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

From parallel study to the acceptance and implementation of animal-free methods

Summary

In April 2020, prompted by the COVID-19 pandemic, the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) presented a proposal (ref. no. NCad-2020-22)[1] to the Minister of Agriculture, Nature and Food Quality to enable parallel studies using innovative animal-free methods that are still in the development stage to be conducted alongside regular studies. This resulted in a request for opinion from the Minister (ref. no. DGA-DAD/20214808), specifying the following tasks:

- Providing insight into which research domains and types of research could benefit from the use of parallel studies in validating animal-free alternative models within the foreseeable future;
- Advising on how the additional costs involved could be financed without negatively impacting other research.

Working method

During the formulation of the recommendations, it soon became apparent that the requests for opinion were difficult to answer in their current form. The terms 'parallel studies' and 'validation' can be interpreted differently depending on the type of research being conducted. From a number of conversations, it also became clear that the idea of parallel studies was not an appealing one, mainly due to their association with the 'classic' explanation that, during parallel studies, an animal-free method is conducted alongside an animal study experiment in order to enable one-to-one comparison of the results. This explanation is considered too restrictive. During the consultations with researchers more interpretations of the term parallel studies were provided. For animal-free methods, very different end points are used than for animal studies. For this reason, the key question is how these results can be validated to enable their acceptance in future safety-, efficacy- and risk assessments. In other fields of research the validation and acceptance of animal-free methods also is not a done deal. For this reason, we shifted the focus of our recommendations to the existing obstacles and what will be required to get animal-free research methods more broadly accepted and embedded. Here and there opportunities to use parallel studies as method to promote this objective have been identified. Whenever applicable, we have mentioned them.

The NCad recommends:

to the Minister of Agriculture, Nature and Food Quality:

1. Investigate the possibilities for establishing early mandatory consultation regarding testing strategies between parties submitting applications and assessors/authorities, so that these comply to the information requirements.
2. Conduct dialogue with companies regarding:
   a. opportunities to remove currently existing obstacles for the large-scale implementation of animal-free testing strategies;
   b. opportunities to make all eligible data available open access;
   c. obstacles that prevent use of the option to submit 3V data alongside regular test results to the European Medicines Agency (EMA).
   d. Investigate opportunities to disqualify certain animal experiments.
   e. Ensure effective training activities in the field of animal-free methods for individuals involved in the assessment of applications for project licenses involving animal studies.

to financiers:

1. Create space for a hybrid approach through extra funding.
2. Make use of target images to identify promising initiatives.
3. Invest in the further development of promising animal-free methods.
4. Set requirements concerning preregistration of research and publication of the results in accordance with the ARRIVE guidelines 2.0.

to education institutions, research institutes and companies:

1. Ensure effective education in animal-free methods for employees involved in the set-up and execution of animal studies.

[1] *Briefadvies Covid-19, dierproeven en proefdiervrije innovatie* (Advisory letter on COVID-19, animal studies and animal-free innovation) | Publication | Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (ncadierproevenbeleid.nl).

Contents

Introduction

In April 2020, prompted by the COVID-19 pandemic, the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) presented a proposal (ref. no. NCad-2020-22)[2] to the Minister of Agriculture, Nature and Food Quality to "enable parallel studies using innovative animal-free methods that are still in the development stage to be conducted alongside regular studies (...) based on the philosophy that innovation of scientific research will better equip us to combat future epidemics". The NCad observed that a variety of promising animal-free models exist that require further development. This pandemic appeared to be the perfect moment to encourage faster further development of alternative animal-free methods within the scope of COVID-19 research. This resulted in a request for opinion from the Minister (ref. no. DGA-DAD/20214808), specifying the following tasks:

- Providing insight into which research domains and types of research could benefit from the use of parallel studies in validating animal-free alternative models within the foreseeable future;
- Advising on how the additional costs involved could be financed without negatively impacting other research.

The terms 'parallel studies' and 'validation' are interpreted differently depending on the type of research being conducted. We have adopted a broad interpretation of this request for opinion. For this reason, we will first establish a definition of these terms. Subsequently, we will give a rough picture of existing opportunities to promote the use of animal-free methods and identified obstacles and needs. This overview will incorporate the opportunities for parallel studies for the development, optimisation, evaluation and validation of animal-free research methods within the various research domains. Subsequently, we will address the funding of parallel studies. Finally, we will issue our recommendations for formulating the preconditions for parallel studies in order to further promote animal-free research.

Working method

Based on scientific literature and telephone interviews, we have created an overview of possible objectives for parallel studies and the obstacles and preconditions involved in these types of study. Furthermore, we addressed the issue of funding. In order to further explore a number of topics in greater depth and identify promising domains and types, we organised panel discussions within three key domains: 1) regulatory/toxicology, 2) translational research and 3) fundamental research.
Translational researchers are also selected based on their expertise in animal-free methods. The results were qualitatively analysed.

[2] Briefadvies Covid-19, dierproeven en proefdiervrije innovatie (Advisory letter on COVID-19, animal studies and animal-free innovation) | Publication | Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (ncadierproevenbeleid.nl).

Parallel studies

The term 'parallel study' can be defined at various levels:

- **Free** (**development**) research during an ongoing project, parallel to research involving animal experiments, e.g. based on human data. These parallel studies give researchers the freedom to develop new animal-free models as an alternative to existing animal studies. Sometimes, a variety of models are used alongside each other in order to answer the research question. In such cases, they are referred to as complementary studies. For example, observations from clinical studies are linked to analyses of material from the same patient group using in vitro methods.

- **Optimisation** of a developed in vitro model parallel to ongoing in vivo research based on results from the clinic/in vivo research. This includes the further development of in vitro tests.

- **In-house validation** of a developed in vitro method parallel to ongoing research and depending on the availability of, for example, human data or animal experimental data. For example, an animal-free method is included alongside the animal models approved by the authorities for products that are to be newly introduced to the market. This will enable researchers to gain experience with animal-free methods and will boost confidence in these methods.

- **Safe harbour strategy:** external assessment – on an informal basis – by authorities whereby data obtained from prescribed tests take precedence. The objective of this parallel study is to demonstrate that the animal-free model and the animal model are equally predictive of the safety and/or efficacy of a product. This strategy will boost the volume of data from non-animal methods and help both the business sector and the authorities to gain confidence and experience in animal-free methods.

- **Formal validation:** a formal procedure, usually within a multi-laboratory framework, in which the results of the animal-free method are displayed in parallel with the test guideline method and in which factors like relevance, robustness and reproducibility are explicitly evaluated.

