How Can the Final Goal of Completely Replacing Animal Procedures Successfully Be Achieved?

Christiane Baumgartl-Simons  
People for Animal Rights Germany—Federal Association against Vivisection (Germany)

Christiane Hohensee  
People for Animal Rights Germany—Federal Association against Vivisection (Germany)

Follow this and additional works at: https://www.wellbeingintlstudiesrepository.org/valamet

Part of the Bioethics and Medical Ethics Commons, Design of Experiments and Sample Surveys Commons, and the Laboratory and Basic Science Research Commons

Recommended Citation
Baumgartl-Simons, C., & Hohensee, C. (2019). How Can the Final Goal of Completely Replacing Animal Procedures Successfully Be Achieved?. In Animal Experimentation: Working Towards a Paradigm Change (pp. 88-123). Brill.

This material is brought to you for free and open access by WellBeing International. It has been accepted for inclusion by an authorized administrator of the WBI Studies Repository. For more information, please contact wbisr-info@wellbeingintl.org.
CHAPTER 3

How Can the Final Goal of Completely Replacing Animal Procedures Successfully Be Achieved?

Christiane Baumgartl-Simons
Deputy Chairwoman, Menschen für Tierrechte—Bundesverband der Tierversuchsgegner e.V. (People for Animal Rights Germany—Federal Association against Vivisection)
baumgartl@tierrechte.de

Christiane Hohensee
Scientific Advisor, Menschen für Tierrechte—Bundesverband der Tierversuchsgegner e.V. (People for Animal Rights Germany—Federal Association against Vivisection)
hohensee@tierrechte.de

Introduction

Article 23 of European Union (EU) Directive 86/609/EEC required that Member States promote the development and validation of alternative technologies and stated that the European Commission (EC) “shall report before the end of 1987 on the possibility of modifying tests and guidelines” (European Parliament, 1986, Article 23). This Directive was replaced by Directive 2010/63/EU on the protection of animals used for scientific purposes, which now requires that Member States develop and validate alternative approaches much more precisely and specifies that the ultimate objective is the “full replacement of procedures on live animals for scientific and educational purposes, as soon as it is scientifically possible to do so” (European Parliament, 2010, Recital 10). However, having followed the initiatives of Member States for more than 30 years, we see that developments to replace animal experiments occur more by accident than by design. Directive 2010/63/EU has not changed this either. This chapter explores the reasons why the development and approval of animal-free methods are not advancing more quickly, and why the numbers of animals used is not declining despite the development of new methods. Undoubtedly, there are complex, multifactorial reasons behind this. Our analysis leads us to the heart of the matter. There is no master plan and there are no responsible project managers who effectively pursue the objectives of Directive 2010/63/EU at a national or EU level.

© BAUMGARTL-SIMONS AND HOHENSEE, 2019 | DOI:10.1163/9789004391192_004
This is an open access chapter distributed under the terms of the prevailing cc-by-nc License at the time of publication.
In 2015, we started collating individual measures in order to tackle the problems we encounter in our day-to-day work as an animal rights organization, defining five categories (or pillars). We were encouraged to pursue our ideas by developments in the United States through concepts, such as *Toxicology in the 21st Century—A Vision and a Strategy* (*Tox21*) (Krewski et al., 2010), as well as by the EU ban on the marketing of cosmetics tested on animals, which came into force in 2013. During this time, the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) presented its plan, *Transition to Non-Animal Research*, referred to here as the *NCad report* (NCad, 2016a), becoming the first EU member state to present a road map for phasing out animal procedures and stimulating innovation without laboratory animals. Unfortunately, no EU Member State, so far, has publicly spoken out in favor of the Dutch initiative. On the contrary, its timeline has been criticized as being unrealistic, risking the safety of medical treatment, and hampering basic research. Only the government of the Brussels-Capital Region has put forward a plan to phase out animal experiments along the lines of the NCad report. Member States should show more support for such initiatives, which aspire to achieve the goal of full replacement stated in Directive 2010/63/EU. At the EU level, unfortunately, there is neither an overall strategy for phasing out the use of animals for scientific purposes nor for monitoring the implementation of the paradigm shift. It is important that changes take place, but how they take place is subject to debate.

Writing from the point of view of a German animal rights organization, the main focus of this chapter is Germany. Although Germany claims to be especially committed to developing animal-free methods, it ranks among the highest in terms of animal experiments in the EU, together with France and the United Kingdom.

1 Part 1: How Seriously Do Member States Take the Tasks and Obligations Stated in Directive 2010/63/EU?

A master plan to end animal experiments requires suitable resources for effectively reducing animal experiments and increasing market-ready animal-free methods. In the following we discuss steps towards that final goal.

On 1 December 2016, the Netherlands became the first EU Member State to present a road map for “phasing out animal procedures and the promotion of innovation without laboratory animals” (NCad, 2016a, p. 3). They are convinced that some uses of animals—currently required by law for safety testing of chemicals, food additives, pesticides, and (veterinary) medicines as
well as the commercial launch of biological products (e.g., vaccines)—can be phased out by 2025, while maintaining existing safety levels. However, their road map recommends that regulatory pre-clinical tests for the registration of new biological substances/products not be phased out by 2025 because there is a lack of replacement methods. Similarly, animal experiments in the field of “curiosity-driven” (NCad, 2016a, p. 38) basic research is not to be terminated by 2025 because there is a basic right to freedom of research; and, as no one knows the subject of research in advance, animal experiments cannot be easily replaced with new animal-free methods. Therefore, it has been necessary to implement 10-year plans for each individual area of research. In the case of animal experiments in applied and translational research (implementation of preclinical research in clinical development), which are also not to be phased out by 2025, the development of replacement methods is to be accelerated. The aim is to reduce significantly the use of laboratory animals for education and training. The planning encompasses transition objectives, transition strategy, and management of the transition. There is every indication that the NCad report, commissioned by the former Minister for Agriculture, Martijn van Dam, has laid out solid project planning with the goal of effecting system change from animal use to animal-free procedures.

This type of planning should have been initiated at an EU-wide level in 2013 when Directive 2010/63/EU came into effect, requiring the phasing out of animal procedures and the acceleration of innovation without laboratory animals. While no Member State has publicly spoken out in favor of the Dutch initiative, the German research association, Deutsche Forschungsgemeinschaft (DFG) and the Allianz der Wissenschaftsorganisationen (Alliance of Science and Research Organizations) issued a critical assessment of the plan, calling its timeline unrealistic and claiming it would endanger the safety of medical treatment and hamper basic research (BfR, 2017a). According to our organization (People for Animal Rights Germany—Federal Association against Vivisection), a good master plan has the following attributes: it includes all stakeholders, including the scientific community, industry, and animal rights/welfare organizations; it has robust monitoring to assess the paradigm shift towards animal-free science; and it has an active commitment of all stakeholders to implement the plan. The master plan for an end to animal experiments requires suitable resources that purposefully pursue this end by reducing animal experiments and increasing market-ready animal-free methods.

The master plan's foundation rests on five pillars:
1.1  **Pillar 1: Increased Research Funding for Animal-free Methods Is Necessary**

Here we refer to animal-free research in the field of applied and basic research. This research field includes methods that can replace animal testing in regulatory animal tests (in a narrow sense). The budget for animal-free research methods should be drastically increased, with the EU and its Member States implementing their own funding programs for replacement methods. Germany, for example, is not a suitable role model: at present, “system-changing” replacement methods compete with “system-maintaining” refinement and reduction methods, because the three options are funded by the same programs.

The EU needs to increase the budget for funding animal-free research. In the current funding period, only one program that is dedicated to replacement, EU-ToxRisk, is funded. This European collaborative project is funded by the EU Framework Program for Research and Innovation, Horizon 2020. The project started on January 1, 2016 and will last for six years. It currently has only €30 million at its disposal (EU-ToxRisk, 2016), with 39 participating groups. By comparison, during the last funding period, the EU Seventh Framework Program provided the project cluster, SEURAT-1, with €50 million in funding for work towards the replacement of *in vivo* systemic dose-toxicity testing (which ran until December 2016)—much more than the current funding.

Special funding programs are essential for creating ready-for-series technologies, i.e., mass production in large amounts with a lower product prize; for example, the characteristics and viability of current cell systems must be improved to recreate the functions of the natural organ as accurately as possible; current organoid biotechnology needs to replicate the behavior of organs more closely; and the functions of the capillary system and immune system still need to be modeled.

National and European budgets, especially for validation studies, are also necessary and the EU Reference Laboratory for Alternatives to Animal Testing—European Centre for the Validation of Alternative Methods (EURL ECVAM) (EURL ECVAM, 2017a) should be provided with adequate resources. This demands a great deal of work. Validation and qualification of the new *in vitro* systems have been discussed in several workshops across the US. These workshops were hosted by the American Institute for Medical and Biological Engineering (AIMBE) and the National Institutes of Health (NIH) at the NIH campus in Bethesda, Maryland. Representatives of the EURL ECVAM and of some European start-ups also took part. The participants determined
the need for a broader definition of validation for the integration of new platform technologies into preclinical safety evaluation. The new in vitro systems, such as the chip technologies (cell and tissue platforms, microelectrode systems with connected measuring devices), biomarkers, and the quality of cell and tissue types derived from human stem cells were also seen as needing evaluation in terms of safety for preclinical applications and efficacy. The pharmaceutical industry, which is the primary customer of the new multi-organ on a chip systems, should also be more involved (AIMBE, 2013).

The EU Member States need to support the concept of Tox21, developed in the US (Krewski et al., 2010). The concept’s goal is to provide a new basis for risk and safety assessments of substances and products (i.e., regulatory toxicology), using new techniques that are human-specific; and to promote the use of animal tests only in exceptional cases (see also the Integrated Approaches to Testing and Assessment adopted by the Organization for Economic Cooperation and Development, OECD, 2017). The OECD now includes 35 countries around the world, across North and South America, Europe, and Asia.

A research focus is also needed on the implementation of cell culture media ready for practical application and for which no animals suffer, i.e., without the use of fetal bovine serum (see Redmond, 2018, in this Volume). Researchers in the US have been able to work with serum-free media in a defined system since 1995 (Schaffner et al., 1995).

1.2 Pillar 2: Animal-free Methods in Teaching and Scientific Research Have to Be Expanded

The goal of Pillar 2 is to establish academic chairs—with corresponding personnel and financial resources for research and teaching and regular professorships—for animal-free research methods, courses of study in the life sciences that do not use animals, a broad range of papers not based on the use of animals, and an increased number of theses and higher qualifications, with a focus on animal-free methods.

