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Putting animal welfare principles and 3Rs into action

Pharmaceutical Industry Report 2022 Update

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*More than medicine - Committing to the science-based phase-in of methods to replace the use of animals for scientific purposes

The impact of the pharmaceutical industry adds value beyond producing medicine and vaccines. We are working with others at the cutting edge of innovation and science and on common issues and challenges, working with health systems to make sure the medicines and vaccines have a positive impact and be responsible in how companies operate. We are doing #MorethanMedicine development.

The pharmaceutical industry understands the importance of ensuring that innovation is undertaken responsibly, with the right processes and governance. We are therefore committed to the science-based phase-in of methods to replace the use of animals for scientific purposes and the deletion of animal tests which are obsolete or redundant.

Advances in science are leading to fewer tests and experiments on animals, and to new ways to reduce the impact on animals. Medicines developers continue to be involved in several initiatives, which affirm the key principles of the 3Rs (Replacing animal experiments wherever possible with substitutive methods, Reducing the number of animals used and Refining experiments to minimise the impact on animals) or change the current research (and development) paradigm. In 2020 the innovative pharmaceutical industry invested an estimated € 39,000 million in R&D in Europe. There has been a steady increase for the past 30 years. In 1990 it was at 7800 Mil, and 2000 at 18000 Mil. However, with a clear increase in R&D investment we are seeing a significant decrease in the number of animals used in industry.

In the EU, the use of animals is impacted across numerous Commission actions including the Pharmaceutical strategy, and as part of the Green Deal, the Chemical strategy for sustainability? We are keen to have a continued dialogue with stakeholders and regulators and together define what is most efficient and appropriate to ensure correct implementation of policies, accelerate development and implementation and access to new methodologies.
Since the adoption of the EU legislation governing animal use, EFPIA and its members have been publishing reports to visibly highlight our actions on putting animal welfare principles and the 3Rs into action. Here we introduce you to our 6th report, Enjoy!

> I support the science-based phase-in of methods to replace the use of animals
>
> **#MorethanMedicine**

KIRSTY REID
DIRECTOR SCIENCE POLICY
EFPIA

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Phasing-In New Approach Methodologies

Medicines developers are required to demonstrate that potential new and commercialized medicines, therapeutics and vaccines are effective and safe in humans. For new drugs, the potential side effects are identified before clinical trials and obtaining the license to go to market. We rely on many different technologies to support the most appropriate testing strategies, which include in vitro assays, in silico methods and at a late stage, animals being used in preclinical development. As well, patient data and clinical research provide additional scientific evidence for research and development of new drugs, therapeutics and vaccines. Research involving the use of animals can provide much important information – for instance they can help to advance basic scientific knowledge, understand the basis of diseases, and to investigate and develop new medicines as they allow for to evaluate effects on a whole organise. However, we also recognise the ethical and animal welfare issues involved. Animal research raises dilemmas not only for scientists and organisations that use animals as part of their research projects, but also for society as a whole. Applying improved biological knowledge, technological advances, computer simulations and innovative (Microphysiological systems, Complex 3D models) methods may lead to significant reduction of the number of animals actually used, however, most of these methods are not yet able to fully replicate/are not sufficient to extrapolate the conclusion of simple models to the complexity and reactions of a living organism especially for systemic and chronic conditions. However, they are already widely used by scientists to inform their research programs and, being complementary the ones to the others, address the scientific questions for the progress of medicine.

Europe must remain a world leader in medical research and innovation to address the unmet medical needs of its citizens and to preserve its capacity to shape its health strategies as well as the unmet medical needs of world regions that do not have the resources or expertise to address their healthcare issues, e.g. addressing Malaria, Ebola. The global health pandemic also highlights this importance. Those conducting biomedical research should have access to the most appropriate technologies to achieve this imperative objective.

EFPIA Members commit to the science-based phase-in of methods to replace the use of animals for scientific purposes and the deletion of animal tests which are obsolete or redundant

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Open Communications
Dialogue | Reporting

EFPIA members support Phasing-In New Approach Methodologies

EFPIA members are committed to the science-based phase-in of methods to replace the use of animals for scientific purposes and the deletion of animal tests which are obsolete or redundant. EFPIA members aim to lead progress on this by engaging in a wide range of practical activities to help drive the development, uptake and promotion of non-animal technologies (NATs) and new approach methodologies (NAMs) so that these can be phased-in as soon as it is scientifically possible to do so.

The pharmaceutical industry members of EFPIA:

- Are fully committed to the principles of 3Rs;
- Continue to support the objectives of the Directive 2010/63/EU on the protection of animals used for scientific purposes which has enhanced animal welfare standards and mandated the application of replacement, reduction and refinement across the EU while ensuring Europe remains a world leader in biomedical research;
- Will continue to strive to go beyond what is legally required and work to develop and validate systems leading to improved 3Rs, animal welfare and high-quality science and technologies in every day practice and ultimately improve the lives of the people and animals that stand to benefit from the research. Training of staff will remain an essential element of good science and good welfare;
- Are committed to continue invest in collaborative research initiatives and projects to improve animal welfare and 3Rs, and support start-ups with expertise in new approaches as we transition from the Innovative Medicines Initiative (IMI – the largest health public private partnership) to the new Innovative Health Partnership (IHI);
- Will continue to work with regulators, the scientific community and civil society to improve implementation of the science and speed up regulatory acceptance of alternative methods in the EU and at a global level;
- Will strive to lead by example by disseminating beyond own department and own establishment to drive improvements in welfare and general quality of science;
- Will improve the systems in place working with academia, CROs, animal breeding and testing facilities to share good practices, new methodologies and lead by example by uptake of high 3Rs and animal welfare standards in the daily activities;
- Will be transparent in telling what we do and how we do it, to explain and justify where live animals are required and used and also inform on the work and commitment of companies to reduce the sectors reliance on animals;
- Will continue to identify, develop and implement their phase-in strategies and communicate on animal use through either dedicated webpages or CSR reports. Open communication and dialogue with the public are key to highlight our contribution to phasing-in replacement methods.

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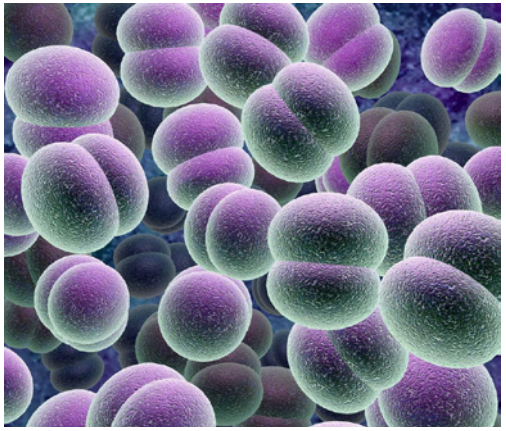
* 3Rs and welfare in everyday practice: Researchers go beyond the regulatory requirements to develop systems leading to improved 3Rs and animal welfare in every day practice
* Science and technology drive 3Rs and welfare – We invest continuously in changing research paradigms
* Staff training is an essential element of good science and good welfare

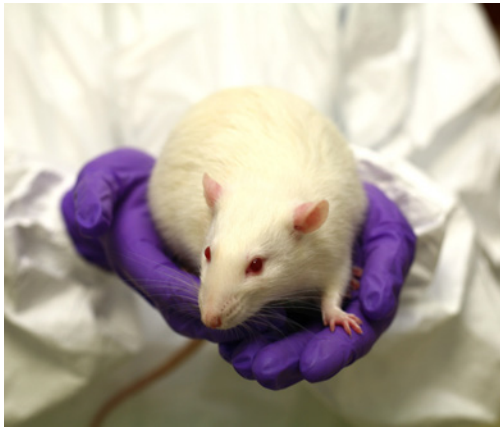
Leading by Example

* Dissemination beyond own department and own establishment drives improvement in welfare and general quality of science
* Full and correct implementation of Directive 2010/63/EC on the protection of animals used for scientific purposes is the responsibility and endeavor of the whole scientific community

Open Communication

* Communication, transparency and dialogue with the public





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3Rs and welfare in everyday practice: Researchers go beyond the regulatory requirements to develop systems leading to improved 3Rs and animal welfare in every day practice

Refinement: Non-invasive repeated urine sampling in female Göttingen Minipigs

Urine is a valuable tool in several contexts, for e.g., diagnostic or scientific investigations. However, it is not as readily accessible as other biological fluids, and urine sampling typically requires housing in metabolism cages or invasive sampling by cystocentesis. A new non-invasive method has been tested to collect voided urine in a voluntary setting. Pigs are gradually accustomed over several training sessions to being handled and touched in the vulvar area. Using food as a positive distraction while doing this helps. In general, no more than 10 short training sessions are necessary for the pigs to become comfortable with the procedure. When this is achieved, an ostomy bag is attached with the hole covering the vulva, so that any voided urine is collected in the pouch. The circumference of the bag is secured to the skin using medical tape. The pig is monitored regularly, and the bag removed when urine is observed in the pouch. Repeated sampling can be performed, as necessary. This method provides a pain-free, non-stressful way of sampling voided urine in non-anesthetized pigs.

© Ellegaard Göttingen Minipigs A/S

Refinement: Mouse Handling Techniques[1]

Laboratory mice need to be routinely handled for husbandry and experimental procedures. Traditionally this has involved lifting the mouse up by its tail. Picking up mice by the tail has been shown to be aversive and stressful as assessed by a range of methods. Research has demonstrated that alternative handling methods such as tunnel handling and cupping are less aversive and stressful. A number of companies have implemented these alternative handling methods across their global sites.

1: Hurst JL, West RS (2010) Taming anxiety in laboratory mice. Nature Methods. Oct;7(10): 825-6. doi: 10.1038/nmeth.1500

Systematic pooling of offspring from different litters and introduction of a 'buddy system' for housing transgenic mice

In small breeding colonies and especially for adult male mice, it can be challenging to have compatible cage mates readily available for social housing. A company has succeeded to reduce the number of individually housed animals in a transgenic breeding colony from 19% to 4% by systematically pooling offspring from different litters at the time of weaning and by the selection of compatible "buddies" for animals that would otherwise end up singly housed. Buddies are animals that don't have the genotype of interest and are maintained to facilitate social housing. The scenarios in which rodents from different cages can be pooled were determined by means of a detailed and structured procedure considering the known information on the strain generated (strain show aggressive behavior? Age pooling possible); gender of animals (females are housed with non-littermates, males demonstrate inter-male aggression, therefore housed with littermate, age of bringing animals together, daily monitoring specifically for aggressive behaviour.

Use of a combined non-invasive activity tracking system with camera recording for an efficient 24/7 behavioral assessment[2]

Before exposing newly developed pharmaceutical compounds to humans, it is important to evaluate, and if necessary, exclude compounds which may cause adverse effects. However, a full assessment of Central Nervous System (CNS) effects is challenging due to limitations in human-animal observational periods. On the other hand, compound effects on the CNS system

2: Frank Cools, Ivan Kopljar, Tekle Fetene, Herman Borghys. (2020) Use of the Actiwatch-Mini® in dog safety studies as an early indicator for drug-induced behavioural changes. Journal of Pharmacological and Toxicological Methods, Volume 104, July–August 2020, https://doi.org/10.1016/j.vascn.2020.106896. Weblink to supplier of actiwatch device: https://www.camntech.com/actiwatch-mini/

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are often reflected in general behavior, activity parameter changes, and unexpected clinical observations. The use of ACTIWATCH, a non-invasive activity tracking system, in combination with continuous camera recording is an appropriate method to follow any activity, behaviour and clinical observation changes during the full experimental period, especially when laboratory personnel are not present in the dog rooms. By addressing concerns about CNS effects of test compounds in advance of going to First in Human (FIH) clinical trials or to later stages of drug development more efficiently in a limited number of animals, this method prevents additional studies and consequently reduces the use of dogs in safety testing and toxicology.

Refinement through continuous digital monitoring of non-rodents in toxicity studies

Evaluation of behaviour in animals is essential to identify potential safety liabilities of drug candidates during pre-clinical development. Manual (visual) recording of behavioral and/or clinical observations in non-rodent toxicology studies, typically dogs and minipigs, performed by well-trained and experienced biotechnicians, has been the traditional standard widely accepted by regulators. These observations usually performed on several occasions during working hours and therefore do not cover animal behavior outside the working day when biotechnicians are not present in the animal vivarium.

To improve the clinical evaluation of non-rodent toxicity studies, a company has implemented Continuous Digital Monitoring, a combination of activity tracking and 24/7 video surveillance, for studies with Beagle dogs and/or minipigs. This approach is a powerful tool that sheds light on the direct observational shortcomings by mapping the awake-sleep activity and revealing time periods for further video analysis. The result is a more comprehensive and objective data set, providing more accurate identification of behaviors, nocturnal recordings, and removing untoward human impact on animal behaviors whilst gathering observations. Moreover, this method enables identification of potential Overall, Continuous Digital Monitoring enriches/improves safety evaluation of non-rodent toxicity studies, increases health monitoring of animals and facilitates decision-making to further refine animal welfare principles (3Rs).

Play Cages for Long Term Rodent Studies

There is an increasing requirement for the long-term housing of rats in telemetry studies. Throughout the literature, it is clear that the general health and condition of rodents is improved when there are more opportunities for them to exercise and display natural behaviors. Animals are provided daily access to a large, tall (three tier) exercise cage. A variety of toys and food items are placed around the cage to encourage climbing and exploring. Enrichment is enhanced by the inclusion of food supplements, in particular vitamin C-rich yoghurt drops which all the animals enjoy foraging for. Destructible enrichment "cocoons" which rats can easily shred to make a nest are also placed in various positions throughout the play cage. Rats also appear to enjoy carrying these cocoons around the cage, thus improving overall enrichment. Tinted plastic play tunnels can easily be placed on any tier of the play cage, these are long lasting, fun to chew and provide an easy view of the animals whilst still providing a refuge. As these play cages have been shown to clearly improve the conditions of rodents, telemetry study animals have started to be housed in this way, whenever possible.

Rat tickling to aid acclimation to inhalation exposure restraint

Assessing the safety of a novel inhalable pharmaceutical requires that rats are acclimated to purpose designed restrainers in which they are held whilst exposed to the new drug.

One innovative project examined how integrating tickling, a positive experience for rats, can help reduce animal stress and increase their acceptance of restraint. The project involved technicians spending approximately 15 seconds gently rolling over and tickling each animal followed by a 15 second rest period, this tickling/rest process is then repeated for up to 2-minutes. This procedure is performed for 3 consecutive days prior to any other procedure. Animals frequently wait for the tickling to continue, try to interact with the technicians' hand whilst still on their back, or wait to be rolled over again. In almost 100% of cases the animals became accustomed to the interaction, they

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would wait by the technician's hand to be rolled over again during their rest period, then explore their environment or try to continue interactions with the technician.

After just 3 days, the rats showed a demonstrable willingness to approach the technician's hand. The technicians involved observed that the animals are much easier to place in the restrainers than previously and exhibit fewer signs of stress or discomfort during the drug administration procedure.

Tail Vein Dosing in Cynomolgus Macaques

Due to the increase in the development of biologically based pharmaceuticals, there is a greater requirement for intravenous infusion administration studies in order to mimic the potential human therapeutic administration. Prior to the administration procedure, animals are acclimated to restraint chairs using positive reinforcement training techniques. Historically, administration of the test material involves insertion of a flexible cannula into a saphenous leg vein. Due to risk of the dosing cannula becoming dislodged, animals must be closely monitored throughout the procedure. If this occurs, a technician is often required to sit in front of the animal and gently hold its leg for the full duration of the test substance administration. Whilst ensuring successful administration, studies have demonstrated that technicians being in such close proximity to non-human primates can cause some stress to the animal.

However, a new tail vein dose administration technique has been introduced, whereby a flexible catheter is inserted into the caudal tail vein and secured in position by surgical tape. Animals are unable to reach the catheter, therefore, there is little risk of it becoming dislodged. Restraint chairs can now be arranged in a semicircle so animals can see each other and view a television screen at the same time as being monitored by technicians stood behind them.

Refinement and Welfare – Housing enclosures for rabbits

One company has ensured refinement measures put in place to improve housing enclosures. Rabbits used in vaccine development are being housed in socially compatible groups in connected cages. To improve rabbits housing conditions, several prototype modules have been developed. Ground-floor housing was initially tested, however, this was not compatible with the type of research conducted. A team of animal technologists, caretakers and the designated veterinarian worked with a supplier for 3 years to design a new housing module. In the final version, rabbits are group-housed on solid floors, with bedding and an automatic water delivery system. A feeder with non-compacted hay, hiding huts and small toys are provided for environmental enrichment. Connecting trapdoors between modules enables researchers to increase the group composition.

Improvements in animal behaviors, as well as more ergonomic working conditions, have been observed by animal caretakers: animals are more relaxed and cooperative. The team were immediately convinced by the values of these new housing devices and are considering using these modules to house other species such as guinea pigs.

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Replacement of fish testing by picoplankton for environmental risk assessment for antibiotics[3]

The availability of effective antibiotics is critical for patient health and the long-term sustainability of the healthcare system. For the regulatory approval of antibiotics an environmental risk assessment (ERA) is required that includes fish testing at the highest severity. Science suggests that since fish are not the target species based on mode of action, they may not be most sensitive organisms that drives environmental protection. A company conducted a meta-analysis of existing environmental toxicology data and cross species comparisons that showed cyanobacteria, the picoplankton responsible for >70% of carbon dioxide fixation on the planet, to be most sensitive taxa in every case. Two publications have led to a change in European Medicines Agency (EMA) legislation removing the requirement for regulatory fish testing.