Validation

Validation means 'declaring or making something valid'. The term has different meanings within different domains of research. Within regulatory research, validation needs a safety perspective, while in fundamental and translational scientific research, it needs a research perspective. In fundamental research, the results do not immediately have to be translatable to humans in order to be valuable to the field of research. On the contrary, for translational and regulatory research, translatability is very important. Within regulatory research, the emphasis is on safety. Within translational research, the focus is on disease processes. So, 'relevance' can have a different meaning in each research domain.

Well-defined criteria[3] exist for the validation of animal-free safety tests, risk assessments and efficacy tests for chemical substances:

- The biological domain (e.g. specific pathways, processes, cell organelles, organs) that a test covers must be clear.
- The reproducibility of test results within and between laboratories must be clear and is a prerequisite for the general applicability of a test.
- The opportunities and limitations of the test due to the specific properties of the substance being tested must be known. For example, some substances cannot be tested a certain testing system, as they do not dissolve well or because they evaporate quickly.
- The predictive value of animal-free tests must be clear.

Animal studies are currently the gold standard for safety and efficacy tests, as a result of which animal studies are used as a reference framework for the development of animal-free methods. The results from the animal-free method must therefore be comparable to the results from the animal study. Once test results obtained using the animal-free method largely match the animal study, the test is considered a valid replacement method.

However, an animal study only serves as a good reference if it has been proven to have a high predictive value for humans, although a large proportion of research is also conducted for the benefit of animals (e.g. environmental research or research into veterinary vaccines or medicines).

Animal models are usually not validated in relation to humans as a first step. When working with a one-to-one replacement of an animal study by an animal-free method, the fact that it is very difficult to simulate the complexity of an entire organism within, and to replace it by, a single model is often given insufficient consideration. In the future, there will be more possibilities combinations of different methods and combination models (e.g. multiorgan-on-a-chip). Developments are currently progressing in this direction[4]. The European Education and Training Platform for Laboratory Animal Science (ETPLAS) also addresses these developments in two e-learning modules[5].

Within fundamental and translational research, an animal method is declared valid if it gives insight into fundamental biological processes that are comparable or if the causes, pathophysiologies or symptoms of disease and/or the responses to treatment are comparable to those in humans[6]. Within scientific research, there is often no gold standard against which a new method can be validated, i.e. it is about new discoveries. A large proportion of research is also conducted without laboratory animals due to the nature of the research question, e.g. because the research is conducted at the cellular or tissue level.

[3] Piersma, A.H. et al. *Validation redefined. Toxicol In Vitro.* 2018 Feb;46:163-165. DOI: 10.1016/j.tiv.2017.10.013.

[4] Van Berlo, D. and Woutersen, J. *Validation of in vitro methods: travelling a long, winding and bumpy road that is littered with deep holes.* TCDD Toxicologie, 2020 (1). Available via 19_2001-TCDD-25.pdf (toxicologie.nl).

[5] EU-52. Searching for (existing) non-animal alternatives. ETPLAS, 2021. Available via Etplas.eu/learn/eu-52 EU-60. Developing in vitro methods and approaches for scientific and regulatory use. ETPLAS, 2021. Available via https://etplas.eu/learn/eu-52/.

[6] Ferreira, G.S. et al. *A standardised framework to identify optimal animal models for efficacy assessment in drug development.* PLoS One. 2019 Jun 13;14(6):e0218014. DOI: 10.1371/journal.pone.0218014. Erratum in: PLoS One. 2019 Jul 22;14(7):e0220325. PMID: 31194784; PMCID: PMC6563989.

For the most fundamental processes, such as DNA replication, the translatability of research using laboratory animals is high. For models of specific diseases and the influence of treatments on it, the picture is more variable. The decision for an animal model is not always made based on systematic evaluation[7, 8]. For this reason, models and databases have been developed with which a weighted decision can be made for a certain animal model (e.g. FIMD[9], the Interspecies Database[10] and comprehensive literature overviews[11, 12]).

Within regulatory research, the predictive value of animal-free methods is often still uncertain[13, 14] and as a result, acceptance of these methods by authorities is low or restricted to being accepted only as a supplementary test. In order to change this, the way we think about validation studies for safety assessments and efficacy tests must be changed. If possible and in the ideal situation humans should be the central focus, as a result of which the validation would be based on data from measurements at humans rather than laboratory animals, on condition that these are available or opportunities to acquire them exist. This is the approach adopted by new research strategies such as Adverse Outcome Pathways (AOP) within the field of safety research. However, this will not always be possible for scientific or ethical reasons. Furthermore, a strong effort must be made to use quantitative rather than qualitative assessment of animal-free research results. In other words, the results must not solely be assessed based on a conclusion of toxicity/non-toxicity, but predominantly on understanding of how these results were achieved. This requires knowledge of mechanisms that result in toxicity or predict efficacy.

Validation of in vitro methods also requires improvement[15]. As a rule, internal validation of the results (an internal inspection) is conducted within a laboratory. However, this inspection is often not extended to other laboratories - also because different laboratories operate different Standard Operation Procedures (SOPs)- and often they do not work in accordance with international guidelines. The Organisation for Economic Cooperation and Development (OECD) – a partnership between 37 countries within which agreements are made concerning accepted safety and efficacy testing, among other things – has issued two guidance documents concerning the validation of in vitro methods[16]. These guidance documents can be used for both regulatory and scientific research, but are still used to an insufficient extent.

7 De Vries, R.B. et al. *Reducing the number of laboratory animals used in tissue engineering research by restricting the variety of animal models. Articular cartilage engineering as a case study*. Tissue Engineering Part B Rev June 25, 2012: 18(6), 427-435.

8 Pound, P., Ritskes-Hoitinga, M. *Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. J Transl Med*. 2018 Nov 7;16(1):304. DOI: 10.1186/s12967-018-1678-1. PMID: 30404629; PMCID: PMC6223056.

9 Ferreira, G.S. et al. *A standardised framework to identify optimal animal models for efficacy assessment in drug development. PLoS One*. 2019 Jun 13;14(6):e0218014. DOI: 10.1371/journal.pone.0218014. Erratum in:
PLoS One. 2019 Jul 22;14(7):e0220325. PMID: 31194784; PMCID: PMC6563989.

