Towards a European science without animal experiments

OPPORTUNITIES FOR THE REPLACEMENT OF
ANIMAL EXPERIMENTS PROVIDED THROUGH
REVISION OF DIRECTIVE 86/609/EEC

An expert report by the Dr Hadwen Trust for Humane Research and the Humane Society International
4 FOREWORD BY DR JANE GOODALL

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We have been led to believe that most of the advances in medical knowledge have resulted from experimental research involving the use of animal models. In fact, careful research into this subject, including studies of medical history, has shown that countless breakthroughs have been due to clinical observation of human patients and epidemiological research. Animals have been used to test the procedures and drugs that resulted only because this is required by law.

Animal testing frequently has not been useful in advancing human medicine due to differences in the biology of humans and even our closest biological relatives, the chimpanzees, whose DNA differs from ours by only just over one percent. For example, although the retrovirus for HIV (1 and 2) will live in chimpanzee blood, chimpanzees have proven poor models for the understanding of HIV-AIDS in humans—they do not develop the full blown symptoms of AIDS. There are many examples of products which, while they did not harm a variety of animal "guinea pigs", subsequently harmed people. At the same time, there are some beneficial drugs that were withheld from humans because they harmed animals during testing.

Much has been done to develop alternatives to testing on animals, such as cell, tissue and even organ cultures, computer simulation, and so on. Unfortunately, even when such alternatives are available, have been shown to be more effective and cost efficient, and have been approved by the government agency responsible, many scientists have been reluctant to use them. After all, technicians and scientists have been trained in animal procedures. And, finally, animal experimentation is a multimillion euro business!

Today, scientists are desperately searching for causes and cures for terrible diseases such as Parkinson’s disease (which afflicted my mother) and multiple sclerosis. As we move into the 21st century, we need a new mindset. Most animal experimenters assert that it will always be necessary to use animals for some procedures, but that, as they now understand the suffering so often caused, they will use as few as possible and treat them as well as possible. I believe that, instead, we should admit that the infliction of suffering on beings who are capable of feeling is ethically problematic, and that the amazing human brain should set to work to find new ways of testing and experimenting that will not involve the use of live, sentient beings. The scientific establishment should actively encourage such research. More funding should be made available for it. And rewards - such as a Nobel Prize - should be given for it.

It is a goal worthy of great energy and scientific ingenuity. It is a goal towards which all civilised nations should be moving.

Jane Goodall, PhD, DBE
Founder – the Jane Goodall Institute
& UN Messenger of Peace

www.janegoodall.org
www.rootsandshoots.org
Medical research and safety testing are responsible for the suffering and death of more than 115 million sentient animals globally each year. The official statistics for the European Union indicate that 12 million animals are used annually. This figure would rise by probably two million or more animals if all significant uses of laboratory animals were included.

Animal suffering touches the human conscience directly, through compassion and empathy; and rationally, through our ability to apply ethical standards to human activity. It is our response to this ethical responsibility that is the mark of a civilised society. As we strive to create a safer and fairer world for ourselves, so too must we apply the values of justice and humanity to our treatment of individual animals.

Surveys and opinion polls have identified that European Union (EU) citizens strongly support improvements in the protection and welfare of animals, and that compassion for animals is important to citizens in all Member States.

For these reasons, the new EU legislation governing the protection of animals used for scientific purposes, and replacing Directive 86/609/EEC, will stand as an expression of European values, and can help to lead the world towards a more progressive and compassionate future.

As well as increasing the level of protection afforded to animals, revision of the law provides a key opportunity to establish new structures at EU and Member State levels to promote the development and use of advanced, non-animal research techniques. These, in turn, will improve progress in the treatment and prevention of human diseases, as well as reducing animal suffering in laboratories.

In order to benefit people and animals, replacing unsatisfactory animal experiments with more relevant and reliable methods must surely be the ultimate goal. To achieve this goal, the EU needs to form a realistic and targeted strategy, utilising every available intellectual, technological, funding, procedural and legislative resource.

This report sets out a practical and achievable framework for the revision of Directive 86/609, putting the ultimate goal of replacement at the heart of the new legislation, and placing the EU in the best position to achieve change.

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1. Currently excluded are invertebrate animals, embryonic and foetal forms, and the generation and maintenance of genetically modified strains of animals.
When considering the animals’ interests, their routine suffering and lack of significant benefit to humans in most cases, it is hard to justify the institutional practice of experimenting on animals.

Peter Singer, Professor of Bioethics, Princeton University, USA.

There are five important reasons why the EU should more actively prioritise and increase its efforts to replace animal experiments with advanced non-animal methods.

A HUMANE SCIENCE THAT AVOIDS ANIMAL SUFFERING

Scientific methods that cause suffering and death to animals are morally unacceptable in today’s Europe. Animal experimentation invariably involves subjecting sentient animals to physical and/or mental pain or distress. Animals are harmed not solely during experiments, but also by transportation, handling, confinement, isolation or over-crowding, excessive noise or light and environmental deprivation. When considering our responsibilities towards animals in laboratories, we should not underestimate the suffering they endure.

An EU-funded study\(^2\) of the Three Rs (Replacement, Reduction and Refinement of animal experiments), conducted by scientists and philosophers, concluded that current thinking is moving away from emphasising differences between humans and other animals, in favour of a less human-centred world view.

It recommended that technological advances must be combined with ethical analyses, to address the justification of animal experiments; the value of animals and their suffering; and the responsibilities of humans to animals.

Ethical constraints on animal experiments

Sometimes decisions are taken to refuse permission for animal research which is considered simply ethically unacceptable. All proposals to conduct animal procedures in the EU should be subjected to detailed and independent ethical scrutiny, before authorisation. This ethical analysis should include an assessment of scientific validity, and a thorough consideration of welfare costs to animals. The ethical panel should be able and willing to refuse authorisation for proposed experiments that they consider are unethical or invalid.
Here are two cases where such decisions have recently been made.

In Switzerland, a request by scientists to conduct research into human depression using marmosets was referred to the Swiss Commission on Animal Experiments and the Swiss Ethics Committee on Non-Human Biotechnology.

