Making **sense** of animal research

REPLACE
REDUCE
REFINE

efpia
European Federation of Pharmaceutical Industries and Associations

Drug Development and the 3Rs

Development Phase	Questions Addressed	Duration	Number of Compounds Tested
Discovery of New Drug	What target enzyme or protein? What test system to show drug-protein interaction? What are chemical lead structures?	3 years	1000'000
Optimization	What is structure-activity relationship? What molecules have not only intrinsic activity but also drug-like properties (absorbability, metabolic stability)?	2-3 years	10'000 100
Preclinical Development	How can kg-amounts efficiently be synthesized? What are suitable formulations? What is pharmacokinetic metabolic and safety profile of the drug candidate?	1-2 years	5
Clinical Testing	What is tolerable dose range? What is dose-effect relationship? Is drug sufficiently safe at efficacious doses?	4-5 years	1-2
Registration	What are sources of variability? What is adverse effect profile?	1 year	0,1

intro

The pace of medical research is fast, but there are still many health challenges ahead. Animals continue to play an invaluable role in meeting these challenges - both in experimental research, and in ensuring maximum safety of treatments before their use in humans.

The process for developing a medicine does not start with animal research, which represents only about 10% of the whole R&D process. It is only undertaken when it is deemed necessary to address a specific scientific question which cannot be answered found through other routes.

Most of the research and development effort goes into non-animal techniques and human clinical trials. But where animals are involved, it is because their role is crucial and indispensable - and will continue to be so for continued improvements in health care.

Progress in science is constantly delivering new and better therapies - for humans and for animals. And the same progress is also providing more and more alternatives to the use of animals.

This publication gives a glimpse of the process of creating new medicines. It also spells out how medicines researchers are at the same time discovering methods to refine, reduce, and in some cases even replace the use of animals.

It discusses what can be done now without having to use animals in research, what cannot yet be done, and what the prospects are for the future. Above all, it illustrates the close link between producing new medicines and discovering new ways of developing medicines. Driving scientific advances forward makes sense for animal research - and paves the way for alternatives.

Tools and 3Rs Principles Applied

Non animal research:
Computer modeling
High throughput screening
Imaging techniques

In vitro studies:
Cell cultures
TissuesOrgans

Animal and in vitro tests:
Ethical review; Improved housing and care;
Refined sampling techniques; Smarter study design; Imaging technologies; Chip technology; Microdosing; Stem cells;
International harmonization; Replacing higher by lower species; etc...

Human clinical trials:
Phase I, II and III (safety, efficacy, variability)

No animal research, no medicines...

...in the past...

Many of the medical advances of the past are taken for granted today. But they would not have happened without animal research. For well over a century, research on rats, dogs and pigs has helped to find new treatments for conditions as diverse as heart disease, infections, brain disorders and arthritis.

Poliomyelitis has been wrecking human lives for ages, and the last century saw epidemics that crippled and killed hundreds of thousands around the world. Now the disease is close to being eradicated worldwide. New medicines have made this transformation possible - vaccines that have been developed over some forty years of research using monkeys and mice.

Breast cancer is the most common cancer among women. Over the last twenty years, successful new treatments have started to emerge. The radically improved survival rates that these medicines offer are the direct result of extensive research including animal studies.

Asthma is the most frequent serious childhood illness in the developed world, where it still causes thousands of deaths every year. But the inhalers commonly seen in schools today deliver medicines that control the condition and allow sufferers to lead a normal life. These medicines are available only because their efficacy and safety have been assured by studies involving animals.

For centuries, the life of diabetics was short and miserable. The disease was inescapably lethal, and death could be deferred only by a strict diet, until the life of a 13-year old in Toronto was saved in 1922 by an injection of a purified extract from isolated pig pancreases. Evaluation of this innovative approach on a female beagle led to the understanding of insulin's effect to lower blood sugar - a finding that has allowed millions of patients since then to survive and lead normal lives.

The success story of transplantation started in 1905 with the first successful human transplant, a cornea, in 1905. A breakthrough was the first transplantation of a human heart by Christian Barnard in 1967. Since then it has become common to replace heart valves, kidneys, hearts and bone marrow. Every transplant surgeon needs to study the complicated techniques in animals prior to conducting surgery on human patients. Also experimental animals were essential to understand the immunological process of transplant rejection and to develop drugs to prevent rejection. Such medicines are still being developed and investigated in rodents, dogs and monkeys.

