



ANIMALS IN DRUG RESEARCH  
**A NEW ERA**



## Fewer animal tests thanks to new research technologies

### Foreword

Tests with animals arouse strong sentiments and cause people to adopt black-and-white positions, whereas the truth lies somewhere in the middle. This is perfectly illustrated by the thalidomide disaster in the sixties. The use of this drug during pregnancy caused malformed limbs in babies. Some saw this as ultimate proof that animal tests were unable to predict the effect in humans and were therefore pointless. That is not correct, however. The truth is that the animal species used in the studies (rats) proved insufficiently sensitive for those 'teratogenic' tests. It is the conclusion that does not tally. The disfiguring effects would have come to light if extra tests had been carried out – for instance in rabbits.

As a worldwide health concern, our family of companies feels responsible to the society we live in. We, too, use laboratory animals, and we, too, look forward to the day when we can do without animal studies.

Until then, however, we are confronted with the moral dilemma presented by the balance between human and animal welfare.

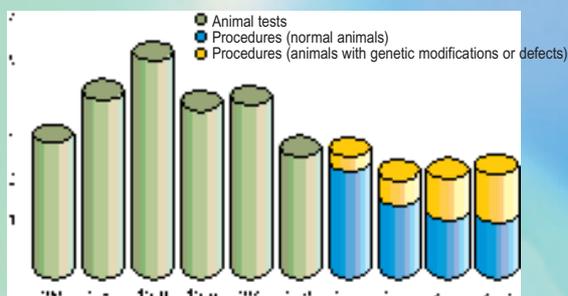
What would you do if a life-saving though potentially carcinogenic drug for you, your parents or your children had not first been tested in animals?

This brochure offers a concise and clear insight into the use of animal tests in the pharmaceutical industry. It sets out the facts and disproves the myths, so that a proper understanding of the rationale for animal tests can facilitate human and animal well-being.

# What?

## Fewer animals used

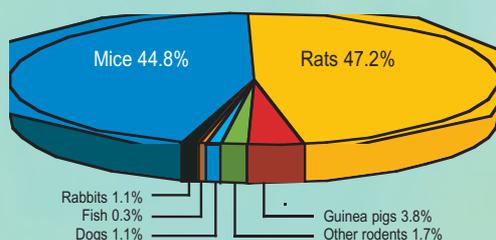
In the last few decades, countless medicines have become available for more and more illnesses. Nevertheless, over the same period, the number of animal tests has halved in countries such as the UK.<sup>1a</sup>



## Almost exclusively rodents

Virtually all our laboratory animals are rodents (97.5%). The rest is made up of rabbits (1.1%), dogs (1.1%) and fish (0.3%). No anthropoids, half-apes or other apes are used.

In the EU, only 0.1% of all laboratory animals are (half-)apes.<sup>1b</sup>



<sup>1a</sup> UK data, <http://www.rds-online.org.uk/>

<sup>1b</sup> Data for all in-house animal studies

<sup>2</sup> Pharma.be brochure 'Biomedical Research and Animal Studies', available at [www.pharma.be](http://www.pharma.be) > Publications and press

The shift to animals of a 'lower order' had already been initiated a century ago. The first Nobel Prizes for Medicine or Physiology went to researchers who conducted studies with cows, sheep, dogs or cats. Rodents first appeared in the sixties and in 2002 even worms.<sup>2</sup>

## Bred

The law is clear: laboratory animals must always be bred in approved establishments in order to guarantee animal well-being and compliance with health regulations. Furthermore, genetic differences between animals from the same population are minimal, so that variations in the test results are limited and more reliable outcomes are obtained. As a result, tests have to be repeated less frequently and fewer laboratory animals are needed. The rumor that stray animals are used is a myth. Laboratory animals such as dogs, cats and primates are even given an indelible marking when they are weaned.

## MICE WITH CYSTIC FIBROSIS

Cystic fibrosis (or mucoviscidosis) affects 1 in 2500 children. They have a life expectancy of only 30 years.

What causes this disease? One faulty gene! In 1992, researchers succeeded in breeding mice with that faulty gene so that at last they could investigate what exactly is going wrong in the body. It has also recently been demonstrated that inhaling 'healthy' mucogens has a positive effect on the lung condition.

The 'cystic fibrosis mouse' is a transgenic laboratory animal, which means that man altered its genetic properties. All its descendants inherit the gene, so that serious human diseases can be minutely studied. In this way, transgenic laboratory animals at last offer hope for all patients suffering from one of the approximately 10,000 congenital disorders.