10 Interspecies database, 3Rs-Centre Utrecht Life Sciences. Available via interspeciesinfo.com.

11 Leenaars, C.H.C. et al. *Animal models for cystic fibrosis: A systematic search and mapping review of the literature - Part 1: genetic models*. Lab Anim. 2020 Aug;54(4):330-340. DOI: 10.1177/0023677219868502.

12 Leenaars, C.H.C. et al. *Animal models for cystic fibrosis: A systematic search and mapping review of the literature - Part 2: nongenetic models*. Lab Anim. 2021. DOI: 10.1177/00236772219906888.

13 Van Berlo, D. and Woutersen, J. *Validation of in vitro methods: travelling a long, winding and bumpy road that is littered with deep holes*. TCDD Toxicologie, 2020 (1). Available via 19_2001-TCDD-25.pdf (toxicologie.nl).

14 Piergiovanni, M. et al. *Standardisation needs for organ on chip devices*. Lab Chip. 2021 Jul 12. DOI: 10.1039/d1lc00241d.

15 Batista, L.S. et al. *Establishing the scientific validity of complex in vitro models*. EUR 30556 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-28410-9 (online), 978-92-76-28309-3 (print), DOI: 10.2760/60/376171 (online). 10.2760/60/399535 (print), KRC122394, *Establishing the scientific validity of complex in vitro models* | EU Science Hub (europa.eu).

16 OECD, Guidance Document on Good In Vitro Method Practices (GIVIMP), OECD Series on Testing and Assessment, 2018. No. 286, OECD Publishing, Paris, https://doi.org/10.1787/9789264304796-en.OECD, Guidance Document for Describing Non-Guideline In Vitro Test Methods, OECD Series on Testing and Assessment, 2017. No. 211, OECD Publishing, Paris, https://doi.org/10.1787/9789264274730-en.

Opportunities and conditions for successful implementation of parallel studies

Research programmes are increasingly using combinations of both in vitro and in vivo research, often as part of multidisciplinary collaborations. Within translational research in particular, in vitro strategies based on patients' material have been fully implemented, often as initial steps within a research programme. Validation of the results is conducted by comparing the results with various models. Although animal-free methods are widely applied, it is difficult to change to completely different research methods within ongoing (animal) studies. In many cases, no animal-free options are available, and in others, the development and validation of animal-free options can require time and money. In practice, these steps are difficult to publicise, and additional training and/or education is required.
Parallel studies can provide an opportunity to run new animal-free methods alongside conventional laboratory animal research. This allows researchers to build experience with animal-free systems, creates opportunities to discover the added value of such systems and allows the emergence of mutual inspiration. Furthermore, it gives direction and room to the further development of new methods, which promotes acceptance and broad implementation of these methods. Parallel studies can also help to build up data using animal-free methods.

'Mandatory' animal studies

A number of widely accepted guidelines[17] are already in place for animal studies designed to test the safety and/or efficacy of new products that a party wishes to introduce to the market, including lists of tests for which the data are accepted by the authorities. Following these guidelines is considered the 'fast lane' for acceptance, but also creates fields of tension. Deviating from these tried and trusted paths can cause problems in the approval process. Within the regulatory domain, sometimes one speaks of mandatory animal studies despite the fact that, in principle, there only are information requirements. The guidelines include accepted tests that provide this information. Part of these tests involve animal procedures. These information requirements differ at the international level. Therefore, testing strategies are not the same all around the world. In the Netherlands, the Medicines Act (*Geneesmiddelenwet*) obliges the Medicines Evaluation Board (MEB) to inform companies that wish to submit a marketing authorisation dossier that they can obtain advice[18] concerning test strategies in advance. The parties submitting the marketing authorisation dossier remain responsible for determining the test strategy, so the advice is non-binding. If the advice is implemented, the application will automatically be processed. However, still the tried and trusted pathways of animal studies are often followed, as this is the quickest way to introduce new products into the international market, even if the advice concludes that certain animal procedures are unnecessary. On the other hand, there are companies that focus strongly on using animal-free methods and are pioneers in this field. Collaborations with network partners in order to explore fields such as animal-free innovations are greatly appreciated by businesses. In this way also the development in the direction of animal-free proof of evidence is followed.
Views within society are divided regarding the established models[19]. This can also be a factor in the conservative attitude adopted within the validation process.

Models have limitations

Both animal models and animal-free models have their limitations. Combined use of models increases the likelihood of complex and new scientific questions being raised. This is illustrated by the following example within the field of COVID-19 research. As the coronavirus (SARS-CoV-2) that causes COVID-19 predominantly enters via the airways, a great deal of COVID-19 research focuses on the airways. Aspects of COVID-19 that occur in humans have also been observed in laboratory animals. However, the results from these animal studies are often not directly translatable to patients due to differences in anatomy and the immune system. Lung organoids created from human tissue can be used as an animal-free method for studying SARS-CoV-2 infections in air sacs, e.g. for screening medicines that combat the development of disease caused by the virus, for which lung cells sensitive to SARS-CoV-2 are particularly important. However, these organoids are less suitable for studying the complex interaction between different types of lung cells or for studying mechanical stress on air sacs. Air sacs remain difficult to cultivate, as type II lung cells change to other types of cells during cultivation. Furthermore, organoids lack blood vessel cells and immune cells. In order to gain a more complete and physiologically relevant picture, complementary researchers

[17] For example, the OECD Test Guidelines for Chemicals and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: OECD Test Guidelines for Chemicals - OECD and ICH Official web site : ICH

[18] Wetenschappelijk en regulatoir advies (Scientific and regulatory advice) | Medicines Evaluation Board (cbg-meb.nl).

[19] Schukken, Y.H. et al. *De Staat van het Dier. Beschouwingen en opinies over de verschuivende relatie tussen mens en dier in Nederland* (The State of the Animal: Reflections and opinions on the shifting relationship between people and animals in the Netherlands). Council on Animal Affairs (RvD), 2019. ISBN; 978-90-830457-0-2 E-ISBN; 978-90-830457-1-9: 37% of people do not believe that we can develop medicines without animal testing, compared to 29% who disagree strongly with this viewpoint. A large proportion of respondents (34%) have no opinion.

make use of slices of fresh lung tissue, 3D in vitro tissue culture and lung-on-chip models. However, studying long-term effects into COVID-19-related damage to the air sacs, such as scarring, remains difficult even when these models are combined[20].

