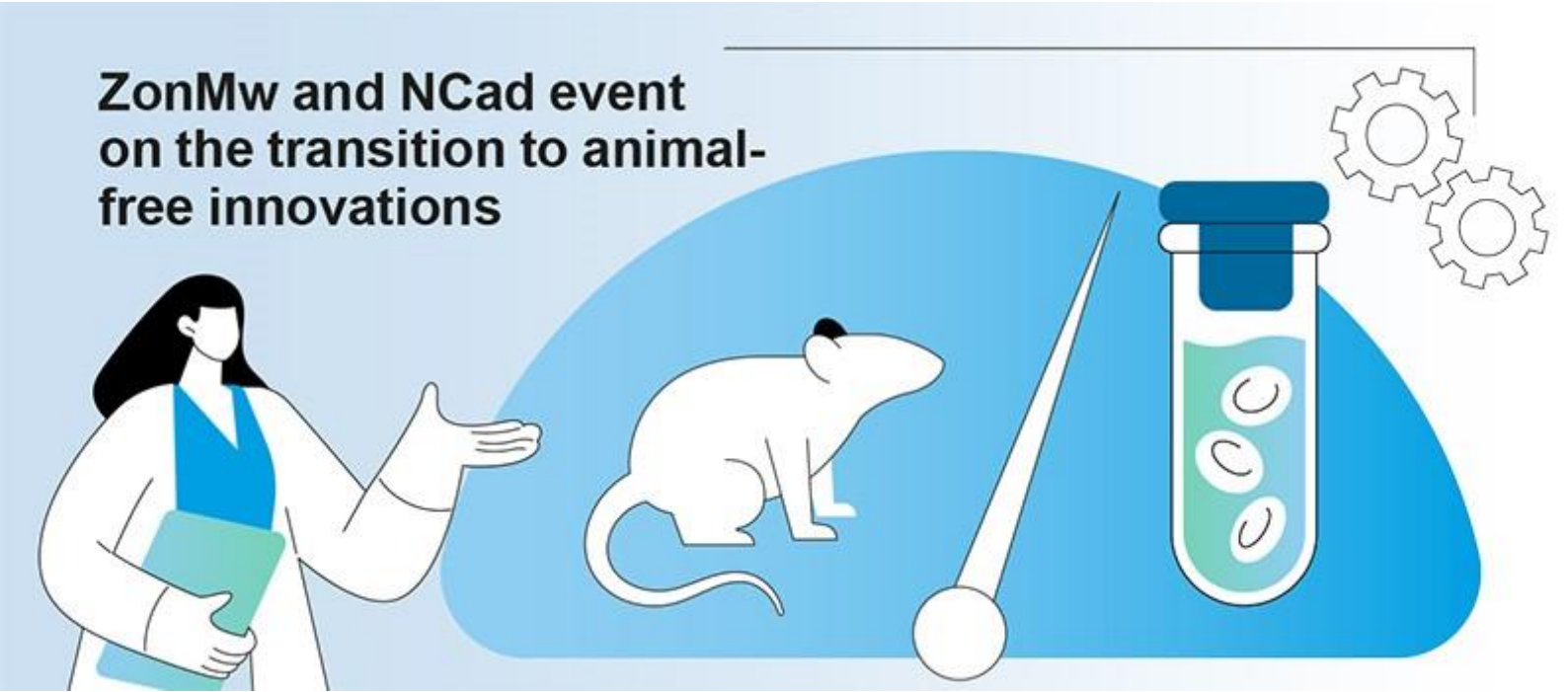
Symposium report

Transition to animal-free innovations: Ambition versus Realism

Report of a symposium held on Tuesday 12th December 2023
at Villa Jongerius in Utrecht

ZonMw

Netherlands National Committee
for the protection of animals
used for scientific purposes



Symposium Report

Transition to animal-free innovations: Ambition versus Realism

2nd February 2024

Symposium on Transition to Animal-Free Innovations: Ambition vs Realism

On December 12, 2023, a symposium was held in Utrecht, organized by ZonMw and the Netherlands Committee for the protection of animals used for scientific purposes (NCad), to discuss the transition to animal-free innovations and animal research. The symposium provided a platform for researchers, policymakers, and other stakeholders to exchange views on the transition to animal-free innovations and animal research. The main goal was to deepen and refine the dialogue between researchers working with animal testing, or animal-free methods. These discussions were facilitated by the findings and recommendations described in the recent ZonMw Knowledge agenda Transition to Animal-free Innovations, as well as the insight from the NCad target images. The symposium emphasized the importance of financial investments, policy guidelines, political involvement, and the impact of public and political perception of animal research. The event provided an excellent opportunity for researchers from different disciplines to discuss the challenges and opportunities in this area.