3: Le Page et al. (2017). Integrating human and environmental health in antibiotic risk assessment: A critical analysis of protection goals, species sensitivity and antimicrobial resistance. Environment International 109; 155-169. Le Page et al. (2019). Variability in cyanobacteria sensitivity to antibiotics and implications for environmental risk assessment. Science of The Total Environment, 695

Refinement through rodent anesthesia[4]

Open chest surgery in rats is a procedure critical for developing cardiovascular (heart and other) medicines. It is technically difficult, and the rats require assisted breathing during the surgery that traditionally has led to complications such as lesions, hypoxia and tissue damage. One team investigated a less invasive technique that used a nose cone rather than a pipe into the trachea. They systematically measured key indicators and demonstrated equivalence. This deceptively simple refinement of technique can improve the care and welfare of rats used in these models.

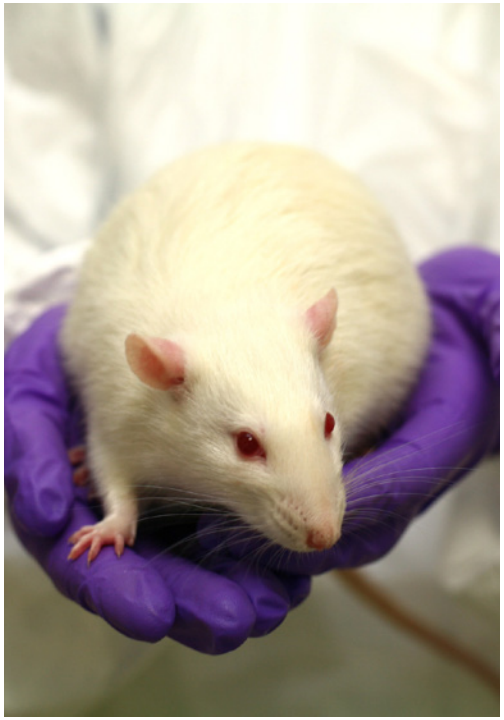
4: Krutrök N, Pehrsson S, Van Zuydam N, Jennbacken K, Wikström J. Ventilation via nose cone results in similar hemodynamic parameters and blood gas levels as endotracheal intubation during open chest surgery in rats. Laboratory Animals. August 2021. doi:10.1177/00236772211031039 https://doi.org/10.1177/00236772211031039

This work has now been shared in the scientific literature demonstrating the biological equivalence in blood gases and cardiovascular function

Reducing, replacing by combining in silico proarrhythmic risk assay with a tPKPD (translational pharmacokinetic / pharmacodynamic model)[5]

A company has developed and utilized a combination of in silico and tPKPD (translational pharmacokinetic / pharmacodynamic model) models on 73 compounds previously tested in vivo in guinea pigs and achieved a 100% sensitivity and 95% specificity to predict heart arrhythmia (QT interval prolongation). The results provide early proof of concept that the combined use of complementary computer-based modeling tools may enable generation of early risk assessment data with reduced resources and animal use in the future.

5: Morissette, Pierre. Toxicology and Applied Pharmacology Volume: 390 (2020)



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Future work to further refine the in vivo:in silico correlations and to expand the relationship between ion channel modeling, tPKPD, and in vivo results for other key cardiac electrophysiology endpoints represents the next step. In summary, the characterization of the in silico and tPKPD models is consistent with the 3Rs. The combined approach demonstrated proof of concept and identified key next steps toward further reduction in animal use. As a result, these efforts, could "reduce" the number of guinea pigs used and, in some instances, "replace" the in vivo model altogether and should enable a faster advancement of new chemical entities into preclinical and clinical development.

Internal Collaboration on Platelet transfusion models to refine animal use[6]

In one company, a collaborative effort between In-vivo Pharmacology, Safety Assessment and Biometrics Research groups resulted in 1) the development of a novel hemostasis (blood coagulation) model which enables concurrent measurement of bleeding time in three vascular sites on an animal, resulting in a reduction of animal usage and study time by 2/3. The effort also resulted in 2) the development of a heterologous transfusion method, in order to use readily available commercial human platelet rich plasma, resulting in a direct savings of approximately 150 monkeys for a study; and 3) results from the study have directly impacted the approval of a novel first-in-class anti-platelet agent, being quoted directly in the final FDA approved product label.

6: Platelet transfusion reverses bleeding evoked by triple anti-platelet therapy including vorapaxar, a novel platelet thrombin receptor antagonist. Cai, Tian-Quan; Wickham, L. Alexandra; Sitko, Gary; Michener, Maria Strainer; Raubertas, Richard; Handt, Larry; Chintala, Madhu; Seiffert, Dietmar; Forrest, Michael. In European Journal of Pharmacology. 5 July 2015 758:107-114. Strainer; Raubertas, Richard; Handt, Larry; Chintala, Madhu; Seiffert, Dietmar; Forrest, Michael. In European Journal of Pharmacology. 5 July 2015 758:107-114

Refining the bile-duct cannulated rat model using a Vascular Access Button™ (VAB)[7]

As part of the development of new drugs, the excretion and metabolism of the drug substance needs to be characterized. If a concomitant investigation of drug excretion via urine, feces and bile is needed, the excretion takes place in conscious, bile-duct cannulated rats.

7: www.sai-infusion.com

Many companies have implemented the use of the VAB. Two catheters are implanted under anesthesia, one in the bile duct of the rat, the other in the upper part of the small intestine (duodenum). The catheters are routed under the skin to the neck of the animal and where they are connected in a loop allowing the return of bile into the intestine.

The standard model required reopening of the skin under anesthesia through a previous suture line, to access the cannulas that were subcutaneously buried for safe transport. Previously, the catheters were connected in the neck with a small metal tube, which was implanted under the skin. On the day of the experiment, a second short anesthesia was required to remove this tube. The catheters were brought through the skin to a harness, which the rats wore throughout the experiment.

A significant improvement has been the implantation of a Vascular Access Button (VAB) under the skin at the back of the animals in the neck with the catheters attached to the VAB internally. An external loop connector ensures the return of the bile into the duodenum in the post-surgery recovery phase. On the day of experiment, a tether is attached to the VAB instead of the loop connector, therefore no second anesthesia is needed. Overall advantages of this model include ease of controlling bile-flow during the post-surgical recovery phase, removing the need for a second anesthesia and associated analgesia. During experiments animals are able to move more freely using the tether system with less risk of injury than the harness, this results in less body weight loss better mobility of the animals and easier handling for oral dosing applications.

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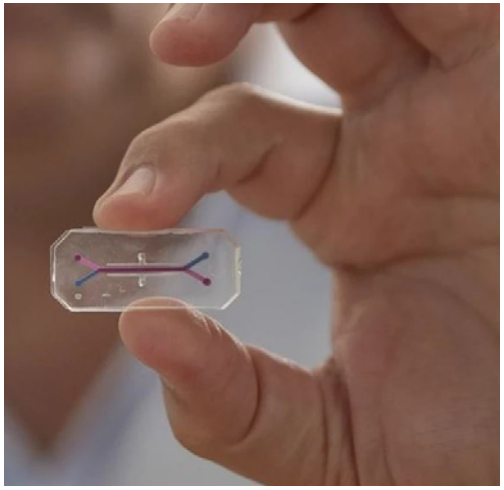
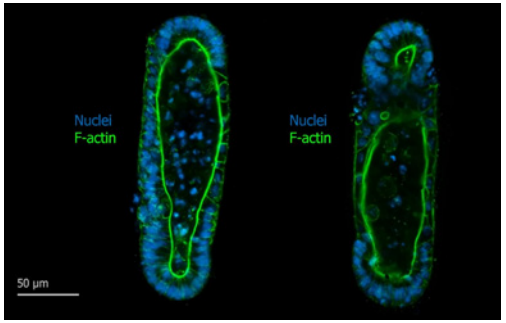
Vascular Access Buttons for multiple blood sampling in Rabbits

The auricular vein and artery are commonly used to collect blood samples from rabbits for pharmacokinetics (PK) & toxicology (TK) studies. However, due to the delicate nature of these vessels it can be challenging to collect multiple blood samples for a typical PK study from a conscious rabbit. The vascular access button is a major refinement allowing for fast, simple and aseptic access to blood sampling with little to no stress to the animal. Additionally, the button is very reliable ensuring blood samples are collected at all the requested time points whereas the previous method would often result in missed time points.

Vascular access buttons (VAB) were originally developed for single channel infusion or blood sampling in rats. The VAB is designed to be implanted under the skin and connect to a catheter, which is inserted into the desired vessel. A miniature external port built into the button allows for fast, simple and aseptic access with a septum needle or tether. A magnetic cap covers and protects the external port. Patency, defined as the ability to aspirate blood, was obtained for up to five months at which time the study utilizing this refinement concluded. When compared to the use of a vascular access port, the VAB had significantly less post-operative complications and was easier to access and maintain.





Organ on a chip development: investigation of mechanisms of thyroid toxicity in humans and rats[8]

Disruption of the thyroidal endocrine system by environmental chemicals is a concern for human health. Currently, animal studies are still requested to fulfill the regulatory requirements for human risk assessment. It is key to understand how an interference of a candidate molecule with the thyroid function translates from rodents, which are particularly sensitive to thyroid misfunctions, to humans. The organ-on-a-chip technology combines 3D cell culture with microfluidics and greatly enhances the current capabilities of reproducing physiological and toxicological interactions between target tissues. One company has therefore developed a functional chip-based in vitro model of the thyroid-liver interaction, for both human and rat, utilizing a commercial multi-organ-chip platform. At its current stage, the thyroid-liver-chip already allows (1) to study biological aspects of both tissues either separately or in combination, (2) to simultaneously investigate direct and indirect mechanisms of potential compound-induced disturbances of the thyroid function in vitro, and (3) to compare treatment responses between the two species.
This model represents a major step towards an assay for studying the human relevance of chemical-induced thyroid toxicity observed in rats. It will moreover enhance the understanding of organ interactions at in vitro level, which is indispensable to reduce and ultimately replace animal experiments in the discovery and development process of agrochemicals or medicines. Therefore, the outcome of this in vitro model development contributes to evidence-based risk assessment and provides a concept to reduce the need for animal experimentation.

8: Kühnlenz et al. (2019): Establishment of a multi-organ-chip based identification platform for endocrine disruptors. Toxicology Letters 314S1 (2019) S293. https://doi.org/10.1016/j.toxlet.2019.09.002. Boehm et al. (2019): Development of a rodent liver-thyroid-2-organ-chip for thyroid toxicity testing. Toxicology Letters 314S1 (2019) S294. https://doi.org/10.1016/j.toxlet.2019.09.002

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New Rodent Surgical Equipment Advancements and Post-Op analgesia in Water

Due to the negative physiological effects of extended anesthesia and the associated risk of increased mortality it is important that heat support and effective analgesia is provided to animals during and following surgical procedures. A company has combined the use of new anesthesia units that provide high quality heat support both intra and post-operatively with analgesia (Carprofen) provided 24 hours prior to surgery and up to 48 hours post-surgery in drinking water instead of gel cups. This technique ensures reliable consumption of analgesic by animals and is a significant refinement in the pre-, intra-, and post-operative care of rodents.

Analgesia is provided 24 hours prior to surgery and up to 48 hours post-surgery to manage wind-up and surgical pain. Analgesia is placed in water instead of gel cups, which ensures reliable consumption by animals. These techniques allow for significant refinements of pre-, intra-, and post-operative care of rodents.

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Experimental design

While animal use continues, Industry has focused on Improvements to experimental design to reduce the number of animals used. There is a need to use sufficient animals to produce robust and reproducible data to give scientists confidence in the results, while with the desire to minimise the number of animals used. If an animal study is not designed to answer the scientific research questions being asked and then the animals and resources used to conduct that study are potentially wasted. Effective experimental design and statistical analysis are critical means of minimising the use of animals and achieving study outcomes. Every effort should be made to improve research studies, using the best available guidelines to ensure that all details are recorded and results reported correctly, which will improve the quality of science and maximize the uptake of 3Rs opportunities. EFPIA members acknowledge that improvements are required across the industry and organised a user workshop in 2019 on experimental design in Industry. It brought together research scientists, preclinical biostatisticians, members of ethics committees and animal welfare bodies. The workshop helped increase awareness across industry of the concept and importance of effective experimental design and identified what is being done while identifying the key factors, challenges and gaps. Since the meeting, members of EFPIA have identified opportunities to implement improved experimental design across their activities.

Reduction by applying machine learning methods to evaluate absorption, distribution, metabolism, excretion, and drug safety in silico

In our industry sector, machine learning and artificial intelligence through in silico modeling are ever-expanding. Knowledge gained and curated from absorption, distribution, metabolism, excretion (ADME) and toxicity data have been used to create integrated data frameworks to guide model optimization and to connect and evaluate data. Using this framework, retrospective compound data can be leveraged by applying machine learning methods to evaluate ADME and safety assays such as phospholipidosis, genotoxicity (MNT), organ toxicity and phototoxicity. Combined in vitro and in silico models can predict blood-brain permeability similarly to in vivo studies. A successful ranking of compounds for in vivo pharmacokinetics studies is thus determined, related to brain penetration, significantly reducing the number of compounds undergoing in vivo studies afterwards.

Manually curated toxicity data from 65 small molecule investigator brochures have also been used to develop an interpreted toxicity database that includes domain expertise. Existing in vitro datasets were collected, generating missing in vitro data for all compounds of interest., developing a robust computational pipeline to connect in vitro and in silico compound features to preclinical toxicity outcomes. Using this pipeline, toxicity outcomes can be predicted and there will be improved understanding of in vitro - toxicity data relationships. The use of in silico approaches to predict animal ADME and safety outcomes in early assessment can complement and reduce the number of animal studies and allow for improved study design. Accurate predictions may eventually be used to fully replace some animal studies.

Improved understanding of toxicities from such efforts may make it possible to design better studies that collect the appropriate endpoints.

Reduction through the application of consistent experimental design principles across R&D

Robust study design of animal experiments is critical to ensure generation of high quality and reliable data, in turn improving reporting and ensuring animal studies are adding to the knowledge base. Animal experiments should include application of statistical principles including blinding, randomization and power analysis to minimize bias and to ensure scientific rigor. Non-Good laboratory Practice (GLP) in vivo studies were categorized by model development, efficacy, pharmacokinetic (PK) studies, safety pharmacology assessment, and toxicology. In order to achieve alignment on the minimum expectations for applying practices across in vivo research and to ensure consistency of approaches across a broad and diverse organization, research statisticians delivered trainings through a series of workshops, enhanced by the internal design of a randomization app. Statisticians are involved

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early in the experimental design process and all studies are scientifically peer-reviewed. Working practices to ensure consistent operationalization of requirements of robust experimental design practices have benefited from a wide network of subject matter experts to share challenges and practical solutions, including influence and knowledge sharing with external partners. Inclusion of additional checks at internal governance reviews highlight the commitment at the institutional level and help to ensure such standards are sustained going forwards.

Reduction by use of cross-over study design and re-use of rodents for pharmacokinetic studies

The pharmacokinetic (PK) profile (the fate of a drug when administered) of a test compound and typically require 3 animals per dose group, with 4 dose groups, requiring 12 animals per study.

Applying a cross-over study design and utilizing microsampling to obtain full plasma concentration profiles from each animal enabled a reduction to 6 animals per study, Whereby the 2 low doses (3 animals/dose) were given first and following a wash-

out period of 1-2 weeks (depending on compound half-life) and veterinary approval, the same animals were reused for the 2 higher dose groups. The animals from the high dose group were humanely euthanized for tissue PK while the 3 remaining animals were reused for another study This cross-over study design and re-use strategy resulted in a reduction of 50% for rodent PK studies while animals remain in good clinical condition.

Reduction of animal use through the application of in vitro dissolution and permeation methodology to pre-select candidates for oral formulations

The implementation of predictive biopharmaceutical tools is a main priority in the field of oral drug formulation development. Biorelevant assay systems are desired as they establish a link for the complex interplay among dissolution, supersaturation, precipitation, and absorption supporting key decisions on early to late-stage drug formulation development.

One company implemented of a combined dissolution and permeation methodology to facilitate preclinical and clinical formulation candidate selection. Due to the different anatomy and physiology of Gastro intestinal (GI) tracts in rat and dog models, the combination of the artificial gastrointestinal tract models, or Tiny-TNO Intestinal Models (TIM), with a Caco-2 test offers a more relevant simulation to human GI physiological conditions and facilitates candidate selection and drug product optimization at late formulation stages.

These combinations reduce animal use requirements in drug formulation development, and more importantly circumvent limitations of animal models in mimicking human GI characteristics for oral drug studies.

Replacement of animal use through application of a microneedle drug delivery system in human skin and artificial skin

Use of animals was removed by careful development of procedures using human skin from cosmetic surgery and artificial skin (Syndaver) materials and innovative solutions like mounting skin on warm liquid reservoirs and stretching the skin were developed to simulate intact, live skin. These tests are considered critical to evaluating design changes that might impact evaluating microneedle performance of a microneedle drug delivery system and microneedle penetration into skin. Identification of a highly standardized artificial material completely eliminated the use of animal testing and reduced associated variability of animal models, while providing an appropriate method to support ongoing quality assessment.