Acceptance of animal-free methods

Many animal-free innovations are not yet sufficiently developed to enable implementation for regulatory validation studies[21, 22, 23]. A few animal-free methods do already give enough information to enable their acceptance as evidence. These are mainly the methods with less complex end points. However, in many cases, it takes a long time before a new method is accepted. In the future, it will be important to build up data on mechanisms that result in toxicity and efficacy in order to facilitate the choice of the most relevant model. The more information is available about the results provided by various substances or categories of substances within a specific model, the clearer the value of that model becomes for safety and efficacy assessments, and the more accurately we will be able to predict how various chemical compounds will behave within the human body without the need for animal studies. Uncertainty regarding the criteria for designating test results as trusted and accepted creates uncertainty. It must be clear what criteria must be met and at what point sufficient quantitative and qualitative data are said to have been provided. For this reason, the focus must shift from 'accepted tests' to 'accepted results'.

Further development of models without the use of laboratory animals

The COVID-19 example outlines the need for opportunities to further develop new methods in order to enable broader application and acceptance of these methods. For example, further development of lung organoids could enable in vitro investigation of air sacs and mechanical stress thereon. Furthermore, it creates opportunities to better establish the value of animal-free methods and to gain experience with these methods (this applies to all promising new methods). It is a process of scientific validation, and in the long run, possibly regulatory acceptance. Insufficient grants are available to fund this further development of new models. As a result, the further development of promising new models is delayed, and they risk getting stranded in the 'valley of death', while investment in the further development of animal-free methods would positively contribute to the broader implementation of animal-free methods in research.

Data sharing

Databases belonging to companies and research organisations often contain a lot of animal experimental data that could be used to compare with the results of new tests, which could reduce the number of animal studies. Data and material from animal-based safety and efficacy studies (e.g. fixed tissue) must be stored for at least ten years and can be requested. However, one factor that must be noted in this regard is that businesses have a pragmatic approach with regard to research: if something does not work, the project will be stopped and the data may therefore be incomplete. Very little investment is made in researching why something does not work.

In order to make use of available data and materials from both laboratory animal research and animal-free methods, it must be known where these data can be found. The availability and findability of data must be improved[24]. Confidentiality statements, intellectual property rights and patents can pose obstacles, although these are not insurmountable, as data can also be used when anonymised and blinded. Retention of ownership is also part of the FAIR principle (Findable, Accessible, Interoperable, Reuseable). Boosting the availability and findability of company data should preferably be done via existing data-sharing initiatives, such as the Personal Health Train method[25], an infrastructure that enables use and reuse of data based on the FAIR principle. Furthermore, (mandatory) preregistration of animal studies may also help to increase findability of data[26].

Data sharing must also be made appealing to companies, as making data available requires time and effort that will not necessarily deliver any concrete value. Recognising the added value of sharing data will require a cultural shift (e.g. perceiving it as a type of corporate social responsibility), and this type of cultural shift will take time. Some companies already publish information about the available data.

[20] Kiener, M. et al. *Human-Based Advanced in vitro Approaches to Investigate Lung Fibrosis and Pulmonary Effects of COVID-19. Front Med* (Lausanne), 2021 May 7;8:644678. DOI: 10.3389/fmed.2021.644678. PMID: 34026781; PMCID: PMC8139419.

[21] European Chemicals Agency. *Non-animal approaches - Current status of regulatory applicability under the REACH, CLP and Biocidal Products regulations*. ECHA-17/R/24/EN, 2017. DOI: 10.2823/000784.

[22] Batista Leite, S. et al. *Establishing the scientific validity of complex in vitro models*, EUR 30556 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-28409-3, DOI: 10.2760/399535, JRC122394.

[23] Kienhuis, A. et al "*New Approach Methodologies" in de veiligheidsbeoordeling van consumentenproducten en voedsel ('New Approach Methodologies'* for assessing the safety of consumer products and food). National Institute for Public Health and the Environment (RIVM), 2021, DOI: 10.21945/RIVM-2020-0093.

[24] Taylor, K. *Recent Developments in Alternatives to Animal Testing*. In: *Animal Experimentation: Working Towards a Paradigm Change*. Leiden, The Netherlands: Brill, 2019. DOI: https://doi.org/10.1163/9789004391192_025.

[25] Personal Health Train by GO FAIR. Personal Health Train – GO FAIR.

[26] Preclinicaltrials.eu, International register of preclinical trial protocols.

Parallel studies can help companies and authorities to gain experience and trust in animal-free methods. As early as 2016, the European Medicines Agency (EMA) established a safe harbour principle concerning acceptance of 3R testing strategies in an EU Directive[27], which enables the sharing of confidential data in addition to laboratory animal data. The objective of this measure is to "create a closed learning platform and pool strengths". So far, no voluntary use has been made of this opportunity, the reasons for which are unknown to the EMA.

Open Science

Data sharing is fully in line with current developments with regard to Open Science[28].
This movement strives to establish a more open and participative research practice by sharing publications, data, software and other types of scientific information at as early a stage as possible and making it available for reuse. To a substantial extent, the decision to select a particular research model to answer the research question concerned determines the usability and reliability of the results as well as the design and execution of a study and the analysis of the results. Openness about both methods and results is vital in order to prevent unnecessary duplication of research, repetition of research that yielded negative results, and bias, as well as increasing opportunities to conduct parallel studies using historical data and to conduct syntheses of evidence[29]. The publication of negative results also helps to boost the reproducibility of research. In this regard, there is also plenty of value to be gained in the field of laboratory animal research. Within ZonMw, a budget is available for the publication (open access or otherwise) of negative results[30]. Guidelines such as PREPARE[31] and ARRIVE[32] provide frameworks for the design, execution and publication of animal experiments and contribute to the Open Science principle. The OECD is developing best practices[33] such as the Guidance Document on Good In Vitro Method Practices (GIVMP)[34], the application of which is not restricted solely to regulatory research. An e-learning module on this theme has also been made available within ETPLAS[35]. Furthermore, within the OECD and the European Chemicals Agency (ECHA), task forces and discussion groups are being organised around case studies. In this way, these organisations are helping to improve the quality and acceptance of animal-free research methods.
ZonMw has in the meanwhile established a grant condition that all research data must be published in accordance with the FAIR principles and that the results must be made available via open access publishing[36]. ZonMw is also currently running a pilot within which one condition for awarding a grant is that essential information from the study protocol of the animal study will be registered in advance (known as preregistration) in an accessible database and that the results will be published in accordance with ARRIVE[37]. Based on learning points from ZonMw's pilot, health funds could also establish criteria for the award of grants for animal studies, such as openness regarding data and mandatory registration of animal studies in a national or European database.