In Germany, for example, individual chairs in some federal states or Bundesländer (Baden-Württemberg, Berlin, Hesse, and North Rhine-Westphalia) have been established to develop methods to replace animal use. Researchers are thus able to offer students a perspective to get closer to the topic and to prepare their theses with non-animal methods. However, there are still only a few chairs in some federal states. There is need for courses of study that address scientific questions using new animal-free methods. So far, students in life sciences courses, such as biology or human medicine, must, with few exceptions, participate in courses that use animals. Furthermore, where platforms
for alternatives to animal tests have been set up, the scientists involved usually investigate 3Rs methods (replacement, reduction, and refinement), which means that the scarce resources are spread across all three areas. Instead, the platforms’ financial resources need to be exclusively available for replacement methods.

The situation is similar in other European Countries. There are a few national centers for alternatives to animal use, such as, the Finnish Centre for Alternative Methods, which teaches only animal-free replacement methods. In the private sector, Altertox Academy (formerly the Center for Alternatives to Animal Testing Academy) offers training for young scientists in replacement methods. Others national centers, such as the Danish 3Rs-Center, the Swedish Toxicology Science Research Center, or the Romanian Center for Alternative Test Methods, were founded to implement the mission of Directive 2010/63/EU and teach not only the replacement of animal use but also reduction and refinement. The range of programs of study for animal-free methods (full replacement) should be expanded. It should be possible to choose courses of studies at European universities that will enable students to do research and develop methods without using animals.

1.3 **Pillar 3: Bans on Animal Testing Must Be Consolidated and Expanded**

Since its review in November 2017, Article 58 of Directive 2010/63/EU has barely led to sufficient improvements for laboratory animals. In the next review, the EC must take into account any progress made in the field of alternative methods that may lead to an end to the use of animals, in particular non-human primates. The first step should be to repeal Article 55 of Directive 2010/63/EU, which allows Member States to provide exceptions from the regulations protecting non-human primates (European Parliament, 2010); it also allows exceptions from Article 2, which stipulates that procedures not be performed on animals if they involve severe pain, suffering, or distress. Therefore, with the approval of the EC, Member States may, for example, use monkeys in experiments that involve severe pain, suffering, or distress.

Further bans should be introduced, including: a ban on patenting genetically altered animals; and a full ban on animal tests for household products and their components, including banning the sale of such goods (analogous to the existing provisions for cosmetic products) is essential.

Viable European and national competency centers should be established, with the following tasks: creating transparency for the public, monitoring adherence to EU animal welfare law, and setting up information offices for all stakeholders.
Closer cooperation/collaboration with scientists and agencies in the US, which is more advanced in technological development. The US regulatory authorities are also not hesitant to search for and accept new animal-free methods. Even pharmaceutical and chemical companies think that the EC should be more orientated towards American models, such as the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) (Ettel, 2018). Another example is the new Interagency Coordinating Committee on the Validation of Alternative Methods plan (National Toxicology Program, 2018).

1.3.1 Suggestions for Implementing Existing EU Law

The EC Department, Directorate-General for Environment, should have a European competency center, for monitoring adherence to EU law and providing up-to-date information on pain, suffering/distress, and fear as well as on animal-free testing methods and other information relevant to this range of topics.

These additional tasks could also be integrated into existing structures, such as EURL ECVAM’s EU Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL), under the umbrella of the EC Directorate-General for Environment. So far, EU-NETVAL comprises laboratories in individual EU Member States that conduct validation studies and assess the reliability and relevance of new animal-free methods (EU-NETVAL, 2018). The European validation authority, EURL ECVAM, could also focus on transparency for the public, provide information offices for all stakeholders, and monitor adherence to EU animal welfare law. It is also vital that a negative list be introduced that will stand up in court, defining tests that may no longer be conducted for ethical reasons.

The establishment of national competency centers as central institutions for informing and educating all stakeholders involved is also necessary. At a national level, these tasks could be completed by the EU National Committees in the Member States. The national information structures established thus far, in accordance with Article 49 of Directive 2010/63/EU, have laid the foundation for such tasks.

The competency centers would be responsible for developing lists of criteria for assessing whether applications for animal experiments fulfill legal requirements, criteria that are lacking, thus far, but are necessary. This requires practicable assessment criteria for qualifying/quantifying the necessity, benefits, and ethical justifiability of the animal procedure as well as specifying the level of distress. The competency centers should also offer education schemes for all
stakeholders. To this end, it would be necessary to develop content and performance records. We recommend developing an education scheme for animal-free testing methods, analogous to the courses of the Federation for Laboratory Animal Science Associations (FELASA, n.d.).

Practical, user-friendly databases should be set up to allow reliable and uncomplicated referencing of animal-free methods.

The approving authorities and monitoring agencies in the Member States should be provided with adequate finances, expertise, and staff.

Steps must be taken to ensure that the Member States implement the requirements set out in Directive 2010/63/EU, without exception. In Germany, for example, although stipulated by Directive 2010/63/EU, the approving authorities currently do not have a comprehensive right for evaluation when assessing applications for animal procedures, due to a decision made by the Higher Administrative Court Bremen (Oberverwaltungsgericht Bremen, 2012). Directive 2010/63/EU stipulates that project evaluation should consist of six evaluation points (European Parliament, 2010, Article 38, Section 2, Subsections a–f). It should include an evaluation of the project objectives, the predicted scientific benefits or educational value, and the severity of the procedures, and a harm-benefit analysis of whether the suffering, pain, and distress inflicted on the animals is justified by the expected outcome, in light of ethical considerations and the ultimate benefit to human beings, animals, or the environment.

1.4 **Pillar 4: Success Monitoring of the Increase of Animal-free Methods and the Reduction of Animal Experiments Is of Particular Importance**

This pillar is especially important. It stands for the need for quality management, as it entails control measures that systematically document the progress of the paradigm shift. Unlike the other pillars, there are no approaches, as yet, to achieve this goal; they have to be established from the start. Definitive parameters should be determined for quantifying and qualifying the reduction of animal experiments and the increase in animal-free methods. A retrospective assessment for all animal tests conducted and the publication of their data crucial. Directive 2010/63/EU expressly provides for such a retrospective assessment (European Parliament, 2010, Article 39).

1.5 **Pillar 5: Complementary Measures Are Necessary**

Complementary measures comprise drastically shortening the time needed for assessing and approving animal-free research methods; researching animal experiment models in order to de-validate them; and introducing a class
Moving from the development of an animal-free method to its implementation in the regulatory framework, which would lead to a broader range of applications, has taken too long. Periods of 12 to 15 years, or even longer, have been a matter of course (Hartung, 2015). There is a lack of financial support for proof-of-concept studies as well as pre-validation and validation studies, so that developers spend a great deal of time seeking financial support. In addition, regulatory authorities become involved in this process at too late a stage (Hohensee and Brüning, 2016; Schöffl et al., 2000).

According to NCad and other researchers, there is a “growing focus on Synthesis of Evidence”, the experimental design and critical reviewing of existing animal models (Cronin, 2017, p. 39; NCad, 2016a; Varga et al., 2010). Just as the new animal-free methods must be validated, it is also necessary for animal testing to undergo systematic review.

Greater demand for national and international coordination of the promotion and acceleration of validation, regulatory acceptance, and implementation of animal-free methods (NCad, 2016a).

2 Part 2: How Has the Change of Course Been Pursued So Far—Nationally, in the EU, and Internationally?

Directive 63/2010/EU provides for a long-term withdrawal from all animal experiments. Nevertheless, for many researchers, the development of replacement methods has not been the priority it should be. It still has a niche existence. Although some research groups focus on replacement, research and budgets at both the national and European levels are too low and should be embedded in a higher-level context. Here, more efforts are necessary.

2.1 Setting up Committees in Accordance with Directive 2010/63/EU

With Directive 2010/63/EU, the EU and its Member States have set the long-term goal of ending animal experimentation and promoting the development of replacement methods for animal experiments. The Directive must be enacted in national law by all Member States. The amended German Animal Welfare Act (Tierschutzgesetz) took effect in July 2013, and the new Animal Welfare Laboratory Animal Regulation (Tierschutz-Versuchstierverordnung) took effect in August 2013. However, while opportunities for achieving a higher level of animal protection should have been put in place, they were forfeited. Although the necessary legal basis exists in primary law, a maximum limit for pain or suffering in animal experiments, and a ban on increasing animal experiments, by
eliminating the exemption clauses, were not enforced in German law (Peters and Stucki, 2012). Furthermore, the comprehensive right of project evaluation pursuant to Article 36 of Directive 2010/63/EU was not implemented and was, instead, replaced by a qualified plausibility check (Hildermann, 2015).

Article 49 of Directive 2010/63/EU states that all EU Member States must establish National Committees for the protection of animals used for scientific purposes (European Parliament, 2010). In Germany, this role is performed by the Federal Institute of Risk Assessment (BfR), which has established the German Centre for the Protection of Laboratory Animals (Bf3R), located at the BfR, as set out in the amendment of the German Animal Welfare Act that took effect on July 13, 2013 (BfR, 2017b). According to Paragraph 45 of the German Animal Welfare Laboratory Animal Regulation, the National Committee advises the competent authorities and animal welfare committees on matters regarding the acquisition, breeding, accommodation, or care of vertebrates and cephalopods (as defined in Paragraph 1, Section 1 of the Regulation); or the use of vertebrates and cephalopods in animal experiments. The National Committee also advises the competent authorities, in accordance with Paragraph 46 of the Regulation, on matters regarding alternatives to animal experiments (Bundesministerium für Justiz und Verbraucherschutz, 2017).

The German Centre for the Protection of Laboratory Animals (Bf3R) was founded in the context of the animal welfare initiative (“Tierwohlinitiative”) of the German Federal Ministry of Food and Agriculture (BMEL) in 2015. While before the establishment of the Bf3R, the Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) was of central importance, it has now been integrated into the Bf3R as one of five areas of competence. The competence areas are research and development of methods to reduce pain and suffering of laboratory animals (refinement); the National Committee; development of alternatives in the field of toxicology; and coordination of research funding. The last two areas of competence were formerly tasks of ZEBET. However, the tasks of all competence areas are predominantly performed by Experimental Toxicology and ZEBET (BfR, 2017b). Therefore, alternative methods are investigated, developed, and validated in accordance with the 3R principles. Thereby, the focus has shifted from the development of replacement methods for animal experiments towards the 3Rs. Today, refinement and reduction of animal use is treated of, at least, equivalent importance.