Reduction of animals used in dose range finding studies

Dose range-finding (DRF) studies are nonregulated studies designed to provide preliminary information on target tissues of toxicity and ensure appropriate dose selection for regulated studies. The general practices is to include contemporaneous

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control animals and 3 test item-dosed groups. Under a new paradigm, large animal controls (Cynomolgus monkey and Beagle dog) are only used for evaluation of in-life findings and clinical pathology parameters and are not terminated, but subsequently returned to the stock colony. Anatomic pathology evaluation (organ weights, macroscopic and microscopic observations) of test item-dosed animals, which is a key study deliverable, is achieved through a combination of efficient utilization of historical data and consistent pathology peer reviews. This is complemented by extensive scanning of historical control animal histology slides and availability of a large digital slide repository. Control animals returned to the stock colony are acceptable for re-use in subsequent studies. Additionally, the new default paradigm includes use of one, rather than two animals per sex per group and the potential to use only one sex when scientifically justifiable. This novel testing strategy was implemented with an average reduction of approximately 120 animals per year.

Reduction of animal use through collecting serial tumor biopsies

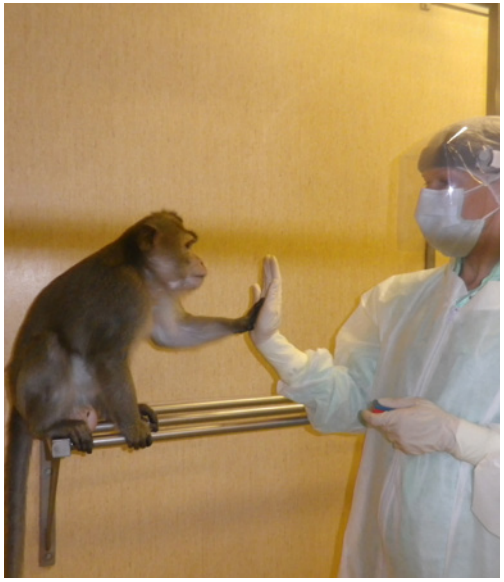
Collection of serial tumor biopsies is a relatively non-invasive survival procedure which can be performed on anesthetized mice whereby a small portion of the tumor is sampled and another biopsy can be collected at a later time and after the previous wound has been allowed to heal. Using this procedure, the tumor environment can be analyzed in the same animal by taking two samples at different time points pre- and post-treatment, and a third at the end of study, enhancing the scientific information derived from the model while enabling a threefold decrease in the number of animals required.

Replacement of animal use through the application of human-induced pluripotent stem cells to generate functional human neurons for in vitro screening

Using human-induced pluripotent stem cells (hiPSCs) enables generation of functional human neurons for in vitro studies. These hiPSC are more relevant to the human disease and were used to replace the use of primary neurons, such as mouse or rat neurons. However hiPSC-derived neurons as a source for functional human neurons that could be used at scale to support screening systems for drug discovery must be developed, optimized, and implemented There are currently no effective disease modifying treatments for neurodegenerative diseases of aging (Alzheimer disease and Parkinson disease) and this research area suffers from lack of relevant in vitro models. This novel screening system replaced the use of mouse/rat primary neurons for in vivo target validation approaches and enabled and through the additional development of a culture system and differentiation paradigm, replaced the requirement of animal–derived cell culture components.

Using acute mouse ocular toxicity model to determine ocular toxicity of antibody drug conjugates instead of monkeys.

Clinical administration of antibody-drug conjugates (ADCs) with microtubule inhibitor (MTI) warheads has been associated with ocular toxicity. This ADC-associated ocular toxicity occurs in the corneal epithelium and may result in eye sensitivity and blurred vision. Based on the commonality of the ocular findings across ADCs targeting different antigens and greater frequency of findings for non-permeable warheads, the toxicity is believed to be the result of non-antigen-dependent ADC uptake in the cornea. Monkey is the species commonly used for ADC toxicology assessment based on antibody cross-reactivity and clinical findings in the cornea. Despite the concordance in ADC-



related ocular findings between monkeys and patients, a different species was preferred due to ethical considerations and test material requirements. The acute mouse ocular toxicity model proved to be representative of initial ocular (corneal) toxicity observed in the cynomolgus macaque and in the clinic, was reproducible, high-throughput and reduced non-human primate use.

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Gene editing approach to reduce and refine animal use

In vivo platforms have been developed to knockout or knockdown targets of interest using clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas-9) technologies. Hematopoietic stem cells were isolated from Cas9 knock-in mice were transduced with CRISPR guide RNA and the infected cells were delivered to recipients to reconstitute animals bearing the desired CRISPR edits. While this system efficiently knocked-down gene expression in vivo, one limitation of the original system was that it was limited to certain disease models because the Cas9 knock-in mice are only available on a C57BL/6 background. To address this challenge, the team evaluated a novel approach to deliver CRISPR/Cas9 editing, namely transfection of CRISPR/Cas9 by ribonucleoprotein (RNP) demonstrating both efficiency of the RNP approach and impact of gene editing in a disease model. The RNP gene editing approach avoids the restriction on using only C57BL/6 animals and makes the in vivo CRISPR system compatible with other disease models requiring different strains of mice, reducing the number of donor animals required by at least half.

In-vitro screen as a replacement of some ex vivo studies

Some compounds may cause severe injection site reactions after repeated dosing in rodents, some due to a pseudo-allergenic response, causing a histamine release. Past practice used post-mortem evaluations of the injections site swelling in rats as correlation for histamine release, thereby enabling selection of drug candidates that don't produce this pseudo-allergenic response. The rat has been previously used in injection site reaction studies, where histamine release also has been the cause of observed changes.

However, Internal in vitro studies show that compounds that caused injection site reactions in rodents also activated the MRGPRX2 (the pseudoallergen) receptor, and that the effect on this receptor correlated with the severity grade of the injection site swelling. The MRGPRX2 in vitro assay enables detection of pseudo-allergenic injection site reactions in animal studies to test if the effect is caused by MRGPRX2 and de-selection of drug candidates, decreasing overall animal use. Approximately 800 rats were saved based on a calculation of "worst-case scenario" for the number of compounds tested in the in vitro MRGPRX2 assay. However, since capacity for in vivo testing would have been limited, the number of compounds tested in vivo would realistically be lower and thus, the estimated number of animals replaced would have been smaller.

This assay can be used for other projects that observe pseudo-allergenic injection site reactions in animal studies to test if the effect is caused by MRGPRX2 and for de-selection of drug candidates.

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Non-animal derived antibodies

The use of animal derived antibodies has become an increasingly political issue in the EU. The Commission Joint Research Centre (JRC) recommends that animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and therapeutic applications and that EU countries should no longer authorise the development and production of antibodies through animal immunisation, where robust, legitimate scientific justification is lacking for animal use in a call for adherence to the legal obligations under Directive 2010/63/EU.

While industry contends that the recommendation is premature, it is important to note that there is a commitment from companies towards identifying opportunities to develop and use the non-animal derived antibodies

Development of in-vitro techniques for high-quality antibody production at large scale

A monoclonal antibody (mAb) is a man-made protein specific to one single epitope - the part of the antigen to which the antibody attaches.

Besides being used as therapeutics (e.g., cancer, autoimmune diseases), they are used for example in diagnostics and analytics. (They can also be used to purify their target compounds from mixtures). Those product's usefulness in several areas has made it necessary to manufacture high-quality antibodies at large scale. Antibody production methods can be divided in two main categories: in-vitro production and in-vivo production. Focus on the ethical aspects of using animal created a demand identify opportunities to move away from animal-derived antibody production methods where possible. This led to the development of in-vitro techniques for antibody production where cells producing the antibodies (hybridomas) are grown in cultures under well controlled conditions.

The development of an innovative in-vitro production process enabled the manufacturing of mAbs by hybridoma cells growth in bioreactors and decreased the number of animals while replacing the traditional in-vivo manufacturing process. Scientists developed new biotechniques of in-vitro production and faced the challenges of in-vitro Monoclonal Antibody Production focusing on 2 main topics:

1. Optimizing Monoclonal Antibody in-vitro Production Systems (bioreactors).
2. Optimizing Culture Media Conditions for Production of Monoclonal Antibodies.

Refinement and Science – Model predictivity by using humanized mice[9]

Monoclonal antibodies (mAbs), are among the fastest growing and most effective therapies for myriad diseases. Multispecific antibodies are an emerging class of novel therapeutics that can target more than one modulator per molecule.

9: Valente, D et al (2020) Pharmacokinetics of novel Fc-engineered monoclonal and multispecific antibodies in cynomolgus monkeys and humanized FcRn transgenic mouse models, mAbs, 12:1 https://www.tandfonline.com/doi/fu ll/10 .1080/19420862.2020.1829337

The combination of different binding affinities and target classes, such as soluble or membrane-bound antigens, within multispecific antibodies confers unique pharmacokinetic (PK) properties. The unique PK behavior of many mAbs results from various factors, including target-mediated drug disposition (TMDD), off-target binding, isoelectric point (pI), glycosylation patterns, neonatal Fc receptor (FcRn) binding patterns, and interaction with anti-drug antibodies (ADAs). Consequently, in vitro models do not recapitulate all these factors and remained not predictive of the PK behavior.

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The most frequently used species for preclinical testing of mAbs and other therapeutic proteins are non-human primates (NHPs), in particular the cynomolgus monkey (Macaca fascicularis). Therefore, in order to refine the use of NHP and to reduce their number in research and development, other models to accurately predict PK behavior in humans needed to be developed. The design of humanized mouse models has been achieved to fill this critical gap for an in vivo predictive PK model. This model (huFcRn Tg32 mouse model) has been validated with reference compounds and internal company compounds combining mAbs, engineered monoclonal and multispecific antibodies. These results could facilitate a screening strategy to select antibodies in Tg32 mice with the most favorable PK characteristics, and could be helpful to characterize the PK behavior of the most promising candidates in NHPs to guide the design of further pharmacology/safety studies in this species. By extension, a first estimation of half-life and linear CL in humans can be directly deduced from this mouse model, not only for mAbs but for all antibodies. Our data could significantly help to accelerate the delivery of novel protein-based therapeutics with a reduced and refined use of NHPs.

Recombinant antibodies platform - an entirely new generation of monoclonal antibodies

A recombinant antibody platform has been established (ZooMAb) which represents an entirely new generation of monoclonal antibodies that offer the superior specificity and affinity of a monoclonal antibody, the reproducibility of recombinant technology, and greener alternatives including sustainability. The technology allows to develop monoclonal antibodies from rabbit blood cells, so there is need to use a animal like in the traditional hybridoma process. However, then all the ZooMAb® antibodies are recombinantly produced in human embryonic kidney) cells or cells from hamsters using a proprietary recombinant expression system, purified to produce robust and highly reproducible lot-to-lot consistency. ZooMAb® recombinant antibodies platform greatly minimize the use of animals associated with traditional polyclonal antibody production processes.

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Collaborative Research

Contributions of cross-discipline collaborative research projects are a vital component to challenge research paradigms in order to improve and accelerate translation from discovery to clinical research and increase focus and development on new approach technologies. The value and spirit of collaboration and sharing of information can help address human health challenges and in so doing reduce the dependence on animal use in areas where it has normally been viewed as necessary and in turn, lead to an overall reduction in the use of laboratory animals.

The Innovative Medicines Initiative (IMI) is one example – IMI is a public-private partnership between the European Union and EFPIA. IMI has since 2009 pursued the goal of developing the next generation of vaccines, medicines and treatments by improving research practice; getting new healthcare solutions to patients faster; and improving health outcomes thanks to new tools, methodologies, research infrastructure and big data. The IMI projects and their different consortia (involving industry, academia, SMEs, patients, regulators, etc.) have contributed enormously to animal welfare.

The next generation of projects will be part of the Innovative health initiative (IHI) - the new public-private partnership (PPP) under Horizon Europe which kicked off in January 2022.

This cross-sectoral Partnership brings together all stakeholders including 5 key health industry associations (COCIR, EFPIA, MedTech Europe, EuropaBio and Vaccines Europe) representing the pharmaceutical, biotech and medical technologies industries operating in Europe. This unique initiative strives to:

- create an EU-wide health research and innovation ecosystem that facilitates translation of scientific knowledge into innovations
- foster the development of safe, effective, people-centric and cost-effective innovations that respond to strategic unmet public health needs currently insufficiently served by industry
- drive cross-sectoral health innovation for a globally competitive European health industry.

The strategic research agenda specifically states that IHI will strive to pursue the aims of Directive 2010/63/EU6 on the protection of animals use for scientific purposes and, in particular, the principle of the Three Rs.

innovative health initiative

FROM IMI TO IHI

IHI PARTNERS

The industry members that make up the new partnership are **COCIR, EFPIA, EuropaBio, MedTech Europe, and Vaccines Europe.** The public member in the partnership is the European Union, represented by the **European Commission**.

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Collaborative Research

Animal use Statistics – Europe's proactive approach in funding alternatives
Excerpts on funding from a blog dated 10.02.20 by Kirsty Reid, Director of Science Policy
https://efpia.eu/news-events/the-efpia-view/blog-articles/animal-use-statistics-europe-s-proactive-approach-in-funding-alternatives

In the EU we have the Framework Programmes for Research and Innovation which address some major societal challenges and have been used extensively to advance the 3Rs. For Framework Programme 6, on average 30 million per year was allocated, for FP7 45 million per year and Horizon 2020, 8th Framework Programme, this amount remains at 45 Mil a year, all towards development of alternative approaches to the use of animals in research. During Framework Programme 7 (FP7), altogether some €200 million have been dedicated to animal-free toxicology projects. H2020 built upon the successes of FP7 and provided further funding opportunities to advance the 3Rs for the benefit of animals and human safety, as well as for the advancement of science. Projects addressed predictive safety testing, including non-animal approaches.

Furthermore, the Innovative Medicines Initiative (IMI) is a public-private partnership between the European Union and EFPIA, it is the largest health partnership globally. With a budget of over 5 billion, IMI is pursuing the goal of developing the next generation of medicines, vaccines and treatments by improving research practice; getting new healthcare solutions to patients faster; and improving health outcomes thanks to new tools, methodologies, research infrastructure and big data. Many IMI projects have contributed enormously to animal welfare.

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NEURODERISK
DERISKING NEUROTOXICITY

IMI NeuroDeRisk project: a comprehensive toolbox aiming at derisking neurotoxicity at early stages of drug discovery while applying the 3Rs[10]

Side effects related to the central and peripheral nervous systems still represent an important cause for drug attrition. Current preclinical models have poor predictivity, meaning that sometimes these side effects are only identified in the first clinical trials. The aim of the NeuroDeRisk project is to deliver a comprehensive set of tools and data that would make it easier for researchers to assess whether or not a compound is likely to be toxic to the brain and the peripheral nervous system, long before first tests in humans. The project focuses on three of the most challenging neurotoxic effects: seizures, psychological / psychiatric changes (such as suicidality or memory impairment), and peripheral neuropathies. For each of these liabilities, various in vitro assays and technologies are used and compared together in terms of predictivity, such as electrophysiology measurements and changes in RNA expression in rodent and human stem cell-derived neuronal cells, or in 'micro-brains on a chip'. An in-silico toolbox is built, for screening new chemical structures against a panel of known neurotoxicophores and predicting their potential adverse effects on the central and peripheral nervous systems. Finally, some animal studies are also conducted using innovative non-invasive methods such as ultra-high definition video monitoring, allowing to detect seizurogenic behavioral changes in rodents housed in their home cages, without the need of invasive surgery and placement of EEG electrodes. After the predictivity of these novel in silico and in vitro models is demonstrated, they should be applied in early drug discovery stages, thereby reducing the number of animals used at later stages and their suffering, as only compounds with low risk of neurotoxicity should progress in animal studies.

10: https://neuroderisk.eu/

Reduction by using of one species for safety assessment

An international expert working group representing 37 organisations including 30 pharmaceutical companies, collaborated in a data sharing exercise to evaluate the utility of two species within regulatory general toxicology studies. The focus of this comprehensive review was to explore whether both a rodent and a non-rodent species are required for general toxicology testing and if opportunities to use or reduce to a single species at different stages of development are being fully exploited, specifically if long-term toxicity data from only one species could be sufficient to support human safety in Phase II clinical trials. Anonymized data on 172 drug candidates (92 small molecules, 46 monoclonal antibodies, 15 recombinant proteins, 13 synthetic peptides and 6 antibody-drug conjugates) were submitted by 18 organisations. The data did show that for many of the compounds that used two species for the first- in-human studies, the toxicities were similar, suggesting that only one species is really required for the next stage of chronic toxicity studies. This option is already accepted by regulators for biotherapeutic drugs. The results published by this expert working group has initiated discussions to encourage this option to become more widely adopted.[11]

11: Helen Prior et al, https://www.sciencedirect.com/science/article/pii/S0273230020300507

EQIPD

IMI EQIPD : Enhancing the quality in nonclinical data with special focus on data from animal studies[12]

Many preclinical data, including those from animal studies, cannot be reproduced due to methodological shortcomings, or issues with internal and external validity of research data, with sometimes far-reaching consequences on drug development and translation to humans. The low drug development success rate is an important concern in the scientific community in general and in pharmaceutical industry. To address these issues, through the Innovative Medicines Initiative (IMI) a consortium of scientists at leading universities, pharmaceutical companies, contract research organizations, technology companies and scientific associations, have come together in a project called the European Quality in Preclinical Data (EQIPD) consortium. The project aims at enabling a smoother, faster and safer transition from preclinical to clinical testing and drug approval by establishing common guidelines to strengthen the

12: https://quality-preclinical-data.eu/

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robustness, rigor and validity of research data, ensuring better reproducibility and improved experimental design.