The two committees produced a report reviewing fundamental ethical viewpoints on primate research. As well as their concern about marmoset suffering, they considered the scientific validity of the proposed marmoset ‘model’ of human depression. Unanimously, they “…questioned the relevance of the marmoset animal model to provide any meaningful findings for research into depression.”

Their final, also unanimous, recommendations were that the marmoset experiments should not be permitted; that the development of alternatives in depression research must be encouraged; and that experiments on great apes should be explicitly prohibited.


In 2007, the German parliament stopped experiments on monkeys at the University of Bremen that had been licensed by the local authorities. The neuroscience research involved inserting electrodes into macaque monkeys’ brains and restraining them in primate chairs while they responded to images on a computer screen.

Source: Nature, 26 April 2007, 446:955

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REFLECTING THE VIEWS OF EU CITIZENS

Without a doubt the public is very concerned about the ethical aspects of animal experimentation.

The 2005 Special EUROBAROMETER 225 Social Values, Science and Technology recorded that 82% of EU citizens believe we have a duty “to protect the rights of animals whatever the cost”.

82% of (EU-25) citizens “uphold our duty to protect the rights of animals whatever the cost”.

The Commission’s 2006 survey also showed the high degree of concern felt for animals. An amazing 42,655 citizens responded, and 93% believe more needs to be done to improve the level of welfare/protection of animals used in experiments by action at EU level. Additionally, 79% believe there is not enough public funding, at European level, for the development and validation of alternative methods to replace animal experiments, and 92% want the EU to play a leadership role in promoting a greater awareness of animal welfare internationally, particularly regarding animal experiments.

Regarding life science research policy, the Commission has stated that:

“Broad public support is essential, and ethical and societal implications and concerns must be addressed. How can Europe deliver effective, credible and responsible policies which enjoy the confidence and support of its citizens?”

The answer is with decisive and committed action based on the views expressed, ensuring that the EU’s research policies actively encourage and enable EU scientists to specialise in advanced, relevant and ethical research methods that can replace animal use.
Animal studies fail to predict human outcomes in 50% – 99.7% of cases, according to independent reviews.

**BETTER QUALITY AND RELEVANCE IN MEDICAL RESEARCH AND SAFETY TESTING**

Scientific reports increasingly show that many animal ‘models’ used to study human illnesses are unreliable. Examples include multiple sclerosis, stroke, rheumatoid arthritis, Parkinson’s disease, Alzheimer’s disease and cancers of the lung, brain and bowel.

An extensive study by Perel and colleagues (2007) examined 221 experiments using over 7,100 animals in research into six different treatments for five human illnesses. For five out of the six conditions, the researchers criticised the quality of the animal research, and they found that half the animal experiments failed to correctly predict human responses to treatment.

A 2005 review, led by Dr Toni Lindl (formerly Professor of Biochemistry at the University of Bonn, and currently Director of the Institute of Applied Cell Culture in Munich), concluded that animal experiments fail to predict human outcomes 99.7% of the time. The researchers analysed 51 series of experiments on 5,000 animals conducted at three universities in Germany. They found that 99.7% of the results were not applicable to humans, and that no medical use had been found for the remaining 0.3%.

Because it is uncommon for other species to naturally suffer from the same diseases as found in humans, medical research on animals relies on conditions that are dissimilar to human illnesses, artificially induced in non-human species. Safety testing is similarly affected by differences in the biology, physiology and metabolism between species. Most animal tests have not undergone formal validation but continue as a scientific ‘convention’, even though there are well-established, serious and unsolved problems: with species differences, dosing discrepancies (between tests and real human exposures), and scaling up results from small, short-lived animals (mainly rodents) to larger, long-lived humans.

To secure the health of EU citizens, the quality and relevance to humans of research and testing must be improved. It is now widely accepted that advanced, non-animal, human-based techniques overcome many of the limitations of outdated animal experiments.
**DRUG AND CHEMICAL SAFETY QUESTIONS ANSWERED MORE QUICKLY AND ECONOMICALLY**

Animal experiments can be very time-consuming and costly. For example, the rodent carcinogenicity assay uses at least 400 – 800 animals per test, takes up to five years to complete and costs more than €1.3 million per test substance.

By contrast, many replacement methods, such as cell-based studies, silicon chip biosensors, genomics, proteomics and computer simulations, can provide fast, reliable answers to medical and safety questions that laborious animal experiments cannot match.

Test-tube safety methods are more precise, versatile and reproducible than testing drugs and chemicals on animals. For example, every batch of insulin used to be tested for safety by the mouse convulsion method, using 600 mice each time. When a non-animal technique replaced the mouse test, faster and more precise results became available. When chemicals were tested for skin irritation using rabbits, the test took 14 days to complete. In comparison, the test-tube methods that now replace rabbits take only 42 hours.

As the Commission acknowledges: “In addition to animal welfare benefits alternative methods also have the potential to provide robust information through quality-controlled, state-of-the-art tests which are faster and less cost-intensive than classical animal-based tests.”

**KEEPING EU SCIENCE COMPETITIVE**

The EU has a head start with its expertise in non-animal research and testing techniques and technologies, which it needs to maintain and develop. For example, new human cell-based techniques to ensure the purity of injectable drugs were recently validated by the Commission’s European Centre for the Validation of Alternative Methods (ECVAM). The new tests greatly enhance patient safety and will replace thousands of rabbit tests each year. They are also a major commercial success with a worldwide market of €200 million, according to the Commission’s Vice-President, Günter Verheugen, who said:

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Alternative methods have the potential to increase the credibility and accuracy of tests as well as the safety of human beings...The development and validation of new methods and strategies will also increase competitiveness of European industry.

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Günter Verheugen,
Vice-President of the European Commission, and Commissioner for Enterprise and Industry.

As research in the development of alternatives is not only beneficial for animal welfare but also encourages the development of new markets for these methods.”

Ensuring that legislation provides an impetus for further development of these world-class skills in modern, non-animal technologies, will facilitate an essential competitive edge for the EU in the fast-moving world of science.