The transgenic laboratory animals themselves are protected from all operations that are necessary to deliberately arouse disease symptoms. If the gene leads to the production of a protein, that protein can even be collected from the animals' milk without having to take blood samples.

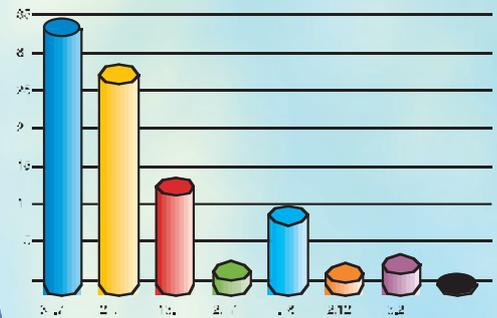


## EU imposes animal testing for safety and efficacy

# Who?

### Goal-oriented

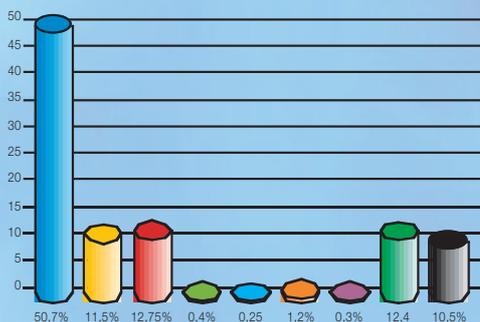
Which investigators in the European Union use laboratory animals? Mainly scientists in fundamental biological research (35%) and in the research and development of medical and related applications (28%). The production and quality assurance of those applications requires 14%, while investigation of their toxicity and safety accounts for another 10%. In the EU in 2002, nearly two-thirds of all laboratory animals were used for research and development in human and veterinary medicine, dentistry and fundamental biological research.<sup>3</sup>



- Fundamental biological research: 34.7%
- Research and development, medical, veterinary and dental applications: 28.4%
- Production and quality assurance, medical and dental applications: 13.6%
- Production and quality assurance, veterinary medicine: 2.47%
- Toxicology and safety: 9.93%
- Diagnosis of diseases: 2.12%
- Education and training: 3.2%
- Other

### If required by law

One-tenth of laboratory animals are used in toxicity and safety tests, 90% of which are required by law in at least one EU Member State. Of that tenth, only 0.25% is needed to test the safety of cosmetics and body care products. This disproves the myth of the indiscriminate use of laboratory animals by the cosmetics industry.



- Medical, dental and veterinary: 50.7%
- Agriculture: 11.5%
- Manufacturing: 12.75%
- Housekeeping: 0.4%
- Cosmetics and body care: 0.25%
- Food additives: 1.2%
- Animal feed additives: 0.3%
- Environment: 12.4%
- Other: 10.5%

<sup>3</sup> Report 5633/05 of the European Union, available in 11 languages at <http://europa.eu.int/comm/environment/>

Our investigators have already synthesized 260,000 original molecules and 80 new medicines.

Nevertheless, there is still no cure for about two-thirds of the 30,000 diseases.

The use of laboratory animals has enabled us to make essential progress in:

- Treatment of various types of cancer
- Inhibition of the AIDS virus
- Testing the first new antibiotic for tuberculosis in 40 years
- Long-acting injections in schizophrenia and psychosis
- Inhibition of autoimmune diseases such as rheumatoid arthritis, psoriasis, Bechterew's disease and Crohn's disease
- Analgesia through the skin.

84% of all animal tests are carried out in research and development, 16% in safety testing required by law, and only 0.2% in education and training.



## High throughput technology, computers, cell and tissue cultures replace animal tests where feasible

# When?

### Computers and technology

Often, thousands of molecules are screened before researchers hit on a substance that potentially has the right action. For example, computers construct theoretical molecules of the desired shape and size to replicate or block the action of an endogenous substance. Before, scientists had no other choice but to use laboratory animals to investigate the particular action of a new synthesized substance. There were no alternatives. This situation has changed with the arrival of high throughput screening. Enormous robot-controlled test batteries select thousands of molecules in one day for their desired effect for a particular disease.

### Cell and tissue cultures ('in vitro')

Human or animal cell cultures are also proving their usefulness. They are used in order to determine in a short time how cells respond to certain molecules. A further step forward is tissue culture – groups of cells – to investigate how tissues or organs react as a whole. For example, which substances cause a blood vessel to contract and to what degree?

Cell and tissue cultures ('in vitro' tests) help to reduce the number of laboratory animals needed and to determine faster whether or not a particular molecule works. Both humans and laboratory animals benefit from this.