[27] European Medicines Agency, *Guideline on the Principles of Regulatory Acceptance of 3Rs (Replacement, Reduction, Refinement) Testing Approaches* EMA/CHMP/CVMP/JEG-3Rs/450091/2012 revised 24/02/2017, *Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches* (europa.eu).
[28] National Programme for Open Science. Wat is Open Science? | Open Science.
[29] Netherlands National Committee for the Protection of Animals Used for Scientific Purposes, 2016. Opinion Synthesis of Evidence in Laboratory Animal Research | Brochure | Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (ncadierproevenbeleid.nl).
[30] ZonMw news. *Hoe kunnen we de transitie naar zo veel mogelijk proefdiervrij onderzoek versnellen?* (How can we optimally accelerate the transition to achieving the greatest possible levels of research without the use of laboratory animals?) 19 February 2020. *Hoe kunnen we de transitie naar zo veel mogelijk proefdiervrij onderzoek versnellen?* (How can we optimally accelerate the transition to achieving the greatest possible levels of research without the use of laboratory animals?) ZonMw.
[31] Smith, A.J. et al. *PREPARE: Guidelines for Planning Animal Research and Testing. Laboratory Animals*, 2017, DOI: 10.1177/0023677217724823 (norecopa.no).
[32] Kilkenny, C. et al. *Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PloS Biology*, 2010; DOI: 10.1371/journal.pbio.1000412
[33] OECD Environment, Health and Safety Publications, *Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA) Series on Testing and Assessment* No. 329, 2 October 2020. ENV/JM/MONO(2020)25. Available via: https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2020)25&docLanguage=en
[34] OECD, *Series on Testing and Assessment No. 286: Guidance Document on Good In Vitro Method Practices (GIVIMP)*, 2018. ENV/JM/MONO(2018)19. Available via http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm,
[35] EU-60. *Developing in vitro methods and approaches for scientific and regulatory use*. ETPLAS 2021. Available via: EU-60: Developing in vitro methods and approaches for scientific and regulatory use – Education and Training Platform for Laboratory Animal Science (Etplas.eu/learn/eu-60).
[36] ZonMw, *Pilot transparant proefdieronderzoek en FAIR data* (Transparency in Laboratory Animal Research and FAIR Data pilot), 2021.
[37] Kilkenny, C. et al. *Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PloS Biology*, 2010; DOI: 10.1371/journal.pbio.1000412.

Disqualification

Article 13.1 of Directive 2010/63/EU on the protection of animals used for scientific purposes[38] specifies that animal studies are no longer permitted in the event that an animal-free method is available that enables the same intended objective to be achieved and that has been certified based on EU legislation. A validated and certified animal-free method should therefore result in EU-wide disqualification of animal studies used for the same objective. However, in practice, this is not the case. Animal models are not just written into directives, but neither are they just removed from them. This is due to the fact that directives reflect agreements made at the global level. Russia, China, Japan amongst others do not accept newly developed products without data from laboratory animal research. Currently, new animal-free methods thus are often used alongside the old methods rather than resulting in disqualification of the animal model (see the added example: the two-generation study). In the short term, formal disqualification of an animal model at the EU level will not result in a reduction in the number of animal studies, but in the longer term, it could help to boost trust in animal-free methods in other countries. Furthermore, these disqualifications could be put on the agenda for meetings of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).
Changing directives is a long-term process. Also, experience tells that continual political and societal pressure is essential[39]. The European Partnership for Alternative Approaches to Animal Testing (EPAA)[40] is a body that strives to maintain this pressure too.
There is therefore demand for the disqualification of animal models and for lists of animal studies with little to no predictive value. The disqualification of models must filter through into regulations, policy, licensing of laboratory animal research and allocation of research budgets. One condition is that people involved in this process must closely monitor developments relevant to their own role.

Example
The two-generation study: example of acceptance of an animal-free alternative alongside the 'old' model.

OECD Test No. 416: The Two-Generation Reproduction Toxicity Study. The objective of this test is to examine the effects of a test substance on the reproductive systems of males and females and on the growth and development of offspring. The substance is administered during the growth and development stage through to the adult stage for two generations in order to demonstrate that the substance does not have any effect on fertility, reproduction and pre- and post-natal development of offspring.

OECD Test No. 443: The Extended One-Generation Reproductive Toxicity Study (EOGERT). This test follows the same pattern as test number 416, except that, in most cases, only one generation is studied.

It was demonstrated that, in the overwhelming majority of studies, the one-generation reproductive toxicity study was just as effective a predictor of toxicity as the two-generation test. Excluding the second generation from the test results in a reduction in animal studies of approximately 40% within this field. However, the one-generation study has been added as a new method rather than replacing the two-generation study.

[38] European Commission. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union 2010 Vol. L276 Issue L276 Pages 33-79. Available via: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32010L0063.

[39] Taylor, K. 'Chapter 24 Recent Developments in Alternatives to Animal Testing'. In: *Animal Experimentation: Working Towards a Paradigm Change*. Leiden, The Netherlands: Brill, 2019. DOI: https://doi.org/10.1163/9789004391192_025.

[40] European Partnership for Alternative Approaches to Animal Testing (EPAA) European Partnership for Alternative Approaches to Animal Testing (europa.eu).

Developments

Within regulatory research, attention is being focused on two tracks: evolution and revolution[41, 42]. Evolution is the improvement of the existing methods, with the animal model as gold standard, and the focus within current legislation. One example is the parallelogram approach, within which the results from various models – e.g. an in vivo rat model versus an in vitro rat-cell model versus an in vitro human-cell model – are compared[43, 44]. Another example is the backwards validation study, during which, for a specific test, it is examined why a large proportion of positive results in animals translated into different results in humans. This results in adjustment or disqualification of the existing tests or models, which improves the predictive value of the tests or models.

The 'revolution' within regulatory research begins from the base with human biology as the gold standard and independent of existing legislation. Various national and international initiatives have been set up in this area. High-throughput analyses – the use of automated equipment to rapidly test millions of samples to examine biological activity within a model organism, cells, pathways or at the molecular level – are being developed to screen for toxicity and efficacy of new chemical compounds and pharmaceuticals[45]. During this stage of the development of these models, the material used is often not exclusively human. Furthermore, it is investigated which pathways are involved in obtaining undesired (negative) results. The combination of this knowledge within a data system enables increasingly better predictability of the safety, risks and efficacy of new substances and the selection of a test battery that is relevant to the risk assessment process of said substances. The recently funded Virtual Human Platform aims to contribute to this objective[46].

