ZESDE ANIMALES-VOORDRACHT

BETTER SCIENCE WITH FEWER LABORATORY ANIMALS:
A techno-moral revolution

Prof. dr. DANIELA SALVATORI

Stichting ANIMALES

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A Techno-Moral Revolution

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BETTER SCIENCE WITH FEWER LABORATORY ANIMALS: A Techno-Moral Revolution

ZESDE ANIMALES-VOORDRACHT

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door

Prof. dr. Daniela Salvatori

Hoogleraar Comparative Anatomy and Physiology
Faculteit Diergeneeskunde Universiteit Utrecht

also on behalf of TPI Utrecht

Stichting ANIMALES

This booklet is a summary of the Foundation Stichting Animales lecture held at the iconic location of Anatomiegebouw in Utrecht on October the 2nd. The lecture held by Prof. Daniela Salvatori was a collaborative effort together with Joaquin Montilla Rojo, Justine Watkins, Jordi Middelkoop, Luc Theunisse, Cristheena Nonis, and Adele Selma Ferrario.

Figure 1. The speakers at the Foundation Animales lecture held at the Anatomiegebouw in Utrecht on 2 October 2024 From the left to the right: Cristheena Nonis, Daniela Salvatori, Luc Theunisse, Joaquin Montilla Rojo, Jordi Middelkoop, Adele Selma Ferrario, and Justine Watkins.

Daniela Salvatori, DVM, PhD, Dipl European College of Veterinary Pathology, CRP/TP, works as Professor of Comparative Anatomy and Physiology and leads the Anatomy & Physiology group at the Veterinary Faculty at Utrecht University. She also leads the Centre of Excellence of Plastination and Virtual Reality, which develops innovative models for training clinical skills and procedures in both humans and animals. She is highly dedicated to animal-free research, and since 2019, she has chaired the Utrecht Transition Programme to Animal-free Innovations (TPI Utrecht), an interdisciplinary group focused on innovation of research and education without using laboratory animals. She is also the Scientific Director of the Center for Animal-Free Biomedical Translation (CPBT), a growing project for which the National Growth Fund has reserved 125 million euro in 2024 to accelerate the transition to animal-free biomedical innovations.

d.salvatori@uu.nl

INTRODUCTION

The relation between animals and humans is complex. The meaning of the human-animal relationship, the meaning of compassion and respect becomes more complex as we are tested in life with all its contradictions. We see a functional diversity of types of human-animal relationships, such as keeping companion animals, farm animals and laboratory animals, each with their own man-made purpose. Different uses allow the same animal to take on different roles: a pig can be a pet, a meat-producing animal or a laboratory animal.

We humans are constantly faced with events that connect and simultaneously confuse the boundaries of these uses and definitions. Herzog (anthrozoologist) has explored the complex psychology of our interactions with other species. He has written a book with a title that summarizes the complexity: *Some We Love, Some We Hate, Some we Eat* (Herzog, 2011). Why It's So Hard to Think Straight About Animals? Not to mention, we as veterinarians have a mission to cure animals; animals are our patients.

This lecture focuses on a particular aspect of this complex relationship with animals: the use of laboratory animals and animal testing.

Why do we still use laboratory animals and why is it time to accept the revolution to animal-free animal-free practices?

This revolution includes research and education, it is closely linked to society and politics and to patients and by the word patients I mean humans and animals.

What is a laboratory animal? We refer to laboratory animals when we perform an act, where an act refers to anything that is done to an animal that can affect his/her welfare. (Directive 2010/63/EU; Commission Implementing Decision 2020/569/EU).

This includes using an animal in a research project, in teaching and the creation or breeding of a genetically modified animal.

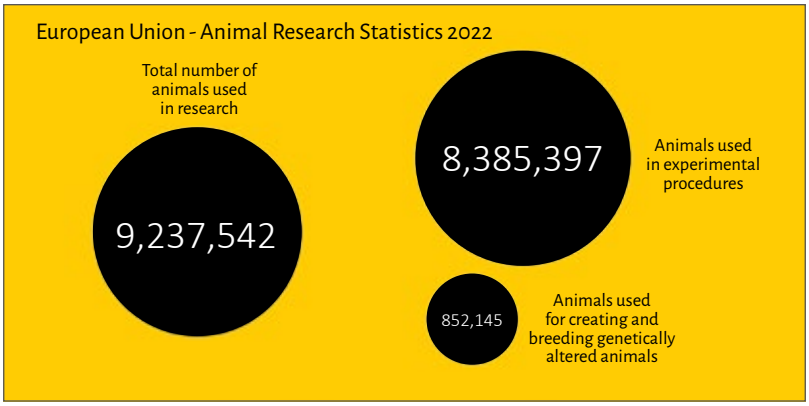


Figure 2. Number of animals used in research in Europe in 2022 (European Union, 2022).

A procedure may involve a single intervention, such as a single injection, or may refer to multiple events, such as inducing, treating and analysing tumours for example.

How many animals do we currently use in Europe? It is not a few thousand animals, but it is millions of animals. The fact is that we use more than 12 million animals a year in Europe alone. If we look at the USA, we are talking about more than 100 million a year (European Commission, 2023).

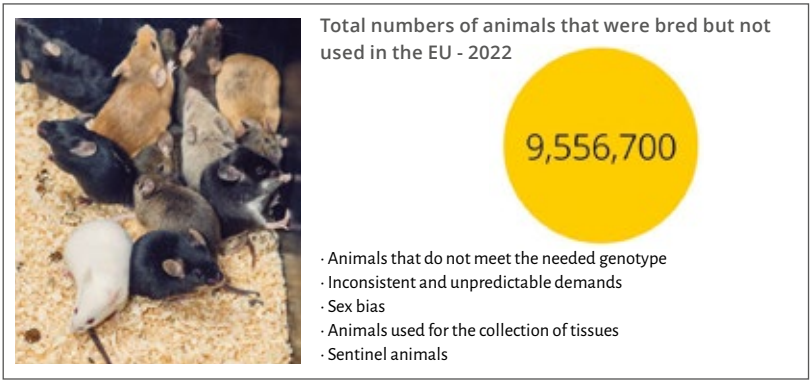


Figure 3. Number of animals bred but not used in research in Europe in 2022 (European Union, 2022).

WHY DO WE USE ANIMALS?

When we talk about basic research, we mean animals used e.g. for oncology and organ systems studies, Translational research and applied research (translational/applied research) involves using animals to study complex human and animal diseases.

Research with laboratory animals is – for the time being – necessary step in the development of new drugs for humans and animals.

The safety, toxicity and efficacy of, for example ingredients in food and chemicals, and the use of medical technology, are studied in animals. 'Regulatory' means: to meet regulatory requirements. A percentage of animals are also used within the education domain.

When we think of laboratory animals, we immediately think about mice, but this is not always the case, ranging from fish, mice, rats to larger animal species such as dogs, sheep, pigs, and so on.