2.2 Has Funding Been Increased in Recent Years?
2.2.1 Funding on a National Level (Germany)
Although an increasing number of projects are funded on a national level, those who conduct research in replacement methods criticize that disproportionate
funding for animal experiments and an exaggerated perception of their importance has led to a decades-long neglect of the development of replacement methods and a reticence to promote them actively (Baumgartl-Simons, 2017; Leist, 2016).

2.3 Programs for the Development of Animal-free Methods: The Funding Has to Be Shared with Refinement Methods

In Germany, projects are funded mostly by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF). BMBF, Bf3R, and the set Foundation together provide approximately €5.7 million per year in funding for research into the development of replacement and complementary methods for animal experiments based on the 3R principles. A few German federal states have established funding programs of their own, including Baden-Württemberg, which provides €400,000 per year; and Rhineland-Palatinate, €70,000 every two years (see Table 3.1). Some individual projects, such as postgraduate programs, are funded by the German research association, DFG; however, this funding is not dedicated to the development of animal-free methods and, therefore, is not listed here.

Note: This table presents an overview, not an official empirical survey.

On a state level, in Germany, it seems that an expansion of research associations and professorial chairs has slowly begun. Some federal states have established research associations or professorial chairs and are providing initial funding for a finite period. For example, Baden-Württemberg funds the Dorenkamp-Zbinden Chair of in vitro Toxicology and Biomedicine/Alternatives to Animal Experimentation with €200,000 to €400,000 annually. However, most of this funding is combined with research for the 3Rs as a whole, so funding for replacement methods alone cannot be quantified. For example, Berlin may soon take over the Berlin-Brandenburg research platform (BB3R), with an integrated postgraduate program; and it plans to establish an institute for alternatives to animal experiments, with €8.6 million, at the Charité University School of Medicine Berlin (Der Regierende Bürgermeister—Senatskanzlei Berlin, 2017). The current BMBF funding has expired and negotiations for funding on a state level are currently in progress. In Frankfurt, a professorship for pharmaceutical technology has been established, with 3R methods as its main research focus. During the next five years, €200,000 will be made available to be shared with another chair for refinement methods. Lower Saxony finances the research initiative R2N—Replace and Reduce, with €4.5 million. North Rhine-Westphalia is funding the Centrum für Ersatzmethoden zum Tierversuch (CERST-NRW), a center for replacement methods for animal experimentation, with €500,000 per year for a period of five years.
### TABLE 3.1 Funding for the development of animal-free methods in Germany

#### A. Chairs and platforms

| Federal state | Subject | Designation | Amount (£) | Financed by |
|---------------|---------|-------------|------------|-------------|
| Baden-Württemberg | Chair | The Doerenkamp-Zbinden Chair of in-vitro Toxicology and Biomedicine | 200,000–400,000 per annum | Baden-Württemberg |
| Berlin | 3Rs platform/ Research Association | Berlin-Brandenburg research platform BB3R with integrated graduate education | 92,000 (2014–2016), negotiations at state level to continue | Federal Ministry of Education and Research (BMBF) |
| Hesse | Chair | Chair of pharmaceutical technology | 200,000 per annum (5 years) | Hesse/Johanna Quandt Foundation |
| Lower Saxony | Research Association | R2N, replace and reduce in Lower Saxony; replacement and complementary methods for trend-setting biomedical research | 4.5 million (4 years) | Lower Saxony |
| North Rhine-Westphalia | Chair | Centrum für Ersatzmethoden zum Tierversuch (CE RST-NRW) | 500,000 per annum | North Rhine-Westphalia |
Since 1981, the BMBF has funded 530 projects for the development of 3R methods (BMBF, 2016). The annual budget totals €5 million (although applicants for funding from this BMBF research budget, and for research prizes awarded for the development of alternatives to animal experiments, include developers of refinement methods for animal experiments, i.e. animal experimenters). So far, more than €170 million in funding has been provided in this area (Hohensee and Brüning, 2016). However, a 2011 study published by the Fraunhofer Institute for Systems and Innovation Research showed that about 30 per cent of the projects funded between 1981 and 2000 focused on refinement (Hüsing et al., 2011). In addition, few institutions or projects receive sufficient funding. For example, the BfR simultaneously supports about ten working groups for up to three years, each with an average of €35,000 per year. By comparison, the
development of the in vitro pyrogen test required €6 million from development to implementation. The working group needed €400,000 per year (Thomas Hartung, personal communication, August 2016). Numerous applicants compete for the low project funds. The funding amount of about €5.7 million from the BMBF, set foundation, and Federal Institute of Risk Assessment is far too small, with no budget available for pre-validation and validation studies. Since the developers of refinement methods can also apply for these funds, even less money is available. Funding for refinement methods should have its own budget. The half-hearted funding is intended to give the impression that Germany actively supports the development of new animal-free methods. However, it is not an expression of the decision to phase out animal experiments, instead, it slows down the development.

2.4 Additional Programs

There are a few complementary funding programs to the main program, Alternatives to Animal Experiments, such as e:Bio-Innovative Competition Systems Biology, which runs until 2020. Systems biology unites complex high-throughput experiments with mathematical modeling of the obtained data, to develop models to predict complex biological processes on cellular, tissue, and organ levels as well as in the entire organism (BMBF, 2011). One of the complementary programs, BioÖkonomie 2030-GO-Bio, aims to further develop innovative research topics in the field of life sciences to the point of making these available for economic implementation, like innovative start-ups (Bundesministerium für Wirtschaft und Energie, 2017). This is interesting for organ on a chip technology developments intended for market launch.

2.4.1 Funding Transregional/On a European Level

There are also a few programs financed by more than one EU Member State, such as InnoSysTox—Innovative Systems Toxicology for Alternative to Animal Testing, a €3 million project financed jointly by Germany and the Netherlands (ZonMw, 2017). The application deadline was December 2014. Another program is the translational funding initiative, Multilateral Collaboration in Computational Neuroscience: Germany—US—Israel—France (BMBF, 2015a). The EU-wide budget is unknown. In Germany, €90,000 – €225,000 in funding was available for a three-year term. The application deadline for the first run was November 2016. However, as previously noted, across the EU there is currently only one program financed by the EU’s funding framework Horizon 2020 dealing with animal-free issues, EU-ToxRisk, which deals with feasibility
studies for methods that most urgently need to be developed in toxicology. In EU-ToxRisk, 39 project participants must share a budget of €30 million.

The research guidelines that define what methods are most urgent are set out by the scientists themselves, and not by the EC. This was the case with project cluster SEURAT-1 (SEURAT-1, 2013), which received €25 million in funding from both the EU and the industrial association, Cosmetics Europe. Cosmetics Europe also contributes to the funding for EU-ToxRisk (amount unknown) (Cosmetics Europe, 2017). Especially urgent areas of research should be stipulated, and the development of animal-free methods must have priority. At the EU level, scientists seem to agree on the methods in most urgent need of development; but in Germany, there is no agency that decides which methods need to be developed and provided with preferential funding. Apparently, a particular bone of contention is whether it is better to fund all research indiscriminately, in the spirit of scientific freedom or originality, or to demand specific research into solutions for important questions (Hohensee, 2015). In the case of achieving the end goal of full replacement, as set out in Directive 2010/63/EU, the answer is clear: Research developments should not be funded indiscriminately, but rather clear priorities are necessary, with replacement of foremost importance.

2.4.2 Funding on an International Level
The US was the first country to take decisive steps towards ending animal experiments, with the concept Tox21 (National Center for Advancing Translational Sciences, NCATS, 2017a). Tox21 is a collaboration among the NIH, FDA, EPA, and the National Toxicology Program at the National Institute of Environmental Health Sciences, with the goal of achieving better assessment of the toxicity of substances by using faster and more efficient human-specific methods in high-throughput technologies. To this end, fit-for-purpose cell models have been developed; and a high-throughput apparatus has been set up, which scans the gene expressions in human cells for alterations after they have been exposed to the test substance. The EPA has established its own National Center for Computational Toxicology, in which prediction models are developed based on the new results (Committee on Toxicity Testing and Assessment of Environmental Agents, 2007; NCATS, 2017b).

A number of organ on a chip start-ups, most of them from the US, have been established to produce lab-scale prototypes. Zhang and Radisic (2017) described the most important 29 start-ups, dividing their work into groups: start-ups developing barrier functions, start-ups developing tissue-interface on a chip, and start-ups developing parenchymal-tissue on a chip. The first scientist to envision the possibility and pioneer the practice of quantitatively-simulating
molecular and cellular biological systems, with *in vitro* devices was Professor Michael L. Shuler of Cornell University, New York (Hurelcorp, 2018). Together with Professor James J. Hickman of the University of Central Florida, Shuler founded the start-up, HESPEROS, in 2014 (HESPEROS, 2017). Hickman developed the first serum-free media for hippocampal neuron cultures in 1995 (Schaffner et al., 1995) and published the first readouts of electrical and mechanical functions of neurons in 1998 (Ravenscroft et al., 1998). Together with Shuler and others, Hickman integrated cardiac, muscle, neuronal, and liver modules in a microphysiological system, under continuous flow conditions in a serum-free defined medium, utilizing a pump-free platform (Oleaga et al., 2016). So far, HESPEROS has successfully tested six organs on a chip (Miller, 2017).

TissUse, a German spin-off from the Technical University of Berlin, was founded by Dr. Uwe Marx in 2010. The initial focus of this company was on the development of two-organ and four-organ models. Today, like HESPEROS, their ultimate goal is to develop a human on a chip, integrating at least 10 organ-like tissue constructs of human origin. Instead of using a gravity-driven flow, like Hesperos, the TissUse platform contains a built-in micropump, driven by an external pneumatic controller. A second microfluidic circuit ensures drainage of the fluid excreted through a kidney's epithelial cell layer. The four-organ chip with intestine, liver, skin, and kidney is already available and can be co-cultured for up to 28 days (Maschmeyer et al., 2015). The Wyss Institute for Biologically Inspired Engineering at Harvard University in Boston has produced 19 university start-ups (Wyss Institute, 2018) and is known for its organ on a chip developments. One of the current research results is a pulmonary thrombosis model on a chip. The research was co-financed by the Defense Advanced Research Projects Agency (DARPA), Janssen Pharmaceuticals (Jain et al., 2018), and funding from NCATS (NCATS, 2018).