This document summarizes the discussions, conclusions and recommendations of the meeting, in particular from the parallel sessions. A short ambience report of this symposium, including pictures of the speakers and participants, was released in December and can be found here.

Opening

The symposium was opened by Prof. Arfam Ikram, chairman of ZonMw, and Henk Smid, chairman of the NCad. They emphasized the importance of the discussion about the use of laboratory animals and animal-free alternatives in biomedical research and the role of the symposium in promoting a balanced exchange of views. They indicated that the commitment and input of many is necessary for this. That is why Smid called the symposium primarily an invitation to conversation and discussion.

Interview with Hugo van Kasteel

After the opening, Ikram and Smid interviewed Hugo van Kasteel, Director at the Directorate-General for Agriculture of the Ministry of Agriculture, Nature and Food Quality, who emphasized the crucial role of the ministry in promoting animal-free innovations. He highlighted the Ministry's support for the Transition Programme for Innovation without the use of animals (TPI) and the importance of this initiative in stimulating methods for animal-free research. Van Kasteel acknowledged that the use of animals in (biomedical) research is expected to continue for the next ten years, despite efforts to accelerate this transition. However, the ministry actively embraces the 'free publicity' for the TPI partner program as a crucial means to promote the development and application of animal-free research methods.

Van Kasteel emphasized the increasing attention in the House of Representatives, where more than ten motions have been passed in recent years regarding this subject. He elaborated upon the ministerial focus points, which include the confirmation of the 3 Rs (Replacement, Reduction, Refinement) with the main focus on replacement as guidelines for the policy, acceleration of the TPI program, and strengthening the position within the European context. To do this, the ministry invests at least 10 million euros per year in animal-free research. In addition, an amount of 125 million euros has recently been reserved for the Growth Fund application for the Centre for Animal-Free Biomedical Translation (CPBT). Finally, Van Kasteel emphasized the need for a change in public opinion and acknowledged that bridging conflicting interests is an essential step in promoting animal-free innovations.

Plenary presentation by Jaap van Buul

Jaap van Buul, Professor of Molecular Cell Biology of Cell Migration at the University of Amsterdam, subsequently offered an in-depth insight into advanced microscopic research focused on the behaviour of white blood cells. His presentation illustrated the journey of white blood cells through the extensive vascular system of the human body and provided insight into the movements of white blood cells upon inflammation. His lively presentation showed the power and importance of in vivo research for a realistic spatial understanding of physiological processes. At the same time, his work indicated the indispensability of in vitro research, including technologies such as vessel-on-a-chip, to understand transendothelial migration and the role of endothelial cells in tissues.
In response to questions from the audience, Van Buul emphasized that in vitro research cannot (yet) completely replace the use of laboratory animals. and underlined the complementary nature of these methods. This led to his plea for collaboration, 'team science', and the sharing of data in open repositories. As such, the presentation by Van Buul offered a fascinating insight into advanced research on cell migration and research methods, highlighting the importance of both in vivo and in vitro approaches in his research.

The parallel sessions

After the plenary program, the participants split up into different groups to discuss various topics from the ZonMw Knowledge agenda or the NCad Target Images. In 2 x 4 parallel sessions, a large number of aspects and perspectives of the transition to animal-free innovation were highlighted and discussed:

1.1 The (un)usefulness of systematic reviews: worthless or worthwhile?
1.2 Target Image Immunology: what will be the next step?
1.3 Target Image Cardiovascular Science: what will be the next step?
1.4 Validation of non-animal methods: What are the opportunities and obstacles?

2.1 Preregistration of animal testing: Why shouldn't we do it?
2.2 What does the transition need: a new mindset or more knowledge and awareness?
2.3 Target Image Education: What will be the next step?
2.4 Target Image Neuroscience: What will be the next step?

