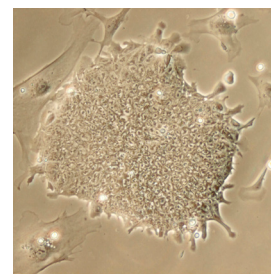
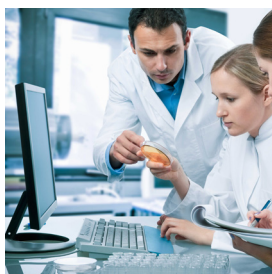


3Rs: Replace, Reduce, Refine

Minimising Impact & Reducing Numbers



COMPUTER SIMULATION IS INCREASINGLY RELIABLE

REDUCTION

Data derived from computer simulation enables a more efficient use of animal models. Also, as the quality of information increases, the best compounds can be selected for further optimisation and development.

Drug Metabolism and Pharmacokinetics (DMPK) refers to the process through which a drug is absorbed, distributed, metabolised and eliminated by the body. **DMPK tests show if the medicine reached the target and for how long.** Traditional methods for testing compounds in animal studies require labour-intensive set up, and a long time is needed to generate and assess the data.

Pharmaceutical companies have therefore developed computer-modelling systems, which can reduce significantly the number of animals used. Computer models consist of several computer simulations that are based on human physiology. When coupled with the data obtained from high-throughput screening robots and previous in vivo data, computer simulation programmes – such as PK Predictor – **deliver data in minutes.** This helps to make a decision on whether to continue with a compound or not, so that animals studies are only conducted for the most promising compounds.



REDUCING ANIMAL USAGE THROUGH ENHANCED EXPERIMENTAL DESIGN

REDUCTION

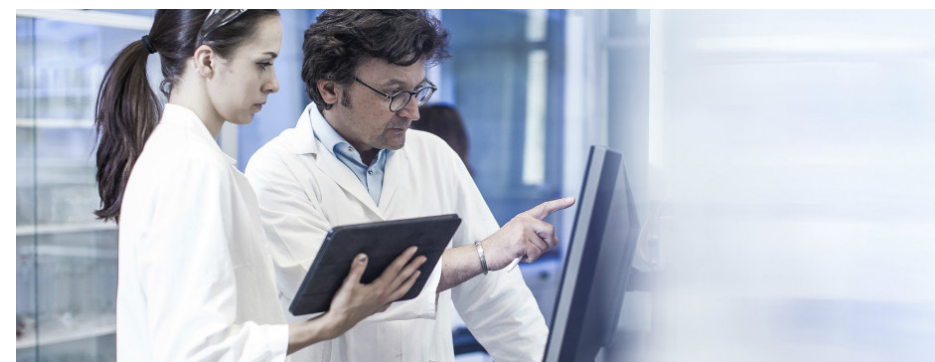
Biological experiments using animals are of great value in helping scientists to understand disease and evaluate new medicines.

However, the statistician supporting the design and analysis of these studies has to balance the inherent inter-animal variability, and therefore the need to use sufficient animals to produce **robust and reproducible data** to give scientists confidence in the results, with the desire to minimise the number of animals used. Variability may be due to any number of factors, and is influenced by both the operator and the environment.

The statistician's approach is to exploit this variation and strengthen the study design, allowing the scientist to better control the biological variability by **removing or reducing the influence of these factors**.

Complex experimental designs are sometimes mistakenly associated with increased animal numbers. In fact, exploring the full potential of alternative designs can help reduce sample sizes.

* **Factorial designs** allow examining simultaneously the effects of multiple independent variables and their degree of interaction in one experiment.



Using a factorial design to understand the underlying cause of a hypothermia side effect in transgenic mice used only 30% of the animals required for a non-factorial design.

