

Research article

Tradition, Not Science, Is the Basis of Animal Model Selection in Translational and Applied Research

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Abstract

National and international laws and regulations exist to protect animals used for scientific purposes in translational and applied research, which includes drug development. However, multiple animal models are available for each disease. We evaluated the argumentation behind the selection of a specific animal model using thematic content analysis in project applications, issued in 2017-2019 in the Netherlands. In total, 125 animal models for translational and applied research from 110 project applications were assessed. Explanations to select a specific model included: the model's availability (79%); the availability of expertise (62%); and the model showing similar disease pathology/symptoms (59%) to humans. Therefore, current selection of a specific animal model seems to be based on tradition rather than its potential predictive value for clinical outcome. The applicants' explanations for the implementation of the 3Rs principles (replacement, reduction and refinement) as to the animal model were unspecific. Replacement was achieved by using data from prior *in vitro* studies, reduction by optimal experimental design and statistics, and refinement by reducing of discomfort. Additionally, due to the need for a test model with high complexity (47%) and intactness (30%), the full replacement of animal models with alternative (non-live animal) approaches was thought unachievable. Without a clear, systematic and transparent justification for the selection of a specific animal model, the likelihood of poorly translatable research remains. It is not only up to the researcher to demonstrate this, as ethical committees and funding bodies can provide positive stimuli to drive this change.

1 Introduction

In the (bio)medical field, the development of new drugs is a journey from “bench-to bedside”, in which effective translation of knowledge takes place from basic science into new treatment options for patients. These treatment options may include options to prevent the onset of diseases, options to (improve) diagnosis, medical devices, or treatment with medicinal products, so called pharmaceuticals. (Woolf, 2008; Fontanarosa and DeAngelis, 2003) However, the translation of knowledge to successful new treatment options, specifically pharmaceuticals, often fails. The failure of drugs in phase II and III clinical trials between 2013-2015 are attributed to lack of safety (24%), lack of efficacy (52%) and for operational, strategic or commercial reasons (24%). (Harrison, 2016) Lack of efficacy is partly attributed to non-predictive animal data. (Godlee, 2018; Pound et al., 2004; van der Worp et al., 2010) The inability of an animal model to predict clinical outcome has several reasons, which can be summarized in poor execution and poor animal model choice. Poor execution comprises poor design, conduct and reporting of the animal studies, leading to false positive results, as well as inadequate feedback of information observed in clinical trials back to the animal model. (Schulz et al., 2016) Poor animal model choice concerns insufficiently taking into account of a different etiology in the animal, animal-human species differences, important clinical endpoints not being available or assessed in the animal, as well as the display of different disease and pharmacodynamic markers in the animal. (Denayer et al., 2014)

The execution issue can be mended by implementing adequate design and reporting of animal studies for which specific guidelines are available. (Percie du Sert et al., 2019; Smith et al., 2018) The animal model choice issue is more difficult to improve as reflected by the following three questions. First, why are specific animal models chosen to test a specific hypothesis? According to the definition of Held an animal model of disease is defined “a living organism in which normative biology or behavior can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans”. (Held, 1980). For most diseases there are many different animal models available, each showing disease pathology and/or symptoms. In these animal models, the disease pathology or symptoms are generated in three main ways: spontaneous, induced (experimental), or via genetic

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modification. In spontaneous models the disease pathology or symptoms develop naturally or as a result of a natural mutation. In induced models the disease pathology or symptoms are induced chemically, biologically or physically in healthy animals. In genetically modified models, the disease pathology or symptoms are a result of genetic modification. (Hau, 2008) Even when similar outcome parameters are assessed, the underlying biology in the animal models is often very different. Therefore, a justification of the selected animal model is warranted. However, the justification for animal model selection in peer-reviewed scientific publications is broad and general, i.e. a model for the disease, a model sharing some markers of the disease, etc. (Veening-Griffioen et al., 2019) This broad wording is of limited value to assess whether the animal model has the highest potential to predict for clinical outcome. Second, is the animal model that is most appropriate to predict clinical efficacy of the intervention selected? Due to the lack of a standardized method to assess which aspects of the underlying biology of the human disease an animal model can simulate, we recently developed a framework to compare animal models. Assessing animal models with this framework allows a more scientifically-grounded justification for the selection of a specific animal model. (Ferreira et al., 2019) However, due to its novelty, this methodology is yet to be implemented. Third, how is responsible use of animals for scientific purposes ensured? Societal pressure to do so has been translated in regulation, like the European Union Directive 2010/63/EU. Article 13 provides guidance on choice of methods and species: *“The methods selected should use the minimum number of animals that would provide reliable results and require the use of species with the lowest capacity to experience pain, suffering, distress or lasting harm that are optimal for extrapolation into target species.”* (European Parliament, 2010)

In order to obtain permission to use animals for scientific purposes in the European Union, a project application must be submitted to the national Central Authority for ethical assessment. About this ethical assessment Directive 2010/63/EU, Article 13 states *“Member States shall ensure that a procedure is not carried out if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognized under the legislation of the Union.”* (European Parliament, 2010). This implicates that during the assessment, animal model should be compared between each other, and procedures are (at least partly) replaced with non-live animal alternative models, i.e. the implementation of the 3R principles replacement, reduction and refinement.