The discussions in these sessions were mostly fueled by the presentation of thought-provoking statements or questions, intended to stir the mind and elicit the formulation of concrete steps forward. Below a summary of each session is presented, including the discussed statements or questions and the main conclusions.

1.1 The (un)usefulness of systematic reviews: worthless or worthwhile?

This session was chaired by Carlijn Hooijmans, assistant professor at the RadboudUMC in Nijmegen, with expertise as a systematic review methodologist. The session aimed to investigate the potential improvements in the structural application of systematic reviews (SRs) and discuss their usefulness, challenges, and cost implications. Systematic reviews have been credited with contributing to better research, improved drug development, and a reduction in the reliance on animal models. They serve the crucial purpose of identifying knowledge gaps and increasing awareness regarding the validity and translatability of the available evidence.

Participants engaged in a dialogue, some questioning the necessity of attending a dedicated course for SRs of animal studies as a requirement to apply for funding. It was noted that one could independently write a systematic review without external funding. Participants emphasized that SR is a tool already mastered by many researchers. The discussion prompted a suggestion to further develop this tool to ensure early scrutiny of the translatability of animal models. Agreement ensued, with the acknowledgement that ongoing efforts are being made to develop methods for in vitro and alternative models, emphasizing the need for validity checks. The participants recognized that these endeavors are still in their early stages, similar to the infancy of initiating methods in other research fields.

1. *In fundamental research you cannot conduct a systematic review.*

During the discussion of the first statement, a large majority of participants expressed disagreement with the assertion that conducting a systematic review in fundamental research is impossible. Those in agreement pointed out the challenges in fundamental research, for example due to the limited or non-existent literature available in some new research areas. The group acknowledged that the feasibility of conducting a systematic review in fundamental research depends on the specific research question. It may be the case that the research question can't be answered using a systematic review, but possibly some aspects of the research questions, such as the decision of which animal model to be used in fundamental research question, can be answered using a systematic review.

Some participants questioned the arbitrary distinction between in vitro and animal models, advocating for openness to all models to achieve the best translation, not only animal studies. They discussed the challenge of incorporating all relevant information into a review without overwhelming the audience.

In the context of conducting systematic reviews of in vitro research, the discussion touched upon the difficulty of identifying all in vitro studies using appropriate filters due to a large variation in in vitro study types and terminology used. Participants acknowledged the similarity in most of the tools that could be used to conduct systematic reviews of animal and in vitro studies. Still, they emphasized that some steps involved in conducting Systematic reviews of in vitro and animal studies would differ and need specific tools, that still need to be developed.

2. *A systematic review costs too much time and is as a consequence too expensive*

The second statement questioning the value of SRs based on their time and cost implications sparked various perspectives and discussions within the group. Some participants dismissed the statement as "worthless," emphasizing that any investment should ideally yield a return. However, the ensuing debate revealed a spectrum of opinions on the topic.

Agreement emerged from a participant who had experienced unexpected challenges during an SR conducted through funding of the ZonMw program More Knowledge with Fewer Animals (MKMD). They expressed that the review ended up costing more time and, consequently, was relatively expensive. Conversely, dissenting voices argued that the upfront investment in time and resources for an SR could lead to significant savings in the subsequent stages of a research project.

Delving into the root causes of time loss in SR, participants identified delays in result comparison between multiple reviewers and delays in retrieving outcome data from the original authors. They also mentioned issues regarding complex data extraction as prominent factors. The discussion turned towards the development of tools to aid in data extraction and speed up other steps of a systematic review through automation. Participants also highlighted the importance of training and education in conducting SRs efficiently.

The discussion shifted towards who would benefit from SRs led to the consensus that any scientist seeking to determine the suitability of proposed animal models would find value. However, concerns were raised about the perceived lack of added value for those without doubts in their research

In conclusion: the session highlighted the potential benefits and challenges posed by systematic reviews in research, particularly in the context of animal models and fundamental research. The participants recognized the need for ongoing efforts to improve the structural application of SRs and the importance of validating research questions and animal models. The discussion also revealed a spectrum of opinions on the time and cost implications of SRs, with some emphasizing their potential return on investment and others highlighting the need for better automation and education in conducting SRs efficiently.