A EQIPD quality system has been developed with a strong link to animal care and use, acknowledging that animal care and use affects the quality of nonclinical data, and that the nonclinical data quality will impact on animal care and use. The project members have developed a concise checklist as part of the EQIPD quality system that allows scientists to check whether their animal care and use processes meets a standard that supports the implementation and maintenance of the EQIPD quality system. Applying this checklist can help in the first step to show potential gaps in the respective Animal Care and Use Program and in the next step to overcoming these gaps. Moreover, the Project members have developed an EQIPD training platform which contains a number of trainings related to animal care and use.

The checklist as well as the training platform directly enhance Animal Welfare.

vac2vac

IMI VAC2VAC : Vaccine lot to vaccine lot comparison by consistency testing[13]

The Innovative Medicines Initiative (IMI) Project "Vaccine lot to vaccine lot comparison by consistency testing" (VAC2VAC) develops and validates alternative in vitro non-animal testing approaches for both human and veterinary vaccines. VAC2VAC brings together a unique One Health consortium of pharmaceutical companies, academia, translational research organisations, Official Medicines Control Laboratories and regulatory bodies with the overall objective to demonstrate proof of concept of the consistency approach for batch release testing of established vaccines. This means that non-animal assays – instead of animal tests – can be used to ensure that each vaccine batch produced is consistent with a batch already proven to be safe and efficacious. It covers vaccine potency, safety and animal welfare.

Every single batch of human or animal vaccines that is manufactured must undergo a series of rigorous tests to ensure it meets certain standards for safety and potency. Today, many of these batch tests involve large numbers of laboratory animals. The vac2vac project, which has 23 partners across 8 European countries, is developing in vitro test methods that can be used to confirm that a batch of vaccines that is newly produced is consistent with and of the same quality as previously quality-controlled batches, and to identify sub-standard batches. VAC2VAC tackles both vaccine quality and animal welfare. It will allow to move away from the traditional paradigm of batch release testing for some vaccines, which relies heavily on animal tests and to accelerate the introduction of the new paradigm of consistency testing, based on innovative, non-animal techniques.

13: https://www.imi.europa.eu/projects-results/project-factsheets/vac2vac

eTRANSAFE

Towards virtual control groups for animal toxicity studies – An eTRANSAFE initiative[14]

The Enhancing TRANslational SAFEty Assessment through Integrative Knowledge Management (eTRANSAFE) is an IMI project that develops an integrative data infrastructure and innovative computational methods and tools that aim to drastically improve the feasibility and reliability of translational safety assessment during the drug development process. The project kicked off in 2017 and will run until end of August 2022.

Sharing legacy data from in vivo toxicity studies offers the opportunity to analyze the variability of control groups stratified for strain, age, duration of study, vehicle and other experimental conditions. Historical animal control group data may lead to a repository, which could be used to construct virtual control groups (VCGs) for toxicity studies. VCGs are an established concept in clinical trials however the idea of replacing living beings with virtual data sets has so far not been introduced into the design of animal studies. Given the fact that toxicity studies usually consist of three dose groups plus one control group, the use of VCGs has the potential for a 25% reduction in animal use. Provided regulatory acceptance can be achieved, this would represent the biggest reduction initiative in pharmaceutical toxicity testing. Prerequisites for such an approach are the availability of large and well-structured control data sets as well as thorough statistical

14: https://etransafe.eu/

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analyses. The IMI projects eTOX and eTRANSAFE have laid the foundation for data sharing among the pharmaceutical industry. Efforts are now being undertaken to share control animal data also from confidential data sets. Since control animal data are not related to the drug candidate and thus pose no IP issues, control group data can be shared without restrictions. Participating companies have started to collect control group data for subacute (4-week) GLP studies with Wistar rats (the strain preferentially used in Europe) and are analyzing these data for its variability. In a second step, the control group data will be shared among the companies and cross-company variability will be investigated. In a third step a set of studies will be analyzed to assess whether the use of VCG data would have influenced the outcome of the study compared to the real control group.

IMI PREMIER - Prioritisation and Risk Evaluation of Medicines in the EnviRonment[15]

IMI PREMIER project - Prioritisation and Risk Evaluation of Medicines in the EnviRonment – This project kicked off in 2020. The aim of the project to contribute to a sustainable future by proactively managing the environmental impact of medicines The current strategy to assess environmental impact of pharmaceuticals in development is impracticable for assessing the 1000+ untested legacy pharmaceuticals that are not under patent and one of those reasons is because it would require intensive animal testing Therefore, one of the set aims is to foster animal welfare by reducing the need for animal testing. A workstream of this project aims to deliver an optimised set of tools for testing and assessing the environmental exposure, effects and risks of APIs where they will generate new in silico and in vitro tools to close the key knowledge gaps to achieve an optimal prioritisation and environmental risk assessment of APIs.

IMI ConcePTION[16]

The information about the risks related to the use of medication during breastfeeding is lacking for most commonly used drugs. The IMI ConcePTION project aims to fill this gap using multiple approaches. Within the project, the pig has been selected as the most appropriate in vivo animal model. In agreement with the application of the "3Rs" principle and international legislations, the consortium have been working on the establishment of cellular lines of porcine mammary epithelial cells as a valid tool to study the mammary epithelial barrier function in vitro.

European Partnership for Alternative Approaches to Animal Testing[17]

EFPIA and a number of its members are founding members of the EPAA – it is a cross-sectorial and multidisciplinary partnership between five European Commission services, 37 companies and eight industry sector associations. The mission of the EPAA is to promote 3Rs in regulatory testing, and facilitate the development and implementation/ regulatory acceptance of alternative testing strategies. EFPIA and its members play important roles on the EPAA work alongside the other representatives with advancements seen in skin sensitisation, vaccines and other biologicals as examples.

Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach

Safety testing of monoclonal antibodies (mAbs) follows ICH S6(R1) guidance and decades of experience with mAb development has shown a relatively benign and well-understood safety profile for this class. Even when safety findings can be anticipated based on pharmacology, animal studies may not yield clinically relevant data and their use should be carefully considered. A consortium of 14 pharmaceutical companies, Medicines Evaluation Board (MEB) and NC3Rs conducted a European Partnership for Alternative Approaches to Animal Testing (EPAA)-funded study to re-evaluate whether a 6 month study is still optimal to evaluate the long term safety of mAbs. The incidence of new toxicities identified in chronic studies, along with impact on mAb development or clinical trial design, was reviewed from data shared by industry participants. To guide the need for

15: https://imi-premier.eu/

16: https://www.mdpi.com/2076-2615/11/7/2012

17: https://ec.europa.eu/growth/sectors/chemicals/european-partnership-alternative-approaches-animal-testing_en

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chronic toxicity studies, an iterative weight-of-evidence model was derived which considers risk factors that influence the overall risk for a mAb to cause toxicity, to drive selection of the optimal duration of toxicity study without defaulting to a study of 6 months duration. This provides a science-based approach that it may be possible to waive chronic studies for some mAbs while ensuring human safety.

Evaluating optimal study designs for toxicity studies with monoclonal antibodies: results from a MEB/Industry/NC3Rs survey

Hsiaotzu Chien [1,5], Helen Prior [2], Fiona Sewell [2], Katrin Schutte[3], Lucinda Weir [4], Peter van Meer [1]
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Abstract 4502
Poster P510

INTRODUCTION

- Non-clinical development of monoclonal antibodies (mAbs) is guided by ICH S6(R1), which allows a flexible approach.
- Typically, packages of studies consist of First-in-Human (FIH)-enabling studies (~1-month) in pharmacologically-relevant rodent and/or non-rodent species to support early developmental phase and a chronic study (to 6 months) in at least one species to support later developmental phase.
- Previous initiatives have focused on optimizing the duration and design of chronic toxicity studies for biopharmaceuticals [1,2] and whether 6-month studies are needed [1, 2, 3, 4].

PROJECT AIM

- Re-evaluate the need for chronic repeat-dose toxicity studies with mAbs.
- Develop a science-driven framework for optimal study designs and duration.

SURVEY & ANALYSIS

A survey containing 3 main sections was conducted:
- Basic product information (species selection, pharmacological relevance); individual study data; short- and longer-term study comparisons.
- Data collection from March-December 2020.

Analysis focused on:
- Species used, pharmacological relevance, study designs for short-term and chronic studies.
- How often were novel adverse events identified in chronic studies; did novel adverse events alter clinical development; could a 3-month study have been sufficient to support further clinical development?

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(1) Clarke *et al.* (2008). Duration of chronic toxicity studies for biotechnology-derived pharmaceuticals: Is 6 months still appropriate? *Regulatory Toxicology & Pharmacology.* 50: 2 – 22. (2) Chapman *et al.* (2012). The design of chronic toxicology studies of monoclonal antibodies: Implications for the reduction in use of non-human primates. *Regulatory Toxicology & Pharmacology.* 62: 347 – 354. (3) Blaich *et al.* (2016). Non-clinical Safety Evaluation of Biotherapeutics e Challenges, Opportunities and new Insights. *Regulatory Toxicology & Pharmacology.* 80: S1 – S14. (4) Sewell *et al.* (2017). Challenges and opportunities for the future of monoclonal antibody development: Improving safety assessment and reducing animal use. *Mabs.* 9(5):742-755.

RESULTS

Overview of dataset

- Surveys for 142 mAbs were submitted by 11 companies
- 59%:41% of studies were conducted pre- or post-ICH S6(R1) revision.
- High variability in study duration (1 day to 13 weeks for FIH-enabling studies; 13 to 52 weeks for studies to support later development)

Pharmacological relevance, species used and group sizes

Group size used in shortest study	Difference in animal use from shortest to longest study		
	Same	Increased	Decreased
2 M + 2 F: 1 mAb	1		
3 M + 3 F: 112 mAbs	42	54	16
4 M + 4 F: 16 mAbs	9	3	4
5 M + 5 F: 1 mAb			1
Other – variable: 2 mAbs	1		1

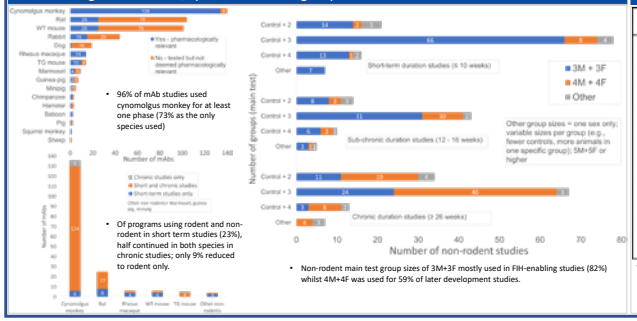
- 44% of mAb studies maintained the same group size, but 54% increased group sizes between the studies.

Data inclusion / exclusion

- 111 standard or modified mAb data sets were used for the analysis of rate of new toxicities*

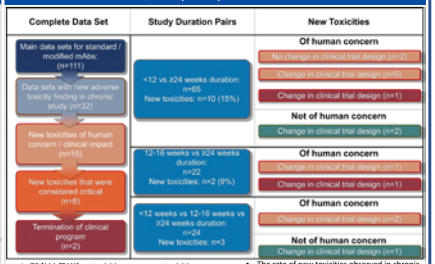
*New toxicity is defined as an adverse finding only observed in chronic studies.

Pharmacological relevance, species used and group sizes



- 96% of mAb studies used cynomolgus monkey for at least one phase (73% as the only species used)
- Of programs using rodent and non-rodent in short term studies (23%), half continued in both species in chronic studies; only 9% reduced to rodent only.
- Non-rodent main test group sizes of 3M+3F mostly used in FIH-enabling studies (82%) whilst 4M+4F was used for 59% of later development studies.

Rate of new toxicities; Risk perception



- In **79/111 (71%)** no toxicities or no new toxicities were noted. For **only 2 mAbs (2%)** were the new toxicities observed in chronic studies considered sufficient to terminate the clinical program.
- The rate of new toxicities observed in chronic studies decreased from 17% to 12.5% to 9% when the duration of shorter-term studies increased from ≤ 4 weeks to 5 –11 weeks to 12 –16 weeks, respectively.

DISCUSSION

- "More mAb programs could follow ICHS6(R1) guidance around using 1 pharmacologically responsive species for later development studies."
- "Increasing group size for chronic studies is not necessary; two test article-dosed groups are often acceptable"
- "For the majority of mAbs (71%) in the dataset, no new findings were identified in chronic studies."
- "Although 15 mAbs (13.5%) had new toxicities of concern, 8/15 new toxicities were considered critical, and only 2/8 resulted in termination."
- "Three-month studies may be more informative compared to one-month studies to support FIH clinical trials."

CONCLUSION

- "The high variability in study design and group size likely reflects case-by-case approaches as outlined in ICHS6(R1) and demonstrates more opportunities to optimize non-clinical packages for some mAbs."
- **"For consideration, a weight-of-evidence approach and a study of 3 months duration may derisk certain mAbs. Further work is ongoing to develop this and will be described in a manuscript in preparation."**

ACKNOWLEDGEMENTS

Special thanks to Lolke de Haan (ADC Therapeutics), Dave Clarke (Lilly), Tim Rowan (EPAA), Leon van Aerts (MEB), and Irene Manou (EPAA). We thank the members of the working group for permission to use the data and for helpful discussions.
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Staff training is an essential element of good science and good welfare

Mechanisms used to promote the development of 3Rs through training The pharmaceutical industry has invested in numerous mechanisms to improve animal welfare and understanding of the necessity for 3Rs implementation through various training initiatives:

- Staff training opportunities, both external and internal, are strongly supported across the company's employees in research and development who handle or care for animals, or develop and agree project proposals.
- Many companies organise veterinary consultations and one on one sessions with scientists.
- Numerous training campaigns have been initiated (e.g. on Bleeding techniques, severity assessment).
- Scientific seminars are organised to inform on methodologies and new technologies.
- Clearly defined terminology and common language is developed for 3Rs-relevant categories and respective definitions for the following: non-animal models, animal models, animal care, animal procedures. For each category, an implementation plan to collect, collate and communicate 3Rsrelevant advances was developed to better inform users.
- Indicators of 'Success' have been established to identify positive change through surveys, training completion, increased discussion and participation in activities.
- Companies have taken on the initiatives to host Internal and external courses by subject matter experts (e.g. handling, training and working with specific species or techniques).

Highly trained staff beyond the minimum requirement Many pharmaceutical companies employ veterinarians, veterinary technicians, operations staff and animal care technicians whose qualifications go well beyond the minimum level required and have extensive additional credentials in order to provide the best care to the animals. For global companies, many of the veterinarians are board certified through the American College of Laboratory Animal Medicine (ACLAM), have completed advanced degrees in addition to their veterinary degrees, and actively participate in annual continuing education. Veterinary technicians often hold varying levels of certification, and may also be certified or licensed veterinary technicians, hold advanced degrees or certifications and participate in continuing education. The majority of surgical and anesthesia technicians are certified through the Academy of Surgical Research as Surgical Research Scientists and/or Surgical Research Anesthetists. Operations staff and animal care technicians may hold advanced degrees, certification with the American Association of Laboratory Animal Science (ALAT, LAT, LATG, CMAR) and participate in continuing education.

Copyright Ellegard Minipigs

Refinement: Implementing blood sampling in a sling – training of staff and refinement of a procedure[18]

Implementing a new technique requires a skilled trainer, dedication and patience from all parties involved. When introducing a new method, it is important to present all the advantages before training commences. It must be explained why the new method is better and remove any insecurity that the new method might induce. We found it was important to ensure a successful experience resulting in the confidence to continue, as it is easy to go back to old methods when insecurity occurs. The level of training or support needed is individual and must be considered.

A 3Rs example, where a standard blood sampling procedure has been refined from V-bench to sling method. A V-bench is commonly used when collecting blood samples from minipigs, which requires multiple staff members, and is very stressful for the minipig and sedation is often required for larger

18: Göttingen Minipigs Magazine 60, 2021 https://minipigs.dk/knowledge-base-1/goettingen-minipigs-magazine

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animals. Blood sampling from a minipig placed in a sling has proven to be less stressful – both for the staff and the animals. Over the course of 12 months all staff members were trained in blood sampling from a sling from young to older minipigs up to 24 months of age. Using older and larger minipigs makes it possible to collect larger batches, thus the number of animals used can be reduced. Also, the minipigs are exposed to less stress, which is an important measure for animal welfare but also ensures high quality blood samples.

Refinement: Training and habituating pigs to scientific procedures

Training and habituating pigs to scientific procedures (e.g. standing completely still for imaging or dosing) is a valuable and efficient way to minimize procedural and contingent stress (e.g. daily routines such as weighing) when working with pigs.

The success of this initiative depends highly on skilled staff (to be trained and work with the animals in the correct way) and on allocating time for these activities. The effort and time spent on training and habituating pigs to relevant procedures pays off very well in the long term.

The interaction between the animal and the technician is furthermore helpful in the socializing process, because the pigs appreciate the activity as they are intelligent beings and working with the animals is rewarding for the technicians, and thus increasing job satisfaction. The training and habituation results in a reduction in the accumulated severity experienced by the pig, and less stressed animals leads to better science in the form of more robust and reliable scientific results.