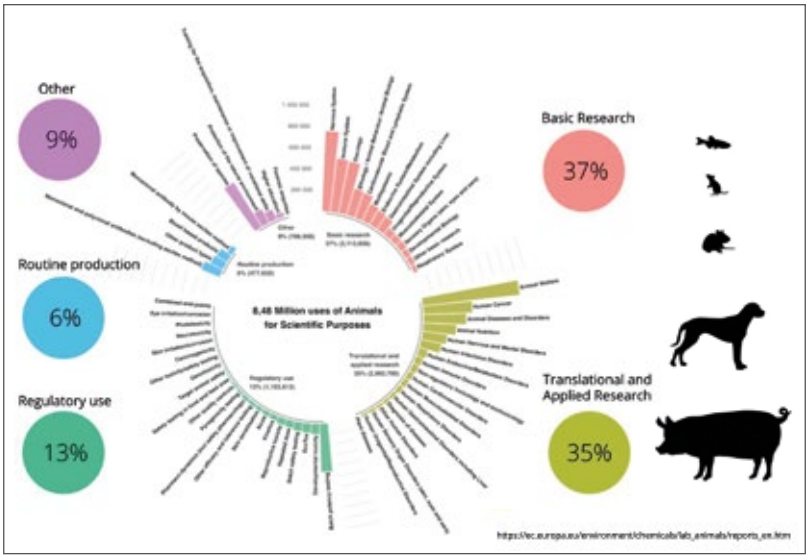


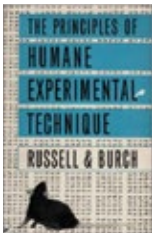
Figure 4. Number of animals used for scientific purposes and fields of use in Europe in 2022 (European Commission, 2024).

REGULATION OF ANIMAL EXPERIMENTATION

Researchers and teachers who use animals must follow EU directives and the Dutch Animal Experiments Act or Wet op de Dierproeven (WoD) in the Netherlands that is based on the EU legislation (Wet op de Dierproeven, 2014). Research and teaching on animals are regulated with the explicit understanding that important new knowledge in this field is obtained without causing unnecessary harm to animals. According to the law, a researcher or teacher must be well informed about the latest non-animal testing methods.

Minimizing harm to laboratory animals is a topic that finds its foundation in the 3Rs: *Replace, Reduce* and *Refine*, first described by Russel and Burch in 1959 (Burch, 1995).

William M.S. Russell (1925-2006) and Rex L. Burch (1926-1996) at the Sheringahan Workshop in 1995.



Cover of The Principles of Humane Experimental Technique courtesy of R. Curren

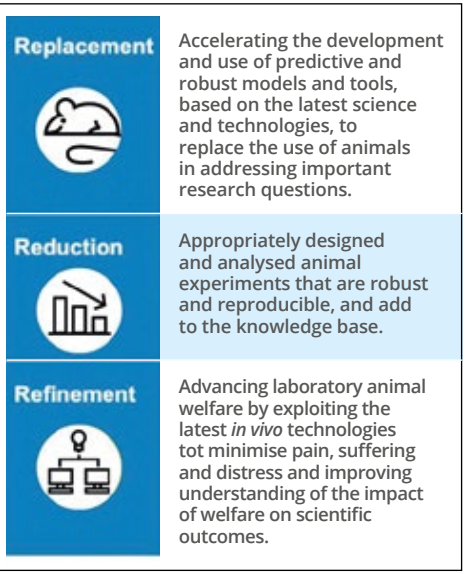


Figure 5. The 3Rs principle: starting from Russell and Burch to the National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs, 2025).

SOCIETY AND ANIMAL TESTING

Animals have been used in studies and research throughout human history. The anatomy and physiology of vertebrates have been studied for millennia, since the beginning of the history of medicine. Physicians in ancient Greece already dissected animals in order to understand the human body. Much of our knowledge on the function of cells, tissues and organs, and mechanisms of diseases has been helped by studies that were performed on animals. They have contributed to many medical treatments, such as drugs, vaccines, and surgical techniques.

Nearly 90% of Nobel Prize research in medicine used animal experiments in their discoveries. For example, the treatment of type I diabetes using insulin was first established in dogs by Banting and McLeod (Nobel Prize in 1921). Animal models have helped to develop vaccines that have saved millions of human and animal lives. In the early 1920s, the introduction of a vaccine against diphtheria significantly decreased child mortality all around the world. This vaccine was at first studied in horses. Many new surgical techniques have been developed and practiced on pigs because their organs are a similar size to those of humans.

Animals are similar to humans, but not identical. Rodents, and in particular mice, are the most common type of mammal employed in experimental studies. Mice are chosen because their genome is similar to that of humans. Over 95% of the genes are the same. In addition, mice are small, easy to handle, and they breed well and in a short time. Cutting edge technology is available to manipulate mouse genes in order to mimic human diseases, so called mouse models of human diseases. Regardless of the similarities, mice do not contract the same genetic diseases as people. Therefore, scientists need to alter their genome to induce human disease. Although many different mouse models are available for studying human diseases, a successful treatment in a mouse does not directly translate into a successful treatment in the human patient.

This is not only due to the genetic differences between mice and humans. In laboratories, mostly inbred strains of mice are used. These are mouse strains produced from brother-sister matings for at least 20 sequential generations, resulting in a highly homogeneous genetic composition. Results can be strongly dependent on the particular type of inbred strain used. A striking example is provided by a study published in November 2014 in Science by a team who reported that some mouse strains are fully resistant to Ebola virus, others die without specific symptoms, while mice from another strain develop fatal hemorrhagic fevers. This example illustrates how animal models must be considered: no single animal model is able to exactly mimic a given human disease.

In addition, it is not possible to recreate all the symptoms of a certain human disease in mice. In aging-related neurodegenerative diseases such as Alzheimer's or Parkinson's disease in particular, one should be cautious when interpreting cognitive, emotional and (of course) language deficits characterizing human disease. This reflects differences in the development and function of rodent and human brains.

It is currently not allowed to test new drugs directly in humans, since it is legally required to try them on animals first (called preclinical trials). This process, undertaken primarily by the pharmaceutical industry, takes an average of 10–15 years and it is extremely costly.

Data from animal research are crucial to researchers in pharmaceutical companies to decide whether a potential medicine will be effective and safe in humans (Fig. use of animals in drug discovery). Despite all efforts, money, time, and animals, the pharmaceutical industry is facing a profound crisis: only 1 in 10 drugs that has a positive effect on animals also work in humans. Only a small fraction of the thousands of human diseases has an approved treatment, and still, many of these cause unwanted side effects.

This is extremely worrying for patients that are waiting to be cured.

Reasons for this non-dismissible global failure have been analyzed and ascribed to faults in study design, analysis and reporting of experiments. Certainly, these can be improved, but this will not solve the differences between humans and animal species. These differences make it impossible to directly translate preclinical results to humans.

The use of laboratory animals is ethically difficult and controversial. The Netherlands has therefore the ambition to significantly reduce the use of laboratory animals over the next few years.

A lot has improved over the past fifty years. The availability of new human-based methods, such as organ-on-chip technologies, has grown enormously. It is, however, often very difficult for young researchers to deviate from the beaten track and choose and position alternatives. It is emotional and difficult, but we need to go through a mentality shift: the way forward is to invest in human relevant research methods and technologies. We have a moral obligation towards patients and animals to humanize medicine (Royal Netherlands Academy of Arts and Sciences, 2021).