For fundamental and applied research, the NCad encourages and facilitates the establishment of target images[47] in which an actual state of affairs concerning the use of animal studies within that field of research is given and opportunities are identified for both the reduction of laboratory animal use and the implementation of animal-free methods.
These developments will ultimately help to refine, reduce and replace animal studies and increase their relevance. Another useful development is the Helpathons[48] organised by the government programme Transition Programme for Innovation without the use of animals (TPI). These creative workshops help to establish the best research models for answering research questions, with animal-free models as their point of departure. For the implementation of good use of the ARRIVE, PREPARE and the Guidance Document on Good In Vitro Method Practices (GIVMP) guidelines, as well as to keep up with developments in animal-free methods, continual education including accreditation is required that also filters through into the curricula of biomedical education programmes.

Multidisciplinary collaboration and broad acceptance

The multidisciplinary community within safety and efficacy research that operates with human biology as the gold standard needs to grow, and the strategy needs to be further harmonised to ensure that it does not remain a series of individual and unconnected initiatives. In this area, the theme of 'building trust' is also key. Demonstrating that this system works, for example via case studies, is vital. Subsequently, working with human biology as the gold standard must become broadly accepted and implemented at the European level. From there it will enable the step towards global acceptance of safety and efficacy assessments without the use of laboratory animals.

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41 Burgdorf, T. et al. *Workshop on the validation and regulatory acceptance of innovative 3R approaches in regulatory toxicology - Evolution versus revolution. Toxicol In Vitro*. 2019 Sep;59:1-11. DOI: 10.1016/j.tiv.2019.03.039.

42 Kienhuis, A. et al *"New Approach Methodologies" in de veiligheidsbeoordeling van consumentenproducten en voedsel* ('New Approach Methodologies' for assessing the safety of consumer products and food). National Institute for Public Health and the Environment (RIVM), 2021, DOI: 10.21945/RIVM-2020-0093.

43 Motwani, H.V. et al. *Parallelogram based approach for in vivo dose estimation of genotoxic metabolites in humans with relevance to reduction of animal experiments. Sci Rep.* 2017 Dec 14;7(1):17560. DOI: 10.1038/s41598-017-17692-5. PMID: 29242644; PMCID: PMC5730592.

44 Knudsen, G.A. et al. *Estimation of tetrabromobisphenol A (TBBPA) percutaneous uptake in humans using the parallelogram method. Toxicol Appl Pharmacol*. 2015 Dec 1;289(2):323-9. DOI: 10.1016/j.taap.2015.09.012. Epub 2015 Sep 24. PMID: 26387765; PMCID: PMC4651786.

45 Thomas, R.S. et al. *The US Federal Tox21 Program: A strategic and operational plan for continued leadership. ALTEX* vol. 35 (2), 2018: 163-168. DOI: 10.14573/altex.1803011.

46 Tinbergen, M. *Veiligheid van chemische stoffen en geneesmiddelen beoordelen zonder gebruik van proefdieren* (Assessing the safety of chemical substances and medicines without the use of laboratory animals). Utrecht University website, 25 November 2020. Available via: www.uu.nl/nieuws/veiligheid-van-chemische-stoffen-en-geneesmiddelen-beoordelen-zonder-gebruik-van-proefdieren.

47 The Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad). *Streefbeelden proefdiervrij onderzoek* (Target image for animal-free research). NCad website, 3 March 2021. Available via: Target images on animal free research | Advice | Netherlands National Committee for the protection of animals used for scientific purposes (ncadierproevenbeleid.nl).

48 Transition Programme for Innovation without the use of animals (TPI). TPI Helpathon. Available via: TPI Helpathon – Home.

Funding of parallel studies

As was also concluded by the exploratory study '*Animal-free innovation in science*'[49], substantial investment is required in order to accelerate the implementation of animal-free methods. Funding of parallel studies will give researchers time and room to identify the advantages and limitations of animal-free models and to further develop the models whenever necessary. Data will be generated using these animal-free methods, and the value of these data can then be explored. This will help to minimise the abruptness of the accelerated transition to increasing levels of animal-free research. Funding of parallel studies must therefore not be seen as funding that has been taken away from other studies, but as an in-depth investment in promising, scientifically relevant and animal-free methods that boost the probability of better translatability, which in turn will result in cost savings and prevent unnecessary suffering of both animals and humans.

Approach

Parties that submit project applications involving animal procedures could be asked about opportunities to also include other, animal-free models within the research design. If it turns out that promising models are available, a supplementary parallel study or validation study can be considered. The researcher must be given the opportunity to add this option to the requested study, and extra funding will be made available. In addition to funding 'in-house' development and validation of new methods, financial support can also be given for the set-up of necessary infrastructure that will enable researchers to make use of key facilities for the implementation of new technology (such as those for microscopy and imaging, flow cytometry, high throughput analysis and in vitro technology, including for the cultivation of organoids, big data storage and analysis of genomics and proteomics facilities). For example, this could include facilities that are already available but often cannot satisfy the demand for project support due to staffing levels at the key facility.
In such cases, extra funding should be set aside for hiring and training specialized people in order to satisfy a growing demand.

Identifying and capitalising upon opportunities

Identifying other possible models without the use of laboratory animals can also be facilitated by arranging Helpathons. These are currently organised by TPI and TPI Utrecht (a task force set up by Utrecht University, Utrecht University Medical Centre and HU University of Applied Sciences Utrecht), although any other research institute could of course also work towards organising its 'own' Helpathons. For this purpose, trained process managers are needed that have completed the Helpathon Masterclass[50].
Extra funding can also be allocated for this purpose. The target images – which the NCad initiates within the context of TPI – can also be used. Target images contain clear and ambitious yet realistic objectives for the transition to animal-free methods within a specific field of research or education. Effectively focused funding can ensure follow up of the recommendations made in the target images. A target image is formulated by experts and has a broad base of support within the domain to which it relates, which establishes positive incentives for research backers. Furthermore, carrying out systematic reviews can also help boost awareness of when further animal studies are unnecessary or when switching to human studies is a better option[51].