1.2 Target Image Immunology: what will be the next step?

This session was chaired by Theo Geijtenbeek, full professor in Molecular and Cellular Immunology at the Amsterdam UMC. Geijtenbeek is one of the driving forces behind the target image on Immunology, which was released the day after the symposium. He started with a short introduction on the topic, followed by a central question focused on the long-term usage of in vitro models. The session was aimed to discuss the challenges, limitations, and potential solutions to transition from animal models to in vitro models in immunological research.

The participants highlighted the evolving trend towards this approach, citing the FDA's preference for shorter testing periods in animal models compared to in vitro models. The discussion touched on the co-culture of T-cells and the complexity of creating organoids with immune cells. Examples from HIV cohorts were provided to illustrate advancements in omics and single-cell sequencing.

1. *What is a major hurdle in the transition to animal-free immunological research?*

The conversation shifted to the challenges of transitioning to animal-free immunological research. Examples were shared, such as the need for donor-matched skin and kidney tissue, easier in UMC than in university settings. Neuroimmunology faces challenges due to the lack of available tissue, leading to reliance on organoids, but encountering resistance from reviewers who demand animal models. A hurdle identified was the international emphasis on animal models, contrasting with a more significant focus on organ-on-chip models in the Netherlands. The discussion emphasized the limitations of certain models and the need for refinement and complementarity between in vivo and in vitro experiments. Suggestions included optimizing the use of certain animal models through comprehensive experiments and improved database connections. Collaboration, funding opportunities, and streamlined infrastructures were considered essential for more efficient experimentation.

Participants expressed concerns about allocating too much budget to transition, leaving limited resources for in vivo research in the biomedical field. The need for education, larger collaborations/consortia, and opportunities for young scientists was emphasized. A paradigm shift was discussed, contemplating a ranking system similar to physics, where authors are listed alphabetically.

2. *What is the most practical strategy to speed up the transition?*

This final question explored practical strategies to accelerate the transition to animal-free research. Participants discussed the potential benefits of combining animal and non-animal labs. Suggestions included creating roadmaps for junior researchers, starting from high school, and addressing funding challenges for in vitro work. Participants also emphasized the importance of knowing who does what in the field through databases and centers of expertise. A call for ongoing dialogue with society and fellow immunologists, with an emphasis on the document being a living document open to updates.

In conclusion: the session highlighted the benefits of transitioning to in vitro models and the challenges and potential solutions to achieve this goal. The participants underscored the need for collaboration, funding, education, and infrastructure to optimize experimentation efficiency. The importance of maintaining a balance between animal and non-animal models was recognized, while anticipating a potential shift in the future.

1.3 Target Image Cardiovascular: what will be the next step?

This session was chaired by Robert Passier, full professor of Applied Stem Cell Technologies at the University of Twente, as well as the LUMC in Leiden. As one of the drivers behind the target image on cardiovascular animal(-free) innovations, he introduced the topic and led the discussion on the use of animals and animal-free innovations in cardiovascular research.

1. *Is there a need to focus on comparing animal and animal-free innovations?*

During the ensuing discussion, the focus was on whether animal-free innovations should be compared to animal models. The group explored the possibility of creating consortia to compare the two models, and whether a new initiative is needed to promote better science. The consensus was that animal-free models are necessary, but multicellularity of organs requires animal models. Additionally, the time course of the disease and similarities to humans must be considered. The funding for animal models is decreasing, but animal models are still necessary for fundamental research.

2. *Should we team up with other initiatives, nationally or internationally?*

The group discussed the importance of collaborating with other initiatives and organizations. A balance must be struck between animal studies and alternative models, and fundamental research is essential for modelling and technological approaches. Multidisciplinary work and collaborations are necessary to minimize the number of animals needed for research. The group also discussed how to find other experts and organizations to collaborate with. Patient organizations are more likely to understand why animal models are necessary, while the general public is often against them.