WHY DO WE QUESTION ANIMAL RESEARCH? AND WHY DOES THIS CRITICISM LEAD TO A QUEST TO MODERNIZE SCIENCE?

The ethical case for animals

Animals can sometimes experience pain, stress and loneliness. Around 90% of animals are killed before or after testing. In addition, there is an almost equally large group of bred animals that are not used but still killed. Ethical opposition to animal testing is based on the moral status of animals as sentient beings; animal experimentation confronts the animal with suffering and death, systematically violating its wellbeing.

In September 2023, for the second time in seven years, more than a million people in Europe called for an end to animal testing by signing a European citizens' initiative. But not only in Europe but also in the US, the percentage of citizens against animal testing has increased markedly over the years.

The scientific case

Animals are similar to humans but not identical. Rodents, especially mice, are the most commonly used mammals for experimental research. Mice are used because of their genome, which is 95% similar to the human genome. Moreover, mice are small, easy to handle and reproduce rapidly. Regardless of the similarities, mice do not always develop the same genetic diseases as humans. Although many different mouse models are available to study human diseases, successful treatment in a mouse does not guarantee the same result in a human (Pound, 2020).

Can the results found in animals predict the outcome in humans?

There are clear differences between animals and humans that cannot be circumvented. This failure is also attributed to errors in the study design, analysis and reporting of the experiments. Although improvement is possible, this does not resolve the human-animal differences. Yet, inadequate quality of published animal experiments is and remains a very painful problem (Fosse et al., 2023).

Using a systematic review tool, we showed that trials on repairing cartilage damage in joints of large animals such as sheep and horses are incompletely reported in scientific journals. We analysed 223 large laboratory animal studies worldwide and found that more than 90% provide incomplete information on anesthesia and pain control (Fugazzola et al., 2022). This is problematic for three reasons: there is no account of how animal welfare was best protected, the study is less reproducible (repeatable), and it is more difficult for other researchers to build on the knowledge from the study. As long as we do animal experiments, they need to be better described.

In this transition phase, there is a lot of focus on building animal-free methods, but too little on phasing out animal testing. I think universities urgently need to work on a policy to properly report on animal testing and reduce the number of unnecessary animal experiments.

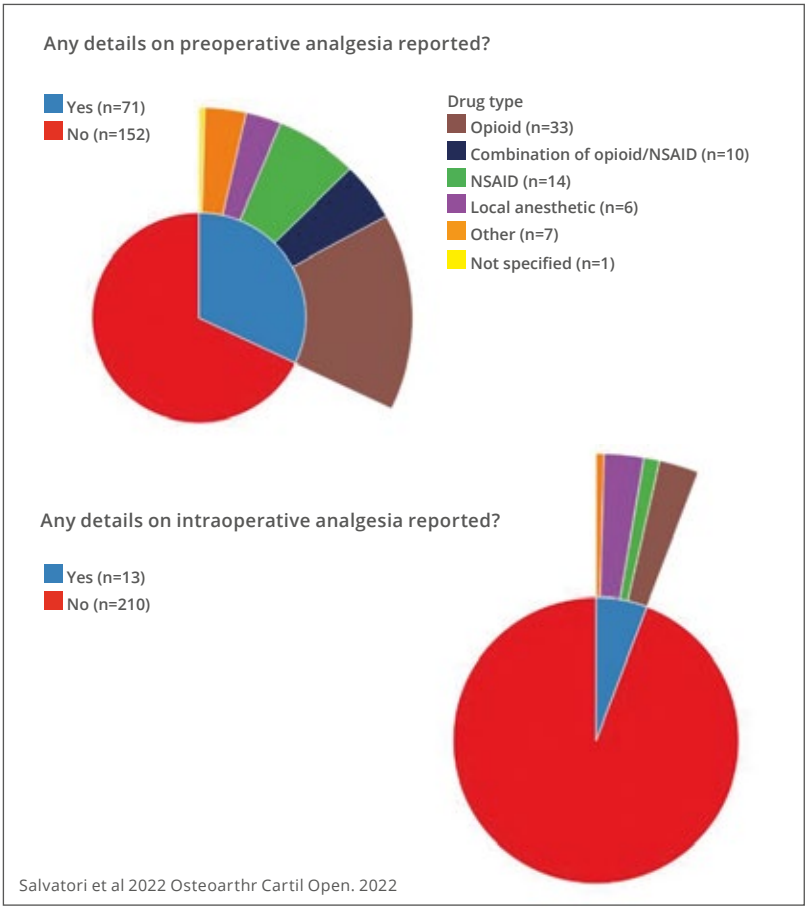


Figure 6. Nontransparent reporting of animal methods. Reporting of anesthesia and pain management in preclinical large animal models showing underreporting of perioperative pain treatment (Salvatori et al.; 2022).

The economic case

We are now going to discuss the economic case of animal free innovation to show that we do not have an appropriate and sustainable business model for producing drugs. Currently, there is an obligation to test new drugs on animals first. This procedure, mainly used by the pharmaceutical industry, takes an average of 10 to 15 years per drug and is extremely expensive. Only once medical studies are completed can a pharmaceutical company seek permission from regulatory authorities to market the drug. In Europe, this regulatory body is the European Medicines Agency (EMA) and in the US, the Food and Drug Administration. Despite all the effort, money, time and laboratory animals, pharmaceutical companies face a serious problem: only one in 10 drugs that have a positive effect on animals also works in humans. The translation from research practice to clinical practice is minimal (Marshall et al., 2023; Langley et al., 2017).

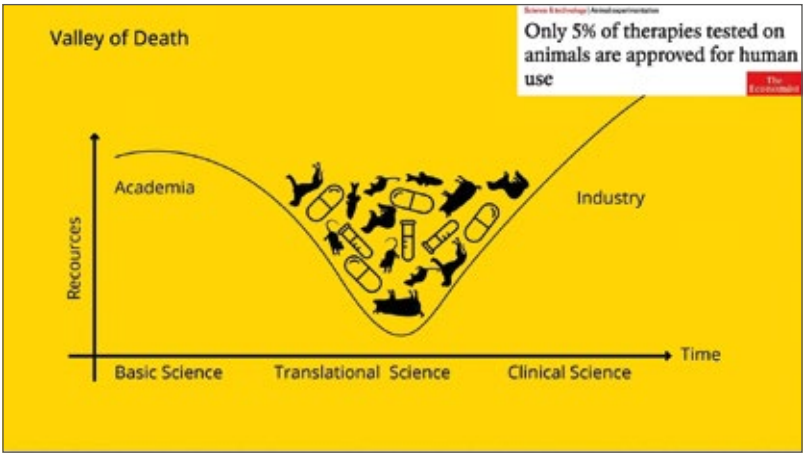


Figure 7. The Valley of Death. Lost in translation: the valley of death across preclinical and clinical phases. The crisis involving the "translation" of basic scientific findings in a laboratory setting into human applications and potential treatments or biomarkers for a disease is widely recognized both in academia and industry.