...and in the present

Many questions can still be answered only by research that makes use of animals. Despite the advances in computer modelling and in alternative methods such as the use of single, isolated cells, the development of diseases or physiological processes cannot always be sufficiently studied with these techniques. Animal studies offer hope to millions of people with conditions ranging from stroke to spinal cord damage, and from sickle cell disease to malaria. The use of monkeys is opening up new possibilities for deep brain stimulation for Parkinson's Disease; mice are making a critical contribution to developing new treatments for adult leukaemia and lymphoma; rabbits and cattle are both playing a part in producing cervical cancer vaccines, and goats in developing blood clotting agents from milk, while bird flu vaccine development is dependent on the use of chickens and ferrets.

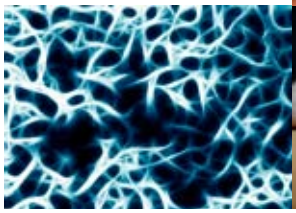
Developing treatments for Alzheimer's Disease requires an understanding of the behavioural changes brought on by the disease. Computer programmes and studies of individual cells are not enough for this. Scientists also make use of animal models, to gain new insights into the causes and to develop new treatments. Mice were used to prove that when injected directly into the brain, antibodies against a plaque protein retarded its growth. Gene therapies that have qualified for clinical trials in humans have first been studied in animals,.

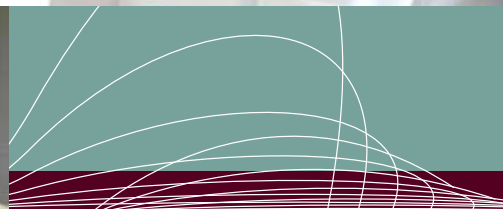
Cystic Fibrosis is the most frequently occurring life threatening genetic disease in Europe. Sticky mucus blocks the lungs and digestive system. One out of 30 Europeans carries the gene causing the disease and 30,000 children and young adults live with it. Some patients live until they're 40 years old, others only 4 years, depending on where they live in Europe. No cure yet exists, but the use of transgenic mouse models is expanding our knowledge of the disease every day. This will be useful for testing lung treatments. Several ways of correcting the fluid transport have already been developed in mice.

Mice are used for initial evaluation of potential HIV vaccines. But since the virus does not infect mouse cells, promising vaccines are tested further on macaque monkeys, which have immune systems more similar to man, allowing an accurate assessment of cellular responses.

Epilepsy is a neurological disorder characterised by seizures that result from excessive electrical activity in the brain. Epilepsy-prone rats have been used to identify a protein that triggers seizures. The researchers used a technique that measures brain activity. Also, a chemical called ethosuximide that protects young animals from seizure has been identified.

Work with mice and dogs is deepening our understanding of muscular dystrophy, which still causes muscle wasting and early death in humans. In particular a naturally- occurring condition in some dogs closely mirrors the human disease mechanism, providing an invaluable model for clarifying how the disease disrupts normal body functions, and for developing potential therapies.

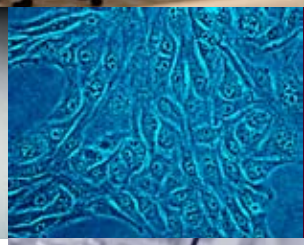




Progress in science leads to progress in **alternative testing**

As part of their search for new medicines, all researchers are also looking for better ways of working. Progress in scientific knowledge and in technology continually offers new opportunities for improving methods and results.

Revolutionary advances over the last 20 years include gene sequencing, the increasing precision of modern MRI scanners, high-speed computer modelling and screening and better understanding of cell behaviour. These techniques have in many cases reduced the need to use animals, or, where animals are still needed, have refined the way that studies are conducted, often both reducing stress and delivering better-quality data.



Research currently underway is exploring the use of stem cells to cultivate human liver and heart muscle cells, allowing replacement and reduction of some animal studies.

Implants for the treatment of bone fractures used to be studied in sheep. Since it became possible to culture cells to create new tissue, much of this work can now be done without using animals. Cultures of bone cores provide tissue that acts like live bone, which can be used for testing strength, and also allows more efficient study of processes in bone tissue.

To develop repellents against ticks, cats were routinely used as host animals so that ticks could feed on their blood. Now scientists have developed artificial feeding membranes that simulate the skin of host animals. In addition, this method makes it possible to make more precise measurements of the effect of different substances added to the blood in these artificial systems.