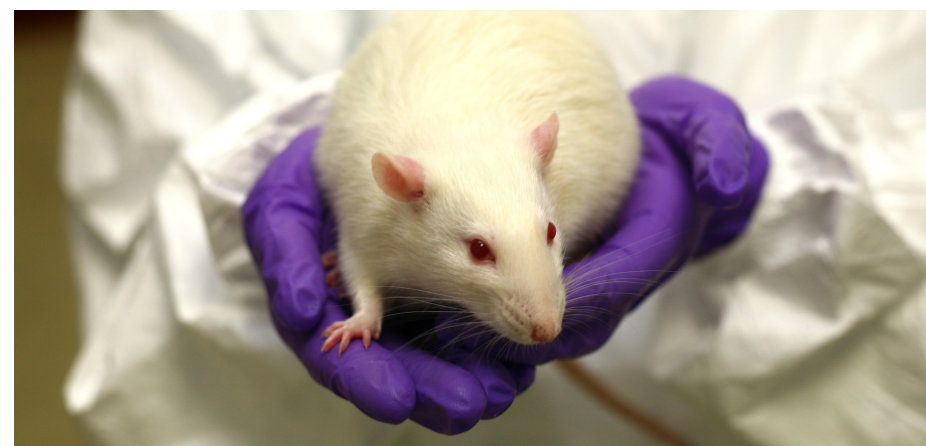
* In **cross-over designs**, frequently used in clinical studies, every animal receives each of the treatments under investigation in sequence, resulting in the use of fewer animals. Using this design for a series of novel anti-psychotic treatments in rats resulted in a 50% reduction in animal usage over a year.

* **Nested designs** allow researchers to investigate factors that may increase variability. When studying brain cell viability in an animal model of stroke, we found that increasing the number of measurements taken for each animal allowed to reduce the total number of animals used by 30%, without compromising the validity of the study.

MEASURING EFFECTS OF ANTI-CANCER MOLECULES WITHOUT THE NEED TO INDUCE DISEASE

REDUCTION, REFINEMENT

Some targets for anti-cancer drugs have specific measurable effects in normal tissues and this can allow researchers to measure their efficacy in animals eliminating the need to induce tumours as well as reduce the number of animals and to provide additional scientific benefit.



The majority of effective anti-cancer drugs currently in use were selected based on their ability to inhibit the growth of tumours in rodents. However, a small number of targets for anti-cancer drugs have specific and measurable effects in normal tissues and this may allow the effectiveness of an anti-cancer drug to be measured in animals without having to induce tumours. If the drug target does play a role that enables simple measurement, we use tests in healthy tissue to assess the effectiveness of new therapies in a normal animal.

Refinement: Tumours do not have to be induced in an animal. The duration of study is often shorter.

Reduction: In a cancer study, to look for activity of a number of potential drugs, blood pressure was monitored in a total of 12 normal rats. This replaced use of 800 mice with tumours that would previously have been required. In addition, the rats received only a single dose per test rather than the 10–14 daily doses to a mouse that would have been required to see tumour growth inhibition.

MOUSE EMBRYONIC STEM CELL TEST (EST) TO DETECT EMBRYOTOXIC POTENTIAL

REDUCTION

The EST can contribute to reducing the number of in vivo studies by helping to exclude from development molecules that could damage the embryo.

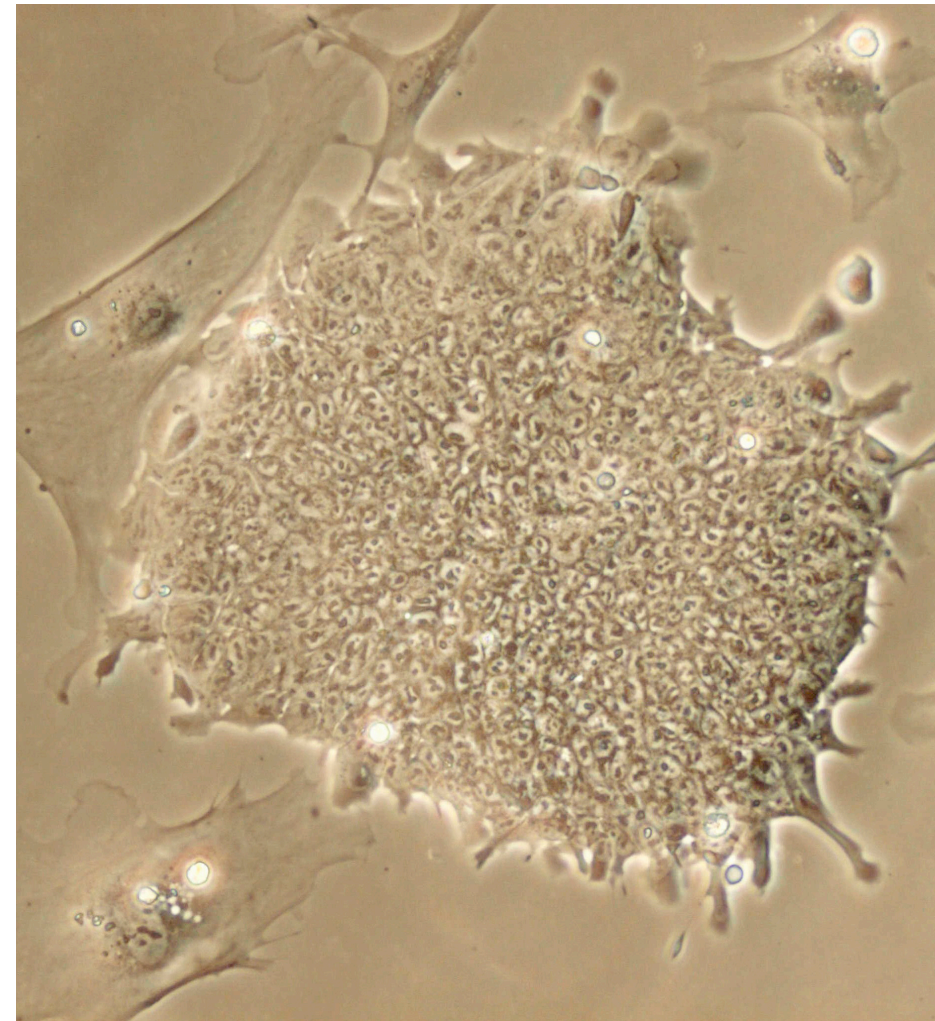
To protect the unborn child, regulatory authorities require that chemical compounds and potential new medicines are tested to assess the risk to the embryo, if the product is to be used by a pregnant woman.