**ADVANCED NON-ANIMAL TECHNIQUES INCLUDE:**

- Gene-hunting tools to find and understand the role of disease-causing genes in people;
- Cell and molecular tests for the safety of chemicals;
- Biosensors that synergise cell research with microelectronics, to study drug metabolism, toxicity and disease biomarkers;
- Ultra-sensitive analytical techniques allow safe, ethical, microdose studies of drugs and chemicals without animal tests, and can enhance safety in the workplace;
- Advanced microscopic techniques for imaging and analysing cell functions in health and disease;
- High-powered computer models that realistically simulate the human body, and its reactions to medicines and chemicals;
- Tissue engineering that re-creates three-dimensional human tissues in the test-tube, for disease research, drug development and safety testing;
- High-technology, safe imaging of the human brain to understand neurological disorders and drug effects on the brain;
- Molecular methods to study disease using human cells in the test-tube.
At the time of going to press, we – and the many millions of animals affected by the current legislation – are still waiting for the proposal, but the political discussions have already begun.

For the reasons outlined in this report, our two organisations – the Dr Hadwen Trust for Humane Research and the Humane Society International – are working to replace all animal experiments. However, for as long as they continue, it is essential that they are strictly controlled and that there is an explicit, pro-active, and vigorously implemented strategy for their replacement.

In this section, our overall goal of replacing animal experiments is set within the broader context of related animal welfare objectives.


\(^\text{12}\) EP Resolution 2001/2259(INI).


\(^\text{14}\) EP Resolution 2006/2046(INI).

\","ANIMAL PROTECTION: KEY THEMES

We see the replacement of animal experiments as one of three key themes to be addressed through the revision:

1 Improved protection of animals
2 Transparency, accountability and enforcement
3 Replacement of animal experiments and implementation of non-animal techniques

Our quest for alternatives to animal testing is leading to quality-controlled and state-of-the-art tests. These methods are quicker, cheaper and – crucially – involve less suffering.

This mission has my fullest support.\(^\text{11}\)

Janez Potocnik,
EU Science and Research Commissioner.
SPECIFIC OBJECTIVES

1 IMPROVED PROTECTION OF ANIMALS

- Extending the scope of the Directive to protect sentient invertebrates and immature (e.g. foetal) animals; as well as animals used in basic research, in education and training, those killed for their tissues, and animals used in the production and maintenance of genetically modified strains.
- Authorisation of research proposals to be dependent on ethical review, successful inspection reports, and satisfactory training and infrastructure.
- Ethical review to include evidence of mandatory application of replacement, reduction and refinement techniques, as well as scrutiny of justification given for requesting authorisation to use animals.
- Carefully defined severity classifications applied uniformly in all MSs, through EU inspectorate liaising with national inspectors.
- Immediate suspension of experiments failing to follow ethical review/authorisation constraints on their purpose or conduct, or those entailing more severe suffering than authorised.
- The breeding, euthanasia and anaesthesia of animals must be strictly controlled so that minimising animal suffering and distress is the key priority.
- Scope for amending severity classifications and other requirements when new information leads to a reassessment of animals’ capacity to experience pain, suffering or distress.
- An end to the use of those species and wild-caught animals whose ethological needs cannot be met while kept in confinement, such as primates.
- A complete ban on experiments deemed to cause severe or prolonged pain, suffering or distress.

2 TRANSPARENCY, ACCOUNTABILITY AND ENFORCEMENT

- Publication of EU and national inspection reports (with names of researchers or institutions omitted or encoded where necessary).
- Commission to collect and publish annual information from MSs on national enforcement measures, including recorded breaches of national law, and evidence of dissemination of information on implementation of reduction, refinement and replacement techniques.
- Publication of prospective and retrospective ethical review statements.
- Publication of animal research project proposals, including sources of funding in the case of publicly funded research.

3 REPLACEMENT OF ANIMAL EXPERIMENTS AND USE OF NON-ANIMAL TECHNIQUES

- EU and MS structures to mandate the development and use of replacement, reduction and refinement (Three Rs) techniques to address all scientific uses of animals.
- EU and MSs to commission, fund and conduct such research.
- EU and MSs to provide training in use of Three Rs techniques.
- Establish an EU database on Three Rs approaches.
- Establish databases of negative and unpublished studies to improve research opportunities, save money and prevent duplication.
- Setting targets for decreasing animal use through application of replacement techniques.
- Regular reporting on the development and use of Three Rs techniques.

Annual publication of detailed statistics relating to animal use, including those relating to severity classifications, production and maintenance of all genetically modified animals and the killing of animals used solely for their tissues.

Animal welfare stakeholder involvement in the ethical review process and national ethical scrutiny bodies.

Reporting on all cases in which authorisations have been revoked due to animal welfare concerns.

Towards a European science without animal experiments
Priorities for replacement: towards non-animal science in Europe

This diagram shows the categories and percentages of animal experiments conducted in the European Union in 2005 under the existing Directive 86/609/EEC. The examples of actual animal experiments in these categories, and the state of progress to replace them, illustrate the urgent need for an expansion of replacement initiatives in Europe, to encompass basic medical research, diagnosis of disease and production of infectious agents, as well as regulatory testing.

1 MEDICINES RESEARCH & DEVELOPMENT:
For medical drugs and devices in human medicine, dentistry and veterinary medicine
- Nos. animals in 2005: 3,746,028
- % total animal use: 31%
- Animals commonly used: Mice & rats, birds & fish, guinea pigs, rabbits, dogs
- Case study: In a Spanish test of a new dental filling material, six dogs were anaesthetised and had root canal treatment on 12 teeth. This involved drilling into each tooth and filling the root canal. A flap was cut into the gums, and a hole drilled through bone to access the roots of the filled teeth. The root tips were cut off and a cavity made in each tooth root. These cavities were filled either with standard silver amalgam or a new polymer material. The dogs were killed after three months. The new filling caused more severe inflammation in bone and gum tissues, and bone growth was no better than with silver amalgam.
- Replacement opportunities: Extracted human teeth are used in vitro to test the sealing ability of root-end fillings. Also, cells can be cultured on polymer materials in the test-tube to assess leakage of chemicals and how much inflammation results. Computer simulations are already used to predict mechanical stresses in fillings caused by bone loss. Further progress with these techniques can replace more animal experiments.