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New and emerging innovative technologies

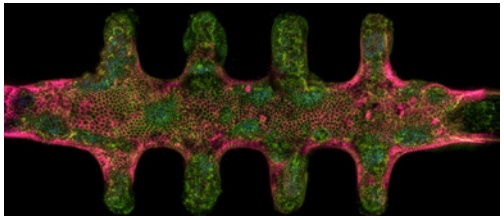
Applying improved biological knowledge, technological advances, computer simulations and innovative (Microphysiological systems, Complex 3D models) methods may lead to significant reduction of the number of animals actually used, however, most of these methods are not yet able to fully replicate/are not sufficient to extrapolate the conclusion of simple models to the complexity and reactions of a living organism especially for systemic and chronic conditions. However, they are already widely used by scientists to inform their research programs and, being complementary the ones to the others, address the scientific questions for the progress of medicine.

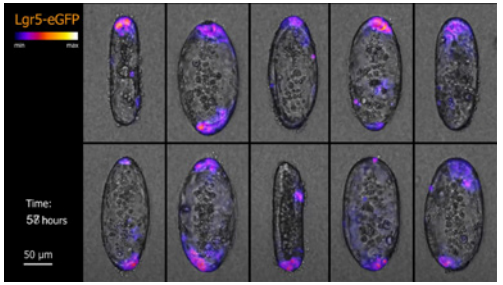
Reduction of use through application of bioluminescence imaging (BLI) to non-invasively monitor longitudinal systemic tumor growth and to determine humane endpoints

Systemic hematological tumor models are considered a better representation of clinical disease over subcutaneous models. While longitudinal tumor burden assessment in a subcutaneous tumor models can be easily measured non-invasively with calipers, disease progression in systemic tumor models requires flow cytometry analyses of tumor cells in harvested organs and tissues. These experiments require large numbers of animals and may lead to significant pain and distress when the disease. In the last 20 years, bioluminescence imaging (BLI) has allowed systemic tumor growth to be monitored non-invasively using a light capturing system in a longitudinal fashion. Ahead of imaging and in vivo model development, the cancer cells must first be stably transduced with a bioluminescence imaging reporter. Once a model is characterized and established, the number of animals required for a longitudinal tumor assessment is greatly reduced, the model more closely mimics clinical hematological malignancies, and the BLI imaging data can be used to set study endpoint criteria for maximum tumor burden and study termination before the disease causes animal pain and distress. Creation of an in vivo systemic BLI tumor library enables establishment of models that better recapitulate clinical disease, longitudinal monitoring of systemic tumors, optimization of tumor burden criteria eliminating animal pain and disease due to advanced disease progression, and reduces the number of animals needed for systemic tumor models.

Refinement through automated continuous observation of the animals to assess lung function instead of invasive techniques

The ability to assess lung function offers an important read-out not only in studies of respiratory disease models but also in safety pharmacology and toxicology testing. Common methods of assessment imply confounding factors and stress to the animals. In previous studies of animal models of idiopathic pulmonary fibrosis (IPF - a condition in which the lungs become scarred and breathing becomes increasingly difficult for humans), disease symptoms such as pulmonary function, have been collected using invasive techniques. The use of an automated and continuous monitoring platform for the observation and study of animals in their home environment without handling or disturbing their natural cycle of rest has enabled the refinement of the animals' experience on study and improved the scientific quality of the resulting data.





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Microphysiological systems

Implementation of a 3D tissue organoid system provides an experimental platform for validation of so-called targets (molecultes that are closely associated with a certain disease). Organoids are miniaturized 3D organ models that work like cells and functions of the organ from which they are generated. Organoids derived from the small intestine and the colon of mice and humans have been used to support validation of early and advanced gastrointestinal (GI) targets and just recently organoid models were created from monkeys (cynomolgus macaques). Intestinal organoids from multiple species contribute to the understanding of species-dependent differences in the outer lining cells' mechanism of action of individual targets by enabling comparative side-by-side studies on organoids derived from mice, monkeys and humans. This reduced animal use by allowing for the prioritisation of targets in vitro prior to studies in animals. Increased in vitro models enabled increased speed at which drug targets move through the pipeline. Gastrointestinal organoids have been used to generate proof of concept/mechanism of action data packages for portfolio targets as well as for biomarker studies and inform differentiation strategies for portfolio targets. Finally, expanding our organoid models into new tissues supports cross-disease area research and allows the organoid platform to become applicable for supporting new disease indications of interest.

Crack It Challenge: Avoid reuse of needs - develop a device using ultrasonic technology[19]

The NC3Rs Crack It Challenge competition funds collaborations between industry, academics and SMEs to solve business and scientific Challenges which will deliver 3Rs benefits, either by improving business processes or developing a commercial product. In response to some of the practical issues with single needle use, a Crack It Challenge was initiated to develop a device using ultrasonic technology which minimises loss of material to dead space and improves animal welfare. The challenge is sponsored by AstraZeneca, GSK and Sheffield University. A prototype has been developed which is currently being tested by the sponsors.

19: https://www.nc3rs.org.uk/sharp-and-point-developing-deviceinjections- mice-avoids-re-use-needles

Using mRNA barcoding to increase lead identification but minimise in vivo studies

False positive and false negative lead identification from in vitro screens can result in poor candidate advancement from subsequent in vivo screens and ultimately the use of more animals. Lipid Nanoparticles (LNPs) are important new drug modalities however, the in vitro high throughput screening to identify leads rarely correlates with in vivo results. A company has developed a method in-house that uses uniquely barcoded mRNA to label the candidates and reduce the in vivo study size five-fold, while increasing the success rate of lead identification.

Novel Artificial Intelligence (AI) approaches contributing to safer drugs through enhanced prediction of safety liabilities[20]

One of the main challenges in the development of new drugs is the high rate (>90%) of drug candidates that do not pass all the tests required to become a marketed drug, partly due to safety issues. It is estimated that 75% of the Adverse Drug Reactions (ADRs) can be predicted by the pharmacological profile of compounds and by identifying their interactions with secondary targets (other than the intended primary target). A poorly selective compound interacting with a lot of these 'off-targets' will have more likelihood of failure at later stages of clinical development. Predicting the safety profile of drugs as early as possible simply based on their chemical structure, is therefore a crucial approach to gain time and efficiency. Over the past years, various data-driven computational efforts have been developed to help optimize drug candidates against some safety liabilities. Among them, one consortium, Effiris), aims to build in silico models to predict effects of drugs on a series of secondary targets known to be associated with adverse effects. This consortium leverages AI to allow the transfer of knowledge from multiple partners into 'federated' models. The advantage is that member data are kept confidential, thanks to privacy-preserving knowledge transfer methods, but all users of Effiris benefit from the shared knowledge and the improved predictivity of the models. It facilitates efficient triage and prioritization of molecules, as well as compound design and by that reduces the number of safety studies in animals.

20: https://www.lhasalimited.org/products/Effiris.htm

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Automated Monitoring of Rat Colony Health and Behaviour[21]

Rat colony housing system allows automated tracking of motion and body weight for objective monitoring of rats while improving animal wellbeing through permission of social interaction and providing a variety of functional areas.

Living together in large social communities within an enriched environment stimulates self-motivated activity in rats. One company has developed a modular housing system in which a single unit can accommodate as many as 48 rats and contains multiple functional areas. This rat colony cage further allows remotely measurement of body weight and continuous measurement of movement, including jumping and stair walking between areas. Compared with pair-housed, age-, strain-, and weight-matched rats in conventional cages, the colony-housed rats exhibit higher body mass indices, have more exploratory behavior, and are more cooperative during handling. Continuous activity tracking revealed that the amount of spontaneous locomotion, such as jumping between levels and running through the staircase, fell after surgery, blood sampling, injections, and behavioral tests to a similar extent regardless of the specific intervention. Data from the automated system enabled identification of individual rats with significant differences (>2 SD) from other cohoused animals. These rats showed potential health problems, as verified using conventional health scoring. Thus, the rat colony cage permits social interaction and provides a variety of functional areas, thereby improving animal wellbeing. Furthermore, automated online tracking enables continuous quantification of spontaneous motion, providing objective measures of animal behavior in various disease models and reducing the need for experimental manipulation. Furthermore, health monitoring of individual rats is facilitated in an objective manner.

21: J Am Assoc Lab Anim Sci. 2017 Jan; 56(1): 18–31. https://pubmed.ncbi.nlm.nih.gov/28905711/

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Dissemination beyond own department and own establishment drives improvement in welfare and general quality of science

Industry Pioneers the Contribution of Substance Data to decrease animal testing[22]

There are several ways and initiatives whereby the pharmaceutical industry contributes to sharing best practices

The aim is to make high-quality, as-yet unpublished, physicochemical, toxicological and ecotoxicological substance data held in the companies' archives available to the public. The publication of this data is intended to extend the variety of publicly available high quality hazard data on chemicals to, among other things, enhance the effectiveness of database-dependent property prediction tools. These data could then be used by academic science or company experts to gradually reduce or to even avoid animal testing of chemical substances, by improving theoretical models of "Structure Activity Relationship" (SAR) and through the opportunities for read-across - which depend on the amount, diversity, and quality of data on which these considerations or calculations are based.

ECHA has agreed to support this initiative and to act as an honest broker and will serve as the neutral platform for dissemination of these data. Read the ECHA press statement **here**.

Ultimately, a program will be created in which additional companies can join and make their archive data available to the public. At this time, the partners (ECHA, EFPIA and the founding-member companies: Boehringer Ingelheim, F.Hoffmann-La Roche, Johnson & Johnson, and Merck KGaA) are running a test phase to flesh out the best ways to run this data contribution initiative.

For more information you can access the industries Q&A **here**.

Positive reinforcement training using Tellington TTouch® as valuable tool for the training tool box

The Tellington **TTouch** method was invented by Linda Tellington-Jones and has been used successfully in many animal species. That said, establishing Tellington TTouch in the field of Laboratory Animals is a very new approach. Positive reinforcement training has been integrated step by step into the training of laboratory animals for several years now and allows many procedures to be performed with voluntary cooperation of laboratory animals. The method is an additional tool in the training-toolbox. It is a very gentle and respectful way of body work which is new to the field of laboratory animals. The method strengthens the well-being of animals in everyday life and at the same time helps to reduce or prevent stress. The method offers effective and easy tools including body touches, but also body wraps and motion exercises that help animals to relax and focus during training and handling and procedure situations. It is very helpful in situations where food can`t be used as a primary reinforcer. Tellington TTouch and the Tellington-bodywraps improve cooperation in animals during many kinds of procedures. Where several animal technicians take care of a group of animals, the same TTouch is used by every technician and helps the animal to quickly relax with less known or new people.
TTouch has been integrated as an easy and effective tool into positive reinforcement trainings and refinement programmes throughout the whole life span of a laboratory animals' life to promote animal welfare and culture of care in the sense of the 3Rs.

Use of hydrophobic bedding for urine collection instead of metabolic cage for mice (and rats)[23]

In non-clinical studies performed during the development of medicines, scientists must follow different parameters to evaluate efficiency and/or toxicity. In some studies, one important parameter allowing to follow the evolution of the pathology can be a urine analysis. This analysis, easy to perform after urine collection under the cages/boxes, has an impact on animal welfare as animals are often maintained in specific "metabolic" cages which are smaller and less comfortable than

22: https://www.efpia.eu/news-events/the-efpia-view/statements-press-releases/industry-pioneers-the-contribution-of-substance-data-to-decrease-animal-testing/

23: https://datesand.com/product/labsand/

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the usual cages, and animals are generally stressed by these abnormal housing conditions. In addition, in mice, as the volume emitted is very low in metabolism cages, it is often insufficient to perform the analysis.
One company has started to use a new bedding type (hydrophobic bedding) which allows collection of a small amount of urine very rapidly after placing animals on the bedding, in their usual box. As this bedding is hydrophobic, the urine forms drops which can be easily collected in thin glass tubes and allow analysis of 1 or 2 parameters depending on the method of analysis. Animals explore this new environment and mark it with urine allowing a rapid collection. The housing conditions for urine collection are similar to their usual conditions and not stressful. This method is applied for mice, and will be considered for use in rats.

Use of a warming cabinet for vasodilation prior to tail vein dosing and blood collection while staying in their home cage[24]

One company has worked to provide a consistent method for warming rodents while reducing negative impact on the animal welfare as well as animal handlers. Placing animals in a slightly warmer environment prior to dosing in the tail vein is usually performed outside the home-cage; the warmer environment. The warmer environment facilitates a vein that is more visible and easy to access. The warming cabinets provide consistent control and monitoring of temperature within the cabinet and enable animals to remain in their home cages, reducing handling and exposure to a novel and potentially stressful environment during warming.

24: www.allentowninc.com

Removal of ovaries in female Minipigs using a minimal invasive method

At the end of a procedure, animals whose welfare is not compromised should be returned to a suitable husbandry system or the animals should be allowed to be rehomed. Minipigs that are proposed for adoption after being used in experiments need to be neutered to prevent further breeding and to allow them to live in stable, sometimes, mixed sex groups. As a refinement for the classical surgical removal of ovaries (ovariectomy), this is now done via a thin flexible tube that has a light and camera at the end (endoscopy), which noticeably reduces the invasiveness and the risks of the surgical procedure, with the result that the animals recover much faster and have less post-surgical complications and over all experience less pain and distress.

Significant first step to replace animal testing - national health authority approval of in-vivo to in-vitro bioidentity assay[25]

Somatropin (a growth hormone) and Gonadotropins (sex hormones) drugs currently require in animals for each batch produced. This is based on the historical requirements for such testing by the U.S. Pharmacopeia (USP). Fortunately, the times are changing and paths to replace animal testing are now also greatly encouraged by health authorities. For example, the relevant USP chapter for Somatropin has been extended to include an alternative to animal testing.

In line with one company's 3R approach to animal welfare, a setup of in-vitro assay alternatives without the use of animals for Gonadotropins and Somatropin in-vivo bioassays was initiated. The good results obtained during the initial experimental phase, allowed the CMC (Chemistry Manufacturing & Control) teams, cross-functional product teams of analytical, manufacturing, supply chain, quality and regulatory experts from various GHO functions, to devise an overall strategy for the submission of these changes to the relevant health authorities.

25: Ph.Eur 2285 and 2286 "Follitropin"; British Ph. "Menotropin" "Somatropin bioidentity tests"USP<126>

The first submissions to health authorities took place in early 2021 and the authority approved the company's variation submission to switch to the proposed in-vitro assay.

Now health authority submissions aiming to replace animal testing also for the Gonadotropin product portfolio will be rolled out.

Group housing of Göttingen Minipigs[26]

Pigs are herd animals with a strong social disposition. They frequently interact with pen mates displaying a variety of social interactions. Therefore, in order to promote natural behaviour and welfare, pigs should be group housed when possible. Females and young males can often be re-grouped successfully, whereas older boars will be too aggressive towards each other if they are re-grouped.

26: Technical Guide- Acclimatisation, Socialisation and Welfare of Göttingen Minipigs (available at https://minipigs.dk/knowledge-base/ educational-package)

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Some notes and tips established on re-grouping pigs:

- A hierarchy will typically be established within the first hour. Some fighting is expected in order to form a pecking order. If fighting is severe or persists, separate the pigs.
- Allow ample space in the beginning- e.g., open up between stalls or use the corridor. Later, pen space can be reduced if necessary.
- If possible, provide visual barriers in the pen (e.g., partial walls or fixed obstacles) to enable pigs to get away from one another.
- Provide interesting new enrichment and food which will serve as distractions in the beginning. Experience is that scented toys (e.g., Bunny Blocks™) and food scattered all over the floor work particularly well.
- Reduce territorial behavior by re-grouping in a neutral pen, i.e., a pen neither pig occupied before.
- If possible, familiarize the pigs with one another before re-grouping them, e.g., allow snout to snout contact by housing the minipigs in adjacent pens in advance.

Re-group in the beginning of the workday to allow for frequent observations and potential intervention throughout the first day.

Replacement of rodent liver damage studies by rodent-derived organoid models to study liver regeneration

Liver disease is on the rise and until now rodent liver damage studies have been the only option to study pathways involved in liver regeneration. With the creation of a rodent-derived organoid model which recapitulates key features of liver regeneration about 99.9 % animals could be saved. The organoids are derived from a single animal and can be expanded and cultured indefinitely. Furthermore, this organoid culture enables genetic modification thereby omitting the generation and breeding of genetically altered animals.

Replacement of animals by in vitro method to screen for potential seizurogenic effects

Until recently, the potential seizurogenic (where seizures are experienced) effects in man of proconvulsant drugs have been tested with the rat hippocampus slice assay. Using this technique, 3-4 animals per compound to be screened were needed. This methodology was replaced by the in vitro screening of human neurons derived from human induced pluripotent stem cells (iPS) (generated directly from a somatic cell and does not require the use of animals) with a microelectrode array technology. In addition, this technology enables a higher throughput as well as a decreased interexperimental variability due to highly standardized cell cultures.tools. Through this, there is an opportunity to improve the hazard characterization of structural analogues, optimize their safe use and can potentially lead to a reduction in animal testing. Mechanisms for the delivery and secure dissemination of the data have already been developed under the REACH regulation and will be used for data donation. As a voluntary non-profit industry initiative, the project is run under the patronage of EFPIA and led through Roche. ECHA is an active partner in this initiative.

Development of an app called Mouse Mapp to assess animal welfare impairments by detecting changes in facial expression and/or body condition[27]

The NC3Rs Crack It Challenge competition funds collaborations between industry, academics and SMEs to solve business and scientific Challenges which will deliver 3Rs benefits, either by improving business processes or developing a commercial product.

27: https://nc3rs.org.uk/crackit/mouse-mapp

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Depending on the Challenge, contracts of up to £1 million for up to three years are available.