### Body in action

Regardless of how effectively cell and tissue cultures can be used to predict certain effects, they still remain isolated systems that are investigated outside their natural environment.

Even the most sophisticated technology cannot possibly predict the many hundreds of

thousands of interactions between the active substance and the cells, tissues and organs in a body (pharmacology).

Does the active substance also have an effect on tissues that have not been investigated? Is it counteracted by another endogenous substance? Are there unexpected or toxic effects? Does the body break down the active substance before it has a chance to work? These are questions that beg for an answer before clinical trials (can) begin in humans.

#### Animal tests ('in vivo')

The human body and that of animals are largely controlled by the same genes. Laboratory animal testing is therefore the best – and for the time being the only – bridge between laboratory research and investigation in humans. Animal tests can provide strong indications as to which substances are likely to be toxic or active in humans as well. In order to determine the acute toxicity of a substance, the toxicity of high doses is measured over a short time, while for the chronic toxicity that of low doses is measured over a longer time. Eventually, 19 out of 20 substances are eliminated in this way before they are tested in humans!

#### Humans and animals

Animal tests are performed painstakingly and consequently have an extremely high predictive value. Nevertheless, it can never be 100% ruled out that an unexpected effect or adverse effect might manifest itself, even after many years of widespread use in humans. Some of the reasons are:

- Differences in bodily action between humans and animals
- Effects that are not visible in animals, e.g. psychological effects
- Averse effects which are so rare (e.g. 1 in 100,000) that the chance of detecting them in tests is virtually nil
- Interactions with medicines that have not been investigated.

## 13 years from molecule to medicine<sup>4</sup>

### Screening of tens of thousands of potential molecules



<sup>4</sup>The stages marked in yellow are those in which animal tests are used.



**Animal testing is only allowed  
if there are no valid alternatives**

## How?

### Strict regulations

European legislation is clear: "Animal tests are only allowed if no reasonable and practical method exists to obtain scientifically valid results without animals".

On the other hand, the law does stipulate that every medicine must be proven safe in animals before it can be used in humans.

### Fifold protection

The regulations governing animal testing are based on five principles:

1. As few animal tests as possible if there are alternatives
2. As few laboratory animals as possible
3. As little pain and/or distress as possible
4. The greatest possible well-being
5. The greatest possible know-how among animal caretakers and investigators.

Monitoring of compliance with all the regulations is ensured by meticulously kept records, approval conditions of laboratories and breeding facilities, and the establishment of an ethics committee at each laboratory. This committee is composed of internal and external specialists. External audits are conducted on a regular basis to verify compliance with the strict international regulations governing housing, care, transportation, etc.

### **Banned tests**

Some earlier tests have now been totally banned, such as LD50, a test that was designed to investigate what the fatal dose was for 50% of laboratory animals.

In most countries, alternatives must now also be used for painful tests such as eye toxicity and skin corrosiveness.

### **Minimum discomfort**

The great majority of trials are carried out without anesthesia for the simple reason that the experiments cause virtually no pain. Examples include a single injection or blood sample, or a change of diet. In all other cases, painkillers or anesthetics are used to minimize discomfort for the animals as much as possible.

### **Optimal well-being of laboratory animals is strictly monitored**

The legislator, the ethics committees, and the investigators themselves see to the greatest possible well-being of the animals. Stress or discomfort is avoided during, before and after the tests. In tests where some degree of inconvenience is unavoidable owing to the nature of the investigation (e.g. development of medication against pain), discomfort is minimized and strictly monitored by objective measurement of stress hormones, for example. We go to this trouble because we owe it to the laboratory animals for the help they give us, but also because the test results are more reliable in animals that are not stressed, and in this way fewer animals are needed.

### **Working with animals**

You can only cater to the needs of an animal if you actually understand those needs. That is why it is very important that everyone who works with laboratory animals receives proper training. In Belgium, BCLAS (Belgian Council for Laboratory Animal Science), the universities of Ghent and Liège, and also some polytechnics and postgraduate training centers provide this kind of training. Nowadays, the subject 'Laboratory Animal Science' is even part of the curriculum of courses giving access to research work, such as Biomedical Sciences, Engineering and Bioengineering, etc.

## **THE STORY OF AN ANIMAL CARETAKER**

"When I started at the laboratory ten years ago, I had problems with animal testing. But once I had talked to a devoted colleague, I soon changed my mind: No medicines without laboratory animals - it's as simple as that. But for that very reason it is our absolute duty to give them the best possible care. Like anyone else who works with animals, I do my job with respect. Sometimes it really hurts when the media once again make unfair accusations.