The Center for Alternatives to Animal Testing (CAAT) was founded in 1981 by Alan Goldberg, a professor at Johns Hopkins University in Baltimore. CAAT was then financed by the American Cosmetic Toiletry and Fragrance Association (CTFA), which was interested in the development of replacement methods for testing their products (CAAT, n.d. a). CAAT’s goal is to create fundamental knowledge regarding possible methods for replacing tests using whole organisms (i.e. living animals) with alternative methods in the development and testing of commercial and therapeutic products. CAAT established its own *in vitro* toxicology laboratory in 1985. In 1988, the cosmetics company, Avon, financed the first program for replacing animal experiments in the field of contact allergies. Since 1989, CAAT has also been supported by government agencies and, as of 1992, by the EPA. CAAT-Europe, at the University of Konstanz in
Germany, the European equivalent to CAAT in the US (CAAT-US), was founded in 2009. CAAT coordinates transatlantic relations between the US and Europe in the field of animal-free methods, by bringing together international stakeholders in congresses and workshops on various topics of non-animal research (Universität Konstanz, 2018). On information days, the public is informed about the latest developments in this field. Both CAAT-US and CAAT-Europe in Germany train young scientists in new animal-free methods (CAAT, n.d. b).

Expanding education and research towards science without animal experiments is crucial, as only a small group of researchers currently work on replacement methods. Despite the aforementioned funded individual professorial chairs, there is still no way of completing a course of studies that would equip students with the professional capacity to develop alternatives to animal experiments. In order to complete a thesis at one of the newly established chairs (see above), the student must first have studied one of the more common courses of studies, such as biology, medicine, veterinary medicine, biochemistry, pharmaceutics, toxicology, or biophysics, which, for the most part, continue to use animals for training (SATIS, 2017a). Only very few tertiary institutions allow the use of alternatives to animal dissection on ethical grounds. Instead, tolerance towards alternatives to the use of animals in training courses has decreased, depending on the course organizers’ attitude. Only four German State Higher Education Acts (North Rhine-Westphalia, Hesse, Saarland, and Bremen) allow the right to decline using animals on ethical grounds (SATIS, 2017b). Most students quickly get used to using animals because they do not want to belong to a fringe group, without prospects of gaining a professional foothold, or they want to keep their options open. The lack of opportunities for attaining qualifications in animal-free courses of studies leads to a lack of qualified young scientists in the area of animal-free methods, so that funding bodies sometimes argue that the reticence in providing finances results from insufficient scientific standards (Hohensee, 2015). The lack of political will means that the subject continues to go in circles.

2.5 The Time-consuming Validation Process
The time taken for the validation and approval of animal-free testing methods urgently needs to be shortened. The broadening of funding by the BMBF program, Alternatives to Animal Testing, to include strategies for implementing newly developed methods as replacements for animal experiments (BMBF, 2015b, Module 11), is a first step in the right direction; but it is by no means enough. Validation studies, in particular, are extremely expensive and time-consuming and go through the bottleneck of the European validation authority, EURL ECVAM, which has limited capacities. There are simply not
enough resources. If a validation study is successfully conducted, the process of recommendation by EURL ECVAM begins with consultations with different committees, including the Preliminary Assessment of Regulatory Relevance (PARERE) and the EURL ECVAM Stakeholder Forum (ESTAF) (EURL ECVAM, 2017b). EURL ECVAM then consults with other EC committees, as well as the other international partners for validation and cooperation on the development of alternative test methods. The general public and companies, who would ultimately implement the development, are given time to submit comments. Only after a long period of time is a Test Guideline drafted, and the method is then included in the annexes of the relevant statutory regulation. This sometimes requires 10 years (Hohensee, 2016a). There are, however, instances where the inventor is not interested in validation because it would tie up resources; for example, Ulrich Stock, who developed a borosilicate chamber with a blood-like solution to test heart valves under human-like conditions in 2011 (personal communication, November 2011). Here an agency is needed to oversee proceedings.

3 Part 3: Why Are There Insufficient Animal-free Test Methods and in Which Areas?

3.1 In Which Areas Are There Insufficient Animal-free Test Methods and Why?

In this section, we focus on the field of toxicology alone. Relevant animal-free methods in the field of toxicology could also be applied to other areas. There has been great progress in the development of replacement methods in the area of local toxicity testing (e.g., skin and eye tests), where many animal experiments have already been replaced (AltTox, 2016). However, there are still no replacement methods for long-term studies of the organism as a whole, for example, in the area of inhalation toxicity or tests for reproductive damage and damage caused to offspring. The development of replacement methods for testing environmental toxicity is also only rudimentary.

The goal of the holistic approach is to be able to depict the entire organism in a simplified and miniaturized form. This is a complicated process because individual organs, their biology, and their interactions within the body are highly complex. In order to replace this complex organism in experiments, scientists aim to create a human on a chip, in which all the vital human organs are combined on a chip of miniaturized scale of about 1:10,000. At present, about eight miniaturized organs can be simulated and interconnected (Ingber, 2017). However, to construct a human surrogate for drug screening, scientists
are convinced that it will not be necessary to replicate a perfect human body but simply to provide a better predictive model than animal models (Wang et al., 2016). For such simplified models, many different technical approaches have been developed; for example, according to chip fabrication techniques, medium composition, delivery systems of media, nutrients, oxygen, metabolites, and so forth, have a strong influence on the quality of cell or organ cultures and the results. Wang et al. (2016) provide an exploratory overview of current developments in multi-organ systems and their pro and cons. Since each start-up presently holds only a piece of the entire puzzle, the authors recommend that the multiple companies should be motivated to join forces to combine their techniques and patents, thereby fostering the continued evolution of more advanced products. In many cases, the viability of organoid system cultures cannot, currently, be guaranteed for long-term investigations.

Depending on the laboratory, there are groups whose cell systems can be utilized for just one week (Hohensee, 2017) and others for three months (Epithelix, 2017). These differences may result from the organs’ different needs and the difficulties of recreating miniaturized human organs, especially in the case of such vital organs as the liver or kidneys. Wang et al. (2016) discuss the need for a common culture medium, with full chemical definition, as a blood surrogate that can maintain the viability and function of various organ models and by the use of extracellular matrices can influence the reproducibility and physiologically-realistic ratio of liquid to cell volumes in the (multi-) organ on a chip system.

Methods in the field of inhalation toxicology are advanced with superficial and deeper respiratory epithelium in vitro and with, in some cases, a viability or usability of more than a month (Epithelix, 2017). Lung models have been in use for quite some time (Esch, Bahinski and Huh, 2015; Huh et al., 2010). One method, having achieved general approval, is currently undergoing a validation study in Germany (Hoffmann et al., 2017). However, it was initially intended for replacing animal experiments in the area of acute toxicity. Developments in the area of long-term toxicity are not as advanced and are being explored in a feasibility study within the framework of the EU project cluster, EU-ToxRisk (EU-ToxRisk, 2016).

Recreating a reproductive tract in a multifluidic system is a particular challenge. Some advancements have already been made, such as the development of 3D cell culture models of animal Fallopian tubes, in which embryos can even develop in the “tubular fluid” (Chen et al., 2017). There are models of the vagina and in vitro test systems with ectocervical cells and fibroblasts, which have been developed to test substances for their irritant, toxic, or endocrine disruptive effects (Ayehunie et al., 2016; Landry et al., 2016). An artificial
reproductive cycle has also been created, using murine ovarian follicles *in vitro*, to investigate the mechanisms of reduced fertility (Zhu et al., 2016). First steps have been taken to culture parts of female ovaries, using human follicles, to study the maturation processes (Laronda et al., 2014). There are also initial developments for studying the hormonal cycle, using human ovarian tissue as well as mice and human follicles (Skory et al., 2015). More advanced is a system (EVATAR) to simulate the 28-day hormone profile of the female reproductive tract to study its influence on reproductive tissue (Xiao et al. 2017). However, due to species differences between mice and humans, the use of murine tissue can only be a first step. The goal is to construct models with human-specific tissue material (for an overview see Eddie et al., 2015). A repro-on a chip could be used in the future to recreate mechanistic developments and disorders in the development of the reproductive organs in the field of basic and applied research but not reproductive behavioral disorders or detrimental effects on offspring. Other solutions would have to be found for these. The project cluster EU-ToxRisk is also conducting feasibility studies in the field of developmental and reproductive toxicology (EU-ToxRisk, 2016). Unfortunately, hormonal regulation (feedback loops with hypothalamus, adenohypophysis, and thyroid, which affect the tissues) and immune defense, which would allow the modulation of a miniature human on a chip, are lacking.

What is appealing about this human on a chip technology is that automation could rapidly shorten the time and cost of development by using large rooms filled with robotic systems to simultaneously run tests on a multitude of chips, with different concentrations of a variety of substances, without the disruption of human factors (e.g., introduction of bacteria, measuring errors, or pipetting errors). This would facilitate more targeted, cheaper, and faster development and production and make it more attractive for industry.

3.2 *Why Aren’t Developments Progressing More Quickly in Germany?*

The development of animal-free methods primarily depends on the advancement of knowledge (Linz School of Education, 1999; Schmiel, 2006) as well as other factors, such as efficient methods of investigation and measurement, high-performance research facilities with a workplace environment conducive to creativity, innovative staff, and, most significantly, sufficient funds. The following observations regarding these factors are restricted to Germany.

3.2.1 *Powerful Methods of Investigation and Measurement*

Modern molecular biological and biochemical methods, insights into cell culture, stem cell research, chip technology, omics technologies, computers of high performance, algorithms, potent imaging techniques, and bioprinters
have considerably advanced the development of animal-free testing methods (BMBF, 2016; DFG, 2017). While these powerful methods should also be available to universities and other research facilities, they would involve considerable cost. Several facilities are currently known to need money for 3D bioprinters in order to advance research on non-animal replacements, including the Institute of Veterinary Anatomy of the Free University Berlin. It is possible that some research remains at a certain level of development due to lack of instruments. This situation has been recognized by the German Research Foundation (DFG, 2017). The problem could be addressed by establishing decentralized method centers, whose services would be available to all authorized research institutes. Collaborations with extramural institutes (e.g., Fraunhofer Institutes) also need to be facilitated and, perhaps, expanded.