Non-invasive imaging techniques are used for research into disease and for in vivo safety assessment of new compounds. They have helped to reduce the number of animals by up to 80%. In addition, these techniques can enhance the quality of data and statistical power of a study. Imaging techniques allow continuous observation of a single anaesthetised animal, and therefore do not require killing a number of animals at different stages of the study for autopsy investigations. They provide information about structure and function of organs, the progress of a disease and how the same technique might function in humans.

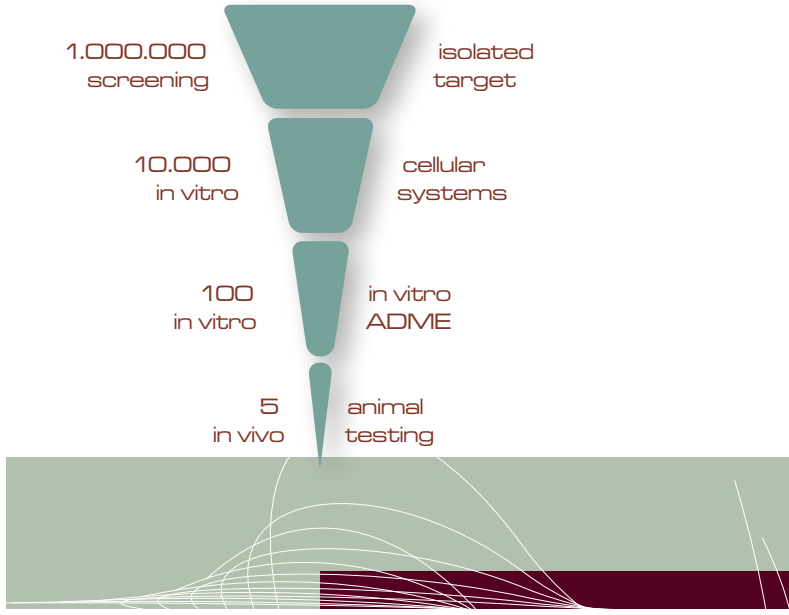
Science is still **moving** ahead...

Studies of the human genome, breakthroughs in high-speed information processing, or the promise of nanotechnology all hold out the prospect of new or improved medicines.

Possibilities of success in meeting health challenges such as emerging diseases, or in new avenues of research to investigate, are revealed only as our knowledge grows.

Against this rapidly evolving context, animals continue to play an invaluable role - both in experimental research, where they allow investigation of a medicine's potential, and in safety evaluation, to reveal any harmful effects before use in humans.

The process for developing a medicine does not start with animal research. It is only undertaken when it is deemed necessary to answer a specific scientific question, whose answer cannot be found through other routes.



...and researchers are still **seeking** alternatives.

As part of the effort to discover new medicines, researchers are constantly seeking ways to improve their research methodology to improve efficiency and results. This means that refinement, replacement and reduction in animal research are part of the scientific process.

It is not in researchers' interest to use animals unless they are absolutely necessary. Using animals in experimentation is very costly - not just because of the breeding and acquisition costs, or the complexity of procedures, but also because of the high standards of husbandry and welfare that animals require and deserve. In addition, the scientific data that have in the past come mainly from animal research can increasingly be derived from new techniques that often provide a higher degree of precision in results, because they are not influenced by the inherent inter-animal variability or by environmental factors. And the use of animals also raises continual ethical questions, requiring the most rigorous justification in personal and professional terms.

So major investments are also made in the development of alternative methods - to reduce the number of animals used, to refine the way they are used so as to minimise discomfort or distress, and, where possible, to replace animals. This widely supported strategy of reduction, refinement and replacement is frequently referred to as the "3Rs" approach.

Work in progress on alternatives

Thousands of initiatives are underway right across Europe to develop alternatives to existing research methods. Some alternatives help to reduce the number of animals used, and even to replace them entirely in some procedures. All of these efforts are aimed at refining the research process, and, where the use of animals remains necessary, at maximising comfort and minimising distress. Recent examples of alternative methods developed by the pharmaceutical industry include the use of in vitro liver cells to assess metabolism and elimination, synthetic animal protein to partially replace animal tissues, and blood cells for pyrogenicity testing. They also include the use of computer technologies in the early discovery process, measuring neuro-chemicals in the mammalian brain using in vivo micro dialysis, and replacing guinea pigs with a cell-based assay to assess the duration of action of drugs for the treatment of asthma.