A company developed a challenge called Mouse Mapp that was successful in the competition. The aim of this Challenge is to develop an application (app) that uses artificial intelligence and machine learning to automatically detect changes in facial expression and/or body condition, to improve the monitoring of mouse welfare. Both facial expression and body condition scoring are currently used as welfare assessment tools in mice. They require training and expertise, take time, can be subjective and are not fully utilized in facilities. An automatic application will aid in consistency and uptake thus improving welfare assessment more widely in mice.

Blog series on the mitigation against reuse of needles[28]

Single-use disposable needles are often used throughout medical research involving animals for injecting substances. Needles might be re-used to reduce time and cost because of the involvement of large numbers of mice and/or expensive test material. However, the re-use of needles results in a loss of sterility and may increase the risk of infection and disease transmission between individual animals and between cages. Re-use also risks dulling of the needle, potentially increasing the pain and discomfort associated with subsequent injections. These concerns create confounding and unnecessary variables which can impact the quality of scientific data collected. To inform about the risks associated to occasional re-use of disposable needles, a series of three blogs have been written for NC3R's by industry partner to highlight the concerns of reuse of these 'single use' needles. The blogs describe the issue and ways of mitigating against reusing needles.

Reduction of animal use by sharing company's knowledge and expertise on animal models and compounds[29]

One company has initiative a Compound Sharing initiative. This is an open innovation initiative driven by scientists where researchers from the external scientific community easily can access selected company compounds. Selected compounds come with a datasheet on the animal models that frequently are used with these compounds. The datasheets include scientific information, such as reagents and vehicles (including a 'calculator' to define number of animals, number of doses etc) and Pharmacodynamic data. Addressing the 3Rs are also included with special emphasis on refinement.

The aspiration is to increase the interaction with the scientific community to accelerate innovation through sharing and potential future collaborations. The initiative encourages the support of a sustainable business by promoting a responsible use of animals and to increase our transparency on work with laboratory animals by sharing our knowledge and expertise. The ambition is to improve science and animal welfare and potentially reducing duplication of studies.

Development of a biobank to allow sharing of tissues and organs

Sometimes animals are produced but not used (these are commonly referred to as surplus animals) or only used as 'vehicle' or control animals whereby they are not exposed to any compound. Simply discarding these animals is a waste, both from an ethical point of view and also from a wish to use resources in a responsible manner.

Some components of tissues and organs are extremely perishable, however, fortunately many tissues and organs can be used after storage under controlled conditions. The availability of these stored biomaterials from laboratory animals represents a valuable alternative to procuring 'fresh' animals and harvesting sometimes only a single organ. A company has therefore established an internal Plasma Bank, where plasma from different animal species used in-house are collected and stored with a complete animal history which governs for the quality of each sample. Accessibility of tissue and organs are systematically tabulated and made easily visible. Requests for specific tissue and organs can be posted. The initiative is estimated to currently save a little more than 1500 animals on an annual basis. The number is expected to increase as more tissue and organs are enrolled in the initiative.

Innovative cage enrichment to reduce aggression in male mice

One company has designed an innovative cage enrichment structure (LUCINE) that reduces the incidence of aggressive behavior in group-housed male mice and that improves animal welfare. LUCINE is a cage enrichment

28: https://www.nc3rs.org.uk/3rs-resources/single-use-needles

29: https://www.novonordisk.com/science-and-technology/bioethics/animal-ethics.html#AnimalEthics

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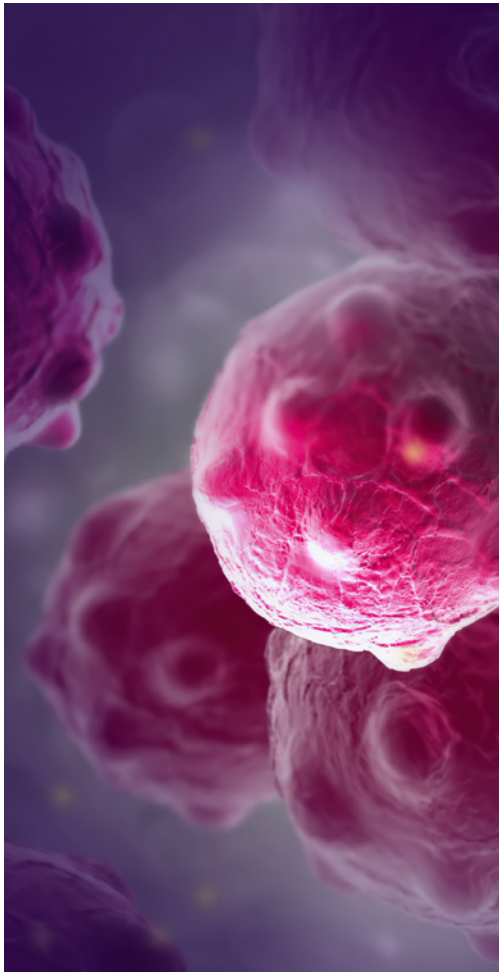
structure that allows to divide any type of mice cage into different compartments. This compartmentation gives the opportunity to submissive animals to escape to a section of the cage where they feel safe from the dominant animal(s) in the group. This new type of cage enrichment also allows mice to climb on the structure and to build nests without compromising visibility for the animal caretakers when they perform the daily behavior and health checks on mice. Observations of behaviors show a positive impact on the welfare of mice. Mice make extensive use of the openings (arches) in the walls and the upper edges of the structure. The nest is usually built in the smallest compartment in the cage. Both observations resemble behavior in nature where mice often walk on fine ridges and make their nests in quite places. In cages where other type of cage enrichment is used, we notice mice are more agitated and chasing each other. The design is easy for animal caretakers and biotechnicians to install and use. Because the structure consists of parts that can be dismantled easily, cleaning and storage are very efficient as well.

In vitro model based on mice primary neuroglial cultures and organoid model to assess neurotoxicity[30]

Investigative Toxicology aims at identifying and predicting adverse functional effects prior to first in human studies. The assessment of neurotoxicity remains a major scientific challenge due to the complexity of the central nervous system. Current strategies to evaluate toxicity of chemicals and drug candidates are predominantly based on ex vivo or in vivo animal studies. These models have limited predictability for neurotoxicity in humans and are not amenable to high-throughput testing. Several non-animal models have therefore been developed and assessed.
An in vitro model based on mice primary neuroglial cultures, able to form a functional network for the explorations of the epileptogenic/ seizurogenic potential of drug candidates (generally for people affected by epilepsy) which is one of the most major adverse drug reactions causing drug marketing failure. This development has been conducted in collaboration with Cellular Dynamics International and Axiogenesis.
The development of neurotoxicity models based on human Induced pluripotent stem cells (iPSC) derived neurons in microfluidic platforms, enables high throughput culture of miniaturised organ models. Examples include where mixed population of neurons with supporting astrocytes have been cultured in 3D, closely representing the physiology of the human brain. In another example, the organoid model has been associated with fast kinetic fluorescence imaging to measure amplitudes and frequencies of the Ca2+ oscillations. The effectiveness of automated imaging assays combined with the organotypic nature of human induced pluripotent stem cell (iPSC)-derived cells opens new opportunities to evaluate the potential drug toxicity of our drug candidates.

The in vitro models of the human brain, especially the microphysiological system, reduces the use of animal models has the potential to better predict adverse effects in humans hence improve clinical development success. Up to now, to limit biased conclusions, the results should be considered in respect with other read-outs in an integrated approach.

30: https://www.mimetas.com/en/products/ https://www.stemonix.com/



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The 11th World Congress on Alternatives and Animal Use in the Life Sciences

The 11th World Congress on Alternatives and Animal Use in the Life Sciences (WC11) took place virtually in 2021. The focus of the conference was on 3Rs in transition – from development to application. It was attended by over 1300 participants - leading international science and policy experts from a number of areas of expertise as well as young scientists eager to share their experiences and learn from others.

The programme covered 4 themes: safety, disease, ethics and welfare and Innovative technologies and offered multiple speakers the opportunity to bring different perspectives to alternatives and animal use in the life science.

As part of the conference scientific program there are a number of key note speakers. Andre Kuipers. He showed what is needed to achieve a very ambitious goal, in his case to live and work in space: courage, expertise, communication, innovative technologies and collaboration. Skills that are also necessary for all workers in the field of alternative methods. Other Interesting keynote lectures covered ethics of innovative technologies, animal welfare and religion, digital twins for better health outcomes, Data quality in translational research technologies, Virtual human-Artificial Intelligence and the potency of human-induced pluripotent stem cells.

For young scientists, there was a special part of the program dedicated to YOU-WC11. It was designed for early career scientists that already work or plan to work in the field of the 3Rs to encourage the dialogue of early career scientists among themselves and with experienced peers that have been working in the field for a long time, thereby creating the opportunity for establishing new professional networks.

During the conference it was very encouraging to see the increased participation of industry, from the pharmaceutical sector and others, and the enormous progress made in the field of alternative methods. Progress made includes collaborations with academia, regulators and civil society organisations to contribute collectively to innovations that will result in better science and in the reduction or even replacement of animal experiments. The new complex in vitro models, including microphysiological systems and in silico methods, and their combination, but also

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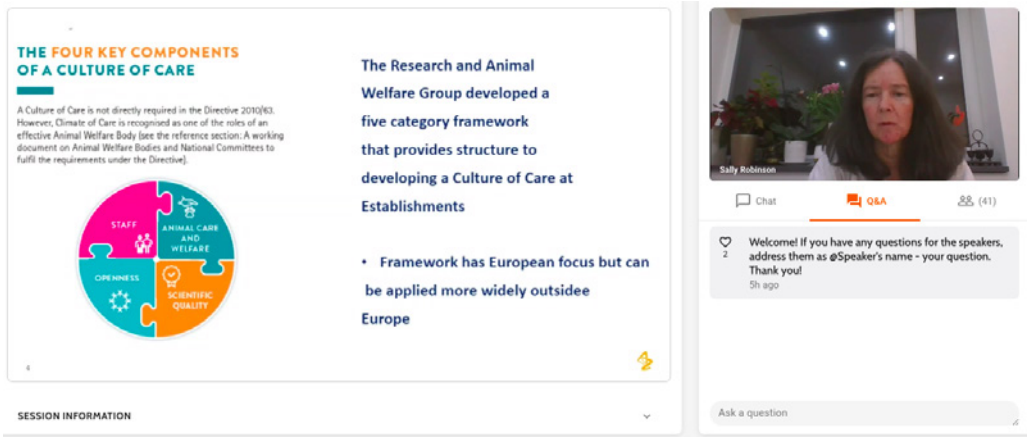
The 11th World Congress on Alternatives and Animal Use in the Life Sciences

insights into the necessary improvements in data quality for translational research were presented. The importance of collaboration between different stakeholders on a global scale, including openness and data sharing, was stressed during the sessions and in the talk shows. Transparent dissemination and communication to the patients, general public, and society, together with good management of the expectations, were mentioned as essential activities to make these groups aware of the state-of-the-science and clear perspectives of the new developments.

Industry engaged early on in the Congress preparations, and was active in the Local organising Committee and the International Scientific Committee. Podcast, and representative key-note speakers.
EFPIA hosted a session on Pharmaceutical Industry initiatives driving the 3Rs where some of the industry initiatives underway within the pharma sector going beyond the legislative requirements for 3Rs where showcased and discussed with participants. Another session hosted by EFPIA focused on Reproducibility in preclinical studies where reproducibility is remains a concern, including a need for improved experimental design. Academic and industry partners spoke on various ongoing initiatives including the IMI project EQIPD

The next congress, the 12th World Congress on Alternatives and Animal Use in the Life Sciences (WC12) will focus on 3Rs over the edge - Regulatory Acceptance and Next-Gen Education. It will take place on the 23 – 27 August 2023 at the Niagara Falls in Canada.



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3R Awards

Grand Prize Winner of the 3Rs Award

One company has recognized and celebrated the 3Rs since the 1980s through its 3Rs Award. They recognize Grand Prize Winner as well as Best of Sector for their 3 sectors (Pharma / Medical Device / Consumer). The 2021 Award Grand Prize recognized work by the company's cardiovascular safety group developing new and innovative human cell-based assays for cardiac hazard identification in early drug safety de-risking. The work form that group was recently published. The 2021 Grand Prize Winners did put the assay into daily practice and is routinely applying it, leading to substantial reduction in animal numbers. Their contribution hopes to impact the industry standard of how this assay is done.

In addition, an affiliation of the company awarded local 3Rs prizes to animal technicians and researchers for outstanding achievements in the 3Rs in the past.

Kopljar I, Lu HR, Van Ammel K, Otava M, Tekle F, Teisman A, Gallacher D (2018)

Annual 3R AWARD

Another company has established an internal 3R Award to appreciate employees who strive to improve the conditions for animals used in research, bringing the company's commitment to the 3R principles into action. The event creates awareness of the many great new 3R initiatives in R&D and emphasizes the importance of animal welfare. All employees are welcome to the 3R Award ceremonies. Staff working within R&D are urged to submit their 3R initiatives. The 3R Award has attracted and inspired many technicians, scientists, veterinarians, licence holders, managers, and many others for more than a decade. Newly onboarded employees in the company gets a a great chance to catch up and learn about the company's approach to animal welfare. The winner of the 2021 Annual 3R Award was "Life is better with friends" – an initiative about improving housing, enrichment and training of obese monkeys which could not have succeeded without a persistent push persuading that this was not a 'mission impossible' but that this could be done.

Global 3R Awards programmes and a 3Rs week

One company has a Council for Science and Animal Welfare, which is an expert decision group responsible for oversight of all animal research in their member organisations.
Each year C-SAW also runs a global awards programme to reward and inspire people who are making a difference in animal research. The company has a shared belief that the 3Rs thrive in a work environment where care is engrained in thier culture, and people are empowered to be open and proud of their work, which is reflected in their award categories.

- ◊ Celebrating our Culture of Care.
- ◊ Recognising achievements in Openness on Animal Research.
- ◊ Rewarding Innovation in the 3Rs.

These awards culminate in a 3Rs Week, where scientists, technicians and invited guest speakers present to a global audience on emerging innovations and practical advice and ideas. This is also an opportunity to showcase all work entered in to the awards programme, for example, every 3Rs poster submission is displayed across the global R&D centres for maximum exposure and adoption.

One company have an Animal Welfare Awards system - Bronze, Silver and Gold level depending on the significance of the achievement, from the local marginal gains to the published efforts with global reach that significantly raise the bar.

The awards are for achievements in the 3Rs, and culture of care has recently been added so efforts that benefit both animals and staff that may not be captured under one of the R's can be formally recognised. The preparing of the roll out the revised process across the company is in progress.

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Full and correct implementation of Directive 2010/63/EC on the protection of animals used for scientific purposes is the responsibility and endeavor of the whole scientific community

The standout legislation in the EU on animal welfare is the Directive 2010/63, on the protection of animals took effect in member states on 1st January 2013. The Directive has enhanced animal welfare standards and mandated the application of Replacement, Reduction and Refinement across the EU and sets full replacement as a long-term vision. It supports research involving animals only when there are no alternative methods, where the potential benefits are compelling, when it is scientifically, legally and ethically justified, and welfare standards are met.

Europe is therefore leading in the 3Rs and the welfare of laboratory animals by integrating fully these principles in all stages of research processes. Thanks to the Directive, questions, tools and regulations are evolving, driven by scientific progress and collaboration. The legislation challenges scientists to change their perspectives and way of thinking, to drive for change.

Nothing is effective if the Directive is not implemented and enforced fully and energy should go into appropriate and effective implementation. EFPIA and its members remain committed to correct implementation of the Directive 2010/63/EU and to enhance the culture of care, and openness for research involving animals.

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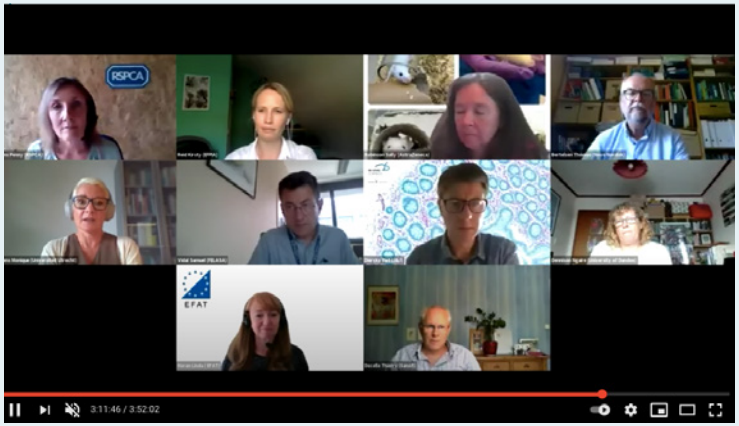
Dialogue with NGOs

Dialogue with NGOs, civil society and other stakeholders is critical to advance science and have critical discussions on animals used in science. Companies proactively engage in constructive dialogue with NGOs. They inform each other and share experiences and expertise through various collaborations on practical and operational issues. EFPIA, jointly with leading animal welfare organisations regularly team up to bring together stakeholder to discuss opportunities for change.

RSPCA/EFPIA Webinar - The Animal Welfare Body - a catalyst for progress

Under Directive 2010/63 it lays out rules setting up Animal Welfare Bodies and their tasks. The AWB plays a vital role in ensuring take up of alternatives and high animal welfare. On the 18 June 2021, EFPIA and the RSPCA (a leading animal welfare organization) joined forces to organize a webinar in collaboration with FELASA and EFAT which aimed to

- help advance and harmonise current good practice among Animal Welfare Bodies (AWBs);
- identify ways of addressing the outstanding issues that are preventing some AWBs from effectively delivering their core and wider tasks;
- enable participants to network and share experiences and good practice; and
- achieve greater openness regarding how AWBs operate and the Directive is implemented.