3.2.2 High-performance Research Facilities with a Workplace Environment Conducive to Creativity

In addition to up-to-date research topics, the workplace environment is also important for the research staff, for example, in terms of hierarchy, recognition, and interdisciplinary collaboration. Recent years have seen progress in this regard. New university courses have also been introduced, some of which are suitable for the development of replacement methods for animal experiments, such as Medical Biotechnology (Technische Universität Berlin, TU-Berlin, 2017), Biomedical Computing (xStudy SE, 2017a), Life Science Engineering (Hochschule für Technik und Wirtschaft Berlin, HTW-Berlin, 2017), Biomedical Engineering (xStudy SE, 2017b), and Biochemistry and Molecular Biology (Universität Bayreuth, 2018). There are increasingly more student exchanges between international partner universities (xStudy SE, 2017a).

3.2.3 Innovative Staff

Understandably, aspiring students and postgraduates, among others, are influenced by their career prospects when choosing suitable fields of study. This choice involves assessing how much return they can actually get on their investment in their course of studies. The best employees go to institutes with the best reputations (“everyone wants to go to Harvard”); institutes that do the best research, who are best known, that have significant influence in the scientific community, that are not economically threatened, and that pay their staff well. For this reason, attractive degree courses in the area of animal-free methods are needed (e.g., Medical Biotechnology at TU-Berlin). There should also be a climate of internationality, enabling an exchange of know-how and strategic thinking in problem solving. At present, there are professorial chairs in Germany, where one can learn animal-free research and testing methods;
however, entire courses of studies are not available. Students often must work with animals or animal organs because they must first complete a standard syllabus (e.g., biology), after which they can attend single courses or complete their theses at the newly installed chairs, in places such as Frankfurt, Düsseldorf, and Konstanz (Buchmann Institute for Molecular Life Sciences, 2017; Hohensee; 2016b; University of Konstanz, 2018).

3.2.4 More Capital from the Corporate Sector Is Necessary

Sufficient capital is important. The development of animal-free test methods in Germany is largely financed by state funding. This means that the risks involved with research and development are borne by society (taxpayers), whilst the returns on successfully developed technologies benefit all stakeholders. It would, therefore, make sense to provide not only national and European funding but also more capital from the corporate sector. European organizations, such as Cosmetics Europe and the European Partnership for Alternative Approaches to Animal Testing (EPAA), are good examples (Cosmetics Europe, 2017; EPAA, 2017). Tax relief for companies that invest in research has been demanded for many years and should be implemented (Verband der Chemischen Industrie, 2017). In the US, there are more ambitious programs leading to better and more innovative scientific outputs. NCATS at NIH and DARPA recently funded a US$150 million program for grants in the field of toxicity testing, drug efficacy evaluation, and disease modeling (Wang et al., 2016). It seems that there is a strong motivation to make the new systems successfully applicable. In Europe, there are no such programs available. Funding programs are fragmented into small individual measures with much smaller budgets. So far, only the Netherlands has clearly expressed its intentions. Other Member States are reserved, expressing themselves at best behind closed doors and referring to their cooperation within the framework of their National Committees. Many researchers who use animal methods likely have little interest in the success of the Dutch plan. Such reservation by Member States could be a barrier towards the success of the plan.

4 Part 4: The Netherlands Makes a Name for Itself

4.1 Why Is the NCad Report a Good Template for a Paradigm Shift?

As of May 2017, NCad has published 108 documents, demonstrating its success (NCad, 2016a); however, the Netherlands’s former Minister of Agriculture, Martijn van Dam, ambitiously pursued “the final goal of full replacement” as set out in Recital 10 of Directive 2010/63/EU (European Parliament, 2010). The
manner in which Dutch politics has responded to the initiative is truly sensational. On April 8, 2016, the Dutch Minister of Agriculture requested that NCad Chairman, Herman Koëter, develops a phase-out timeline for procedures involving animal use. The plan, Transition to Non-Animal Research, was published on December 15, 2016 (NCad, 2016a). The history of its development is as interesting and groundbreaking as the plan itself.

4.2 The Development of the NCad Report

The NCad plan is not the result of a whim but rather the product of years of expert preparation, in which all stakeholders were involved. In June 2014, the Dutch Ministry of Economic Affairs commissioned the expert group, The Think Tank on Supplementary Financing for Alternatives to Animal Testing (De Denktank Aanfullende financiering alternatieven voor dierproeven), to develop recommendations for additional funding for the development of “innovations without laboratory animals” (NCad, 2016a, p. 42,). The Think Tank presented its 140-page report, In Transition! The Netherlands leads the way in laboratory animal-free innovations, in October 2015 (Henneman et al., 2015). Based on this report, on April 8, 2016, the Minister of Agriculture, Martijn van Dam, assigned NCad the task of presenting a strategy for phasing out animal procedures. Van Dam specified that the strategy should involve the National Institute of Public Health and Environmental Protection (Rijksinstituut voor Volksgezondheid en Milieu Netherlands, RIVM). RIVM has significant expertise in toxicity testing and is both national and international coordinator for 3Rs methods. van Dam instructed NCad to name specific phasing-out targets and stated that the legally required toxicity tests should be phased out within 10 years, which would reduce the number of animals used in experiments by 10% in the Netherlands. He also emphasized the goal, formulated in the Think Tank’s recommendations, that the Netherlands become the world leader in laboratory animal-free innovations by 2025 (NCad, 2016b).

NCad conducted two expert workshops on June 9 and July 7, 2016 in cooperation with the RIVM (NCad, 2016a, p. 13). In August 2016, the LinkedIn group, Towards a Future of Scientific Progress Without the Use of Experimental Animals, was founded (Koëter, 2016). As of November 17, 2016, the group had 245 members, but unfortunately it delivered little of substance (NCad, 2016a, p. 51). A public consultation was held on September 8, 2016 in Den Haag, where a broad range of organizations commented on specific recommendations, such as “It is possible to move away from the regulatory animal procedures within the next ten years” (NCad, 2016a, p. 51). The report, Transition to Non-Animal Research, was presented to van Dam and published on December 15, 2016.
4.3 **What Facts and Figures Are Named in the Phase-out Timetable?**

In its report, NCad divided animal experiments into different areas and assessed the possibility of reducing them by 2025. The Netherlands aims to be an international leader in the field of innovation without laboratory animals by 2025 and sees a realistic chance of achieving this goal. Areas and possibilities of reduction, as noted in the NCad report, are:

1. **Regulatory tests:**
   - “The use of laboratory safety testing for chemicals, food additives, pesticides, and (veterinary) medicines can be phased out by 2025 whilst maintaining the existing safety level” (NCad, 2016a, pp. 3, 17).
   - “The use of laboratory animals in regulatory tests for the release of biological products, such as vaccines, will be phased out by 2025 whilst maintaining the existing safety level” (NCad, 2016a, pp. 17–18).
   - Regulatory preclinical tests associated with the registration of new biological substances/products cannot be phased out by 2025. “At this stage, however, due to the complex composition of these products and generally complex mechanism of action, the regulatory preclinical research associated with the registration of new biopharmaceuticals (such as vaccines or monoclonal antibodies) cannot be phased out at the same pace” (NCad, 2016a, pp. 17–18).

2. **Basic scientific and medical research:**
   - Animal experiments in the field of “curiosity-driven basic research cannot be phased out by 2025. Therefore, individual ten-year plans are necessary for each area of research” (NCad, 2016a, p. 15). The complex procedures and interactions in an organism as a whole cannot be simulated at the current time.
   - “Within the field of fundamental scientific research, the reduction or phasing out of the use of animals is not realistic in the short term in all areas of research” (NCad, 2016a, p. 18).

3. **Applied and translational research:**
   - While animal experiments in applied and translational research (implementation of preclinical research in clinical development) cannot be phased out by 2025, the development of replacement methods can be accelerated. This includes investing more in human-specific models and less in animal models. The Netherlands aims to become “an international leader” in this respect (NCad, 2016a, p. 19).

4. **Education and training:**
   - “By focusing on animal-free practices and actively reflecting on the use of laboratory animals in education, the use of laboratory animals for education and training can be significantly reduced” (NCad, 2016a, p. 19).
4.4 Transition Objectives, Transition Strategy and Management of the Transition

NCad has found that there is a realistic chance of completely phasing out animal experiments in the areas of regulatory safety tests (for chemicals, food additives, pesticides, and veterinary and human medical products) and regulatory tests for the release of biological products (e.g., vaccines) by 2025. This requires a transition strategy and management of the transition. The development will not take place on its own. Therefore, we strongly recommend the development of NCad’s transition objectives, transition strategy, and program for transition management.

4.4.1 Transition Objectives

The transition objectives refer to a paradigm shift away from existing mindsets and practices, which are combined with animal use, to a strong focus on innovations without laboratory animals. In regulatory research, this means a significant reduction in the use of laboratory animals; in the field of basic research, the development of a ten-year vision for each area; and in the field of applied and translational research, more rapid progress, for example, through the development of human models for human diseases. Furthermore, the use of animals in education and training can be significantly reduced (NCad, 2016a, pp. 3–4).

4.4.2 Transition Strategy

NCad has stated that the following is necessary for a good transition strategy:
- the use of human data
- international cooperation for a new approach to risk assessment
- multidisciplinary cooperation on the development and approval of replacement methods
- monitoring of the evaluation and dissemination of replacement methods
- monitoring and evaluation of the reduction of animal procedures (since the contribution replacement methods make towards the reduction of the number of animals used in the Netherlands cannot currently be proven)
- development of an innovation index for replacement methods (data warehouse, directory) in collaboration with other countries (NCad, 2016a, p. 24).

4.4.3 Transition Management

The NCad report states that the key to success is (international) collaboration among all stakeholders. The Minister for Agriculture would have the guiding role but also involve other ministries. The existing Interdepartmental Working Group on Alternatives to Animal Procedures would be transformed into an
Interdepartmental Management Group, with the involvement of representatives from several ministries. The Management Group would draft an agenda for the new replacement methods that need to be developed in consultation with all stakeholders.

4.5 Evaluation and Conclusions
An analysis of the 108 reports that led to the decisions presented in the NCad report, showed that the phase-out timeline is not an unrealistic idea. The phase-out timeline is based on the results of two years of intense consultation and work, involving representatives of stakeholders from the fields of science, applied research, contract research, laboratory animal science, medicine, replacement of animal testing, and animal welfare (NCad, 2016c, p. 2). The phase-out timeline was developed with all participating groups; as such, the road map is certainly ambitious but by no means unrealistic. It is, therefore, disconcerting that other EU Member States have either ignored or rejected the Dutch plan, and none have expressed support.