REFINEMENT

▸ reducing stress in animals

Laboratory mice and rats spend their lives in a cage. Enriching their environment, with shelters, shredded paper, pieces of wood, or climbing opportunities, allows them to explore, to build nests, and to find refuge - to exhibit normal activities and thus to reduce stress. Enrichment has been shown to reduce inter-animal variability in response to experimental conditions, and in some cases may reduce the number of animals required to provide information.

REDUCTION AND REPLACEMENT

▸ greater accuracy from in vitro systems

While in vivo models allow reliable measurement of alterations in articular cartilage, the cellular mechanisms controlling those changes are difficult to examine. In-vitro models offer a more direct way to study the cellular response to articular loading under controlled circumstances. Because such in-vitro studies are a closed system, the experimental conditions can be controlled more precisely, and inter-individual variability can be reduced.

REFINEMENT, REDUCTION AND REPLACEMENT

▸ ensuring ethical procedures

Companies operate their own internal ethical review systems that often exceed any legal requirements; they establish high standards for housing of experimental animals; they set up supervision mechanisms for all animal research; and they fund research into viable alternatives, and promote awareness of alternatives through awards and education.

REFINEMENT AND REDUCTION

▸ smarter study design

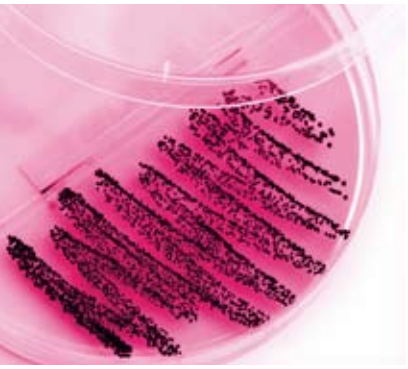
The design of studies involving animals is constantly being improved, for instance by the prior definition of a humane endpoint - the point when the test can be stopped because its objectives have been met and before the animal suffers significant distress. This presupposes adequate methods for measuring distress, and the attention of fully trained personnel. Careful consideration of study design can also reduce the number of animals required for a trial. The use of statistical support helps to ensure that the number of animals is as small as possible, while providing scientifically meaningful results.

REFINEMENT AND REDUCTION

▸ information from implants

Small implants allow remote monitoring of heart rate and blood pressure in animals used in research throughout an experiment. This means animals don't need the more stressful (and laborious) removal from their social environment for repeated scanning, and boosts the quantity and quality of data, thus reducing the number of animals required to produce meaningful results.

REDUCTION



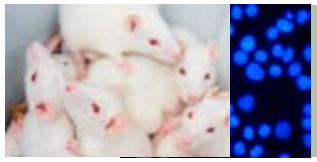
▸ early in vitro safety testing

To protect the unborn child, new medicines need to be studied to make sure they would not damage the embryo if used by a pregnant woman. Current international guidelines require studies on pregnant animals, but some companies are employing an in-vitro embryonic stem cell test. This cannot replace the formal test on live animals, but it can reduce the number of such tests, by helping exclude compounds that are not suited for further development and thus avoiding unnecessary testing.

REFINEMENT AND REDUCTION

▸ gentle pain measurement

To investigate the capacity of new medicines to relieve pain and improve mobility in arthritis, companies use a new system of imaging and analysing how an affected animal walks, so as to immediately detect any subtle changes following treatment, before the changes progress to a more painful condition. This is less stressful for the animal than older methods, and can provide more accurate data using a smaller number of animals.



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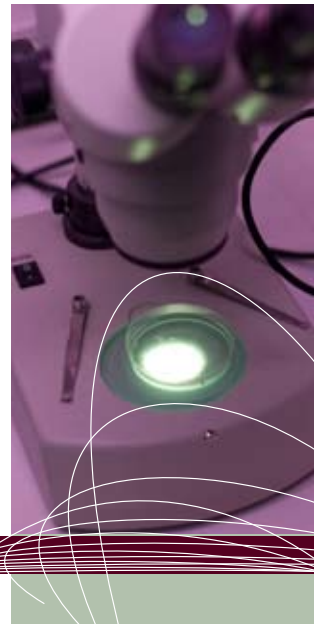


▸ early elimination of unsuitable compounds

Primary cell cultures are increasingly used to rank or select compounds in the early development stage, making it possible to discard unsuitable compounds before any animal research is undertaken. In vitro human hepatocyte cultures are used to assess how a compound will be metabolised and eliminated from the body, for instance, and new automated tests also improve accuracy. As a result, compounds are more precisely selected at the early stage of research, reducing the need for animal studies.