According to current international guidelines this developmental toxicity testing involves the treatment of pregnant animals, mainly rats and rabbits.

A mouse in vitro embryonic stem cell test was implemented in some companies in pre-clinical development units (where medicines are tested before they are first given to human patients).

The EST uses the **potential of embryonic stem cells to specialise into different functions** in the body, such as beating myocardial cells. This type of cell was chosen primarily because embryotoxic effects should be first seen in the heart tissue.

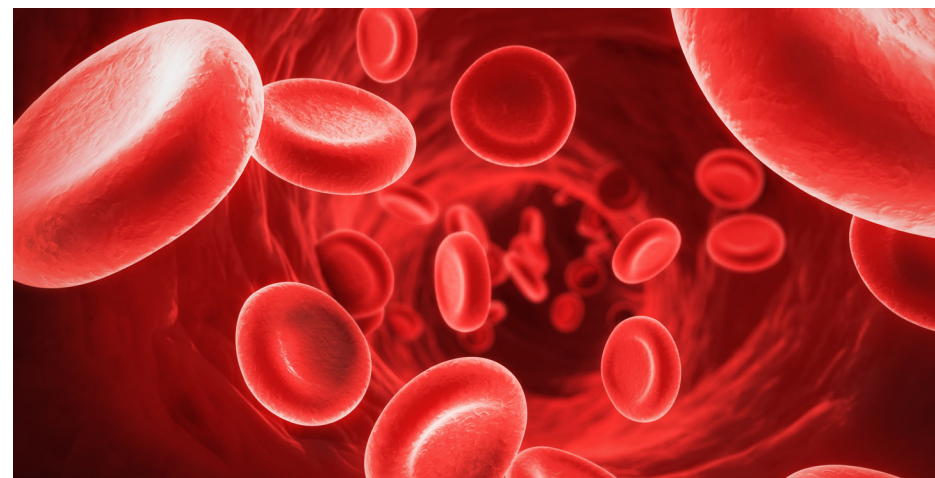
Using this test means only the most promising compounds are tested in animals, reducing the number of animals used.



NEW BLOOD SAMPLING TECHNIQUE HELPS REDUCING NUMBERS OF ANIMALS

REDUCTION

One specific area where the pharmaceutical industry has been involved in developing and exploring the potential of a new technology is micro-sampling. This ability to collect and analyse blood samples as small as 5–20µl has led to re-examination of the way in which blood samples are taken in both the clinic and in animal studies.



Technical developments in measuring equipment (e.g. high performance liquid chromatography mass spectrometry HPLC–MS/MS) and increased sensitivity of detection have allowed for the collection of much smaller blood samples from animals. This has led to an opportunity to refine the way in which the animal studies required prior to human dosing can be conducted. An example of where this technology has contributed to 3Rs progress is:

- * When applied for Toxicokinetic (TK) or pharmacokinetic (PK) sampling in rodent studies, these techniques serve to reduce animal use and also add scientific advantages by allowing a **direct comparison of potential adverse effects with compound exposure in the same animals**. To date, the implementation of these micro-sampling approaches in exploratory nonclinical studies and in some regulatory studies has the potential to enable a reduction of 60% in the number of mice required for stand alone to TK/PK studies and of 80% in the number of samples to analyse.
- * These efforts associated with bioanalytical micro-methods will avoid the need to use satellite animals in regulatory toxicology studies and there is currently an ICH S3A Q&A document in consultation that supports the use of micro-sampling as well as outlining its potential limitations.