Consider for example our brand-new Drug Safety Evaluation Center, where the animal tests are carried out for toxicity and 'in vitro' tests are developed to replace animal tests.

I am pleased that our laboratory animals can live there in the very best conditions. For example, the air is completely refreshed there 15 to 20 times an hour, which is once every three minutes! Bright lighting is avoided, the temperature is ideal, and the cages are spacious. As a result, we amply meet European standards. This makes our job most enjoyable too, so there is always time to stroke our animals."

## No new drugs without laboratory animals



# Why?

## Safety

### Stringent laws

The law requires that a potential medicine must first be proven safe in animals before it can be tested in humans. Different animal species are used for this purpose in order to avoid a repetition of the thalidomide disaster in the sixties. The use of that product during pregnancy caused deformed limbs in babies. Why did this happen? The drug had only been tested in rats, a species which afterwards turned out to be insufficiently sensitive to those 'teratogenic' tests. If extra tests had been done with rabbits, the development of that medicine would have been stopped at once.

### No alternative

We are currently busy finding alternatives to replace animal tests as much as possible (see also under 'Where?'). Nevertheless, no matter how reliable those alternatives may become, they remain isolated testing systems that can never fully replicate the complex interactions in the human body. Only animal tests can provide evidence of important adverse effects such as high blood pressure or liver damage, or threats to the unborn child (see also under 'When?').

### 99.5% Reliable

Animal tests are extremely efficient in detecting adverse effects. This is demonstrated by the small number of medicines that have to be taken off the market. Since 1961, of 2000 drugs in the United States, the United Kingdom, France and Germany, only 10 have had to be withdrawn. That is a success rate of 99.5%!<sup>5</sup>

### Ethics

Every investigator at our company looks forward to the moment when animal tests are no longer needed. But until then, it remains a matter of weighing up the pros and cons, of reconciling human rights and animal rights, and animal rights and animal well-being. That well-being is guaranteed not only by the strict regulations, but even more so by the respect that our investigators show towards their laboratory animals and the care with which they treat them.

### Cost

The use of laboratory animals is avoided as much as possible for financial reasons too, because animal testing is a very expensive business. In the UK, only 10% of the budget for pharmaceutical research is spent on animal tests.<sup>6</sup>

### MEDICAL BREAKTHROUGHS BY ANIMAL TESTING

Medical breakthroughs would have been virtually impossible to achieve without animal testing. Here are a few examples:

#### TECHNIQUES

Heart valve surgery  
Anesthesia  
Transplantation  
Blood transfusion  
Kidney dialysis  
Chemotherapy  
Burns  
Cataract  
Heart-lung machine

#### DISORDERS

Diabetes  
Tumors  
Leukemia  
AIDS  
Antibiotics  
Burns  
Epilepsy  
High blood pressure  
Asthma

#### VACCINES

Polio  
Measles  
Tetanus  
Mumps  
German measles (rubella)  
Whooping cough  
Hepatitis  
Diphtheria  
Meningitis

<sup>5</sup> Spriet-Pourra C & Auriche M (1994) Drug Withdrawal from Sale. 2nd edition. PJB Publications Ltd (Scrip Report)

<sup>6</sup> [http://www.rds-online.org.uk/pages/home.asp?i\\_ToolbarID=8&i\\_PageID=152](http://www.rds-online.org.uk/pages/home.asp?i_ToolbarID=8&i_PageID=152)



## Research

### Medical needs

The human race is afflicted by 30,000 illnesses, of which only 10,000 can be treated (fully or partially). Moreover, new life-threatening diseases keep cropping up, such as AIDS, Creutzfeld-Jacob and SARS. Others are becoming increasingly prevalent (e.g. Alzheimer's) or better diagnosed (e.g. cancer).

Most human illnesses also occur in one or several animal species, and this helps us to gain a better understanding of what exactly goes wrong in the disease process, or vice versa. For example, the muscles of rats with muscular dystrophy are affected less quickly than those of patients. Understanding why this happens may help us to find a treatment for this fatal condition.

Antibiotics save the lives of tens of millions of people each year. Nevertheless, germs sooner or later become immune to the existing antibiotics. Thanks to tests with mice, we hope to have the first new antibiotic against tuberculosis in more than 40 years ready within a few years. This is excellent news for the 300,000 patients who are infected each year by a multi-resistant tubercle bacillus.

### Animals help animals

Animals help man to combat his diseases and to improve his quality of life. It may look like one-way traffic, but it is not. Humans and animals have a lot of illnesses in common and, fortunately for animals, quite a few of the drugs for human use also find their way into veterinary medicine. This is how antibiotics, anti-inflammatory drugs, painkillers and dozens of other medicines find a double usage. In this way, man gives animals a better health in return for their contribution to the necessary tests.