REDUCTION

▸ sharing data between researchers

Unnecessary duplication of animal studies is being reduced by collaboration between European pharmaceutical companies and animal welfare charities. They are centralising existing but widely dispersed data on the characteristics of the excipients used as vehicles for test substances, and making these data available to scientists who are planning animal studies. This will significantly reduce the need to study known excipients in animals.

The application of 3Rs programmes is an integral part of the pharmaceutical industry's day to day research activity. Every company involved in drug development is keen to reduce reliance on animal data, and to maximise the value of data by improving welfare and by standardising quality procedures when animals have to be used. Many companies require each of their research sites to create an annual plan of enhancement, covering improved housing conditions, less stressful or invasive research techniques, or better study design to reduce animal use.

Complex science and complex processes

In medicine, as in all sciences, nothing is simple - and neither is the development of alternatives to the use of animals in medicines research. The limitations are scientific, technical, regulatory, and logistical.

The use of cell cultures instead of live animals offers huge scope as an alternative test method. But for scientific validity, some studies have to be conducted in a complete living organism, since cells do not experience anxiety or endure diarrhoea or suffer from neuro-behavioural disorders.

Any reduction in the number of animals used in a particular procedure also has to be made in a way that does not reduce the scientific validity of the test. If too few animals are used, the results may be invalid, and the test will have to be conducted again - resulting in the use of more rather than less use of animals. Statistical input can help to optimise study design to deliver the information required using the minimum number of animals.

Even where valuable alternatives exist in principle, technical limitations may sometimes prevent their implementation in practice. For instance, scanning offers huge potential for refinement, reduction and even replacement of some animal testing procedures. Until recently scanners were developed principally for use with humans, and were therefore of a size and design unsuitable for rodents. Technological advancements in recent years helped to miniaturise these devices for use on mice, with excellent results.

Regulations can also be a barrier to the use of alternatives. Animal study requirements are clearly described and controlled in Europe - and so is the use of alternatives. Once an alternative has been developed and validated, it still has to be formally accepted in the context of the international registration of a medicine.

It can take 5-10 years to validate and implement a new method for legally required studies (and cost more than €500,000), and 3-5 years to validate and implement a new method for basic research. The outcome of these long and expensive processes is not always successful.

Legislation is not static. The relevance of regulatory requirements is also reviewed. For instance, the LD50 test (a study to find the dose of a compound that causes death in 50% of animals treated) is now no longer required. More recently, pharmaceutical companies have demonstrated that single-dose acute toxicity studies on animals are no longer necessary before first administration of a new medicine to man, because the data they provide can be obtained from other sources. As a result they have already reduced the use of this method in their internal procedures. However, this requirement still has to be formally removed from regulations before it ceases to be a mandatory elements in the authorisation procedures for new medicines.

European and national pharmaceutical laws set specific regulatory obligations for safety, quality and efficacy that can currently only be fulfilled with the results of animal studies. Safety and regulatory requirements are continuously being reviewed, and this unfortunately has frequently resulted in an increase in requirements.

Europe's legislation protecting laboratory animals sets very high standards. Additional requirements can be justified only when they bring tangible improvement in animal welfare and where they do not compromise patients' access to essential treatments. Undue tightening of regulation in Europe could make things worse. For instance, banning the use of non-human primates in Europe would not stop this type of research, but would merely drive it to more distant territories where Europe has no mandate to influence the standards of animal care and use.