3. *Is early involvement of industry/biotech or regularity bodies necessary? How can this collaboration ensure equal partnerships?*

The group agreed that early involvement of industry and regulatory bodies is essential in research. The industry needs models for specific processes, but the models are often designed for one purpose only. An important role in initiating fruitful collaborations was considered for Health~Holland. However, as industry is often rather careful when it comes to competitors, the question was raised on how realistic it is to entice them for such collaborations and involve them from the start? Moreover, it was also mentioned that there is usually not enough possibilities in such programs to accommodate adequate animal research.

4. *How can awareness of innovative technologies, methodologies and initiatives be enhanced?*

The final topic of discussion was how to increase awareness of innovative technologies, methodologies, and initiatives at an earlier stage. The group suggested starting with education and providing a realistic view of opportunities. Scientists themselves often polarize, so education is essential. Animal models have a negative view, and universities and medical centers should publicly state that animal models are still needed. A higher level should put out a statement that high-quality research requires animal models and that removing or allocating animal testing centers will not solve the problem.

In conclusion: animal models are still considered necessary in cardiovascular research, but animal-free models are also essential. Fundamental research is necessary to create better models and collaboration with other initiatives and organizations is essential. The earlier involvement of industry and regulatory bodies is necessary to ensure equal partnerships. Education is essential in increasing awareness of innovative technologies, methodologies, and initiatives, and universities and medical centers should publicly state that animal models are still needed.

1.4 Validation of non-animal methods: What are the opportunities and obstacles?

During a session chaired by Janny van den Eijnden-Van Raaij, managing director of hDMT, the Dutch Organ-on-Chip (OoC) consortium, the topic of validation of animal-free innovations was discussed. Van den Eijnden expressed the importance of common terminology as she introduced the advantages and complexity of OoC technology.

1. *Validation prevails over qualification*

The discussion began with a debate on whether validation or qualification should be used as a term. While some argue that validation is too strict, others say qualification is too vague. It was argued that qualification is related to assays, and that validation is a different term to show that a model is fit for purpose. Others indicated that qualification is used for regulatory purposes to demonstrate the reliability of a method for a specific purpose, while validation is more related to the pharmacological field.

It was agreed that both terms are necessary, but the context of use should first be defined to ensure relevance or reliability for a certain purpose. Validation of a new model by comparison with an animal model often fails, as animal models are not always reliable. The conclusion of the discussion was that specification and clarification of the terms 'Qualification' and 'Validation' are needed.

2. *Data from OoC models should only be benchmarked with human data, not with animal data.*

Regarding the benchmarking of data from OoC models, one-third of the audience agreed that the data should only be benchmarked with human data, not with animal data. However, it was noted that the best available data should be used. Some had doubts about the usefulness, quality and/or reliability of available human data. Others indicated that science is complicated, and medicine even more. The best data of today is likely no longer the best data in 20 years. It was stated that outcomes from OoC models should be verified in patients via clinical trials.

3. *Qualification of OoC models does not need standardization to ensure reliability and robustness.*

The need for standardization to ensure reliability and robustness was debated. Some argued that if a model is qualified for use and fit for purpose, there is no need for further validation. Others pointed out that standardization is necessary to reduce the variation in models resulting from variability in protocols to create and use them.

4. *Regulators are not willing to collaborate with developers and end users.*

The final statement was about the willingness of regulators to collaborate with developers and end-users. Some people found it difficult to get in touch with regulators and have proper discussions, but it was suggested that there are multiple ways to get in touch with them, such as through the Innovation Task Force of the EMA. It was agreed that regulators are willing to open discussions and interact with researchers, although the process could be improved and made more transparent.

In conclusion: it was emphasized that common terminology, validation, qualification, benchmarking, standardization, and collaboration are all necessary to end up with robust and reproducible models that are useful and reliable for the patients who need them.

2.1 Preregistration of animal testing: Why shouldn't we do it?

Preregistration of animal study protocols was the topic of discussion in a session chaired by Julia Menon, daily director of Preclinicaltrials.eu, a platform dedicated to pre-registration. Menon held an extended introduction on the topic of preregistration and highlighted its scientific and ethical advantages.

1. *Have you ever heard of preregistration or have you preregistered a study?*

Only a few participants replied affirmatively to this initial question. Preregistration of animal experiments seems favorable at Utrecht University and Radboud University, where work protocols from animal welfare body applications can be easily transferred to the preregistration repository.