The webinar was an engaging endeavor attended by 395 participants from 21 countries represented industry, academia, regulators, veterinarians, European Commission and NGOs. These learning and sharing opportunities are essential in driving change.

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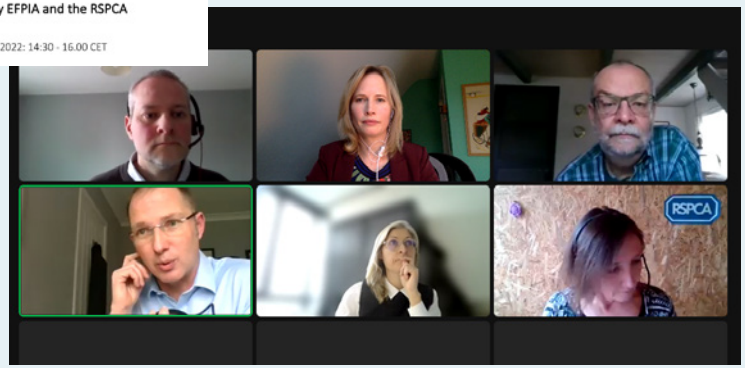
Accelerating Global Deletion of the Abnormal Toxicity Test (ATT) - Planning common next steps

The ATT results in the unnecessary and unethical use of animals since there is no scientific rationale as to how that test would be able to fulfil its objective of detecting contaminations. The networks across the industry, NGOs and regulators have for the past years coordinated cooperation in order to remove requirements to use the ATT test.

An open workshop was organized on October 14th 2021, by the Animal Free Safety Assessment Collaboration (AFSA), the Humane Society International (HSI), and EFPIA, in collaboration with the International Alliance of Biological Standardization (IABS). The workshop saw the participation of over a hundred representatives from international organizations, pharmaceutical industries and associations, and regulatory authorities from 15 countries. Participants reported on country- and region-specific regulatory requirements and, where present, on the perspectives on the waiving and elimination of the Abnormal Toxicity Test. The participants also discussed specific country and global actions to further secure the deletion of ATT from all regulatory requirements worldwide.

How the pharmaceutical industry is tackling 'severe' suffering in animals used in science.

On the 26 January 2022, EFPIA cohosted along with the RSPCA, an on-line event. The webinar was attended by over 500 participants, representing industry, academia, the European Commission and national regulators. There is widespread support within the scientific community for working to reduce, and ideally end, severe suffering. Industry representatives shared and showcased some of the positive and practical steps that have been taken by the pharmaceutical industry aimed at avoiding or reducing 'severe' suffering in animals used in research and testing, followed by discussion on current challenges and how these are being overcome.



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Open Communications | Dialogue | Reporting

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Culture of Care

Thomas Bertelsen, DVM
Novo Nordisk
Member of the EFPIA Research and Animal Welfare Group
Lead of the Culture of Care Network

Culture of Care (CoC)[31] is a fairly new concept in relation to the use of laboratory animals. However, the term has been used in the health-care nursing sector for 40 years or so. The health care sector works with patients who frequently are not able to take care of themselves – likewise laboratory animals are entirely relying on our care and empathy. The meaning of the term "culture" has many interpretations. In the context of this brochure, CoC means what we think, what we do and how we collaborate to achieve a positive impact on animal welfare.

The current and extensive legislation on the use of laboratory animals tells us how to do things right. CoC is about doing the right things by going beyond and above the requirements of legislation and to put emphasis on the intended outcome of being compliant with the legislation. This is why a CoC is such a powerful enabler to achieve our goals because it is outcome-driven and has a positive impact on animal welfare.

There are many ways to work with CoC. EFPIA members have been reflecting on the concept of a CoC and how it is understood and applied across research institutions and companies in Europe - some of our members have published a "checklist" to help engage in or enhance discussions on a CoC within the individual establishments.

EFPIA and many of its members are also part of the International Culture of Care Network, which was established in 2016. The network acknowledges the value of the diversity that the individual establishments have in terms of its own characteristic CoC, which is why also e.g. NGO's and regulators are members. The network encourages an approach that is going beyond and above legislation, and ideas and initiatives are freely shared within and outside the network.

This section describes initiatives that are directly related to EFPIA members' efforts in working with Culture of Care, but I trust that you will recognise the 'fingerprint' of CoC in a lot of the examples that are presented in other sections of this brochure.

When you go through the examples of this brochure, you might think "in my establishment we cannot do all of this", - and you're absolutely right! No-one can do everything, but we can all do something, and I hope that this brochure will give you a nudge in the right direction and inspiration to develop your own Culture of Care.

I hope that this brochure will give you a nudge in the right direction and inspiration to develop your own Culture of Care

31: https://norecopa.no/more-resources/culture-of-care

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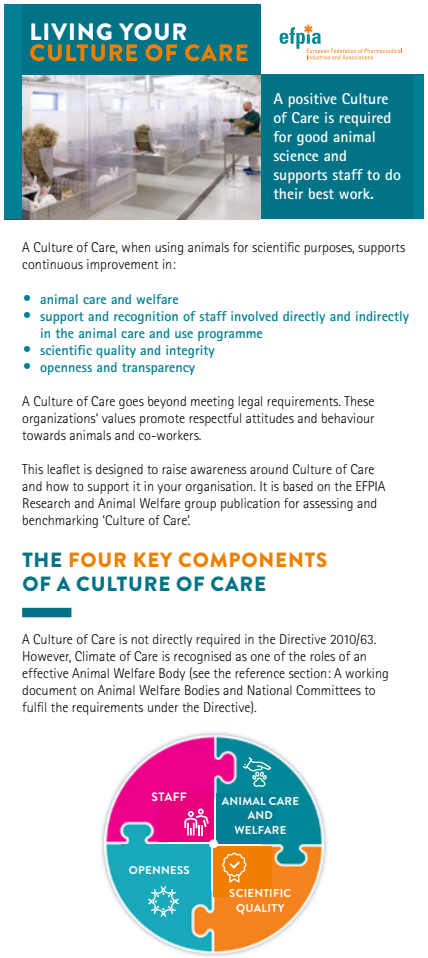
EFPIA and its members driving forward Culture of Care

The European Federation of Pharmaceutical Industries and Associations' Research and Animal Welfare group (EFPIA RAW) members reflected on the concept of a Culture of Care in relation to animal care and use and on differences in its understanding and application across European pharmaceutical companies. The term 'Culture of Care' is used across different regions and organizations but rarely with any defined indicators to support working practice. EFPIA's Research and Animal Welfare group developed a framework to help organizations identify gaps or potential areas for improvement in support of a positive Culture of Care. The framework is a tool that identifies five areas of focus for a Culture of Care: company values; strategic approach at establishment level; implementation structures; staff support; and animal care and procedures.

The framework is intended as an aid for continuous improvement, highlighting where indicators of good practice are present. The goal is to provide points of reflection and ideas for those looking to implement a Culture of Care in a structured way, while facilitating a professional and strategic approach. To prevent it supporting a 'tick-box' exercise, the framework must not be used as an auditing tool, but as a starting point for consideration and discussion about how care manifests within the context and constraints of individual establishments.



To further promote the Culture of Care concept and help establishments, both within industry and academia, to further consider and take up Culture of Care initiatives, EFPIA prepared a **leaflet** to raise awareness around Culture of Care and provide advice on how to support it in different organisations. It is freely available to download by all interested parties. **Read more**.

Culture of care- technicians specifically in mind[32]

Developing and sustaining a Culture of Care can lead to staff satisfaction and improved Animal Welfare. A Culture of Care should involve all of those working directly and indirectly with animals. However, it is particularly important to recognise and support those who carry the burden of care i.e. the staff that conduct procedures on and care for animals used in research. This article provides practical examples and ideas of how animal technologists and care staff can contribute to the Culture of Care in their workplace and methods by which they can be supported to do this e.g. by their managers. For example the paper touches on the role of the Animal Welfare Body, Recognition, Openness and Training and Development.

Improving culture of care through maximising learning from observations and events[33]

A recent published paper describes a systematic process for reporting observations and events that have the potential to help with continuous learning, improving animal welfare and supporting staff. The process took learning from a philosophy from the safety, health and environment arena on accident prevention and this philosophy is Human and Organisational Performance (HOP).

32: https://www.atwjournal.com/thejournal

33: Robinson S, White W, Wilkes J, Wilkinson C. Improving culture of care through maximising learning from observations and events: Addressing what is at fault. Laboratory Animals. eptember 2021. doi:10.1177/00236772211037177. This paper was also covered in the October 2021 3minute 3Rs podcast link below 3 Minute 3Rs October 2021 | 3 Minute 3Rs on Acast

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The two key aspects were (a) the systematic logging of observations and events; and (b) the learning approach of HOP to following up on observations. This allows for an environment to promote continuous improvement for both animals and staff by recognising, rewarding and sharing good practice, as well as where near misses are openly reported and learnt from. Supporting animal welfare, staff welfare, improving scientific quality and transparency are the four key pillars of a positive culture of care. Using a system and learning approach rather than focussing on blaming people is critical to developing open reporting and a positive culture.

The inclusion of welfare-enhancing initiatives in the daily life of a dog

Companies are dedicated to implementing a culture of care within their establishments. It plays a crucial role in the continuous refinement of daily procedures regarding the welfare of lab animals. An example from one company, in the last decade, great efforts have been made to keep on improving the daily life of the dogs in their facility. In this context socialization programs were initialized and/ or optimized - a dog walking program and an adoption program for dogs.

On top of internal playing areas, a company has installed an outside playing area in 2017. Ever since, dogs can play outside in a fenced and equipped sandy field, enjoying playmates and animal technicians in open air. A second outside playing area was created in 2021 to give other dogs the opportunity to play outside on a regular basis. Two times a week these outside playing areas are the starting point for weekly walks with volunteers. Colleagues from any department on Campus can sign up and join during their lunch break for a walk with the dogs in the nearby forest.. It is seen as a great success, not only for the dogs but also for the volunteers who can stretch their legs and network during the walk. This initiative initially started 5 years ago as one of the preparatory steps for dogs going for adoption but nowadays more dogs can be included in the dog walking program.

By bringing dogs outside on a regular basis, they and the work they are involved in are given more visibility within the Campus and reinforce the open communication of the culture of care.

Tunnel handling – a process for successful implementation.

The introduction of new standards for housing and handling of laboratory animals is sometimes at risk of not being taken up sufficiently due to a lack of attention to perceived and actual barriers. These barriers can be cultural ("we have always done it this way" or a perceived criticism of current standards), scientific (impact on scientific endpoints, severing the link to historical data) or practical (it takes longer time or is more burdensome). This may especially be true in a large and complex organisation with many stakeholders.

In the case of introducing tunnel handling/ cupping of mice the different types of barriers were addressed.

Cultural: staff was informed that the change was not due to a current wrong practice (handling by the tail), instead the animal welfare implications of the new versus the old method were discussed. Trainings were provided and tunnels were made easily available.

Scientific: Dialogues were initiated with scientists to discuss animal welfare and scientific benefits with the new methods and solutions to the scientific barriers were cleared.

Practical: increase resource spends (staff time) and equipment (tunnels) were supported by with relevant managers from Line of Business. The efforts to break down or reduce the barriers required considerable resources in terms of time to 'negotiate' the introduction of the initiative but is has proven to be a necessity for a successful implementation of an animal welfare improvement.

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Culture of Care, - making it efficient[34]

Culture of care is a strong enabler as a means to achieve an effective and efficient goal in terms of improving animal welfare within laboratory animals. However, Culture of Care is a very ambiguous concept and in a given user establishment it cannot stand alone, it needs supporting structures and it needs clearly defined goals and aims for achievements. One company have designed a working model that integrates these three elements. The company has assessed the Culture of Care by using an in-house designed multi-level survey tool focusing on 'what we think', 'what we do' and 'how we work together'.

The supporting structures, e.g. Implementing early Humane Endpoints, the Animal Welfare Body, access to 3R information, housing and care refinements are brought into play according to which aim or goal it must support.

The aims or goals are selected by an iterative process, encouraging continuous improvements. Examples of aims or goals are: minimizing animals with an actual harm classified as 'severe', percentage of animals training or habituated to procedures, optimizing use of 'surplus' animals by making these available for organs, tissue etc.

These three elements – culture, supporting structures and aims must be united in a formalised, coherent and seamless structure.

Culture of care in times of crisis

The Covid-19 pandemic has the potential to contribute to "compassion fatigue" in both, human and animal care staff. Compassion fatigue is characterized by emotional and physical exhaustion leading to a diminished ability to feel compassion for others (or animals). The current global pandemic increases the demands on the staff and therefore the risk of developing compassion fatigue, and as such was an area of focus in two recent Science and Nature journal articles. Fortunately, the Pharmaceutical industry was well prepared for a wide range of emergency scenarios, including ensuring appropriate animal care and welfare practices during a pandemic, that are continuously evaluated to ensure the smooth operation of animal engagement even in the event of a crisis. As a result of this preparation, animal care and welfare was, and continues to be, maintained at its pre-pandemic high standard. In the context of a commitment to further growing an excellent culture of care focusing on wellbeing of the associates as well as the animals, one member company organized a global presentation for the employees who work with the animals. The presentation aimed to raise awareness for the potential for compassion fatigue, offered practical strategies to prevent and minimize the development of compassion fatigue and included details demonstrating the company's commitment to the highest animal welfare standards.

Mouse handling as an example of Culture of Care

The key to non-aversive methods of handling lies in determining what capture method will create the least anxiety in an individual mouse: tunnel, cupping or another method. For example, it was observed that some mice refused to enter the tunnel by aiming their noses away from it or pushing against it. The next step is to find the method that best suits the individual mouse. A hierarchy of capture methods were adapted, starting with the tunnel, followed by the cupping method and finally asking staff to use other non-aversive alternatives if either of the methods were not appropriate. If the same approach is used by all handlers it is likely to further reduce the anxiety of the mice. Examples of methods used are: herding the mouse onto a grid surface, using other enrichment items in the cage such as the mouse house, and using a cage label to herd them into the tunnel, this method works well with handlers who are concerned about being bitten. Encouraging handlers to devise, share and use alternative methods helps further improve the wellbeing of laboratory mice.

Culture of care - Rodent Home Cage Monitoring

Home Cage Monitoring enables us to remotely monitor mouse activity in a cage via a sensor board under each cage position on a rack. Each board contains 12 electromagnetic sensors which continuously track and record spontaneous mouse activity. This enables animals to remain in their home-cage environment whilst providing an independent objective assessment of activity during experiments. This not only impacts positively on the welfare of the animal (e.g. reduction in stress by allowing the animal to remain in its home cage), but it also improves the quality of the data generated. The animals are monitored from under the cage so no reduction in environmental enrichment is needed

34: https://doi.org/10.1177/00236772211014433

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and monitored activity wheels can be included. Home Cage Monitoring can not only monitor the animal activity and inform staff of any prolonged activity changes of the cage occupants but also inform relevant staff when bedding needs changing; water and food are low or bottles are missing and when cages are incorrectly placed on the rack. This type of 24 hour cage monitoring is likely to bring improvements to both housing, care and welfare for mice that are housed on it.

Culture of care in rodent handling

Handling of rodents can be a daunting experience for staff and when people feel uneasy it can create tension in the animal too. Part of the difficulty is to find good resources that give clear guidance on how to handle animals. One company has developed an internal website for mouse and rat handling which has links to external resources and clear pictures and videos as well as explanatory text. These resources have been translated in Italian and Spanish to enable their global community to have a consistent message in terms of why we should handle animals in a specific way (avoiding the tail for both mice and rats) and showing people how to pick the rodents up and who to contact for further help and guidance.

Progressing a Culture of Care in a Global CRO

Culture of Care in animal welfare has become a frequently used phrase within the laboratory animal science and research community, including within contract research laboratories (CRO). Over the past 4 years a leading Global CRO has embarked on an innovative Culture of Care immersion programme across multiple sites. At the heart of this employee led project were the core principles of inclusiveness and embracing innovative advances in the 3Rs.

With Senior Leadership support and encouragement, the project gathered colleague feedback from across this global organisation resulting in the development, celebration and launch of new employee commitments supported by innovative and engaging training completed by over 5000 colleagues globally. Since their launch, further opportunities are being created for employees to celebrate their animal welfare achievements, discuss their new commitments, share ideas on what a Culture of Care means and how they would like to support its progression.

Culture of Care - Caring for People, Caring for Animals

A Culture of Care program encompasses a wide range of topics at the forefront of the animal research field, such as: excellent animal care and welfare, implementation of the 3Rs, re-homing of research animals, support of organizations through fundraising/ outreach, and nourishing a culture of respect. A successful program creates an inclusive environment and encourages both caring for one another and supporting each other's passions, priorities, innovative ideas, successes, and disappointments.

Companies want everyone to feel proud of what they do and feel comfortable educating both internal and external communities about the compassion held for their animal heroes, the immeasurable value of animal research, and the importance of animal welfare. They act with compassion, responsibility, transparency, dedication, and welfare in mind, and strive to empower those around us to be involved and offer creative ideas and innovations.