2 BASIC BIOLOGICAL RESEARCH:
Studying the structure and function of living beings in health & disease
- Nos. animals in 2005: 4,035,470
- % total animal use: 33%
- Animals commonly used: Mice & rats, birds & fish, amphibians, pigs
- Case study: Pigs were used in Italy to test whether stem cells can repair heart damage. The pigs underwent surgery where an artery was tied off so that they suffered a heart attack. Thirty days later, they had further surgery where genetically modified stem cells were injected into a heart vein. The pigs were dosed with immunosuppressive drugs daily for a month, to prevent rejection of the stem cells, before they were killed. The injected
cells had moved to the area of damaged heart tissue, but did not develop into heart cells.

**Replacement opportunities:** Stem cells can develop into heart cells in the test-tube, and their characteristics (beating rate, electrical and biochemical activity, etc.) can be studied in vitro. These cells can also be assessed in vitro to predict their function after implantation. In further test-tube studies, the cells can also be placed into heart slices (damaged in one area to mimic a heart attack), to see whether they move to the injured tissue.

### 3 SAFETY TESTING:
Testing the safety of drugs and chemicals for humans, animals and the environment

- **Nos. animals in 2005:** 1,026,286
- **% total animal use:** 8%
- **Animals commonly used:** Mice & rats, guinea pigs, birds & fish, rabbits, dogs, primates

**Case study:** In animal trials of a new drug called TGN1412, macaque monkeys were given 500 times the dose subsequently given to healthy human volunteers. The monkey tests showed no adverse affects, but six volunteers nearly died from a massive immune reaction causing multiple organ failure, emphasising the difficulties of interpreting test results from other animals.

**Replacement opportunities:** In vitro human cell studies are more relevant, and could have avoided this tragedy. Test-tube studies carried out after the event revealed a major difference in the reactions of human and monkey cells to this drug. A novel microscale cell culture model of the human immune system is now available to study immune reactions to drugs in vitro. And, sensitive analytical methods now permit ultra-safe ‘microdose’ studies in human volunteers, at an early stage of drug development.

### 5 DIAGNOSIS OF DISEASE:
Animals used in methods to diagnose human and animal diseases

- **Nos. animals in 2005:** 272,014
- **% total animal use:** 2%
- **Animals commonly used:** Mice & rats, rabbits, birds & fish, guinea pigs

**Case study:** Internationally, animals are used to identify the strains of microbial pathogens causing illnesses. For example, the bacteria Peptostreptococcus can lead to abscesses in the brain, liver, breast and lungs of sick patients. The pathogenic strains are typically identified by injecting guinea pigs with the bacteria, causing abscesses on their back or legs.

**Replacement opportunities:** Mass spectrometry is a method of chemical analysis which, combined with laser technology to develop MALDI-ToF mass spectrometry, now enables scientists to identify many pathogenic bacteria without using animals. The technology can be adapted to speedily identify many other infective pathogens, replacing the routine use of guinea pigs, rabbits and other animals in diagnostic tests.

### 4 OTHER:
Experiments not counted elsewhere, including the production and maintenance of infectious micro-organisms

- **Nos. animals in 2005:** 984,238
- **% total animal use:** 8%
- **Animals commonly used:** Mice & rats, birds & fish, rabbits

**Case study:** In laboratories worldwide, rats undergo injections of chemicals to damage their immune system and are then infected with pneumonia-causing fungi. These fungi particularly affect AIDS patients, children and the elderly. The fungi are grown in the rats, who are used as living ‘bioreactors’, causing them to suffer from pneumonia. When they become seriously ill they are killed and the fungi are removed to be used in research.

**Replacement opportunities:** In vitro culture methods are being developed by the Dr Hadwen Trust to bypass the use of animals to breed the fungi. These non-animal systems have the advantage of enabling scientists to study the pneumonia fungus that infects humans, rather than the one that infects rats. The replacement technique is therefore much more relevant for human patients than the animal method.

### 6 EDUCATION AND TRAINING:
Use of animals at all levels of education, plus training of surgeons and laboratory personnel

- **Nos. animals in 2005:** 198,994
- **% total animal use:** 2%
- **Animals commonly used:** Mice & rats, amphibians, pigs, birds & fish

**Case study:** Frogs (amphibians) are still used in school- and university-level education for many students worldwide. In a university course in France teaching embryology, frogs are injected with hormones to stimulate over-production of eggs and sperm. The male frogs are killed and their testes removed, and the female frogs have their eggs removed by students so that they can perform in vitro fertilisation. The female frogs’ ovaries are also removed for study before they are killed.

**Replacement opportunities:** Interactive CDs are available to teach students the development of the amphibian eggs and embryos. High-quality videos, animations and images of microscopic sections of embryos are included in the CDs. Anatomical models are also available showing frog embryology from fertilisation to late-stage development. Students can be taught dissection and observational skills using plants instead of animals.

### 7 PRODUCTION/QUALITY CONTROL OF BIOLOGICAL MEDICINES:
Routine production of antibodies, and tests to ensure the purity and potency of medicines and vaccines for humans and animals

- **Nos. animals in 2005:** 1,854,553
- **% total animal use:** 15%
- **Animals commonly used:** Mice & rats, rabbits, guinea pigs, birds & fish, hamsters

**Case study:** In Lethal Dose 50% (LD50) tests, groups of animals are injected with different amounts of a test substance to identify the dose at which 50% die. LD50 tests are conducted routinely on thousands of mice each year to standardise the potency of batches of Botulinum toxin. This toxin is used medically to treat neuromuscular conditions (and cosmetically as an anti-wrinkle treatment). Some mice used in these tests develop paralysis, first in the legs but then in the breathing muscles, causing them to die from suffocation.