Only a fraction of the thousands of diseases in humans have an approved therapy, which in many cases also causes unwanted side-effects. This is very worrying for all patients, who hope for a cure. The experience with Covid-19 shows that more alternatives were used and accepted for vaccine development and production; human studies started earlier and these ran in parallel with animal studies; and regulators have accepted historical data from previous vaccine research.

The Food and Drug Administration has recently made proposals to encourage a programme focused on relevant human data. The aim is to yield findings that are more relevant to humans and reduce the production costs. This means that this way of drug development is not economically sustainable: Too expensive, it takes too much time, and it yields little.

And finally, we have a more complex ethical problem that goes beyond laboratory animals but has implications for patients (human and animal) and ultimately for societal benefit (Han, 2023).

Governments and other organizations that use public funds to finance medical research therefore have an ethical duty to human beings to support methods that are most likely to lead to progress. There is a global need for better and more affordable medicines at a time when the burden of disease is rising faster due to an ageing population and the increase in chronic diseases. In other words, we need to strive towards a better biomedical system.

The Netherlands wants to be a frontrunner in the field of animal-free but abolishing all animal testing within 2025 as said by State Secretary for Economic Affairs Van Dam in 2016 will not succeed. The use of laboratory animals has not decreased in the last 5 years.

In 2021, the European Parliament approved a plan of action to end the use of laboratory animals. The EU document contains two key points: 1) the 3Rs have not been enough, and 2) there is urgent need to embrace new animal-free technologies (European Union, 2021/2784(RSP), 2021).

Replacement, Reduction and Refinement: have been the guiding principles for the ethical use of laboratory animals for years. There seems no doubt that we need to provide as much protection as possible, that animal welfare is paramount. We must know how to ensure the best possible care. As a veterinary pathologist specialized in laboratory animals, I know the incredible effort of researchers, technicians and animal carers to ensure optimal care for animals.

But The 3Rs miss an important ethical dimension: research on animals is only ethical if it is valuable for science and society, and it is precisely this element that is not represented by the current 3Rs.

The evaluation of project proposals does not always include a proper assessment of scientific validity that focuses on the research question. Scientific validity should be leading. Here we call on the Central Committee on Animal Experiments and animal welfare bodies to be forward-thinking and fully embrace scientific validity in their ethical review. This is not contrary to the 3Rs but it is an upgrade of the 3Rs.

Science is people work: '*We Work Differently than We Think*' in many ways (I paraphrase Prof Ellemers). A recent work by PhD student Désirée Veening-Griffioen showed that the current choice of a specific animal model seems to be based on tradition rather than its potential predictive value for clinical outcome. The article shows that scientists often make the choice of animal model in a non-specific way. This gives the possibility of non-valid results (Veening-Griffioen et al., 2021).

The way forward: if we acknowledge these facts, we have strong arguments for finally modernizing medical research for the benefit of humans and animals.

The toolbox of animal-free models is extensive and shows the potential to increase our understanding of diseases and accelerate the discovery of effective treatments.

New Approach Methodologies (NAMs) are defined as '*new scientific approaches that focus on human biological processes to investigate diseases and potential treatments, using human cells, tissues, organs and existing data*'. They also use artificial intelligence and organ-on-a-chip technology.

Such a chip consists of a plastic plate with microchannels and chambers in which complex tissues of cells grow. By adding drugs or foods, for example, researchers can monitor the effect live under the microscope. The future is to get the whole body on a chip (Schmeisser et al., 2023).

INTERNATIONAL AND NATIONAL SUPPORT FOR MODERNISING MEDICAL RESEARCH

The Netherlands wants to lead the way internationally in innovations that make animal tests unnecessary. Therefore, the central government encourages the development and application of methods for research without testing animals. This is done with the partner programme Transition to Animal-free Innovation (TPI). TPI operates under the direction of the Ministry of Agriculture, Nature and Food Quality. (Ministerie van Landbouw, Visserij, Voedselzekerheid en Natuur, 2025).

The TPI process is shaped by various parties such as e.g. KNAW, RIVM, ZonMw, NCad and others. TPI central inspired the Utrecht Life Sciences deans to establish TPI Utrecht.

TPI is so special because the veterinary school, the only one in this country, is part of it. Together with my colleagues from the University, the Academic

Hospital and Hogeschool Utrecht, we started TPI Utrecht, a truly interdisciplinary group. We wanted and still want to create an inclusive community. We have not always had meaningful discussions about animal testing in recent years. The discussion too often became a kind of arena where scientists had to take a stand. Scientists who used animals were sharply confronted with the moral criticism towards animal use and people perceive moral criticism as harmful, so people find it difficult to accept that information. The reality is that a lot of researchers are in transition; I am also a researcher in transition. I have reviewed many project proposals as a designated veterinarian where there is also a lot of focus on animal-free methods. Within TPI Utrecht, we applied the concept of 'cultural humility'. We tried to understand our own assumptions and those of others, prejudices and values.

But we also provided practical support: opened a helpdesk, organised many roundtables with many research groups but we also made many relationships physically and online in the animal research centres. Scientists are good at what they do, but communicating with journalists, activists or policymakers can be a different story. There is a plethora of social media. The field of animal-free innovation is more political and important to a lay audience than many others.

Fortunately, at TPI Utrecht, we have constant help from communications experts and that has been essential. This is how we help each other: by sharing successes and frustrations, we get more opportunities to move forward and know we are supported.

We wanted to create an inclusive community and I think we succeeded, but now we are at a crucial point: we need to allow TPI Utrecht to act as a user committee, since validation and acceptance of non-animal models are key factors. But we have bigger plans; we want to channel this energy and work into a well-organised innovative centre. The new **Centre for Animal-Free Biomedical Translation** (CPBT in Dutch: **C**entrum voor **P**roefdiervrije **B**iomedische **T**ranslatie) will shape this approach, creating new

business opportunities around animal-free technologies and biomedical translation. This allows the Netherlands to distinguish itself globally and realize its ambition of becoming a leader in animal-free innovation.

EDUCATION IS THE CATALYST FOR ANIMAL-FREE INNOVATIONS

The modernisation of science cannot happen without the help of education. This requires education to actively participate in the transition process and traditional practices to be scrutinised. A recent EU status report on non-animal methods in science and regulation includes the following call to students and teachers: 'education and training programmes are a crucial endeavour towards the ultimate aim of abandoning animal testing' (Halloway et al., 2021).

As new methods are constantly being developed, there is a constant need for education and training in all aspects of non-animal testing science to disseminate the new methods and enable them to be applied in practice; therefore, it is very important to create education plans aimed at students and professionals.

The ambition statement (see QR code) advocates for reducing laboratory animal use in higher education by promoting innovative, animal-free teaching methods. It emphasizes policy changes, increased awareness, and the establishment of a national hub for non-animal testing education (Ministerie van Landbouw, Natuur en Voedselkwaliteit, 2023).