[49] ZonMw. *Proefdiervrije innovaties voor de wetenschap; versnellen en kansen verzilveren* (Animal-free innovation in science: acceleration and capitalising on opportunities). ZonMw website, 2021. Available via: https://www.zonmw.nl/nl/actueel/nieuws/detail/item/proefdiervrije-innovaties-versnellen-en-kansen-verzilveren/

[50] Transition Programme for Innovation without the use of animals (TPI). *Masterclass: learn to organize and facilitate your own helpathon*. Available via: MASTERCLASS: LEARN TO ORGANISE AND FACILITATE YOUR OWN HELPATHON - TPI Helpathon Online - Give a Day (impactdays.co)

[51] ZonMw. *Systematisch literatuuronderzoek vervangt, vermindert en verfijnt proefdieronderzoek* (Systematic literature study replaces, reduces and refines laboratory animal research). ZonMw website, 10 November 2020. Available via: https://www.zonmw.nl/nl/actueel/nieuws/detail/item/systematisch-literatuuronderzoek-vervangt-vermindert-en-verfijnt-proefdieronderzoek/

Conclusion

The term 'parallel study' can be interpreted in a variety of ways. In regulatory research, it refers to studies in which animal-free alternatives are used alongside approved animal models. The purpose of the parallel study – also referred to in this domain as a validation study – is to demonstrate that animal-free models deliver the same predictive value as animal models with regard to safety and efficacy. Since a few years a new route has been started in which the human situation is the central focus and new methods are developed to assess safety and efficacy in humans. This approach focuses on assessing the safety and efficacy of chemical substances and pharmaceuticals without using laboratory animals. Within translational and fundamental research, parallel studies are conducted for the optimisation and in-house validation of methods or as a supplement to other studies conducted to answer the same research question. Parallel studies are already commonplace within these research fields, although this does not mean that everyone is optimally capitalising upon the opportunities offered by animal-free methods.

Parallel studies can be useful for exploring opportunities and building experience and trust in new animal-free methods. Parallel studies can also promote the further development of new models. Within the funding and licensing system for animal studies and the curriculum of biomedical education programmes, greater attention should be paid to educating people about developments concerning animal-free methods, including efficient search strategies.

In all domains, the validation of new methods must comply with the general principles of validation, meaning their predictability, relevance and reproducibility must stand up to scrutiny. There is still a great deal of uncertainty regarding the criteria with which new methods must comply in order to ensure sufficient confidence in the methods. The lack of available data generated via animal-free methods also plays a role. Also, within the regulatory domain, not all countries are as yet willing to phase out laboratory animal testing, and animal-free methods are therefore often included alongside tried and trusted models in guidelines and directives. However, this is at odds with Directive EU/2010/63.

Funding of parallel studies can contribute to exploration of the benefits and limitations of animal-free models and allow experience and trust in these models to be boosted. Funding also promotes the further development of these models and the generation of animal-free data, which in turn can help to accelerate the use and acceptance of animal-free methods. Publication and registration requirements can be included in the award criteria for this funding, which will boost research quality and data availability.

Recommendations

The requests for opinion were:

1. providing insight into which research domains and types of research could benefit from the use of parallel studies in validating animal-free alternative models within the foreseeable future;
2. advising on how the additional costs involved could be financed without negatively impacting other research.

It soon became apparent that it was difficult to provide an answer to the requests as formulated, as the terms 'parallel studies' and 'validation' can be interpreted in a variety of ways. For this reason, the NCad has adopted a broad approach that addresses how parallel studies can be used to accelerate the implementation and acceptance of a switch to animal-free methods and under which preconditions . For the second question, the NCad can only issue judgements on the way in which funding of parallel studies can contribute to this acceleration.

Recommendations to the Minister of Agriculture, Nature and Food Quality

Investigate the possibilities for establishing early mandatory consultation regarding testing strategies between parties submitting applications and assessors/authorities, so that these comply to the information requirements.
A paradigm shift is required in order to change the information requirements at the global level. By obliging parties submitting a marketing authorisation dossier for a new medicine, a new chemical compound, etc. to request scientific advice regarding the testing strategies, blind trust in traditional testing methods is prevented. Furthermore, this will promote the generation of data from animal-free methods, as the authorities will be the first party to assess the animal-free data and will know exactly which of the tests provide sufficient information for assessment. Investigate whether such an obligation can be implemented into existing legislation in consultation with the relevant ministers.

Conduct dialogue with companies regarding:

- opportunities to remove currently existing obstacles for the large-scale implementation of animal-free testing strategies;
- opportunities to make all eligible data available open access;
- obstacles that prevent use of the option to submit 3V data alongside regular test results to the European Medicines Agency (EMA).

For companies it is vital that incentives are in place to promote animal-free testing and that barriers that block this are removed. Research has been conducted into these issues, and suggestions[52, 53] have been made at the international level. Make use of the results to enter into discussions with companies regarding opportunities to remove the obstacles.

Despite the EMA having offered the safe harbour option since 2016, no parties have as of yet made voluntary use of it. The reasons for this are unknown. Possibly this is due to insufficient awareness that the option exists, or it could be that 3R methods are currently mostly considered as effective ways to screen medicines in the early stages of the development process, rather than as a way to further develop these methods in order to replace animal procedures within the regulatory process. As providing 3R test results will help to gain experience and build trust in animal-free methods (among other methods), it is important that businesses are asked what would encourage them to make use of this option.

Investigate opportunities to disqualify certain animal experiments
Within regulatory research, new animal-free methods are usually added to the list of approved tests specified within guidelines. This has to do with the international scope of these lists. Within fundamental and translational research as well, there may be animal models and animal procedures that can be disqualified. For this purpose, make use of research data and suggestions already made by other organisations, such as:

- the *European Partnership on Alternative Approaches to Animal testing* (EPAA)[54];
- the in depth study put on NCad's agenda, exploring which animal procedures could be eligible for disqualification.

Within Europe it should be insisted that these results must be implemented.