**Replacement opportunities:** The SNAP-25 test is a non-animal replacement that is faster, cheaper and more sensitive than the mouse assay. The replacement test, which has already saved thousands of mice, uses a synthetic protein in the test-tube and has been approved for final potency testing of Botulinum toxin batches.
Non-animal research has already delivered countless success stories, making a real difference for people and animals - here are just a few:

UNDERSTANDING NEUROLOGICAL DISORDERS

Research to understand human neurological disorders often uses animal surrogates, including monkeys and rats, in invasive experiments that routinely involve physical restraint, implanted brain electrodes, chemical injections and inflicted brain damage.

The research aims to understand the structures, connections and functions of the human brain, but for decades, more was known about the brains of monkeys than humans. Findings from these experiments were of unknown relevance to patients suffering from conditions such as Alzheimer’s disease, multiple sclerosis, epilepsy, chronic pain, stroke and schizophrenia.

The advent of safe imaging technologies, such as magnetic resonance imaging (MRI) and magnetoencephalography (MEG), has revolutionised understanding of neurological conditions by making it possible to study in detail the brains of human volunteers – healthy subjects and patients with serious conditions that urgently need researching.

These imaging techniques and related approaches, such as transcranial magnetic stimulation (TMS), have already replaced distressing long-term experiments on monkeys. By enabling real neurological problems to be researched in humans, medical progress in these important areas has already accelerated15.

Replacing animal experiments with non-animal alternatives isn’t just an ideal for the future: science and medicine have already benefited from the implementation of these advanced techniques.

Case studies: Replacement success stories

Advances in technology mean that state-of-the-art brain imaging of humans can now directly replace some invasive animal studies.

Professor Paul Furlong, Professor of Clinical Neuroimaging, Aston University, United Kingdom.

Non-animal research has already delivered countless success stories, making a real difference for people and animals - here are just a few:

15 See, for example, the Dr Hadwen Trust’s Science Room website: www.scienceroom.org/human-brain-networks
REPLACING ANIMALS AS ‘PROTEIN FACTORIES’

Monoclonal antibodies are manufactured proteins used widely in medical research, in diagnosis, for identifying or purifying compounds, and as medical therapies.

For two decades, these special antibodies were manufactured by a process that involved injecting cancerous cells into mice, rats or rabbits, who developed large and painful abdominal tumours as a result. At frequent intervals, a needle was inserted into the abdomen and liquid containing the antibodies was withdrawn. Animals continued in a state of pain and distress for several weeks.

In Britain alone, 17,000 animals a year were once used as ‘living factories’ in painful procedures to mass-produce these proteins. Techniques were perfected to manufacture the antibodies in cell cultures in test-tubes and, following pressure from animal protectionists, the British government advised that the animal method should be replaced by cell cultures.

Since 2003, in Britain no animals have been subjected to this inhumane procedure – which nevertheless continues in other EU countries, causing pain and death for tens of thousands of animals each year.

SAFER INJECTABLE MEDICINES

A recent success for the European Centre for the Validation of Alternative Methods (ECVAM) is a suite of novel human cell-based tests to ensure that medicines administered into the bloodstream are safe.

These injected medicines must be tested to make sure they are free of bacterial contamination. The standard animal test, introduced in the 1940s, measures fever response in rabbits restrained in stocks. Some rabbits suffer fever, respiratory problems, organ failure or fatal shock. Additionally, the rabbit test is relatively insensitive, time-consuming and costly, and unsuitable for important new kinds of medicines.

Better understanding of the human fever reaction, together with advances in cell biology, led to the development of the new test-tube techniques which use human blood cells.

Now fully validated by ECVAM, the new tests are more reliable (avoiding species differences), more sensitive and accurate, quicker, more adaptable and less costly.

As a result, patients are safer and an estimated 200,000 rabbits will be spared from suffering every year in EU laboratories. Yet until recently, few people believed that a complex whole-body reaction such as fever would ever be transferred to the ‘test-tube’.

SKIN SAFETY WITHOUT RABBIT TESTS

In 2007, research and development of non-animal tests to check the safety of chemicals and cosmetics on the skin, yielded success. Using small discs of artificial human skin in the test-tube, the new methods will save the lives of an estimated 20,000 rabbits who were due to be used for testing the skin irritancy of chemicals and cosmetics throughout Europe.

As well as sparing rabbits from painful skin inflammation, the new tests will also improve chemical safety, as they’re considered more accurate than the rabbit tests they replace.

ECVAM has validated and endorsed the fast, simple test-tube methods saying they “...will completely replace the regulatory Draize skin irritation test, a classic test...”
The field of 3-D in vitro modelling, using human tissues and fluids, will lead to major advances in cancer and neurological diseases, overcoming many of the disadvantages of animal experiments. — Professor Geoffrey Pilkington, Professor of Cellular & Molecular Neuro-oncology, University of Portsmouth, United Kingdom.

introduced into safety tests for drugs and chemicals 60 years ago”.

Adoption of these new ECVAM-validated methods in the EU will encourage their use internationally and their acceptance by the Organisation for Economic Co-operation and Development (OECD) for regulatory use in its member countries. The new techniques, therefore, are likely to end – finally, and globally – the use of rabbits in painful skin irritation testing.

BETTER MEDICINES, FASTER

All novel medicines are tested on animals to try and predict their behaviour in humans. Tests are conducted in rats, dogs and monkeys to gauge how a drug is absorbed, distributed, metabolised and excreted – known as its ADME characteristics.

Currently, 92% of new medicines passing animal tests are subsequently rejected, wasting time and money and putting the health of human volunteers at risk16. A frequent reason for failure is that animal tests do not sufficiently predict ADME characteristics in humans.

Now there’s an alternative. Medicines regulators (including the European Medicines Evaluation Agency) now accept results from safe, early human ADME studies, using tiny drug doses that are far below toxic levels, known as microdoses.

Human microdosing studies use ultra-sensitive analytical methods, such as AMS (Accelerator Mass Spectrometry), to study the ADME characteristics of experimental drugs. By understanding early in the development process how novel medicines behave in humans, time and money is saved – and animal tests of unsuitable drugs are avoided.

