requirements and issues. Researchers in the first phases need to be more aware of the needs of those working in the last phases of the research process in obtaining and giving regulatory approval, which in many cases now depend on animal experiments.

Much can be achieved in advancing the adoption of 3R alternatives with more interaction between parties and coordination within the research chain by government, research, and industry. Critical success factors in this respect are making risk assessment of chemical substances more transparent by sharing data between organisations and joint capacity building of researchers and expert assessors.

Quality control of pharmaceuticals and biologicals (serums and vaccines)

- **Implement a consistency approach to batch release of vaccines**
- **Collect material from retrospective studies and pilot projects using innovative 3R methods.**

The distinction between fundamental research for medicines and quality control is rather arbitrary. Up until now, animal tests have been used in quality control before market release. In the foreseeable future, the consistency approach is likely to gain momentum for the obligatory legal approval of vaccines for human use, and will lead to considerable reduction in the use of animal experiments.

In the consistency approach, quality of the batch is related to the safety and quality of the reference batch. It involves demonstrating consistency in production and production processes in relation to the reference vaccine. The consistency approach is now applied to new generation vaccines and according to the experts is suitable for classic vaccines, the production of which is standardised with process control and quality systems such as Good Manufacturing Practice. To this end, acceptance by regulatory authorities at international level needs to be intensified and investment made in sensitive measuring equipment and applicability for complex molecules.

Regulatory authorities, however, have a reputation of being somewhat reluctant to accept 3R alternatives. Perhaps because of cultural differences within international organisations, they are slow to reach consensus and agreement is reached only after extensive testing. For instance in the development of a new product with the application of nanotechnology, more in vivo studies may be carried out than scientifically or even legally necessary. Thus in facilitating a switch to innovative 3R methods, experts propose inventories of retrospective studies be made and pilot projects undertaken to gain experience with alternative test methods. Pilot studies would be valuable in areas where as yet alternatives accepted by regulatory authorities are not available, and then risk evaluations could be carried out with material obtained from these tests.

Much goodwill can be generated by making the process of regulatory acceptance and implementation more transparent, right now it is perceived of as a ‘black box’.

Stimulating 3R alternatives

The study led to a number of recommendations to stimulate wider acceptance and adoption of 3R alternatives that fall into two categories as follows:

- To create a more favourable research environment
- To stimulate an integrated chain approach in an international setting.

Create a favourable research environment

Further acceptance and implementation of 3R alternatives will require a change in mindset by individual researchers and by the scientific community worldwide. This refers to the creation of a research environment in which replacing, reducing, and refining animal experiments is the norm rather than seen as an additional burden imposed externally by research funders and others.

Changing attitudes

The process of changing attitudes takes time and must start with the raising researchers' awareness of the advantages and opportunities that 3R alternatives offer over conventional methodologies in gaining insight into biological processes. In this respect, 3R alternatives need to be incorporated into basic science education, and researchers provided with continuous and structured information and training on 3R applications.

Individual scientists committed to 3R alternatives can do much to stimulate further acceptance and adoption. Senior scientists who are experts in their respective fields can act as ambassadors by consciously and practically endeavouring to get valid results using 3R methods. In publishing their research, scientists need to be stimulated not only to present the scientific impact but also to make the societal impact with regard to 3R alternatives measurable and visible. If they are encouraged to publish negative results of animal experiments, for instance in literature reviews, overlaps and useless experiments could perhaps be prevented. In addition, research organisations such as medical faculties could be stimulated to set out 3R focal points for issues on animal experiments. Researchers need to be encouraged to participate in social debate on the value of animal experiments and in doing so to become more aware of the societal impact of their actions.

Innovative technologies

Key opportunities in 3R alternatives will come from the further development and adoption of innovative technologies such as stem cell culturing and "-omics". This requires an increasingly multidisciplinary approach that creates 'push-pull' incentives for the development and validation of these 3R techniques. A shift to more interdisciplinary research would increase opportunities of developing new paradigms. This must be done by forging links enabling researchers from different disciplines to work closer together. However, this is a challenge that will require a change in mindset because publishing and international recognition in the current research environment tends to favour the specialist.