2. *What is your first impression of preregistration? Do you have any concerns?*

The group was asked about their first impression of preregistration and whether they had any concerns. Some members expressed doubts about its implementation, while others cited clinical trials as a successful example of mandatory preregistration.

The group discussed the administrative burden of preregistration, the need for a streamlined system, and the variety of preclinical research. The challenge of making the system easy to use and delivering consistent outcomes between preregistration and manuscript was also discussed. The group acknowledged the value of the platform, which is funded by the Ministry of LNV until 2026, and the need for additional sponsors to maintain its societal benefit.

The discussion highlighted that preregistration works in ClinicalTrials.gov, because it is mandatory and about longer research. However, there was less agreement on its value and ability to implement it in preclinical research. Some members saw the value of preregistration for drug research, while others felt that it was not applicable for exploratory research. Post-registration (i.e. registering a protocol once the study has already started or has been completed) was also discussed as a possibility.

The group agreed that preregistration creates more workload, and filling out more forms and templates could be a challenge, despite the existing transfer options of work protocols. They also acknowledged that a forced obligation could lead to resistance from researchers. Journals are not yet ready to make preregistration mandatory, and the group discussed the need to prove its effectiveness.

The discussion highlighted the need for a clear understanding of the value of preregistration in preclinical research. The participants acknowledged that it could be useful for drug interventions and translational research, but its implementation should not be forced upon researchers. The success of preregistration in clinical trials was attributed to its mandatory nature, and the group questioned the need for a similar approach in preclinical research.

In conclusion: The discussion on preregistration of preclinical studies protocols pointed out the administrative burden and the need for a streamlined system. The group acknowledged the value of the platform, but questioned its applicability in exploratory research. The platform Preclinicaltrials.eu was acknowledged as a valuable resource, but additional sponsors are needed to maintain its societal benefit.

2.2 What does the transition need: a new mindset or more knowledge and awareness?

This session on the importance of mindset in the transition towards animal-free innovations was chaired by Henriëtte Bout, an ethicist, philosopher, and the driver behind the NCad report '*Transition to animal-free research*' (December 2016). The group delved into the ethical considerations associated with animal research and the subsequent application of these ethics to alternative methods.

Participants subsequently engaged in a thought-provoking exploration of varying perspectives on the moral relevance of animals. The debate included anthropocentric, zoocentric, biocentric, and holistic/ecocentric views on this. It was mentioned that in society a shift can be observed towards a more biocentric view from an anthropocentric/zoocentric view. There are also different beliefs among scientists regarding the moral status of the (laboratory) animal, while at the same time, the Dutch law regarding animal testing is zoocentric. More insight into these different moral beliefs (also among scientists) can help in the conversation about the moral acceptability of the use of laboratory animals and the need to replace animal testing.

The group then discussed the notion of using animal models exclusively when the research question necessitates it, as this is where the representatives of all moral approaches can agree. Emphasis was placed on the importance of employing alternatives when applicable and fostering the development of new alternatives to enhance research methodologies.

1. *As long as journal reviewers are still asking for animal testing, new animal-free alternative methods will not be used in the first place in biomedical research*

Debate arose regarding the role of journal reviewers in perpetuating the use of animal testing. While some argued for advanced researchers to challenge such requests, concerns were raised about the practicality of this in the competitive landscape of research funding and publication.

2. *Laboratory animal science courses should change the name and content of their course*

The need for more comprehensive courses educating scientists on alternative methods was highlighted. The group explored whether current courses adequately address alternatives or if improvements are needed in curriculum design. Some noted that these adjustments are necessary, because there is a lot of focus on the responsible use of animals and not enough on alternative methods.

3. *We chose the mouse, because 'we have a lot of experience with this model' researchers often write on their application → is this logical or not?*

Participants emphasized the importance of scientifically justifying the selection of specific animal models. To "merely" invoke the habit, is no longer a sufficient argument in today's time, as also recognized by the DECs and CCD. The group emphasized that researchers are often cognizant of alternative methods, but may opt for animal models based on the specific nuances of their research questions.