The EU’s Microdose AMS Partnership Programme17 is already boosting Europe’s expertise in microdosing and AMS. Most of the world’s top drug companies have started to implement these techniques, which are fast, versatile, highly relevant to patients – and save animals.

According to many experts, including the world’s largest drug regulatory agency, the US Food and Drug Administration in their 2004 report, Challenge and Opportunity on the Critical Path to New Medical Products.

http://www.eumapp.com/
Techniques using human cells in the test-tube are yielding new discoveries about diseases, without harming animals. For example, advanced three-dimensional cell culture approaches allow experiments in the test-tube that are shedding new light on the underlying processes that lead to breast cancer and sepsis.
The majority of Europe’s Three Rs work focuses on the minority of animal use – regulatory testing. Most animal experiments (up to 66%) are in non-regulatory areas. Most animal experiments (up to 66%), however, are in non-regulatory areas, including medical research where, despite immense potential, replacement efforts have received insufficient attention and funding. Extending intensive replacement efforts to medical research would enable it to move away from potentially misleading animal surrogates, and improve understanding of human biology and illnesses through the use of 21st century research techniques.

In outlining some existing replacement initiatives in the EU and beyond, our aim is to ensure that proposals for new national and EU structures devoted to promoting the replacement of all animal experiments, are rooted in real experience and based on best practice.

**EUROPEAN UNION**

The European Centre for the Validation of Alternative Methods (ECVAM) was created by the Commission in 1991, in direct response to Directive 86/609/EEC. Prior to that, the development of alternatives to animal experiments was largely conducted by charitable organisations.

Based at the European Commission Joint Research Centre in Italy, ECVAM focuses mainly on regulatory testing and undertakes the following activities:

- Acting as an international reference centre, especially developing, evaluating and validating non-animal regulatory test methods
- Organising international validation studies and promoting regulatory acceptance for Three Rs tests

Case studies: Replacement initiatives in the EU and beyond
Facilitating information exchange

Creating and maintaining a database on alternative regulatory test methods

Enabling dialogue between legislators, industry, scientists, consumer organisations and animal welfare groups to promote the Three Rs.

ECVAM has successfully validated more than 24 replacement, reduction and refinement methods, 14 of which have already gained regulatory acceptance. These tests address the safety of chemicals, drugs, vaccines and other products regarding skin irritation, fever, genetic damage, eye irritation, acute poisoning, light-induced reactions and more.

Thanks in large part to ECVAM’s leadership and expertise, the EU now leads the world in replacing, reducing and refining animal safety tests for regulatory purposes.

These are some of the in vitro replacement projects funded through FP6 and FP7:

- ACuteTox – an in vitro test strategy for predicting human acute toxicity.
- BioSim – using modern simulation techniques to create a more rational drug development process and a reduction in animal experiments.
- carcinoGENOMICS – to develop in vitro methods for assessing the carcinogenic potential of substances.
- Sens-it-iv – to develop test-tube alternatives to animal tests for assessing chemicals that may cause skin or lung allergies.
- ReProTect – to explore the field of reproductive toxicology in order to reduce the number of animals used.
- PREDICTOMICS – short-term test-tube assays to predict long-term toxicity.
- liintop – optimisation of liver and intestine cell studies for pharmacokinetics and pharmacodynamics studies.
- TOXDROP – highly parallel cell cultures in nanodrops, a new format for cell-based toxicity assays to replace mice.
- Biosensors Based on Molecular Organization – development and validation of alternative techniques to replace animal testing in drug screening and environmental control protocols.
- VITROCELLOMICS – reducing animal testing of drugs by use of human cells.
- INVITROHEART – reducing animal experimentation in drug testing by using human cells.
- Comics – will provide in vitro assays for screening chemicals for genetic toxicity and so help limit animal experimentation.

EU FRAMEWORK PROGRAMMES FOR RESEARCH

EU research funding is allocated according to priorities proposed by the Commission and agreed by the European Parliament and Council every five years. The current Seventh Framework Programme (FP7) of the European Commission for research and technological development for the period 2007 to 2013, was adopted on 18 December 2006 and has been allocated a total budget of €51 billion. FP7, along with previous research programmes, allows for considerable investment into the development of Three Rs techniques. Under FP6 (2002 – 2006), a total of 21 projects were allocated funding totaling over €63 million with the aim of creating technological platforms for the development of alternative testing strategies.
GERMANY

In Germany, the Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) was established by the government in 1989 with the explicit goals of replacing (and reducing) animal experiments, especially in regulatory safety testing, to alleviate the suffering of animals used in experiments19.

ZEBET is a unit within the Federal Institute for Risk Assessment (BfR) whose main activities include:

- Running a database and an information service on Three Rs methods at national and international levels
- A research programme actively developing replacements for animal experiments
- Conducting validation studies, nationally and internationally
- Co-operation with validation centres and institutions worldwide to promote research in the Three Rs
- Forum for information exchange on Three Rs methods, for scientists, animal protectionists, politicians, media and the general public.

As an example of a Member State (MS) institute, ZEBET has played a unique leading role within the EU, in the development and validation of successful non-animal tests.

Additionally, the German Federal Ministry of Education and Research (BMBF) also prioritises the replacement of animal tests, and since 1980 has provided €70 million for more than 230 research projects. The Ministry believes this effort has significantly reduced animal tests worldwide, by an estimated one million animals annually (www.bmbf.de/en/1040.php).

The requirement to replace, reduce or refine animal experiments may sometimes have been difficult to achieve, because conventional internet search engines are not specifically designed to search and retrieve information about the Three Rs. The first worldwide ‘knowledge-based’ search engine, called Go3R (www.Go3R.org) has just been launched as a co-operative effort by Transinsight GmbH (Dresden), the Technical University Dresden and ZEBET. All around the world, scientists will now more easily find the information they need, with time savings of more than 90%. The Go3R initiative illustrates what can be achieved when motivated experts are able collaborate to benefit animals and science.

UNITED KINGDOM

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) was established by the government in 2004. It is funded mainly by the government, with additional input from the pharmaceutical and charity sectors.