USING FISH LARVAE TO REPLACE RODENTS

REPLACEMENT

Rats, mice and guinea pigs are needed to study the pharmacological effects of a molecule. The pharmaceutical industry has developed a model using zebrafish which, under certain circumstances can offer a viable alternative to the use of rodents at certain stages of the research programme.



At some stages of research, it is possible to use zebrafish larvae to replace higher-order animals (mice, rats, etc.) to study targets and mechanisms in intact organisms. The reliability of these studies for man is very similar to the one offered by rodent species for some studies.

Zebrafish larvae are complex "lower" vertebrates. They emerge from their eggs 48 hours after fertilization with a complete central nervous system, as well as endocrine, gastro-intestinal and cardiovascular systems. This allows simultaneous monitoring of behaviour and various physiological parameters.

During the first six days after hatching, the larvae are less than 1 mm long, meaning handling and housing are much easier than for rodents.

Using zebrafish larvae brings direct "3R's" benefits:

Replacement: This method makes it possible to evaluate compounds early in drug discovery in lower-order animals, instead of rodents.

Reduction: Studying the effects in a complex organism makes the early selection of compounds much more reliable, lowering the overall number of mice or rats used in subsequent research phases

PUTTING ANIMAL WELFARE PRINCIPLES AND 3RS INTO ACTION

Promoting good science and animal welfare, as well as increasing understanding of how the two are intertwined for the benefit is an essential part of promoting and enabling high quality research and 3Rs achievements. The progress we make in this area is one step in enhancing benefits for patients. This poster presents a non-exhaustive inventory of examples from the pharmaceutical industry and its collaborations within the research field.

1. Beyond policy principles: the EFPIA working group on Research and Animal Welfare

- Fosters **on-going exchange of information and good practice** within the pharmaceutical industry and beyond (ongoing active engagement in dialogue with the public, legislators, policy makers and interested parties) within and beyond EU borders;
- Works to **reconcile research needs** with animal welfare imperatives;
- Promotes **development and uptake of 3Rs approaches** in research and testing across all stakeholders;
- Has published a **report showcasing tangible examples** of their 3Rs activities going beyond regulatory compliance;
- Has established **links with US counterparts** (3Rs leadership group of International Consortium for Innovation and Quality in Pharmaceuticals) to coordinate and avoid duplication of effort.

2. Results of joining forces and sharing information

- **Microsampling and dried blood spot methods** to reduce and refine animal use in toxicokinetic and pharmacokinetic studies for human dosing;
- **Recommendations for significant refinement of short term toxicity studies** based on data on body weight loss from fifteen pharmaceutical companies;
- **Welfare assessment tool** (cognitive bias testing and cardiovascular analysis in dogs) to measure experimental refinement, and focus training on individual animals;
- **Educational tools** for training for non-human primates, dogs, goats and pigs to voluntarily cooperate with scientific, veterinary and husbandry procedures.

3. Beyond compliance: improving practice and leading by example

- **Regular monitoring** of animal welfare standards and **independent audits**, internally and of more than 500 external partners;
- **Ongoing review and best practice sharing** of animal welfare standards and 3Rs recommendations across member companies, with contract research organizations and other stakeholders;
- **Animal welfare guidelines and recommendations** for non-English-speaking animal caretakers and lab staff;
- **Promotion of information, education and training** to implement the EU Directive on protection of animals; in various Expert Working Groups and Scientific Coalitions (e.g. UK Bioscience Coalition);
- **Participation** in Center for Alternatives to Animal Testing (CAAT) and European Partnership for Alternative Approaches to Animal Testing (EPAA) activities.