4. *If you use a NAM in your research, you must also run animal experiments next to it. In this way, you can demonstrate the added value of the NAM.*

The group supported the idea of running parallel animal experiments alongside non-animal methods to validate and qualify in vitro experiments. The discussion also underscored the significance of open-access publication for ensuring external validity. Furthermore, as the specific NAM is related to the research question, it may not be possible to validate a NAM by running an animal test in parallel. Hence, the question that you ask and the method that you use are critical.

5. *What do we, as funders, need to do?*

The consensus leaned towards encouraging researchers to publish their work openly, making outcomes and validity accessible to the broader scientific community. Additionally, suggestions were put forth for ZonMw to engage in global discussions and extend support beyond national boundaries.

In conclusion: This complex topic is still evolving, and a more comprehensive understanding of the challenges and opportunities is essential. The group recognized the societal shift towards a more biocentric perspective, shaping the future landscape of research.

2.3 Target Image Education: What will be the next step?

This session on the target image on innovation in higher education with fewer laboratory animals was chaired by Daniela Salvatori, full professor in Comparative Anatomy and Physiology at the University of Utrecht and chairperson of TPI Utrecht. As one of the drivers behind this target image, she introduced the topic and led the discussion.

1. *Is our higher education system failing to train students and professionals in NAMs?*

Participants generally agreed that the higher education system is failing to train students and professionals in animal-free techniques. It was concluded that students lack sufficient training in the fast-developing field of NAMs. It is crucial to teach them how to be critical when it comes to scientific research, and that the focus should be on learning how to choose the best method. Moreover, it was acknowledged, with regards to life-long learning and train-the-trainers, that also established researchers and staff of animal facilities should be enrolled in continuing educational programs on NAMs.

2. *Is it important that MBO, HBO and/or Wo students work together? If yes, why, and how to make it happen?*

It was agreed upon that there is merit in students from these different education levels to work together when it comes to learning about animal research and the use of NAMs. However, the curricula are not yet flexible enough to facilitate collaboration between MBO, HBO, and WO students of different disciplines.

3. *Are we giving the best possible education in terms of methods (for use of simulators/continuing education etc.) and approach? Identify problems and solutions.*

On the topic of whether the current education scheme is offering the best possible methods and approaches when it comes to NAMs, it was suggested that not everyone doing a LAS (Laboratory Animal Science) course needs all the specifics. As such, the LAS course could be restructured into a two-step approach: a theoretical part accessible to a wider target population, and a practical part on species and procedures that is only for those who are going to perform an actual experiment themselves. Furthermore, it is recommended to facilitate more collaboration with experts from other universities to set up better protocols for the same experiments.

4. *Stop using animals now! Make it mandatory in every bio-medical bachelor and master's program to implement animal-free methods.*

It was agreed upon that innovation in animal-free methods should be used as much as possible in education, and whether animals should be used is situation-dependent. However, it should also be evaluated if animal-free methods are appropriate for each setting and research question. It was also recommended to invest in and establish funding for innovation in animal-free teaching methods and technology, while assessing their effectiveness in an evidence-based manner. Animal-free models should be positioned as the golden standard in teaching and practical animal work should only be allowed when skills have first been proven to be sufficient in simulation methods.

5. *The educational hub for animal-free innovations: Do we need it? Which goals should this hub have?*

Finally, it was agreed upon that an educational hub for NAMs is valuable, focusing on the training of bachelor and master students, but also PhD students and other professionals, organizing workshops and networking within different areas and together with companies. This would lower the hurdles to connect and work together.

In conclusion: This session addressed important questions about the current education system with regards to teaching both the new and current generation of researchers on animal-free innovations in biomedical research. The recommendations made emphasized the need for collaboration, investment in innovation, and continuous education in the rapidly changing field of animal-free innovations.

2.4 Target Image Neuroscience: What will be the next step?

This session on the target image on Neuroscience was chaired by Elly Hol, full professor on Glia biology of brain diseases at the University Medical Center Utrecht. As one of the drivers behind this target image, she introduced the topic around the question of how we can increase the impact of animal-free models, including computer models and artificial intelligence, in neuroscience. The follow-up discussion with the participants was organized around 4 statements.