In a pragmatic way, TPI Utrecht started Helpathons, Challenge Based Learning courses and many other elective courses for students and professionals. A Helpathon is "A space where you can ask questions… about an existing or new research project, where you can go for all questions and all possible answers that can contribute to innovative research, preferably animal-free." It is an intervention connecting people and ideas from different back-

grounds and sometimes conflicting views. There were several Helpathons, e.g. for finding animal-free solutions for liver diseases or setting upnew education. This showed mutual trust and respect: the beginning of a very frank collaboration.

We started challenge-based learning courses supported by the Alliance with TU/Eindhoven, Wageningen University and Academic Hospital, and Utrecht University of Applied Sciences. Students from different disciplines are working with each other and with social partners and companies on different aspects of the transition to animal-free.

Some of these courses are already embedded in curricula of the Faculty of Veterinary Medicine and the GSLS and in the lifelong learning programme, but a more comprehensive programme for students and professionals, with national and international partners, is urgently needed.

In any case, let us not forget that laboratory animals are also used within curricula in education; I am going to describe 3 different situations; 1) the biomedical Ba and ma courses, 2) the veterinary curriculum, and 3) the post-graduate training of researchers who actually conduct animal experiments (namely the laboratory animal science course). Recently, a national working group (of which I am a member) published an ambition document for fewer laboratory animals in education, commissioned by the Univ. of Ned. & the Ned. federation of univ medical centres in which we substantiated that in some courses there is a clear preference for the use of laboratory animals without clear explanations and specified learning objectives.

I advocate to stop killing animals in teaching in all biomedical Ba and Ma courses now. Moreover, animals in this category are mainly used to gain knowledge about the anatomy and physiology of organ systems, and as a source of material for training molecular/cell biology techniques. However, for the above disciplines and their learning objectives, many effective animal-free methods are already available, evidence-based published.

There is no need to kill animals to build knowledge and critical thinking skills. Moreover, students who are required to perform painful techniques

and terminal surgeries have been shown to show signs of stress and 'compassion fatigue'. This results in reduced empathy for others and can reduce the quality of medical care provided to animals. Methods also in education should not be based on tradition and students have the right to use non-animal methods.

Veterinary education is a more complex case.

The goal of veterinary education is to provide professionals with the necessary skills and knowledge needed to provide effective, safe and ethical animal care and treatments while safe guarding public health. Our veterinary students should be given ample opportunities to practice in a safe environment, without stress and with sufficient time and opportunities to receive feedback.

Teaching clinical skills can be done in a variety of ways: From textbooks to e-learning, from simple to more complex models, to live animals, to the patient.

We already have an undergraduate virtually without the use of laboratory animals. Our anatomy and physiology courses are done without laboratory animals.

Many non-animal methods have already been developed and organised in the veterinary skills labs. We are making exciting plans for a modern skills lab, where models, can be combined with computer simulations and modern technology such as virtual reality. Our skills lab will be the central place where education and training are offered to students, veterinarians and other professionals working with animals. To enhance the educational benefits of a skills lab, underlying educational theories and a well-designed learning environment must be part of the approach.

Many published articles directly compare training on live animals with training with models. This assumes hat training with live animals is the ideal method (the so-called gold standard). According to this thinking, replacing animals with simulators is only possible if there is sufficient evidence that simulators provide a more effective learning experience.

However, we should focus on achieving relevant learning goals and not simulators versus animal. Success should be determined by setting adequate learning targets.

Together with animal-free, we set up the animal donor codicil. If a pet has unfortunately reached the end of his/her life, you can choose to make its body available to science. Thanks to this animal donor codicil, experimental animals are no longer needed for anatomy education. We make hard and soft plastinates not only for anatomy but also to practise clinical and surgical procedures and, in the future, combine them with virtual reality.

The technique of plastinate-making offers a unique way to preserve body parts or the whole body of animals and humans. The result is a realistic and detailed anatomical image of organs, tissues and fine structures. Pllastinated specimens last for decades.

In the human medical field, VR is becoming an important tool to train surgical skills. VR provides a fully interactive 3D projection ('avatar' or holographic) of an anatomical model in which all structures (such as bones, muscles, blood vessels) are annotated and visibility can be changed to show underlying structures. The package is pre-designed to include animated 3D movements, commands and feedback, and elements of serious gaming. Since computer- and VR-based simulations are not location- or time-dependent, they can be shared with many partners outside the faculty.

Because of the ethical, economic, social and scientific discussion, I advocate including the transition to animal-free innovation in national and international sustainability policies.

Sustainable development includes animal, human and nature

We offer some showcases prepared by students, showing their research work and tangible tribute to animal-free innovations.

THE GLOBAL EDUCATION HUB FOR ANIMAL-FREE INNOVATIONS

Adele Selme Ferrario and Cristheena Nonis

Adele Selma Ferrario is a passionate PhD student at UMC Utrecht and Utrecht University's Faculty of Veterinary Medicine. Her research focuses on developing education and training for knowledge exchange within the CPBT project consortia, collaborating with researchers from academia and industry, legislators, and the public. She holds a master's degree in Toxicology and Environmental Health, with a profile in Science Communication and Education, and has completed the Young Innovators Honours Programme at Utrecht University. Driven by a strong passion for change-making and the ethics of science, Adele applies her transdisciplinary background to advance educational and communication initiatives that promote animal-free testing, reflecting her commitment to innovation and ethical research practices in research and education.

Cristheena Nonis is a second-year master's student in Drug Innovation at Utrecht University, with a background in Biomedical Sciences. Cristheena currently works as a student assistant at TPI Utrecht, where she contributes to the development of the Global Education Hub in collaboration with PETA. Her role in communications is key to advance innovation, as she works to raise awareness and foster collaboration. Cristheena is particularly motivated by the potential to make a meaningful impact in research and drug development. By promoting animal-free models, she aims to ensure that patients receive human-relevant medicines, reduce clinical trial failures, and prevent the waste of valuable resources and investment. This commitment to improve the future of healthcare fuels her work, and she remains dedicated to contributing to these advancements in every aspect of her career.

The Global Education Hub, launched in November 2023 by TPI Utrecht TPI Utrecht and Peta UK, is a collaborative platform dedicated to advancing animal-free education and research methods worldwide. This initiative is aimed at the co-creation and sharing of educational resources to promote animal-free innovation. Through this project, the hub strives to advance animal-free education and education about animal-free research methods, emphasizing the significance of innovative non-animal models and the importance of unified efforts in shaping the future of education. To date, the Hub has held four plenary meetings, with the latest on October 28, 2024, and has established four working groups:

1. Regulators – enhancing education for regulatory professionals
2. PhD and Postdocs – looking at specialised training for emerging researchers
3. High School, Bachelor's & Master's – focusing on formal education across secondary and higher education
4. Funding – currently inactive; to support projects as needed in the future.

The Hub has published a foundational paper to publicly share its mission and goals as it continues to define its identity:
DOI: https://doi.org/10.14573/altex.2411251

We welcome new members and contributions – please reach out if you are interested in joining our efforts to promote animal-free education!