4. 3Rs-focused research for quality of science and animal welfare

Replacement of monkey kidney primary cells with L20B cell line for inactivation testing of polio vaccines	→ Reduction of number of animals used by ~95 % for this specific purpose
Rat aortic tissue based assay for unwanted blood pressure effects	→ Reduction of number of animals used by ~95 % for this specific purpose
End-point grid for clinical signs to stop study or decrease dose	→ Refinement in dose finding studies
Smaller needles for blood sampling in non-rodents	→ Less trauma
Study investigating influence of oestrous cycle in pharmacokinetic studies	→ Shift from male to female mini-pigs enables group-housing
Software to stimulate absorption of drugs through skin in humans and animals	→ Reduced reliance on animal studies
Reduction of fasting time in rats and mice for charcoal meal studies	→ Group housing enabled by reduced aggressive behaviour
Validated & integrated method to identify potential effects of drugs on male fertility	→ Spares 80 male rats for every compound tested
New safety test for three new monoclonal antibody anti-infectives	→ Reduction of number of non-human primates by 57 % for this specific purpose
Method for keeping myocytes healthy two weeks instead 24 h	→ Many more compounds can be tested on cells supplied from a single dog
Validation of 3T3 cytotoxicity test to identify non-toxic chemicals with ECVAM	→ Reduction of number of animals by 80 % for this specific purpose
New fish liver cell model with spheroid cells being alive 40 instead of 4 days	→ Reduce and replace live fish in regulatory required bioaccumulation tests
Potential model in fish to assess hearing loss in safety assessments	→ Significant reduction of number of animals used in preclinical testing

5. Biomedical research delivers 3Rs benefits: examples from the Innovative Medicines Initiative (IMI)

Project	Objective	3Rs benefits
StemBanCC http://stembancc.org	Generate and characterise high quality human induced pluripotent stem (iPS) cell lines to study a range of chronic diseases (e.g. diabetes and dementia) and test for drug efficacy and safety	Liver, heart, nerve and kidney cells for toxicology testing
EUROPAIN http://www.imieuropain.org	Elucidate the mechanisms of pain, using novel experimental models, human volunteers and clinical data of pain patients to improve treatment of chronic pain	Improved or reduced animal models
ABRISK http://www.abirisk.eu	Understand factors behind immune response which may decrease effectiveness of products based on proteins or monoclonal antibodies and aid in the creation of new, safer biopharmaceuticals	Tools for determining patient response directly, i.e. without the use of animals
OrBiTo http://www.orbitoproject.eu	Understand how orally administered drugs are taken up from the gastrointestinal tract into the body, and create new tests and computer models that better predict the performance of e.g. capsules or tablets in patients	Non-animal tests and computer models

DEFINING METRICS TO ASSESS THE PROGRESS OF 3Rs INVESTMENTS AND ACTIVITIES



Background and Challenges

Members of the European Federation of Pharmaceutical Industries and Associations (EFPIA) launched an **unprecedented collaborative attempt** to define how to measure **investments in 3Rs and their impact**.

Such **key performance indicators (KPIs)** are necessary to assess effectiveness and to provide evidence-based communications about industry efforts in 3Rs and other animal welfare initiatives.

The following challenges were identified and addressed:

→ Direct links of 3Rs initiatives to business projects are **often not visible**; therefore these investments and efforts are not always adequately communicated internally or externally.

→ EFPIA companies conduct research in a **wide range of therapeutic areas**. One set of KPIs may not fulfil all needs.