1. *Human in vitro models rapidly increase in impact if they are validated with animal models*

In reply to this statement, some participants argued that it depends on the research question, and in vitro models are useful for cellular research questions, but not for behavior and cognition-related research questions. Others suggest that animal models don't fit well for human situations, and it makes sense to validate in vitro models only with human biology or data. However, some argue that while phasing out animal research and coming up with better-predicting models, validating against animal models is necessary to convince traditional researchers. Overall, the conclusion is that both animal and human in vitro models are necessary depending on the research question.

2. *All primary neuronal cultures can be fully replaced by iPSC-derived cultures*

Most of the participants disagreed with this statement due to various reasons such as the difficulty of working with brain tissue, the problem with the epigenetic background of iPSC cells, and the fact that iPSC-derived cells are not the same (i.e. immature) compared to cells isolated from post-mortem tissue. In addition, it was brought up that neuron-glia cultures from rodent pups form more mature synapses than the current iPSC-derived neuron-glia cultures, which do not represent physiological neurons and glia. Some people were in the middle and didn't know enough to make a decision. It was also mentioned that standardized protocols for iPSCs are improving, but some people still use tumor-like cell lines as a model for neurons and glia, which is not ideal. Overall, the discussion suggested that the replacement of <u>all</u> primary neuronal cultures by iPSC-derived cultures is not feasible at the moment.

3. *The Netherlands is a front-runner in brain-on-a-chip models.*

In response to the question of whether The Netherlands is a front-runner in brain-on-a-chip models, some of the participants looked upon the country's progress positively, whereas others were more sceptical. The group also discussed whether investing in brain-on-a-chip models is worthwhile, with some saying that it depends on the research questions that need to be answered. When the research is focused on translational work, we need more investments in optimizing the iPSC-models; however, currently cannot fully model the complexity of the brain. Furthermore, ethical implications of brain-on-a-chip models were also discussed, including concerns about the sentience of the models and the need for ethical discussions as the technology develops. The group emphasized the importance of having a strong rationale for investing in a technique and making sure it complements existing in vivo models.

4. *Brain-on-a-chip can replace all other disease models*

All participants disagreed with this statement, as it was concluded that other models are still needed. It was argued that animal models are still necessary for certain questions that cannot be answered without in vivo models, and that the focus should also be on making in vivo models less discomforting for animals. The follow-up question asked who is worried about their research, and there were various responses, including concerns about the lack of funding for animal facilities and the need for better legislation to facilitate research with human tissue. Overall, it was emphasized that while alternative models should be developed, in vivo animal models are still necessary for certain research questions.

In conclusion: This session highlighted the importance of using animal and in vitro models in brain research depending on the research question. While animal models are necessary for certain questions that cannot be answered without in vivo models, in vitro models are useful for cellular research questions. The replacement of all primary neural cultures by iPSC-derived cultures is not feasible at the moment, but standardized protocols are improving the quality and maturity of the iPSC-derived cultures. The Netherlands is making progress in brain-on-a-chip models, but investing in the technology is needed, as several research questions can currently not be answered by the existing technology. Overall, the discussions emphasized the importance of having a strong rationale for investing in a technique and making sure it complements existing models.

Closing:

After the parallel sessions, the participants gathered for the final plenary part of the meeting. Prof. Sue Gibbs, full professor in Skin and Mucosa Regenerative Medicine at the Amsterdam UMC received the Willy van Heumen oeuvre award for her commitment to animal-free scientific research throughout her career. Gibbs, who was completely surprised and overwhelmed with his recognition, has been involved in many ways in the successful development of animal-free therapies and testing strategies, for which she is widely recognized.

Finally, initiators and organizers Leane van Weereld (NCad) and Martijn Nolte (ZonMw) summarized the main outcomes of the eight parallel sessions and thanked everyone who had contributed to this special event. The planned time for drinks and informal networking afterwards was greatly exceeded. This was illustrative of the involvement and excellent atmosphere on this afternoon.

Colofon:

This symposium was jointly organized by the ZonMw program More Knowledge with Fewer Animals (MKMD) and the Netherlands Committee for the Protection of Animals Used for Scientific Purposes (NCad), supported by the Team Events from The Netherlands Enterprise Agency (RVO).

For more information, please contact us via e-mail: mkmd@zonmw.nl or NCad@minlnv.nl

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Netherlands National Committee
for the protection of animals
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