The NC3Rs currently has no research laboratories of its own, but awards research grants for Three Rs research, importantly both in medicine and toxicology. In 2007 it awarded grants worth €3.14 million. Its income from the government is €3.14 million and is due to double by 2010.

The NC3Rs provides essential information resources through its website20, and organises workshops and conferences to promote the implementation of the Three Rs. With the authority of government behind it, the centre benefits from the co-operation of leading scientific bodies as well as the pharmaceutical and chemical industries.

The Dr Hadwen Trust for Humane Research is a charity established in 1970 on the basis of an ethical opposition to animal experiments. It funds a portfolio of exclusively non-animal studies to replace animal experiments, mainly in fundamental medical research, thus benefiting people and animals.

The charity is supported almost solely by the general public and receives no government assistance. It has achieved many successes in replacing animal experiments in a very wide range of medical and related fields (www.scienceroom.org). The Dr Hadwen Trust now awards €626,000 every year to researchers developing replacement methods, a sum that has increased in recent years as a result of the growth in public support of its work.

19 See ZEBET’s website: www.bfr.bund.de/cd/1591
20 See NC3Rs’ website: www.nc3rs.org.uk

Towards a European science without animal experiments
Facilitate and provide guidance on validation criteria and processes

Facilitate the acceptance and awareness of scientifically valid test methods

Consider petitions from the public for review and evaluation of new and revised test methods that have evidence of scientific validity

Make available to the public ICCVAM final test recommendations

Prepare reports on ICCVAM progress and accomplishments and make these available to the public.

ICCVAM follows a very different model from that established in the EU, as it does not provide laboratory facilities or experimentally validate new methods. ICCVAM’s work has sometimes actually delayed rather than accelerated progress, as ICCVAM is prone to initiating costly and duplicative re-evaluations of methods already validated to international standards by others.

SWITZERLAND

The 3R Research Foundation in Switzerland was established in 1987 by collaboration between the public, pharmaceutical and animal protection sectors, and is supervised by the Federal Department of Home Affairs.

Research funds are jointly provided by the Federal Veterinary Office and pharmaceutical partners. In 20 years the Foundation has awarded €8.71 million Swiss Francs to 99 research projects aimed at replacing, reducing and refining animal experiments. In the last decade, the Foundation says its research has made a major contribution to reducing the number of animal experiments in Switzerland, and improving research and testing methods too.

Unlike ZEBET, the 3R Research Foundation has no research capacity of its own and its information resources and outreach activities are limited.

ICCVAM (USA)

The Interagency Coordinating Committee on the Validation of Alternative Methods was established pursuant to the NIH Revitalization Act of 1994. In 1997 ICCVAM was established as an ad hoc standing committee to harmonise the development, evaluation, validation and acceptance of alternative methods, and in 2000, ICCVAM was established in law as a permanent interagency committee.

The functions of ICCVAM are to:

- Co-ordinate the review and evaluation of new and revised test methods
- Submit ICCVAM test recommendations to appropriate US Federal agencies
- Facilitate interagency and international harmonisation of test protocols that encourage the reduction, refinement and replacement of animal tests
- Facilitate and provide guidance on validation criteria and processes
- Facilitate the acceptance and awareness of scientifically valid test methods
- Consider petitions from the public for review and evaluation of new and revised test methods that have evidence of scientific validity
- Make available to the public ICCVAM final test recommendations
- Prepare reports on ICCVAM progress and accomplishments and make these available to the public.

ICCVAM follows a very different model from that established in the EU, as it does not provide laboratory facilities or experimentally validate new methods. ICCVAM’s work has sometimes actually delayed rather than accelerated progress, as ICCVAM is prone to initiating costly and duplicative re-evaluations of methods already validated to international standards by others.
OTHER INITIATIVES

The European Partnership for Alternative Approaches to Animal Testing (EPAA) and other industry contributions (European Union)

The EPAA was founded in 2005 due to growing public awareness of animal suffering caused by regulatory testing, and because of a general acknowledgement that those companies using animal tests have important expertise to facilitate the development and validation of Three Rs methods. The EPAA is a partnership between seven industry associations (covering chemicals, cosmetics, pesticides, biocides, pharmaceuticals, veterinary medicines and detergents) and the European Commission.

While the industry contribution has the potential to make a huge impact on reducing the number of animals used in regulatory testing, the EPAA’s progress has been disappointing in some areas, while more promising in others.

Central to the aim of the EPAA was a transfer of data and reference chemicals from industry to ECVAM for use during validation studies. This has been slow, and by late 2007, the Commission reported that no data had actually been transferred21.

However, industry and EPAA initiatives are responsible for some major advances. The contribution made by individual cosmetics companies to bring alternative methods into use in time for the deadlines set by the Cosmetics Directive, continues to put other sectors to shame.

The US National Research Council vision for toxicology (USA)

In June 2007, the US National Research Council (NRC) published a report22 calling for a paradigm shift in toxicology: “...from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin.”

Consequently, the US Environmental Protection Agency, National Toxicology Program and National Institutes of Health Chemical Genomics Center recently signed a five-year ‘Memorandum of Understanding on High Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings’, aimed at building the scientific and technological basis for the paradigm shift envisioned by the NRC.

The Johns Hopkins Center for Alternatives to Animal Testing (USA)

The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) is a non-profit centre in the Johns Hopkins University. Dedicated to improving the health of people and animals, CAAT supports the creation, development, validation and use of alternatives to animals in research, product safety testing, and education.

The functions of CAAT are to:

- Promote and support research in the development of in vitro and other alternative techniques
- Foster discussion among diverse groups leading to creative approaches to facilitate acceptance and implementation of alternatives
- Provide reliable information on the science, philosophy and public policy of alternatives to academia, government, industry and the general public
- Educate and train in the application of alternatives.

The Centre works with scientists in industry, government and academia to find new ways to replace animals with non-animal methods, reduce the number of animals used, or refine methods. CAAT hosts Altweb, a large, free and publicly accessible website devoted to alternatives.
Three dimensional cell cultures are being developed and applied in medical research such as breast cancer, brain tumour invasion and liver disease, as well as in regulatory testing. This complex Phenion® full-thickness skin model is a realistic human skin equivalent for product development and safety testing.
Drawing on the various Three Rs initiatives outlined so far, we have formulated a ‘wish-list’ for EU policy makers.