5 The Way Forward
What has to be done to rigorously pursue the “phasing out of animal procedures and the stimulation of innovation without laboratory animals” (NCad, 2016a, p. 3)? With the NCad report, Transition to Non-Animal Research, the Netherlands has not only presented an opinion but has developed a concept for purposefully affecting a paradigm shift. The NCad report contains clear transition objectives, a transition strategy, and a program for transition management. Our association, People for Animal Rights Germany, proposes that the following can be achieved, based on the Dutch concept and including our five pillars and our demand for a master plan for phasing out animal experiments:

- The EU Member States’ National Committees (European Parliament, 2010, Article 49) should endorse the NCad plan. The national responsible ministers (especially the ministers responsible for the animal welfare, science, and research portfolios) should advocate for the adoption of the NCad concept, to encourage the governments of the Member States to implement the Dutch plan in their own countries. Parallel to this, we recommend that the responsible national ministers vote in their respective Councils of Ministers to support the NCad plan at an EU level.
- Based on the national governmental resolutions and the resolutions of the EU Council of Ministers, the EC should adopt the NCad concept as a principle for action for reducing animal testing and funding animal-free
procedures. Political action is also urgently needed to stop the different stakeholders working against each other.

- These objectives can be achieved by the EU Members States’ ministers responsible for the animal welfare, science, and research portfolios, by developing a new approach to assessing the risks posed by substances and asserting this internationally beyond the EU. The pivotal issue is the actual risk (risk assessment) and not the substances’ overall hazard potential (hazard assessment) (NCad, 2016a, p. 20).

- In the short term, the Netherlands intends to compile a priority list for areas of regulatory testing that lack animal-free methods. Other EU Member States should actively support the Netherlands in compiling this list. The EU Member States and the EC should then approve funding programs for these procedures. Funding programs should only be available for animal-free testing methods and provide continuous funding, from development to final validation.

- It is necessary that the responsible ministers in the EU Member States optimize the validation process. The results of methods to be validated must be compared with human data and not with data from animal experiments (NCad, 2016a). The time needed for the validation process should also be shortened.

- The responsible ministers in the EU Member States should agree on the introduction of a monitoring system for quality assurance during the transition process, which would monitor the development and application of animal-free procedures and the phasing out of animal procedures, allowing timely intervention in the case of negative developments.

- The Dutch Minister of Economic Affairs will set up sub-domains in order to draw up ten-year plans for the different areas of basic research. The plans will name realistic objectives for “innovations without laboratory animals” (NCad, 2016a, p. 3). This task is a particular challenge, as basic research has, until now, been regarded as sacrosanct. This makes it all the more important that all EU Member States actively participate in the drawing up of these plans.

6 Final Remarks

We strongly recommend transforming the current plan, *Transition to Non-Animal Research*, proposed by NCad, from an uncoordinated single process to a targeted joint project, in which all stakeholders stand behind the same goals and actively pursue them based on an overall plan. The NCad report describes crucial objectives: ending animal procedures for regulatory tests; listing
and prioritizing the animal-free procedures that need to be developed; and
drawing up ten-year plans for phasing out animal procedures in the different
areas of basic research. Under the leadership of politics, the principles that
underlie the NCad report should be implemented in the EU Member States,
supported by the EC, and ideally in step with international regulatory authori-
ties and scientific bodies. The implementation should be ensured by national
and international project management, accompanied by a monitoring system,
and made transparent to the public. A joint pursuit of the Dutch concept by
the other EU Member States would work towards achieving the goal of Recital
10 of Directive 2010/63/EU to replace animals for scientific and educational
purposes. This requires a common will and joint preparation of individual
plans in European (or even international) workshops with all major stake-
holders, in particular science, industry, and public authorities. The successful
phase out of animal testing in the field of cosmetics, which is gradually tak-
ing place worldwide, as well as current multi-organ on a chip developments
across many countries, have shown that it is possible to achieve these goals
together.

References

AIMBE (2013). Third AIMBE/NIH Workshop – AIMBE. [online] Available at: http://
aimbe.org/events/third-aimbe-nih-workshop/ [Accessed 11 January 2018].

AltTox (2016). Validated & Accepted Alternative Methods. [online] Available at: http://
alttox.org/mapp/table-of-validated-and-accepted-alternative-methods/ [Accessed 6
August 2017].

Ayehunie, S., T. Landry, K. LaRosa, M. Klausner and J. Sheasgreen (2011). Identifica-
tion of Endocrine Disruptors Using an Organotypic Vaginal Tissue Model. SOT 2011,
Abstract #2373. [online] Available at: https://www.mattek.com/reference-library/
identification-of-endocrine-disruptors-using-an-organotypic-vaginal-tissue-
model/ [Accessed 6 August 2017].

Ayehunie, S., Landry, T., Hayden, P. and Klausner M. (2016). Pre-validation of in vitro - in
vivo assays for vaginal irritation. In: Society of Toxicology 2016 Annual Meeting.
[online] Available at: https://www.mattek.com/join-us-at-sot-2016/ [Accessed 6
August 2017].

Baumgartl-Simons, C. (2017). Das Ende muss endlich wirksam verfolgt werden.
Tierrecht, 02, p. 4. [online] Available at: https://www.tierrechte.de/images/stories/
Presse_und_Magazin_Tierrechte/Magazin_2-17_.pdf [Accessed 11 January 2018].

BfR(2017a). NationalerAusschuss Tierschutzgesetz – BfR. [online] Available at:
http://www.bfr.bund.de/de/nationaler_ausschuss_tierschutzgesetz.html [Accessed 2
August 2017].
BfR (2017b). *Fragen und Antworten zum Deutschen Zentrum zum Schutz von Versuchstieren (Bf3R)*. [online] Available at: http://www.bfr.bund.de/cm/343/fragen-und-antworten-zum-deutschen-zentrum-zum-schutz-von-versuchstieren-bf3r.pdf [Accessed 4 August 2017].

BMBF (2011). *Bekanntmachung des Bundesministeriums für Bildung und Forschung von Richtlinien zur Förderung „e:Bio – Innovationswettbewerb Systembiologie“ Vom 17. Dezember 2010*. [online] Available at: https://www.bmbf.de/foerderungen/bekanntmachung.php?B=608 [Accessed 4 August 2017].

BMBF (2015a). *Bekanntmachung des Bundesministeriums für Bildung und Forschung von Richtlinien zur Förderung der “Multilateralen Zusammenarbeit in Computational Neuroscience: Deutschland – USA – Israel – Frankreich” Vom 16. Juli 2015*. [online] Available at: https://www.bmbf.de/foerderungen/bekanntmachung-1068.html [Accessed 4 August 2017].

BMBF (2015b). *Bekanntmachung: Richtlinie zur Förderung von “Alternativmethoden zum Tierversuch“*. Bundesanzeiger vom 24.12.2015. [online] Available at: https://www.bmbf.de/foerderungen/bekanntmachung.php?B=1124 [Accessed 6 August 2017].

BMBF (2016). *Lebenswissenschaftliche Grundlagenforschung Alternativen zum Tierversuch*. [online] Available at: https://www.bmbf.de/de/alternativen-zum-tierversuch-412.html [Accessed 6 August 2017].

Buchmann Institute for Molecular Life Sciences (2017). *BMLS – Drug Delivery and 3R-models*. [online] Available at: https://www.bmls.de/Drug_Delivery_and_3R-Models/people.html [Accessed 6 August 2017].

Bundesministerium für Justiz und Verbraucherschutz (2017). *§46 TierSchVersV – Einzel­norm*. [online] Available at: http://www.gesetze-im-internet.de/tierschversv/__46.html [Accessed 4 August 2017].

Bundesministerium für Wirtschaft und Energie (2017). *Förderdatenbank – Inhalts­verzeichnis*. [online] Available at: http://www.foerderdatenbank.de/Foerder-DB/Navigation/Foerderrecherche/inhaltsverzeichnis.html?get=ea6079646dcbff078c1252af29g6d;views=document&doc=13310 [Accessed 4 August 2017].

CAAT (n.d. a). *CAAT History*. [online] Available at: http://caat.jhsph.edu/about/history.html [Accessed 26 July 2018].

CAAT (n.d. b). *About Us: Center for Alternatives to Animal Testing*. [online] Available at: http://caat.jhsph.edu/about/index.html [Accessed 26 July 2018].

Chen, S., S.E. Palma-Vera, M. Langhammer, S.P. Galuska, B.C. Braun, E. Krause, A. Lucas-Hahn and J. Schoen (2017). An Air-liquid Interphase Approach for Modeling the Early Embryo-maternal Contact Zone. *Scientific Reports, 7*, 42298 [online] Available at: https://www.nature.com/articles/srep42298 [Accessed 12 February 2018].

Committee on Toxicity Testing and Assessment of Environmental Agents (2007). *Toxicity Testing in the 21st Century: A Vision and a Strategy*. [online] Available at: http://
dels.nas.edu/resources/static-assets/materials-based-on-reports/reports-in-brief/
Toxicity_Testing_final.pdf [Accessed 5 August 2017].

Cosmetics Europe (2017). *Promoting Science and Research.* [online] Available at: https://
www.cosmeticseurope.eu/how-we-take-action/promoting-science-research/
[Accessed 4 August 2017].

Cronin, M. (2017). *Non-animal Approaches — The Way Forward.* Report on a Europe-
ean Commission Scientific Conference. Brussels, Belgium. December 6–7, 2016.
[online] Available at: http://ec.europa.eu/environment/chemicals/lab_animals/3t/
pdf/scientific_conference/non_animal_approaches_conference_report.pdf
[Accessed 26 July 2018].

Der Regierende Bürgermeister – Senatskanzlei Berlin (2017). *Senat beschließt Entwurf
des Charité-Vertrages 2018–2022.* Pressemitteilung [online] Available at: https://www.
berlin.de/rbmskzl/aktuelles/pressemitteilungen/2017/pressemitteilung.617676.
php#search=%22Tierversuch*%22 [Accessed 4 August 2017].

DFG (2017). *Hochdurchsatzverfahren: DFG fördert moderne Forschungsinfrastrukturen
in den Lebenswissenschaften.* [online] Available at: http://www.dfg.de/service/
presse/pressemitteilungen/2017/pressemitteilung_nr_28/index.html [Accessed 6
August 2017].