In order to create and foster this culture, one company hosts events and work collaboratively on their internal sharing sites to highlight this. They also have an annual Veterinary Sciences Animal Research & Welfare 3Rs Competition and awards ceremony. Outside of the competition, people are encouraged to share innovative and impactful ideas that have been implemented throughout the year so they can be promoted throughout the institution. They also provide continuing education in the form of various resources, webinars, and training opportunities on the Global internal sharing sites for Culture of Care and 3Rs Initiative. Inclusion in the Culture of Caring is an important component for everyone to be comfortable to offer and share their perspective.

Leading by Example

3Rs in vaccine and biologics development and production

Vaccine developers, as with medicines developers, are required to demonstrate that potential new medicines and vaccines are effective and safe in humans, and that potential side effects are identified before they get a license to produce and go to market. For vaccines, the use of animals in previous research has led to successful vaccines for polio, several types of meningitis, typhus, whooping cough, smallpox, tetanus, measles, cholera, etc. However, in these modern times, advances in science are leading to fewer tests and experiments on animals, and to new ways to reduce the impact on animals.

For vaccines, there is extensive ongoing work to decrease and, in some cases, remove the use of animals in both the research and development phases and the production phase.

Collaborations are key in driving forward the shift towards replacement. Bringing together experts and scientists in the field from different key players drives this change. For this reason, industry engages across different partnerships on focused topics. The European Partnership for Alternatives to Animals (EPAA) is one of these such partnerships. Projects of relevance include focus on Clostridium septicum vaccine validation of alternatives to in-process control tests (tox/residual toxoid toxicity – MLD, and toxoid antigenicity - TCP) which has been ongoing since 2013 (and builds on from a previous EPAA Vaccines Consistency Approach project (2010-2015). The ultimate goal is the regulatory acceptance of the validated in vitro assays through their inclusion in the Ph. Eur. Monographs to achieve replacement of the current animal tests. Another EPAA project ongoing is that of the Human Rabies Vaccines focusing on Potency test replacement of in vivo rabies potency test by in vitro methods.

Vac2Vac is an Innovative Medicines Initiative (IMI) project where the public and private sector consortium members are working on vaccine batch to vaccine batch comparison by consistency testing and they aim to develop and validate quality testing approaches for both human and veterinary vaccines using non-animal methods.

During the recent World Congress on Alternatives and Animal Use which took place virtually, attended by 1300 participants, there were a number of very interesting presentations given on numerous on-going activities focusing towards moving away from animal research and testing for vaccines. These presentations included the IMI Vac2Vac project mentioned previously, and also specific company strategies working towards a full in vitro approach for certain vaccines, including DTaP (Diphtheria, Tetanus, Pertussis) potency testing, whereby the aim is to have the animal approach removed from regulatory requirements worldwide. Furthermore, strategies are also being implemented for meningitis, pyrogenicity and human rabies vaccines where the focus is again towards a complete animal free process. Great progress has been made across the board, whereas intermediate steps reduction and refinement processes have already been taken up.

In a webinar in October 2021, EFPIA teamed up with the Animal Free Safety Assessment Collaboration (AFSA) and Humane Society International (HSI), in collaboration with the International Alliance of Biological Standardization (IABS) to organize a virtual workshop on "Accelerating Global Deletion of the Abnormal Toxicity Test - Planning common next steps". The participants discussed concrete ways forward to the global deletion of the Abnormal Toxicity Test from regulatory requirements for human vaccines and biologicals.

In addition to the gradual replacement of animal testing methods by alternative methods and in anticipation for all the alternative methods to be made available, which may still take up to 20 years, Vaccines Europe in particular continues to call on Europe in compliance with 3R and the Official Control Authority Batch Release (OCABRs) for vaccine batch release to reduce or even eliminate redundant animal tests carried out by European Official Medicines Control Laboratory (OMCLs), in accordance with what the European OCABRs already authorised and recommended.

In addition, in accordance with the WHO guidelines recommending the development of control and release strategies based on an approach based on risk management

Leading by Example

(for the benefit of patients) and in a manner comparable to what is already in place in the United States and Canada, we have recommended "reliance" to see the extension of mutual recognition agreements with these countries to control and release of batches (in particular for in vivo methods) in order to prevent these tests on animals already performed redundantly by the authority of the exporting country, to be repeated for the third time by European OMCLs for vaccines from the US and Canada.

The overall plan is therefore to accelerate the development of analytical methods to replace animal testing, and in the meantime to support drastic reduction and repetition of redundant tests by the authorities to the bare minimum required to protect public health.

Focusing primarily replacement, applying 3Rs[35]

Biologicals such as vaccines, cytokines, enzymes, and hormones are tested extensively post-licensure as part of routine quality control and batch release testing to ensure safety and potency. The World Health Organization (WHO) has established international standards for this purpose and their guidelines carry significant influence, being adopted by most global regulatory authorities. However, a review of the animal testing requirements within these guidelines has never been conducted and there may be opportunities to further implement the 3Rs.

The UK National Centre for the 3Rs (NC3Rs) has been tasked by the WHO and funded by the Bill & Melinda Gates Foundation to carry out an independent review of WHO guidelines for biologics to determine:

- Which animal tests are recommended for the batch release testing of biologics.
- What 3Rs principles are already encouraged, what opportunities exist for better implementation of 3Rs, and to make recommendations to WHO on how this could be best achieved.
- What barriers exist in different regions which may hinder the adoption of 3Rs approaches by manufacturers, national regulatory authorities, and control laboratories that are responsible for the testing and release of biologicals.

Approximately 8 million animals a year are used worldwide in routine quality control and batch release tests of biologicals. This puts a significant financial burden on manufacturers and national control laboratories, it is time and resource intensive, and the methods themselves can cause significant pain and distress to the animals.

The project is being overseen by an expert international working group (including WHO staff and members from national regulatory agencies, national control laboratories, manufacturers, and other relevant organisations) to support the guideline review and develop recommendations to be submitted to the WHO Expert Committee on Biological Standardization (ECBS) for their approval and implementation. The project also includes a number of stakeholder engagement activities including surveys and regional workshops.

Replacing the in vivo rabbit pyrogen test

An animal free safety release assay for vaccines has been developed in one company starting more than 10 years ago to replace the current in vivo rabbit pyrogen test (RPT). The monocyte activation test method (MAT) which is part of the European Pharmacopoeia since 2011 is now getting more acknowledged worldwide and has been accepted by several Health authorities as a replacement of the RPT outside of Europe. This required parallel testing to proof equivalence to other pyrogen assays. In the last 5 years several studies demonstrated the superiority of the monocyte activation test in comparison with the RPT regarding more rapid tests enabling a release in shorter time to the patients without compromising on the safety of lifesaving vaccines. Consequently, the MAT is the preferred safety assay in vaccine development and together with the endotoxin tests it allows to retire the rabbit pyrogen test in vaccine release. MAT adds value whenever the endotoxin test reaches its limitations. This allowed, in the last 5 years, to reduce the amount of Rabbit Pyrogen Tests by more than 95%. The aim is to test with the two alternatives only before 2025, presuming acceptance of the health authorities. MAT is therefore a key contributor to the strategy to replace all animal assays for the release testing of vaccines. The EDQM is foreseeing to remove the RPT test from all European Pharmacopeia within the next 5 Years.

35: https://nc3rs.org.uk/review-animal-use-requirements-whobiologics-guidelines

Leading by Example

Replacement of horseshoe crab assay by using a non-animal-derived reagent for endotoxin detection in vaccine products

Animal tissues (Blood, cells, organs and tissues, antibodies…) represent important reagents for laboratory activities. Their replacement by non-animal derived sources is considered whenever possible. Replacement of Limulus amoebocytes by recombinant proteins is one such opportunity:
Endotoxins, heat-stable lipopolysaccharides from Gram-negative bacteria, are potential contaminants that can be introduced during manufacturing of pharmaceutical products, including vaccines. Parenteral pharmaceutical products undergo endotoxin testing because endotoxins are pyrogenic in humans and can induce severe physiological reactions. Currently, animal-derived Limulus amoebocyte lysate (LAL) assays are widely used. This requires blood sampling in horseshoe crabs, which is an endangered species.

To address the biodiversity issue, assays using recombinant Factor C (rFC), a non-animal-derived reagent, have been proposed as alternatives. The strategy of the vaccine division is to replace the LAL assays by the rFC assay. As some components in the matrices of pharmaceutical products can interfere with these assays, a company compared two LAL- and two rFC-based assays for endotoxin detection in four complex human vaccine matrices. It was demonstrated that both LAL and rFC assays are adequate for testing and releasing four vaccine products. The rFC assays offer a number of benefits (lot-to-lot consistency, more robust, less interference, security of supply), including compliance with the principles of the 3Rs by safeguarding animal welfare and promoting more ethical and sustainable use of animals for testing. After full validation, pending acceptance by drug agencies, they could be considered as suitable replacement assays for the detection of endotoxin in the manufacturing processes of vaccines.

The company has received the Frederick Simon Award for the best Scientific Publication 2020 published in the PDA Journal of Pharmaceutical Science & Technology.[36]

36: https://journal.pda.org/content/74/4/394

Leptospira in vitro potency assays can replace currently standard in vivo potency test in hamsters[37]

Leptospira is a bacteria that amongst other things causes liver disease in dogs. The quantification of antigenic mass by ELISA as a new in vitro potency test supports the 3Rs concept and is in accordance with European Pharmacopoeia Monograph 0447 (Canine Leptospirosis Vaccine [Inactivated]). The two corresponding test methods (sandwich ELISAs) are based on monoclonal antibodies specific for immunodominant leptospiral lipopolysaccharide (are the main antigens responsible for immunity in leptospirosis a bacterial disease in humans and animals) epitopes. Protection in passive immunization experiments demonstrate that these monoclonal antibodies recognize key protective antigens in currently licensed human and veterinary whole cell Leptospira vaccines. The high precision and robustness render the two ELISAs much more reliable correlates of potency in dogs than the hamster potency test. The recent approval of these assays for a new canine leptospirosis vaccine is an important contribution to the 3Rs in quality control testing of Leptospira vaccines.

38: Development of Leptospira in vitro potency assays: EU/ industry experience and perspectives. Klaasen, H. L. B. M.; van der Veen, M.; Molkenber, M. J. C. H.; Bruderer, U.. BIOLOGICALS; SEP 2013; 41; 5; p315-p322,

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Communication, transparency and dialogue with the public

Corporate statements - Communicate on the 3Rs through dedicated sections in publicly available Corporate Social Responsibility (CSR) reports.

Disseminating openness and transparency - Many companies have signed transparency agreements and their websites are important tools in making available information and explaining the use of animals in experiments and including videos of housing and animal handling.

Open laboratories - The general public, NGOs and policymakers are offered the opportunity to visit laboratories or take virtual tours to see the housing and learn about the uses of animals and multiple efforts of industry to enhance the 3Rs in daily practice.

Dialogue with NGOs - Companies proactively engage in constructive dialogue with NGOs. They inform each other and share experiences and expertise through various collaborations on practical and operational issues.

Openness across the industry on animal use and 3Rs

The use of animals in research and testing is a controversial subject and to support an open dialogue on the use of animals for scientific purposes a high degree of transparency and openness is important. The corporate websites are important tools to EFPIA members to making information about the use of animals easily accessible. Telling what we do and how we do it, is crucial to explain and justify why the use of live animals is still an indispensable requirement to develop drugs for serious diseases or chronic illness. One way this is done is by posting on company websites the actual numbers of the different species used and presenting pictures or videos of housing and even a virtual tour in an animal research laboratory .

3Rs Promotion and openness

Companies are making considerable strides in communicating not only what is happening in animal research facilities but also why the work is required. In order to increase transparency with colleagues, animal facilities have opened up in a variety of ways.

- At many workplaces, it is traditional to participate in the annual "take your child to work day". During this day many activities are arranged across the site to help employees' children engage in science and to learn more about what their parents do every day.
- Global 3Rs awards have become customary in pharma industry. Some companies communicate openly about these awards. One example is a blog written for the NC3Rs that describes this company's 3Rs award programme and how an awards programme can promote 3Rs innovation. https://nc3rs.org.uk/news/using-award-scheme-promote-3rs-innovation
- Biomedical Research Awareness Day (BRAD) - BRAD was launched in 2016 by Americans for Medical Progress (AMP) in the US and takes place every third Thursday in April. This day is an opportunity to inform and raise awareness among the company's employees about the need for and benefits of animal research for the development of new medications and therapies. Companies use the day to inform through numerous on-site events, presentations and workshops, as well as the presentation of new digital technologies. Information on company's animal welfare and ethical oversight policy and standards as well as the high quality of care for the animals is also communicated during the various events.

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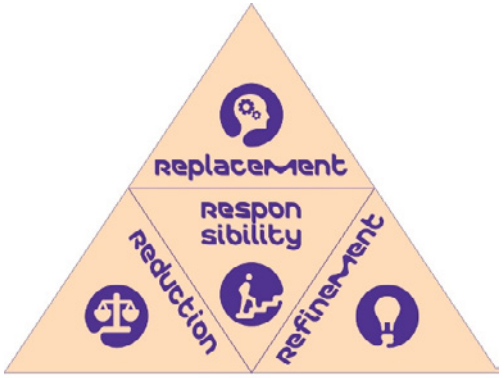
◊ The Animal Welfare 4R Day and 4R Awards for pioneers in animal welfare
One company has added a fourth "R", Responsibility to the well-established 3R principle. To celebrate and promote their 4 Rs, 4R Days were organized and a 4R award issued. For each of the 4 categories REPLACEMENT, REDUCTION, REFINEMENT and RESPONSIBILITY, the company celebrated pioneering winning projects out of an impressive list of fantastic applications in a ceremony during their 4R Days.

◊ The REDUCTION category was awarded to a project to eliminate the use of guinea pigs for adventitious agents testing.

◊ The REPLACEMENT award for their efforts to push for in vitro cytotoxicity testing to replace animal testing during plastic material qualification for Mobius™ single-use components.

◊ The REFINEMENT award recognized the creative development of a large group-housing system for mice that significantly improves their quality of life by allowing them to establish functional areas and chose with whom they want to spend their time.

◊ Finally, but not lastly, one group demonstrated outstanding Responsibility as they demonstrated significant impact on animal welfare by creating awareness for animal usage in non-in vivo personnel of their project. They educated the entire team and in particular those who were not involved in the in vivo part of the project what it means to work with animals and how important it is to honor their sacrifice for a project which, in turn, will support treatment of diseases in human beings.



Close collaboration between Veterinary teams, 3Rs subject matter experts, trainers and researchers ensures the latest refinement techniques are used to improve animal welfare during studies. Examples may include:

◊ Ensuring researchers are utilizing the latest in anesthesia and analgesia modalities while minimizing the impact on the proposed research.

◊ Staying current with the latest in blood sampling techniques and dosing techniques.

◊ Proactively planning experiments in a way that minimizes animal impact, such as reducing the number of sampling time-points, strategically utilizing sampling sites or using advanced vascular access port systems or remote sampling systems.

◊ These collaborations allow for prospective discussions on how 3Rs can be utilized at all stages of the study.

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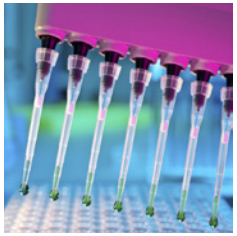
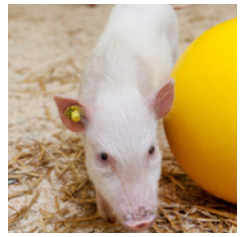


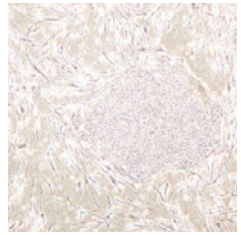
In addition to participating in international events and platforms, the pharmaceutical industry is communicating on the 3Rs through their websites and Corporate Social Responsibility (CSR) reports. Often, there is a dedicated section on animal welfare and the 3Rs are included in these reports. Furthermore, some member companies produce their own annual 3Rs report illustrating industry's commitment to applying the 3Rs principles in animal research and to enhancing scientific advances leading to the implementation of one of the 3Rs.

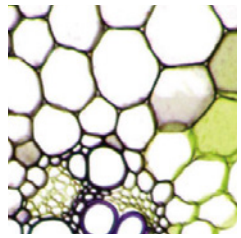
A few examples can be found here:
* Astellas
* AstraZeneca
* Bayer
* Johnson & Johnson
* Novartis
* Novo Nordisk
* Merck
* Roche
* Sanofi
* UCB

EFPIA Partners in Research
* Labcorp

EFPIA have produced five previous reports on Putting animal welfare principles and 3R into action







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Useful Links

Accreditation of Laboratory Animal Care International (AAALAC) - **www.aaalac.org**

Alternatives Approaches to Animal Testing (EPAA) - **www.ec.europa.eu/growth/sectors/chemicals/epaa_en**

Altertox Academy - **https://academy.altertox.be/**

European Centre for the Validation of Alternative Methods (ECVAM) – **www.eurl-ecvam.jrc.ec.europa.eu**

European Commission - **www.ec.europa.eu/environment/chemicals/lab_animals/home_en.htm**

Federation of Laboratory Animal Science Associations (FELASA) - **www.felasa.eu**

Innovative Health Initiative (IHI) - **www.ihi.europa.eu**

Institute for Laboratory Animal Research (ILAR) - **www.dels.nas.edu/ilar**

National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) - **www.nc3rs.org.uk**

Norecopa - **norecopa.no/**

3R Foundation - **www.forschung3r.ch**

For more information, contact

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