What is more, laboratory animals help other animals in a direct way as well, because drugs that are developed specially for veterinary use have to be tested in laboratory animals first.

Research into typical animal diseases such as mad cow's disease or bird flu can only be carried out with those animals.

The result is animals that are happier because they are healthier, and epidemics that can be prevented.

### XENOTRANSPLANTATION

Various disorders, whether congenital or not, are capable of destroying organs. In those cases, organ transplantation makes the difference between life and death. Unfortunately, the success of the operation is determined by the degree of similarity between the old organ and the new one.

More than 150,000 people worldwide are on a waiting list for organ transplantation; one-third of them in vain. Another half a million patients who cannot live without a kidney machine would benefit from an organ transplant.

Pigs could offer a solution to this shortage. The pig's organs and proteins closely resemble those of humans. Hundreds of thousands of diabetics have already been helped with purified pig's insulin, while the heart valves of pigs are capable of giving a diseased human heart a new lease of life.

Unfortunately, the use of live animal organs leads to acute rejection (within a few minutes) because the body immediately identifies one protein on the cell surface as an intruder. In 2002, scientists managed to eliminate the gene for this protein (one of the pig's 100,000 genes!). In this way, a new pedigree pig is bred whose organs do not provoke acute rejection. The medium- and long-term risk of rejection still remains to be treated, however.

The process opens up entirely new perspectives for the transplantation of skin, pancreas cells for treating diabetes, kidneys, spleen and liver. Our investigators therefore continue to keep close track of developments in this area of research.<sup>7</sup>

<sup>7</sup> [http://www.rds-online.org.uk/pages/home.asp?i\\_ToolbarID=8&i\\_PageID=161](http://www.rds-online.org.uk/pages/home.asp?i_ToolbarID=8&i_PageID=161)



# Where?

## 3R's

Both the pharmaceutical industry and animal rights groups are committed to maximizing animal welfare. Progress is being made on three fronts – the three “R” principles:

### ■ REDUCTION

Using the fewest number of laboratory animals possible.

### ■ REFINEMENT

Developing test procedures that limit the potential for discomfort to animals.

### ■ REPLACEMENT

Substituting alternative methods without animals wherever possible.

**It takes time to replicate three billion years of evolution in a test tube**

### Reduction

Test results are only useful if they are 'statistically significant'. In other words, they reasonably exclude the possibility that a result may be attributed to coincidence. The idea is to use as few animals as possible and still obtain reliable results. Otherwise the test has to be repeated.

The use of genetically identical animals (see the 'cystic fibrosis mouse' under 'What?') also rules out quite a lot of variability, so that equally reliable results can be obtained with fewer animals. The same applies for the care of laboratory animals: the better the care, the smaller the risk of infections and other disorders distorting the results.

Furthermore, the competent government authorities and the pharmaceutical industry in America, Europe and Japan are working on a harmonization of the legal requirements for drugs in order to avoid obligatory repetition of virtually identical tests.

### Refinement

By refining the research and breeding methods, animal discomfort is avoided as much as possible. Where a particular test calls for repeated readings of a certain hormone, multiple blood sampling is avoided by introducing a small appliance under anesthesia which continuously reads the hormone levels.

Another example of refinement is the abolition of the LD50 test (the dose at which 50% of laboratory animals die). It has been replaced by the 'Fixed Dose Procedure', which yields equally reliable results with a fixed dose, which is not lethal and for which fewer laboratory animals are needed.

### Replacement

Naturally a great deal of attention goes to methods that entirely obviate the need for animal tests.

High throughput technology, computer models, cell and tissue cultures have already been discussed under 'When?'.

When a drug is developed, we investigate whether it irritates the eyes. To this end, we used to apply the substance directly in the eye of a rabbit (Draize test). Fortunately, we now use bovine eyes that are obtained from abattoir offal. The BCOP test (Bovine Corneal Opacity & Permeability Test) is used to determine whether the cornea lets through more light and liquid after contact with the substance. The values that are measured are combined to obtain an in vitro score, which is a measure of the in vivo eye irritation.

These tests are sometimes labeled as 'alternative' methods, which is not exactly the right choice of words, since it suggests that those methods can replace animal tests. This is usually not the case. For that reason it is better to speak of 'complementary' methods. Although no expense or effort is spared to make progress, it turns out time and again how difficult and even impossible it is to 'imitate' the body in a test tube. This should hardly come as a surprise: after all, it has taken the human body three billion years to evolve into its present state!