BARRIERS TOWARDS CRUELTY-FREE BOTULINUM TOXIN POTENCY TESTING

Jordi Middelkoop

Jordi Middelkoop is an inquisitive research assistant at the Utrecht University Faculty of Veterinary Medicine, focusing on writing a journal article on the ongoing transition towards the animal-free quality control testing of 'Botox' products. He holds a Master's Degree in Drug Innovation and completed the profile 'Translational Life Sciences', where he worked on a project exploring the opportunities in acceleration the adoption of animal-free methods in science. He applies an analytical approach and views problems from multiple perspectives in order to promote sustainable and ethical practices in scientific and regulatory testing.

Botulinum neurotoxin, commonly known as 'Botox', has been used for decades in the treatment of various neuromuscular disorders. It has also proven to be effective in the aesthetic treatment of facial wrinkles, as localised injections of Botox can temporarily relax the facial muscles that cause these wrinkles. In recent years, the annual number of aesthetic Botox treatments has grown significantly. Since botulinum neurotoxin is a dangerous biological product harvested from bacteria, the potency of each batch needs to be tested during manufacturing to ensure the quality and safety of the final product. The 'gold standard' test for Botox potency to this day is the mouse lethality bioassay. This is a LD_{50} (lethal dose 50) test where groups of mice totalling about one hundred are injected with different doses of Botox. Over the course of multiple days, mice are slowly paralysed, receive blurry vision, have trouble breathing, and finally die of asphyxiation. The dose at which 50 percent of mice die is used to determine the final dose of the Botox product for use in humans. The mouse lethality bioassay is a cruel test, with the mice suffering for days and dying

a slow and painful death. Humane killing of the mice is often employed when it is clear that they are suffocating, but far too late to eliminate most suffering. Not only does the bioassay raise ethical concerns, but there are also concerns regarding the reliability of the results. Mice need to hydrate and feed themselves frequently, and muscle paralysis can lead to death by dehydration or malnutrition as a result. This may be the reason why results differ between testing facilities for the same Botox product. Since this test needs to be performed for every single batch of Botox that is produced, there is a clear need for animal-free alternatives to prevent mice from suffering for routine testing purposes. Significant progress has already been made by three major European manufacturers of Botox, as they have developed cell-based assays for testing their own products. Despite this, it has been estimated that over 400 thousand mice were killed in 2019 for Botox potency testing in Europe alone. The regulatory guidelines in the European Pharmacopeia have accepted the use of cell-based assays, but demand that potency is calculated relative to a Botox sample calibrated in product-specific LD_{50} units. This means that no manufacturer is able to produce Botox without animal testing yet. Additionally, the lack of a standardised cell-based assay has resulted in new Botox products that are still solely tested on mice being approved for the European market. Use of the alternative test cannot be enforced if a manufacturer has not developed their own version yet. Mice should not be subjected to the cruel bioassay as a part of routine testing, especially considering the widespread use of Botox for vanity purposes. Therefore, there needs to be a co-ordinated effort between scientists, regulators and manufacturers to ensure that Botox potency testing can be performed entirely without the use of animals.

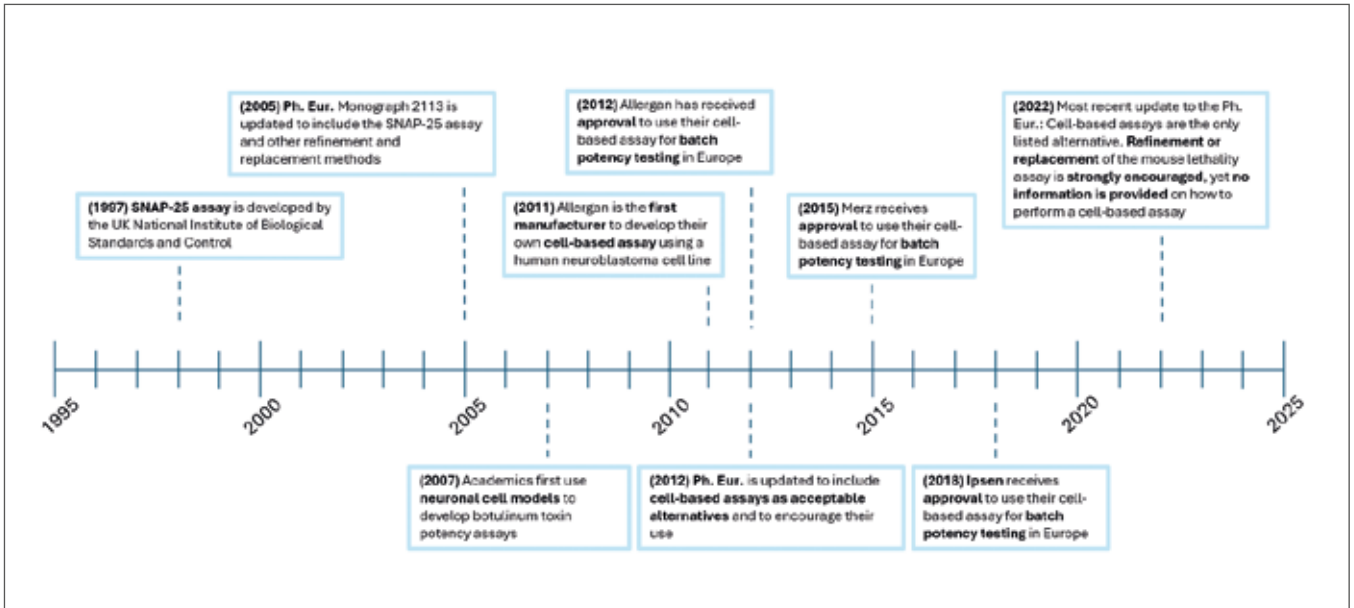


Figure 8. Timeline highlighting the length of the ongoing transition towards the cruelty-free potency testing of botulinum neurotoxin. Important timepoints include updates to the guidelines in the European Pharmacopeia (Eur. Ph.), development of relevant replacement methods, and the implementation of these methods by manufacturers Allergan, Merz and Ipsen.

THE PYROGENICITY PARADIGM SHIFT

Justine Watkins

Justine Watkins is a dedicated PhD Student at Utrecht University, IRAS Toxicology faculty of Veterinary Medicine. Her current project is a part of an NWO funded initiative to increase the acceptance of animal free methods in regulatory toxicology. The consortium, SAFE (Safety assessment through animal free evolution), seeks to use transformative governance and action research to accelerate the acceptance of non-animal methods in the EU and USA. She is focused on developing strategies to implement non-animal new approach methods (NAMs) into a new risk assessment framework: NGRA (Next generation risk assessment). She hopes to make an impact on animal welfare and animal rights, such that animal testing becomes a thing of the past. j.c.watkins@uu.nl

Efforts to develop ethical, human-centric methods for assessing health risks have grown, fostering opportunities to move away from animal testing. Despite this, nearly 1 million animals were used for regulatory testing in Europe from 2018–2022, with over half for product quality control (QC). In batch safety and potency testing, non-animal alternatives offer potential, but adoption faces historic, scientific, and regulatory barriers.