Ensure effective training activities in the field of animal-free methods for individuals involved in the assessment of applications for project licenses involving animal studies.
In accordance with Article 38 of Directive 2010/63/EU and Section 14(c) of the Experiments on Animals Act (*Wet op de dierproeven*), animal welfare bodies are obliged to "advise the staff on the application of the requirement of replacement, reduction and refinement and keep it informed of technical and scientific developments concerning the application of that requirement". Animal ethical committees and the Central Authority for Scientific Procedures on Animals (CCD) must "possess expertise in the

[52] Project Platform | Internal Market, Industry, Entrepreneurship and SMEs (europa.eu).
[53] Schiffelers, M.J.W.A. (2016), *ANIMAL TESTING, 3R MODELS AND REGULATORY ACCEPTANCE Technology Transition in a Risk-averse Context*. Thesis, ISBN 978-90-393-6567-0; https://dspace.library.uu.nl/handle/1874/334103.
[54] European Commission, Internal Market, Industry, Entrepreneurship and SMEs – EPAA Alternative approaches to animal testing Project Platform. Website available via: Project platform (europa.eu).

scientific fields and scientific applications for which the animals will be used, including replacement, reduction and refinement in the fields in question" (Section 18 of the Experiments on Animals Act). This can be promoted via continual further training in the form of lifelong learning.
A guideline in this area is already available for animal keepers, biotechnicians and researchers that work with laboratory animals[55]. Of course, the need for further training also applies to members of animal welfare bodies. These matters are currently being elaborated within the professional field, including the safeguarding of skills and an appropriate registration system featuring periodic study credit targets, which is comparable to the registration system used within the medical profession.
Members of animal ethics committees and the Central Authority for Scientific Procedures on Animals, for example, could be obliged to obtain a specific number of study credits in their field of expertise in order to retain their membership. Central coordination and financial support will be required for this purpose.

Recommendations to financiers (both government and public-private)

Create space for a hybrid approach through extra funding
Within regulatory, fundamental and translational research, there is a need for creativity without the results having to immediately have a translational application. By giving researchers the opportunity to add (promising) animal-free methods to the requested research project involving experimental animals, a hybrid approach involving both animal studies and freedom for the further development and characterisation of animal-free methods is created. In this regard, multidisciplinary collaboration should also be encouraged. For this purpose, a predetermined amount of the research grant should be made conditional for this purpose. Also, for this extra funding should be made available.

Make use of target images to identify promising initiatives
The hybrid approach can be more effectively implemented by capitalising on promising developments identified within the target images[56]. A number of target images are currently in development. These provide suggestions for promising developments within a field of research that can be leveraged with the aid of funding.
Given the manner in which the target images come about, the opinions and opportunities presented therein will have a broad base of support within the field of research concerned. This will help grant providers to make decisions in applying focus within the grant programmes.

Invest in the further development of promising animal-free methods
Current grant policy often focuses solely on the development of new innovative methods, while the subsequent steps required to achieve acceptance and implementation of the methods frequently draw the short straw. There is great demand for further development of promising animal-free models to allow broader implementation. In other words, attention must be paid to bridging the well-known 'valley of death'[57] that can often hinder innovative developments. This could be done by providing funding in stages. For example, during the first stage, the development of a new animal-free model could be funded. In a later stage, conditional investment could be made in the further development and qualification of models that show promise in the first stage.

Set requirements concerning preregistration of research and publication of the results in accordance with the ARRIVE guidelines 2.0
Implementing preregistration and/or usage of preregistered reports and the ARRIVE guidelines 2.0 help to boost the quality of animal studies and publications, which will directly improve the reproducibility and value of the research results. Preregistration of animal studies will encourage openness concerning animal procedures, increase pressure to publish results (including negative results) and reduce the current levels of publication bias. ZonMw is currently running a pilot[58] in which preregistration and publication in accordance with ARRIVE guidelines 2.0 is established as a condition for the award of grants. The primary goal of this pilot is to explore practical implications, including a possible extra administrative load for grant providers, researchers and publishers of scientific journals. Once the pilot is complete and insight has been gained into the practical implications and possible solutions thereto, then – provided the results of the pilot justify doing so – these conditions can be adopted by the funds, which will help to establish preregistration and publication in accordance with ARRIVE guidelines 2.0 as the standard. In this regard, the use of preregistered reports should be accepted as an alternative for preregistration. All of the above should also be applicable to animal-free methods.

[55] 3Rs Centre ULS, IVD-Platform and the NCad. A Guide to Continuing Professional Development in Animal Experimentation, 2019. Available via: *Guide to Continuing Professional Development (CPD) in animal experimentation* | Publication | Netherlands National Committee for the protection of animals used for scientific purposes (ncadierproevenbeleid.nl).
[56] Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) *NCad helpt bij het starten van streefbeelden proefdiervrij onderzoek* (NCad helps to formulate target situations for animal-free research). NCad website, 26 November 2019. Available via: https://english.ncadierproevenbeleid.nl/latest/news/19/11/26/ncad-helps-to-start-outlooks-on-animal-free-research.
[57] The 'valley of death' is the gap between the development of a concept and its implementation. Many innovative ideas run aground during this process.
[58] ZonMw. *ZonMw zet in op meer transparantie van dierproeven en evalueert eigen Open Science beleid* (ZonMw devotes attention to greater transparency in animal studies and evaluates its own Open Science policy). ZonMw website, 30 November 2020. Available via: ZonMw.

Recommendations to education institutions, research institutes and companies

Ensure effective education in animal-free methods for employees involved in the set-up and execution of animal studies

To encourage optimal use of available and for the research on hand relevant animal-free research methods, knowledge of the available models and their possible applications is required.

The current curriculum of biomedical education programmes must be updated with the latest developments. Implementation of 'lifelong learning' is desired, as part of which researchers and members of animal welfare bodies continually work to update their knowledge of developments in the field of animal-free methods. Basic requirements for working with laboratory animals are already in place, and the laboratory animal science course (in compliance with Section 9 of the Experiments on Animals Act) covers these requirements. However, more emphasis should be put on up-to-date knowledge of animal-free methods, synthesis of evidence and the guidelines for responsible experimental design and usage of laboratory animals. The animal welfare bodies must ensure general awareness of guidelines such as ARRIVE and PREPARE and actively encourage their usage. For this purpose, usage can be made of all available educational materials, such as those made available by ETPLAS. It is vitally important that establishment licensees provide their staff involved in the design and execution of animal studies (including the members of the animal welfare bodies) with sufficient freedom to participate in lifelong learning activities. The umbrella organisation DALAS[59] is already working on an up-to-date list of available courses and training programmes. Alignment with these efforts could be beneficial. Also, an accreditation system could be linked to it. A variety of accreditation systems for the fields of human and veterinary medicine and of the experimental animal are already up and running in the Netherlands and in other countries. The experiences gained with these systems can be used to set up a system as proposed here.

In addition, opportunities must be created to experiment with animal-free methods in parallel with animal studies where chances are signalled. These types of parallel studies can facilitate the adoption of a new approach to research questions in a positive way. Specific questions can be addressed via Helpathons, which can also be organised locally, as TPI Utrecht is already doing. In this way, researchers search for the optimal model based on the research question.

[59] Dutch Association for Laboratory Animal Science (DALAS). Available via: Home - DALAS.

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