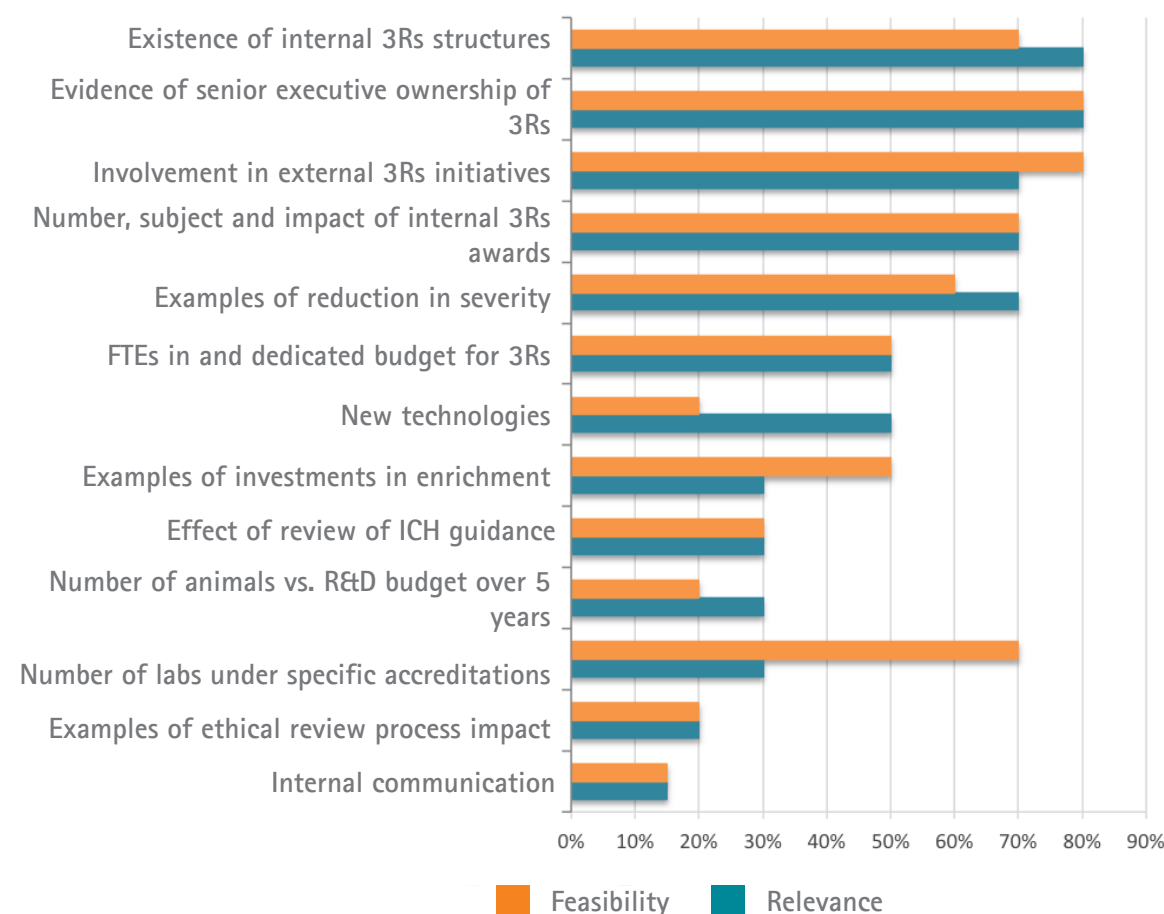
→ Number of animals used in research is not appropriate as a KPI, as it is influenced by **multiple variables** (closure of sites, discontinuation or launch of projects, etc.).

→ There are **several facets** to 3Rs – ethical, scientific, costs; requiring multiple and sometimes qualitative KPIs.

Results

Companies discussed a wide range of potential KPIs. These were then assessed for **relevance** and **feasibility** within individual companies.

Latest results of the ongoing work to define a common set of indicators to provide evidence of the investments in and benefits of 3Rs implementation:



Conclusions

1. Numbers of animals used alone **would not give accurate picture** of 3Rs efforts.
2. Assessing the impact of and communicating about 3Rs efforts/investments requires a **set of quantitative and qualitative KPIs**.
3. Companies may **measure 3Rs and welfare investments in different ways** due to their diverse ways of operating.
4. A menu of **options assessed for their relevance and feasibility** has been identified in order to allow companies to communicate internally and externally about their efforts and achievements.
5. There is a large amount of agreement on the **relevance and feasibility of many of the qualitative KPIs**.
6. The attempt to collaboratively define meaningful 3Rs metrics is an unprecedented effort, demonstrating EFPIA members' **ongoing determination** to further progress the enforcement of the 3Rs principles.

EFPIA represents the pharmaceutical industry operating in Europe and is the voice on the EU scene of 2,000 companies committed to researching, developing and bringing to patients new medicines that improve health and the quality of life around the world. Its working group on Research and Animal Welfare contributes to EU and international debates and dialogue on welfare of laboratory animals, enhances sharing information and good practice, works to reconcile research needs with animal welfare imperatives and promotes development and uptake of 3R approaches in research and testing both within the industry and beyond.

Poster prepared with the support of the member companies and associations of the Research and Animal Welfare Group: Abbvie - ABPI - AstraZeneca - Bayer - Boehringer Ingelheim - Bristol Myers Squibb - Covance - EARA - Eli Lilly - Ellegaard Göttingen Minipigs - Farmindustria - FELASA - Grünenthal - GSK - GSK Vaccines - Hoffmann La Roche - IFAH - Interpharma - Johnson & Johnson - Marshall BioResources - Merck KGaA - MSD - Novartis - Novo Nordisk - Orion - pharma.be - Pfizer - Sanofi - Sanofi Pasteur - Servier - Shire - UCB Pharma - Understanding Animal Research - vfa

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