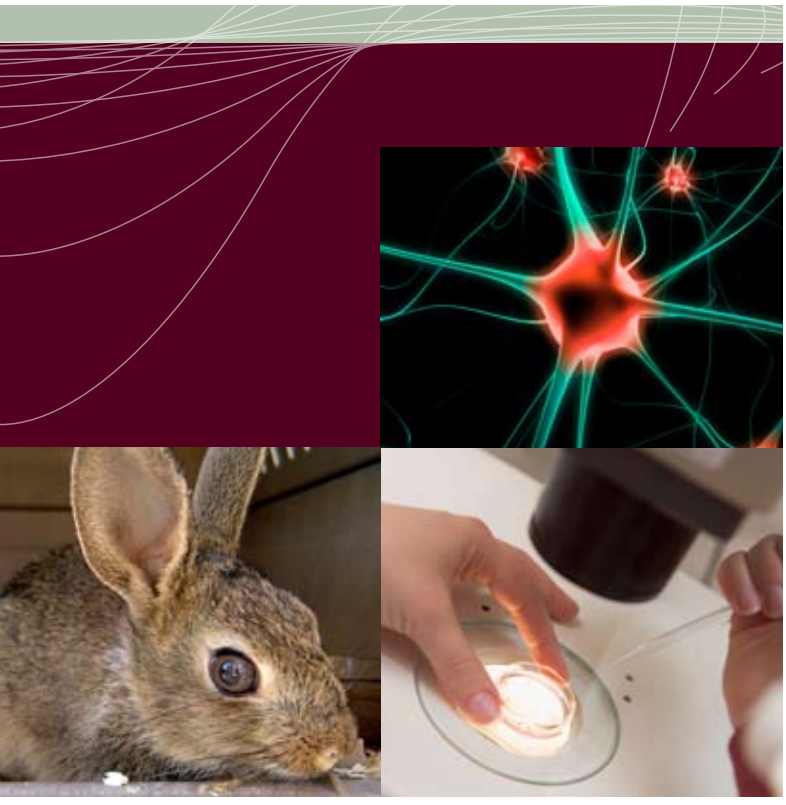
Over-regulation might severely reduce the research and development of new medicines in Europe. Indeed, although it represents a small part of the ten years or more normally required to develop a new medicine, the use of animals in pre-clinical studies is pivotal. Driving animal research out of Europe would also drive out other high value added R&D processes - and reduce the EU's science base, exporting research jobs, and leading to research conducted outside the EU's regulatory scrutiny.

Where next for **alternatives?**

The prospects are good for alternatives to play an increasing role in the research and development of new medicines. Scientific and technological advances are generating an ever-wider selection of methods to refine, reduce and replace the use of animals. Mechanisms are also in place to speed regulatory acceptance of alternatives in safety and efficacy evaluation, and to standardise test requirements more widely.

But there is currently no prospect of an end to the use of animals in medicines development. Mice and rats are contributing to development of stem cells for spinal cord and heart repair; work with mice holds out the prospect of oral or inhaled insulin for type 1 diabetes, of angiogenesis inhibitors for cancer and blindness, of gene therapy for muscular dystrophy; and mice and monkeys (e.g. macaques) are being employed in the development of malaria vaccine.

In addition to current unmet needs in the treatment of disease, new diseases continue to emerge.



Meanwhile, pressures for ever-safer treatments tend to strengthen and broaden safety assessment requirements. Testing of each batch of vaccines to be released on the market is still mandatory for public health reasons. In many cases this has to be carried out in live animals. Until the law is changed, therefore, animals will continue to be necessary.

And because the pursuit of research is an open-ended exercise, it is possible that new developments will further boost demand for the use of animals in testing procedures. Dramatic advances in biotechnology have made it possible to design genetically modified animals, which can be purpose-designed for specific test procedures. Transgenic animals are helpful and sought-after models, particularly in basic research and especially since the mapping of the human genome. Researchers have now identified all the human genes, they still do not know the functions of many of them. Genetically altered animals can help find the answers, because many human genes are found in a similar form in mice. The same technologies are creating the prospect of radical new treatment options through cell and gene therapy.

The availability of these techniques creates opportunities for new research - to overcome continuing challenges such as cancer, AIDS and cardiovascular disease. This simultaneously drives the need for using animals, in basic and applied research, and in safety testing.

The inescapable **dilemma**

If researchers could dispense with the use of animals in developing new medicines, they would. No scientist wishes to inflict any distress or discomfort on any living creature. But at present new medicines depend on animal research. It is unethical and illegal to test or use a new substance in humans without first ensuring maximal possible safety.

So scientists, regulators, industry, and society at large face an ethical dilemma: is the use of animals justified in the interests of a greater benefit? At present, the societal consensus is that it is justified where it is indispensable and where possible harm is minimized.

But each new development in science brings with it new questions of an ethical nature. As effective alternatives are developed and validated, they should be employed. However, before then can effectively be used, they need to be accepted by the regulators.

Industry and researchers too are constantly reassessing the balance between the development of new medicines and the development of alternatives to animal research.

Advances in science lead to new opportunities, new challenges, new medicines, and to new alternatives - and some of these new avenues can mean using fewer animals, while some may involve the use of animals in new ways. Everyone involved - and that also includes society at large - must play a part in reflecting on how this inescapable ethical dilemma is resolved.

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Leopold Plaza Building
Rue du Trône 108 - box 1
B- 1050 Brussels
Tel: +32 (0)2 626.25.71
e-mail: info@efpia.org

www.efpia.eu