Financing incentives

One way to stimulate multidisciplinary and innovative research that focuses on 3R alternatives is through financing incentives. For instance, conditions for funding biomecial research by government should provide for the development and application of 3R methods. Sensitive to financing arrangement, senior scientists would be responsive to 3R alternative approaches and could be invited to make their efforts more visible in this respect. Young scientists would benefit from training in 3R alternatives because this new knowledge can become a success factor in winning research budgets.

In addition, companies carrying out contract biomedical research could be remunerated for contributing to the development and application of 3R methods, for instance in giving perspective to regulatory implementation of such methods. However, such measures require long-term planning and cooperation between policy makers, companies and other stakeholders.

Stimulating an integrated chain approach

Acceptance of 3R alternatives requires continuous dialogue and feedback between all partners in the chain from development to implementation including validation and acceptance by regulatory authorities. The development and implementation of 3R alternatives requires an integrated approach throughout the research chain from fundamental, applied, and regulatory approval. Feedback and dialogue are essential between and across these chains.

The chains vary between fundamental and applied research and within these research processes. The largest chain includes preliminary investigations, research and development, independent review, and implementation. For products to be launched on the market such as pharmaceuticals, the chain also includes validation, and regulatory approval. In all cases, structured feedback is needed with those involved in implementation.

An integrated approach across research areas would also facilitate more multidisciplinary approaches because 3R alternatives will depend increasingly on building and sharing knowledge and techniques across disciplines. The implication is that much closer links need to be forged between regulatory authorities and the scientific community, and between government, research organisations, industry, and society. An integrated approach implies that all partners - government, research, industry, and society - have shared responsibility for 3R alternatives.

Creating more interaction will require a change in mindset because partners in a chain have different research backgrounds, interests and speak another language with terms such as validation and implementation having different meanings. Incentives are needed to foster an open attitude and willingness to invest jointly in new developments.

To make better use of 3R alternatives in fundamental research, scientists must be up to date with new developments and actively participate in these processes. While the main responsibility rests with researchers themselves,

stakeholders such as subsidy providers, clients and peer reviewers play a role. In the regulatory approval of a new medicine, government has an influential role.

An essential part of the process is early assessment of the potential application of specific 3R alternatives. This requires closer interaction between research and practice, for instance by investing in translational research to bridge the gap between fundamental research and the end point of improved and safe products and treatment methods. This is particularly the case in quality control of human medicines and risk assessment of harmful substances. Retrospective evaluation of animal experiments should include both positive and negative results and impacts.

Evaluated results of assessments about the usefulness and need for animal experiments are becoming increasingly accessible to researchers and other interested parties – both positive and negative results. Dissemination of such data, however, requires an active policy as there is no market for evaluations of such results in the research world. Such evaluation research projects should preferably be stimulated and facilitated by government in the framework of socially responsible actions.

International dimension

Fundamental research, which is the birthplace of 3R methods, is an international activity and the findings are published in international peer-reviewed journals. To ensure that knowledge generated is made available for practical application, researchers, international advocates, regulatory authorities need to be involved in processes leading to priority setting and programming of research.

Validation and implementation of 3R alternatives take place in the international arena and are complex, time-consuming processes that require openness with data by all parties involved. International harmonisation and cooperation are essential and particularly so in the priority areas identified in this study. This is especially so because of the large investment required by companies involved and lengthy regulatory processes.

As well as technical validation, 3R alternatives require international acceptance. In this respect, international legislation that applies to the safety of products for patients and consumers including the use of animal experiments is being harmonised and implemented in national legislation including the EU. International acceptance is complex in that agreements are needed on the degree of acceptable risk to patients and consumers. Many international accepted systems are based on animal experiments and gaining acceptance for innovation in 3R alternatives takes time. There has been a breakthrough with the Cosmetics Directive that comes into effect in the EU in 2013 prohibiting substances that have been tested in animal experiments.

While many legal frameworks do not specifically restrict the use of animal experiments, the EU legalisation REACH for toxicity analysis of chemical substances offers explicit opportunities for 3R alternatives.
There are opportunities for 3R alternatives in safety testing which could lead to a reduction in animal experiments by preventing overlaps and repetition of experiments. This will require close cooperation in designing safety studies and tests between governments, between government and industry, and between industries. There are also opportunities for 3R alternatives in Integrated Testing Strategies in which different types of data are integrated for risk assessment of a specific substance. Optimal use of 3R alternatives depends on databases of good quality data and advanced models, and clear agreements with regard to access.

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