This is our vision for creating the changes needed to further medical progress and ensure the safety of EU citizens, while at the same time reducing and replacing animal use.

Implementation of our wish-list would help to address the concerns of EU citizens, as expressed through the Dr Hadwen Trust-initiated petition (Replace EU Animal Tests) presented to the Chair of the European Parliament’s Petitions Committee on 28 May 2008. The petition has been supported by animal protection groups across the EU including Austria, Britain, Croatia, Denmark, Finland, France, Germany, Italy, the Netherlands, Norway, Portugal, Spain and Sweden.

**AT EU LEVEL**

An over-arching EU Centre of Excellence for Three Rs Research would co-ordinate and help to set strategy for Member State bodies, charitable institutions and industry contributors dedicated to replacing all scientific uses of animals. The Centre would also co-ordinate the dissemination of information from and between national centres, and promote the development and use of replacement techniques internationally.

Within the Centre, ECVAM’s specialist contribution in conducting validation studies and promoting the use of alternatives to regulatory tests would be retained, and strengthened to include other areas of animal use.

A combination of laboratory facilities and personnel with top-level policy expertise would ensure that the EU Centre takes a worldwide leadership role, and that research is focused on those areas offering most potential to further medical progress, enhance safety and replace animal use.

The Centre of Excellence would report regularly on its targeted replacement activities, and the resulting medical and safety advances.

**AT MEMBER STATE LEVEL**

National centres should also work flexibly with charitable organisations and commercial companies where possible, and should be funded through national research programmes. They should undertake some or all of the following functions, depending on expertise:

1. Provide leadership, co-ordination, multi-disciplinary strategies and focused expertise for national and international initiatives to replace animals in research and testing

2. Acquire new funding for researchers to develop, optimise, pre-validate and validate non-animal replacements for animal research and regulatory animal tests

3. Plan and commission key projects to overcome barriers and achieve step-changes in the replacement of animal use

4. Conduct essential in-house research where required, particularly as research partners in co-ordinated EU-wide or international research projects

5. Provide training in non-animal techniques for academics, toxicologists and small and medium enterprises

6. Raise the profile and scientific status of replacement initiatives, including through promotion of this principle to the academic and industrial research and animal-use communities.
The revision of the animal experiments Directive 86/609/EEC provides an opportunity to establish the Three Rs as the focus of new EU structures and policies. In particular, replacing animal experiments with non-animal research and testing methods will avoid large-scale animal suffering; implement the wishes of EU citizens; enhance medical progress and improve safety; and help keep the EU science base competitive in the global arena.

While animal experiments continue, however, they must be regulated strictly in a transparent and accountable manner so that the protection of animals from pain and distress is paramount. A new EU Centre of Excellence in Three Rs Research, hosting an expanded ECVAM, could ensure a world leadership role for the EU in cutting-edge, advanced non-animal science. The Centre would network with national centres established in each Member State. This would provide a research and development framework for an EU-wide strategy to achieve the goal of replacing all animal experiments, to the benefit of people and animals.
This report has been written and produced by the Dr Hadwen Trust for Humane Research and the Humane Society International. A web version and further information are available at: www.endeuanimaltests.org

THE DR HADWEN TRUST

The Dr Hadwen Trust is the UK’s leading medical research charity that funds and promotes exclusively non-animal techniques to replace animal experiments. Our vital work benefits humans, with the development of more relevant and reliable science, whilst also benefiting laboratory animals.

Our charity’s guiding principle, that excellence in medical research and safety testing can and should be pursued without animal experiments, is realised through our portfolio of high-quality, peer-reviewed and innovative research. The Dr Hadwen Trust has funded research in a range of fields including epilepsy, cancer, asthma, diabetes, drug testing, arthritis, lung injury, vaccine testing, dentistry, heart disease, fetal development and pregnancy, and brain tumours. Internationally recognised as a leading authority on replacing animal experiments, the Dr Hadwen Trust also actively promotes the concept and practice of non-animal research through publications, workshops, debates and the media.

DR GILL LANGLEY

MA, PhD, MIBiol, CBiol

Gill is the Dr Hadwen Trust’s Science Director. For her first degree, Gill studied physiology, cell biology and zoology at Cambridge University, and then gained a doctorate in neurochemistry, also from Cambridge. As a research fellow at Nottingham University she studied neurochemistry in human cell cultures.

Gill served for eight years as a member of the British government’s Animal Procedures Committee which advises the Home Secretary on animal experimentation, and has also advised the government on the introduction of the new EU chemicals legislation, REACH. She has been an invited expert in several initiatives of the European Commission and of the Organisation for Economic Co-operation and Development (OECD). Gill is currently a member of the Replacement Advisory Group of the British National Centre for the Replacement, Refinement and Reduction of Animals in Research.
With nearly ten million members and constituents globally, Humane Society International (HSI) is one of the largest animal protection groups in the world. The international arm of the Humane Society of the United States (HSUS), HSI works to reduce suffering and to create meaningful social change for animals by advocating for public policies to protect animals, investigating cruelty and working to enforce existing laws, educating the public about the issues, and conducting hands-on programs, such as assisting animals when disasters strike. HSI offices in Asia, Australia, Costa Rica, Canada and Europe help carry out field activities and programs.

Emily McIvor

Emily is EU Director of the HSI, and has worked on EU policy relating to the welfare of animals used in research and testing for many years, including through membership of DG Environment’s Technical Expert Working Group on the revision of Directive 86/609, and co-ordination of responses to both the Commission’s experts’ consultation and the impact assessment questionnaire. In addition, Emily has been a regular contributor to the REACH political negotiations and implementation planning, having been a member of RIP 3.4, co-ordinated animal welfare input to RIP 3.3, and participated in both the SPORT and PRODUCE initiatives. Ms. McIvor is also a member of the EPAA Mirror Group.