Pyrogenicity testing exemplifies progress in overcoming these challenges. Historically this test was reliant on the Rabbit Pyrogen Test (RPT), but its ethical and scientific limitations spurred the development of *in vitro* alternatives like the Monocyte Activation Test (MAT). MAT is now validated and accepted in Europe, United States, Russia, and India. However, so far, only the European Pharmacopeia (Ph. Eur.) has phased out RPT, reflecting MAT's integration as a compendial method. On the other hand, in the US, RPT is still considered the gold standard, and MAT is not considered

compendial to RPT. This illustrates that MAT adoption remains inconsistent globally. Regulatory inertia and slow progress in phasing out RPT are highlighted as disparities.

PLASTINATED ANIMAL SPECIMENS FOR EDUCATION AND RESEARCH

Tim van Olmen

Tim van Olmen is a taxidermist and technician in Anatomy & Physiology. He is involved in the preparation and plastination of animal specimens for educational purposes. Preparation involves making specific anatomical structures – such as nerve pathways, the respiratory tract, or reproductive organs – visible by carefully removing surrounding tissue. These structures are then preserved and made suitable for education through plastination. The resulting plastinates can be either hard or soft. Soft plastinates retain some natural flexibility, allowing for manipulation, while hard plastinates are more rigid but also slightly less vulnerable. In addition to plastination, he supports researchers and PhD candidates at RMU in the Hubrecht laboratory, where his expertise in histology and immunohistochemistry plays a valuable role.
t.j.s.vanolmen@uu.nl

The plastinates presented after the lecture are mainly used for educational and training purposes. The plastinates originating from animals that have been donated after they died to the Faculty of Veterinary Medicine by means of the *animal donor codicil*. There, the animals are initially prepared by professionals. In this way, specific parts of the animal, such as muscles, nerves or the position of organs, can be made visible. The prepared animals are then plastinated. This process keeps the animal very durable and safe to touch. Because no casts or other artificial means are used, the plastinates look exactly the same as a living animal, with all the fine structures still present. This means that the quality is very high and very useful for the purposes mentioned. A distinction is made between hard and soft plastinates. Hard plastinates are processed in such a way that

they are no longer movable after completion. This can be applied well to samples where, for example, the position of certain structures is important to preserve. With soft plastinates, the samples remain flexible enough to be able to move. For example, the abdominal organs can be placed aside to study the underlying structures.

These techniques ensure that fewer laboratory animals need to be used for education and training. We notice that this is increasingly being taken up by companies that are looking for ways to train their staff without using live animals. A nice and important step towards reducing laboratory animals.

DEVELOPING AN IN VITRO MODEL OF THE TERATOMA ASSAY FOR ASSESSING THE MALIGNANCY OF HUMAN PLURIPOTENT STEM CELLS

Joaquin Montilla Rojo

Joaquin Montilla Rojo is a biotechnologist specialized in regenerative medicine, I have developed a great interest in the safety of stem cell-based products for their use in clinics, topic of my current PhD project that I am undertaking at the Regenerative Medicine Centre of Utrecht. I aim to shed light into what makes a stem cell-based therapy or product (un)safe for their use in patients, always aiming to do so using an animal-free methodology that could benefit not only the patients but also the future of biomedical research in our nation-wide goal to avoid animal use in research. j.montillarojo@uu.nl

Human pluripotent stem cells (hPSCs) have a myriad of potential clinical applications due to their capacity to self-renew and differentiate towards any of the embryonic germ layers. These has driven the onset of personalized medicine, with the promise of the development of stem cell-based therapies for multiple diseases such as pulmonary disorders, heart disease, diabetes mellitus type I and Parkinson's disease, among others. However, hPSC-derived cell products have shown to have the risk of tumor formation when transplanted *in vivo* into patients. To circumvent this issue, the safety of their use needs to be addressed, which includes the evaluation of the intrinsic hPSC malignant potential *in vitro*. Due to our limited knowledge on the underlying mechanisms causing this malignant potential, this assessment has traditionally been performed focusing on the histological traits of malignancy by xenografting the cells into immunodeficient mice (teratoma assay), for later evaluating the tissues found

in the resulting tumor. Although informative, this assay faces scrutiny due to the lack of standardization of the procedure, the long waiting times and high related costs as well as the limited predicted value and questionable ethics behind animal testing. As part of our work, we aimed at developing a reliable, standardized and fully animal-free *in vitro* model able to recapitulate the teratoma assay for addressing the histologic features of hPSC malignancy in an efficient manner. We generated embryoid bodies (EBs) as basic models of unbiased cell differentiation from validated malignant and safe stem cells, and exposed them to animal-free and animal-derived culture conditions. These were then compared on the basis of their capacity to generate structures displaying tissues with the same histopathological traits as those observed in the teratoma assay. Similar tissues were obtained in EBs from malignant stem cells, safe hPSCs, and iPSCs with impaired differentiation, respectively, cultured in both animal-derived and -free conditions, although with low efficiency. Our results suggest that it is possible to develop an *in vitro* system able to recapitulate (immature) teratoma formation using animal-free differentiation conditions, although efficient tissue formation should be improved potentially by better mimicking the mouse injection site microenvironment through co-culture and hydrogel embedding conditions.

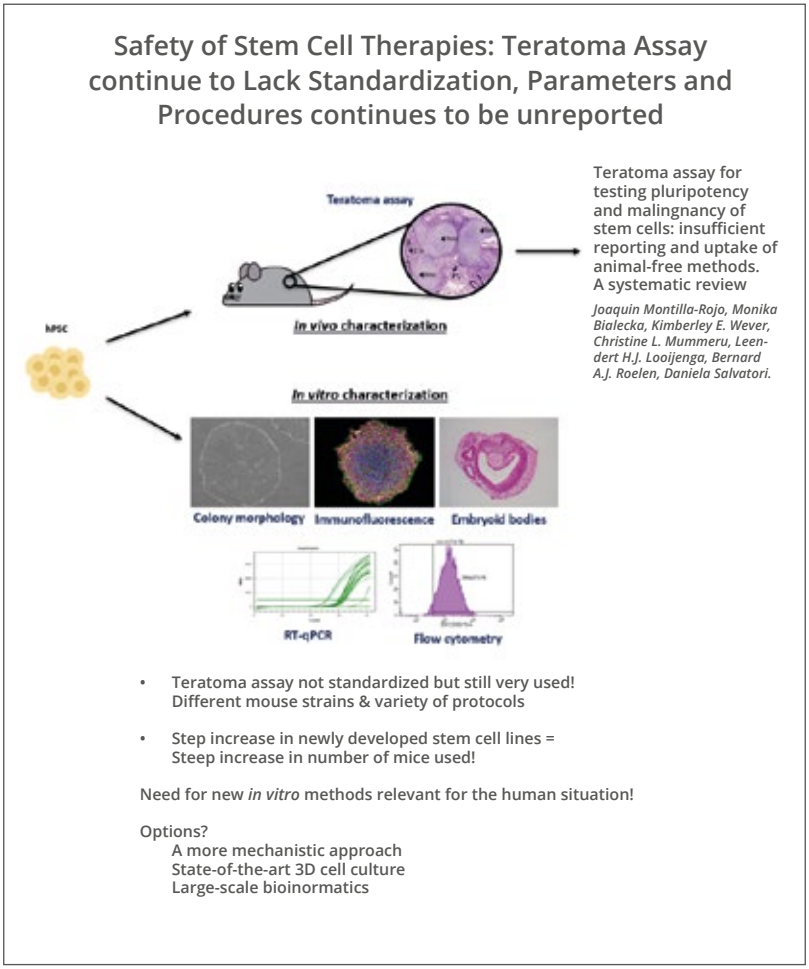


Figure 9. Approaches for the characterization of human pluripotent stem cells for their safe use in clinics. Human pluripotent stem cell malignancy is a key factor hampering their potential use in clinics. It is currently evaluated using animal experiments, but these entail ethical and scientific issues such as the lack of standardization of the technique and low translatability of the results. The development of animal-free methods is then a logical next step and could include the combination of multiple approaches such as using 3D culture models and bioinformatic tools.