Eddie, S.L., J.J. Kim, T.K. Woodruff and J.E. Burdette (2015). Microphysiological Mod-
eling of the Reproductive Tract: A Fertile Endeavor. *Experimental Biology and
Medicine,* 239(9), pp. 1192–1202.

EPAA (2017). *European Partnership for Alternative Approaches to Animal Testing –
Wachstum – European Commission.* [online] Available at: https://ec.europa.eu/
growth/sectors/chemicals/epaa_de [Accessed 6 August 2017].

Epithelix (2017). *Toxicity of Inhaled Products: Acute, Long Term or Repeated Dose Test-
ing of Drugs/Formulations or Xenobiotics on Reconstructed Human Airway Tissues
(MucilAir)*. [online] Available at: http://www.epithelix.com/services/toxicity-of-
inhaled-products [Accessed 6 August 2017].

Esch, E.W., A. Bahinski and D. Huh (2015). Organs-on-Chips at the Frontiers of Drug
Discovery. *Nature Reviews Drug Discovery* 14, pp. 248–260.

Ettel, A. (2018). *Es wird nicht möglich sein, Tierversuche zu ersetzen.* Welt. [online] Avail-
able at: https://www.welt.de/wirtschaft/article1734933400/Tierversuche-Laengst-
nicht-alle-Tests-dienen-der-Forschung.html [Accessed 13 Feb. 2018].

EU-NETVAL (2018). *EU-NETVAL* (European Union Network of Laboratories for the
Validation of Alternative Methods). [online] Available at: https://eurl-ecvam.jrc.
ce.europa.eu/eu-netval [Accessed 12 February 2018].

EU-ToxRisk (2016). *EU-ToxRisk — An integrated European “Flagship” Pro-
gram Driving Mechanism-based Toxicity Testing and Risk Assessment for the
21st Century.* [online] Available at: http://www.eu-toxrisk.eu/ [Accessed
2 August 2017].
EURL ECVAM (2017a). *European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM).* [online] Available at: https://eurl-ecvam.jrc.ec.europa.eu [Accessed 4 August 2017].

EURL ECVAM (2017b). *EURL ECVAM Recommendations.* [online] Available at: https://eurl-ecvam.jrc.ec.europa.eu/eurl-ecvam-recommendations [Accessed 6 August 2017].

European Parliament (1986). Council Directive of 24 November 1986 on the Approximation of Laws, Regulations and Administrative Provisions of the Member States Regarding the Protection of Animals Used for Experimental and Other Scientific Purposes (86/609/EEC). *Official Journal of the European Communities,* L 358/1, p. 5. [online] Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31986L0609&from=EN [Accessed 5 March 2018].

European Parliament (2010). 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 Communities,* L 276, p. 33–79. [online] Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063 [Accessed 3 August 2017].

FELASA (n.d.). *FELASA.* [online] Available at: http://www.felasa.eu/ [Accessed 3 August 2017].

Hartung, T. (2015). Food for Thought ... The Human Whole Blood Pyrogen Test – Lessons Learned in Twenty Years. *ALTEX,* 32 (2), pp. 79–100. [online] Available at: http://altweb.jhsph.edu/altext/32_2/FFT.pdf [Accessed at 12 Feb. 2018].

Henneman, P., van P. Megchelen, M. Pijnappel, H. Smid, J. Wijnja and M. Zuidgeest (2015). *In transitie! Nederland internationaal toonaangevend in proefdiervrije innovaties.* [online] Available at: http://library.wur.nl/WebQuery/title/2102149 [Accessed 26 July 2018].

HESPEROS (2017). *Team.* [online] Available at: http://www.hesperosinc.com/team/ [Accessed 10 January 2018].

Hildermann, L. (2015). *Die EU-Tierversuchsrichtlinie und ihre Umsetzung in nationales Recht: unions- und verfassungsrechtliche Aspekte.* Dissertation. Universität Trier.

Hochschule für Technik und Wirtschaft Berlin, HTW-Berlin (2017). *Bachelor-Life Science Engineering.* Official Website Hochschule für Technik und Wirtschaft Berlin. [online] Available at: http://lse-bachelor.htw-berlin.de/studium/ [Accessed 6 August 2017].

Hoffmann, S., N. Möhle, M. Auferheide, D. Steinritz, A. Tsoutsoulopoulos, A. Breit and O. Krischenowski (2017). *Validation of the CULTEX RFS Method to Assess the Acute Inhalation Toxicity of Dusts.* [online] Available at: https://www.researchgate.net/project/Validation-of-the-CULTEX-RFS-method-to-assess-the-acute-inhalation-toxicity-of-dusts [Accessed 6 August 2017].
Hohensee, C. (2015). Fachgespräch zu Tierversuchsetzverfahren im Deutschen Bundestag. [Blog] InVitro+Jobs. Available at: http://www.invitrojobs.com/index.php/de/neuigkeiten/news-archiv/item/1835-fachgesprach-zu-tierversuchsetzverfahren-im-deutschen-bundestag [Accessed 4 August 2017].

Hohensee, C. (2016a). Berlin: Podiumsdiskussion in der Urania. [blog] InVitro+Jobs. Available at: http://www.invitrojobs.com/index.php/de/neuigkeiten/news-archiv/item/2148-berlin-podiumsdiskussion-in-der-urania [Accessed 6 August 2017].

Hohensee, C. (2016b). CERST – Auftaktveranstaltung in Düsseldorf: Vernetzung vorantreiben. [blog]. InVitro+Jobs. Available at: http://www.invitrojobs.com/index.php/de/neuigkeiten/news-archiv/item/1986-cerst-auftaktveranstaltung-induesseldorf-vernetzung-vorantreiben [Accessed 6 August 2017].

Hohensee, C. (2017). Arbeitsgruppe im Portrait: Leibniz-Institut für Arbeitsforschung an der TU Dortmund. [blog] InVitro+Jobs. Available at: http://www.invitrojobs.com/index.php/de/forschung-methoden/arbeitsgruppe-im-portrait/item/2745-arbeitsgruppe-im-portrait-leibniz-institut-fuer-arbeitsforschung-an-der-technischen-universitaet-dortmund-ifado [Accessed 6 August 2017].

Hohensee, C. and C. Brüning (2016). Förderung tierversuchsfreier Verfahren: Gesamistrategiefehlt. Was bringt das Förderprogramm "Alternativen zum Tierversuch"? Interview at tierrecht.de, 03, p. 10. [online] Available at: https://www.tierrecht.de/images/stories/Presse_und_Magazin_Tierrecht/Magazin_3-16.pdf [Accessed 4 August 2017].

Huh, D., B.D. Matthews, A. Mammoto, M. Montoya-Zavala, H.Y. Hsin and D.E. Ingber (2010). Reconstituting Organ-level Lung Functions on a Chip. Science, 328(5986), pp. 1662–1668.

Hurelcorp (2018). Leadership. Michael L. Shuler, Ph.D. [online] Available at: http://hurelcorp.com/shuler.php [Accessed 10 January 2018].

Hüsing, B., F.P. Gruber, H. Reinhold and S. Wydra (2011). Evaluation des Förderschwerpunkts Ersatzmethoden zum Tierversuch. Abschlussbericht 24. Feb. 2011. Karlsruhe: Fraunhofer-Institut für System- und Innovationsforschung (Fraunhofer ISI) Available at: https://www.ptj.de/lw_resource/datapool/_items/item_2542/schlussbericht.pdf [Accessed 4 August 2017].

Ingber, D.E. (2017). Human Organs-on-Chips as Replacements for Animal Testing. [Talk] Science and cocktails. Available at: https://www.youtube.com/watch?list=PL2c_ujuXhAvQOt8KrtERRMjAkJgr-PQ1k&time_continue=193&v=HGoxeVaWzdQ [Accessed 6 August 2017].

Jain, A., R. Barrile, A.D. van der Meer, A. Mammoto, T. Mammoto, K. De Ceunynck, O. Aisiku, M.A. Otieno, C.S. Louden, G.A. Hamilton, R. Flaumenhaft and D.E. Ingber (2018). Primary Human Lung Alveolus on a Chip Model of Intravascular Thrombosis for Assessment of Therapeutics. Clinical Pharmacology and Therapeutics, 103, pp. 332–340.
Koëter, H. (2016). *Blog van de voorzitter.* [Blog] Nationaal Comité advies dierproeven. Available at: https://www.ncadierproevenbeleid.nl/actueel/weblog/weblogberichten/2016/september-2016 [Accessed 6 August 2017].

Krewski, D., D. Acosta, M. Andersen, H. Anderson, J. Bailar, K. Boekelheide, R. Brent, G. Charnley, V. Cheung, S. Green, K. Kelsey, N. Kerkvliet, A. Li, L. McCray, O. Meyer, R. Patterson, W. Pennie, R. Scala, G. Solomon, M. Stephens, J. Yager, L. Zeise and Staff of Committee on Toxicity Test (2010). Toxicity Testing in the 21st Century: A Vision and a Strategy. *Journal of Toxicology and Environmental Health, Part B,* 13(2–4), pp. 51–138.

Laronda, M.M., F.E. Duncan, J.E. Hornick, M. Xu, J.E. Pahnke, K.A. Whelan, L.D. Shea and T.K. Woodruff (2014). Alginate Encapsulation Supports the growth and Differentiation of Human Primordial Follicles Within Ovarian Cortical Tissue. *Journal of Assisted Reproduction and Genetics,* 31, pp. 1013–1028.

Leist, M. (2016). Ohne ausreichende Forschungsförderung kommen Alternativmethoden zu Tierversuchen nicht voran. Eine Debatte der Leibniz-Gemeinschaft: Braucht Forschung Tierversuche?. *Tagesspiegel.* [online] Available at: https://causa.tagesspiegel.de/opendebate/leibniz-debattiert/braucht-forschung-tierversuche/ohne-ausreichende-forschungsfuerderung-kommen-alternativmethoden-zu-tierversuchen-nicht-voran.html [Accessed 4 August 2017].

Linz School of Education (1999). *Der wissenschaftliche Erkenntnisfortschritt. Kriterien der Bewahrung und Verwerfung von Theorien. Methodologien wissenschaftlicher Forschungsprogramme.* Johannes-Keppler-Universität Linz, Abteilung für Bildungsforschung. [online] Available at: http://paedpsych.jku.at:4711/LEHRTEXTE/Schmid99.html [Accessed 6 August 2017].