VIRTUAL REALITY AVATARZOO

Beerend Hierck and Daniela Salvatori

Beerend Hierck, PhD is a researcher, educational innovat and head of the veterinary SkillsLab. He has been workin at the Utrecht University Veterinary Medicine faculty since November 2021. Before that, he worked as a human anatomy teacher at Leiden University Medical Center. There, he received various awards as best and most innovative teacher at the Medical School as well as of Leiden University. In 2016 he started focusing on innovative methods to teach and learn spatial anatomy. He developed DynamicAnatomy, an augmented reality application to learn about the 3D anatomy of the human lower leg and about the rotation of the ankle joints. When he transferred to Utrecht he started, togeth.. with Prof. Daniela Salvatori, a research group at the Department of Anatomy and Physiology, Clinical Sciences, which focuses on using eXtended Reality (XR) technology to improve spatial knowledge acquisition ("3D-learning") and clinical competence. He developed AvatarZOO. As educational innovator and head of the veterinary SkillsLab he is in a good position to focus on simulation technology for veterinary education. b.p.hierck@uu.nl

Boost learning in full 3D! AvatarZOO is an initiative of Beerend Hierck and Daniela Salvatori who developed an XR application for veterinary, medical, and general life sciences education that allows for the effective acquisition of spatial knowledge. AvatarZOO is a price-winning XR application currently used for Microsoft HoloLens 2, that allows students to interact with a digital 3-dimensional anatomy model of various animal species. Access various fully reviewed anatomy models and interact with them in your own learning environment. Quickly explore spatial relationships between muscles, bones, and joints, and learn anatomical names easily and intuitively. In addition, Avatar ZOO now also supports clinical competence development, without the need for (experimental) animals. Research projects at the vet faculty focus on 1) Personalized implementation into

the curriculum, 2) Determining cognitive load while working in a digital 3D learning environment, and 3) Clinical competence development.

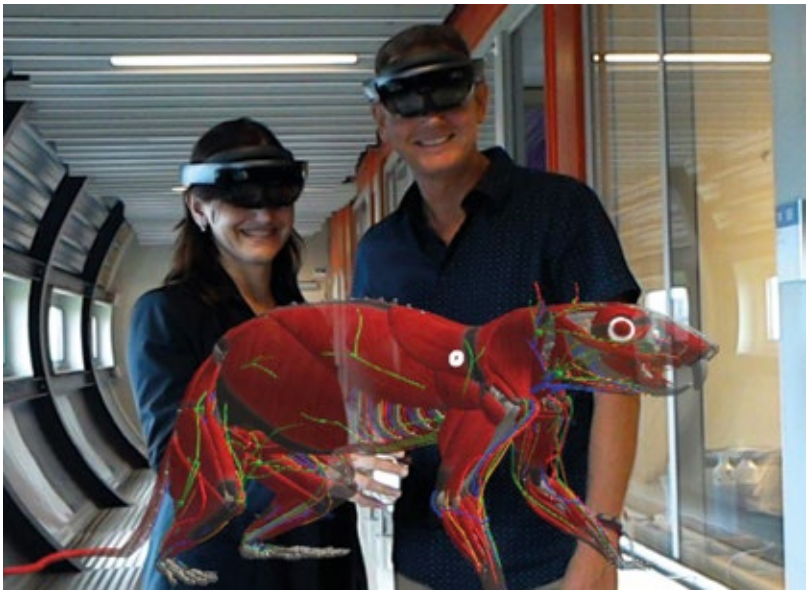


Figure 10. From the left to the right: Daniela Salvatori and Beerend Hierck using AvatarZOO.

Learn more about our vision on advanced and innovative education minimizing the use of experimental animals and maximizing animal welfare, on our research into the efficacy of learning with AvatarZOO, and on the development of AvatarZOO (Utrecht University, 2024).

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Stichting ANIMALES

De Stichting Animales werd op dertien november 1997 opgericht met als oorspronkelijke doelstelling "het verlenen van hulp aan bedreigde dieren in de ruimste zin des woords". Het werkterrein van de stichting was vooral gericht op Latijns Amerika, met name Venezuela, omdat twee van de toenmalige bestuursleden daar enige tijd beroepsmatig werkzaam waren. In verband met de verslechterde politieke situatie aldaar, werd in 2014 besloten de stichting om te vormen tot een vermogensfonds met ANBI-status en de doelstelling te wijzigen in "het bevorderen van dierenwelzijn bij voorkeur in Nederland, alles in de ruimste zins des woords". De stichting tracht haar doel te bereiken door:

a. Het stimuleren van op dieren gericht wetenschappelijk onderzoek op het gebied van welzijn, zoals bijvoorbeeld voeding, medisch handelen en cognitie en emotie.

b. De overdracht van kennis over dierenwelzijn aan dierhouders en beheerders te stimuleren.

c. Het bij voorkeur jaarlijks organiseren van een "Animales-Voordracht" om het belang van dierenwelzijn onder de aandacht te brengen.

d. Het mede financieel ondersteunen van onderzoek op het gebied van dierenwelzijn.

Op de website www.animales.nl vindt u verdere gegevens over de stichting, zoals de samenstelling van het huidige bestuur en kunt u zich aanmelden voor het bijwonen van de Animales-Voordrachten.

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Daniela Salvatori, DVM, PhD, Dipl European College of Veterinary Pathology, CRP/TP, works as Professor of Comparative Anatomy and Physiology and leads the Anatomy & Physiology group at the Veterinary Faculty at Utrecht University.

She also leads the Centre of Excellence of Plastination and Virtual Reality, which develops innovative models for training clinical skills and procedures in both humans and animals.

She is highly dedicated to animal-free research, and since 2019, she has chaired the Utrecht Transition Programme to Animal-free Innovations (TPI Utrecht), an interdisciplinary group focused on innovation of research and education without using laboratory animals.

She is also the Scientific Director of the Center for Animal-Free Biomedical Translation (CPBT), a growing project for which the National Growth Fund has reserved 125 million euro in 2024 to accelerate the transition to animal-free biomedical innovations.
d.salvatori@uu.nl