Maschmeyer, I., A.K. Lorenz, K. Schimek, T. Hasenberg, A.P. Ramme, J. Hüblner, M. Lindner, C. Drewell, S. Bauer, A. Thomas, N.S. Sambo, F. Sonntag, R. Lauster and U. Marx (2015). A Four-Organ-Chip for Interconnected Long-Term Co-culture of Human Intestine, Liver, Skin and Kidney Equivalents. *Lab Chip,* 15/12, pp. 2688–2699.

Miller, N.S. (2017). UCF Startup's Product Could Bypass Drug Tests on Animals. *Orlando Sentinel,* [online] Available at: http://www.orlandosentinel.com/health/os-ucf-body-on-chip-hesperos-20170302-story.html [Accessed 14 January 2018].

National Toxicology Program (2018). *A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States.* [online] Available at: https://ntp.niehs.nih.gov/pubhealth/evalatm/natl-strategy/index.html [Accessed 13 February 2018].

NCad (2016a). *NCad Opinion: Transition to Non-Animal Research.* [online] Available at: https://english.ncadierproevenbeleid.nl/latest/news/16/12/15/ncad-opinion-transition-to-non-animal-research [Accessed 6 August 2017].

NCad (2016b). *Opinion Provided by NCad as to How the Netherlands Can Become a Pioneer in Non-Animal Research.* [online] Available at: https://english.
HOW CAN THE FINAL GOAL OF COMPLETELY REPLACING ANIMAL

ncadierproevenbeleid.nl/latest/news/16/12/15/ncad-opinion-transition-to-non-animal-research [Accessed 6 August 2017].

NCad (2016c). Request for Roadmap for Reducing Animal Procedures. [online] Available at: https://english.ncadierproevenbeleid.nl/documents/publications/16/9/30/letter-ministry-economic-affairs [Accessed 6 August 2017].

NCATS (2017a). About Tox21. [online] Available at: https://ncats.nih.gov/tox21/about [Accessed 6 August 2017].

NCATS (2017b). Tox21 Operational Model. [online] Available at: https://ncats.nih.gov/tox21/about/operations [Accessed 5 August 2017].

NCATS (2018). Tissue Chips for Disease Modeling and Efficacy Testing. [online] Available at: https://ncats.nih.gov/tissuechip/projects/modeling [Accessed 11 January 2018].

Oberverwaltungsgericht Bremen (2012). OVG: 1 A 180/10; 1 A 367/10. Bremen: Supreme Administrative Court, p. 24. [online] Available at: https://dejure.org/dienste/vernetzung/rechtsprechung?Gericht=OVG%20Bremen&Datum=11.12.2012&Aktenzeichen=1%20A%20180/10 [Accessed 3 August 2017].

Oleaga, C., C. Bernabini, A.S. Smith, B. Srinivasan, M. Jackson, W. McLamb, V. Platt, R. Bridges, Y. Cai, N. Santhanam, B. Berry, S. Najjar, N. Akanda, X. Guo, C. Martin, G. Ekman, M.B. Esch, J. Langer, G. Ouedraogo, J. Cotovio, L. Breton, M.L. Shuler and J.J. Hickman (2016). Multi-organ Toxicity Demonstration in a Functional Human In Vitro System Composed of Four Organs. Scientific Reports, 6, p. 20030. [online] Available at: https://www.nature.com/articles/srep20030 [Accessed 5 August 2017].

Organisation for Economic Co-operation and Development, OECD (2017). Integrated Approaches to Testing and Assessment (IATA). [online] Available at: http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm [Accessed 03 August 2017].

Peters, A. and S. Stucki (2012). Rechtsgutachten zu verschiedenen Fragen im Zusammenhang mit der EU-Tierversuchsrichtlinie. University of Basel. [online] Available at: http://www.digit.de/system/files/106/original/Rechtsgutachten_Umsetzung_EU-Tierversuchsrichtlinie.pdf [Accessed 26 July 2018].

Ravenscroft, M.S., K.E. Bateman, K.M. Shaffer, H.M. Schessler, D.R. Jung, T.W. Schneider, C.B. Montgomery, T.L. Custer, A.E. Schaffner, Q.Y. Liu, Y.X. Li, J.L. Barker and J.J. Hickman (1998): Developmental Neurobiology Implications from Fabrication and Analysis of Hippocampal Neuronal Networks on Patterned Silane-Modified Surfaces. Journal of the American Chemical Society, 120(47), pp. 12169–12177.

Redmond, C. (2019). When is an Alternative Not an Alternative? Supporting Progress for Absolute Replacement of Animals in Science. In: K. Herrmann and K. Jayne, eds., Animal Experimentation: Working Towards a Paradigm Change, Vol. 22. Brill Human Animal Studies Series, pp. 656–674. Leiden: Brill.
SATIS (2017a). SATIS – für humane Ausbildung “Ethik-Hochschulranking”. [online] Available at: http://www.satis-tierrechte.de/uni-ranking/ [Accessed 6 August 2017].

SATIS (2017b). Was sagen die Hochschulgesetze?. [online] Available at: http://www.satis-tierrechte.de/wp-content/uploads/2017/01/Was-sagen-die-Hochschulgesetze-1.jpg [Accessed 6 August 2017].

Schaffner, A.E., J.L. Barker, D.A. Stenger and J.J. Hickman (1995). Investigation of the Factors Necessary for Growth of Hippocampal Neurons in a Defined System. Journal of Neuroscience Methods, 62(1–2), pp. 111–119.

Schmiel, U. (2006). Erkenntnisfortschritt in der betriebswirtschaftlichen Steuerlehre. In: Fortschritt in den Wirtschaftswissenschaften. Wiesbaden: Deutscher Universitätsverlag, p. 148.

Schöffl, H., H. Spielmann, F. Gruber, H. Appl, F. Harrer, W. Pfäffler, H.A. Trithart, eds. (2000). Forschung ohne Tierversuche 2000. Wien: Springer, pp. 123–124.

SEURAT-1 (2013). Seurat-1: Towards the Replacement of In Vivo Repeated Dose Systemic Toxicity Testing. [online] Available at: http://www.seurat-1.eu/pages/background.php [Accessed 4 August 2017].

Skory, R.M., Y. Xu, L.D. Shea and T.K. Woodruff (2015). Engineering the Ovarian Cycle Using In Vitro Follicle Culture. Human Reproduction, 30(6), pp. 1386–1395.

Technische Universität Berlin, TU-Berlin (2017). medbt: Studium. [online] Available at: http://www.medbt.tu-berlin.de/menue/studium/ [Accessed 6 August 2017].

Universität Bayreuth, Lehrstuhl für Biochemie (2018). Lehre. [online] Available at: http://www.biochemie.uni-bayreuth.de/de/teaching/index.html [Accessed 6 August 2018].

University of Konstanz (2018). CAAT-Europe, Activities. [online] Available at: https://cms.uni-konstanz.de/leist/caat-europe/activities/ [Accessed 26 July 2018].

Varga, O.E., A.K. Hansen, P. Sandøe and I.A.S. Olsson (2010). Validating Animal Models for Preclinical Research: A Scientific and Ethical Discussion. Alternatives to Laboratory Animals, 38, pp. 245–248. [online] Available at: https://www.infrafrontier.eu/sites/infrafrontier.eu/files/upload/public/pdf/Validating%20animal%20models.pdf [Accessed 12 Feb. 2018].

Verband der Chemischen Industrie (2017). Mehr Mut zur Innovation spürbar – Verband der Chemischen Industrie e.V. (VCI) [online] Available at: https://www.vci.de/themen/bundestagswahl/blog/mehr-mut-zur-innovation-spuerbar-editorial-cr-04-2017.jsp [Accessed 6 August 2017].

Wang, Y.I., C. Carmona, J.J. Hickman and M.L. Shuler (2016): Multiorgan Microphysiological Systems for Drug Development: Strategies, Advances, and Challenges. Advanced Healthcare Materials, 7(2), p. 1701000.

Wyss Institute (2018). Wyss Institute, Wyss Institute at Harvard. [online] Available at: https://wyss.harvard.edu/ [Accessed 26 July 2018].

Xiao, S., J.R. Coppeta, H.P. Rogers, B.C. Isenberg, J. Zhu, S.A. Olalekan, K.E. McKinnon, D. Dokic, A.S. Rashedi, D.J. Haïsenleder, S.S. Malpani, C.-A. Arnold-Murray, K. Chen,
HOW CAN THE FINAL GOAL OF COMPLETELY REPLACING ANIMAL

M. Jiang, L. Bai, C.T. Nguyen, J. Zhang, M.M. Laronda, T.J. Hope, K.P. Maniar, M.E. Pavone, M.J. Avram, E.C. Sefton, S. Getsios, J.E. Burdette, J.J. Kim, J.T. Borenstein and T.K. Woodruff (2017). A Microfluidic Culture Model of the Human Reproductive Tract and 28-day Menstrual Cycle. *Nature Communications*, 8, 14584.

xStudy SE (2017a). *Applied Biology (B. Sc.). Hochschule Bonn-Rhein-Sieg, Sankt Augustin / Rheinbach / Hennef.* [online] Available at: https://studieren.de/applied-biology-hochschule-bonnrheinsieg-sankt-augustin-rheinbach-hennef.studienprofil.t-o-a-101.c-33409.html [Accessed 6 August 2017].

xStudy SE (2017b). *Biomedical Engineering, Master, Universität Lübeck.* [online] Available at: https://studieren.de/biomedical-engineering-universitaet-zu-luebeck.studienprofil.t-o.a-446.c-259.html [Accessed 6 August 2017].

Zhang, B. and M. Radisic (2017). Organ-on-a-Chip Devices Advance to Market. *Lab on a Chip*, 17(14), pp. 2395–2420.

Zhu, J., Y. Xu, A.S. Rashedi, M.E. Pavone, J.J. Kim, T.K. Woodruff and J.E. Burdette (2016). Human Fallopian Tube Epithelium Co-culture with Murine Ovarian Follicles Reveals Crosstalk in the Reproductive Cycle. *Molecular Human Reproduction*, 22(11), p. 756–767.

ZonMw (2017). *Meer Kennis met Minder Dieren — In huis workshop systematisch literatuur onderzoek.* [online] Available at: https://www.zonmw.nl/fileadmin/zonmw/documenten/Corporate/Subsidies/PDF_s/Meer_Kennis_met_Minder_Dieren_-_In_huis_workshop_systematisch_literatuur_onderzoek_2.pdf [Accessed 4 August 2017].