Due to the availability of many different animal models for the same disease, we were interested in the reason why researchers choose a specific animal model to answer their research question. We hoped to identify specifically which aspects of the animal models are important in the domain of translational and applied research for new treatment options for humans.

We evaluated how the considerations for the use of animals for the purpose of translational or applied research (Directive 2010/63/EU, Article 5), particularly the choice of a specific animal model, are reflected in project applications. We selected project applications in the Netherlands¹ as illustrative case within the European Union. However, the project applications forms¹ for animal procedures in the Netherlands (Fig. 1) lack a specific question on the justification (“why”) of the animal model selection. Nonetheless, the application form provides applicants room to explain their choices, e.g. in section 3.4.2 Research strategy in the Main Text: *“Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed”* and in Appendix section 2B Description of animal procedures – Animals: *“Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices”*. The justification of animal model selection is also absent in application forms in other European Union Member States, as confirmed by the National Contact Points (European Commission, 2020). Exceptions are the United Kingdom (*“Explain your choice of animals, model(s) and method(s)”*) and Italy (*“Justify the animal model adopted”*). For other Member States, similar to the Dutch question, a justification for the choice of species is required, i.e. Estonia (*“What is the most appropriate animal species for the purpose of the animal experiment”*), Greece (*“Justify the choice of this species”*), Sweden (*“Motivate the choice of species”*). Outside the European Union, the USA² also asks (*“Justify the appropriateness of the species selected”*).

Recently, the European Parliament published a revised Implementing Decision 2020/569/EU, Annex I, where in the Non-Technical Summary, section Refinement, applicants should specify *“With the new knowledge obtained from this project, are the animal models used still the most appropriate? Please specify per species/model, where appropriate.”* (European Parliament, 2020)

In the Netherlands, the Non-Technical Summary of each project application is published anonymously online³. The corresponding full project applications are proprietary of the individual license holders, and therefore not publicly available.

We aimed to get a better understanding on why (justification) applicants selected a specific animal model as model for the human disease.

2 Material and methods

2.1 Project Applications for Scientific Procedures on Animals

In each European Union Member state, the Non-Technical Summary, in the local language, of each project application is published by the National Central Authority. The requirements for the Non-Technical Summary is described in the Directive 2010/63/EU, Article 43. (European Parliament, 2010) A general outline of the project application form is shown in Figure 1.

The Non-Technical Summary does not contain information on the justification for the choice of the animal model. Therefore, we searched on the institutional websites of all license holders in the Netherlands, listed in the Annual report of the National Inspectorate (Nederlandse Voedsel- en Warenautoriteit, 2018), for availability of full-text publication of the institutional project applications.

¹ <https://www.centralecommissiedierproeven.nl/onderwerpen/aanvraag-vergunning/documenten/formulieren/15/5/18/manual-applying-project-licence> (accessed 7/1/2020)

² <https://olaw.nih.gov/guidance/obtaining-an-assurance.htm> (Accessed 01/02/2020)

³ www.centralecommissiedierproeven.nl

Non-technical summary	Project proposal: Main Text	Appendix: Description of animal procedure
<ol style="list-style-type: none"> 1. General information <ol style="list-style-type: none"> 1.1 Title of the project 1.2 Duration of the project 1.3 Keywords 2. Categories <ul style="list-style-type: none"> Basic research Translational or applied research Regulatory use or routine production Research into environmental protection in the interest of human or animal health or welfare Research aimed at preserving the species subjected to procedures Higher education or training Forensic research Maintenance of colonies of genetically altered animals not used in other animal procedures 3. Project description <ol style="list-style-type: none"> 3.1. Main objective (scientific and/or social relevance) 3.2. Project returns 3.3. Species and estimated numbers 3.4. Anticipated negative consequences for the welfare of the animals 3.5. Anticipated level of discomfort 3.6. Destiny of the animals 4. Replacement, reduction and refinement <ol style="list-style-type: none"> 4.1. Replacement 4.2. Reduction 4.3. Refinement 4.4. General measures taken to reduce negative consequences for the welfare of the animals 	<ol style="list-style-type: none"> 1. General information <ol style="list-style-type: none"> 1.1 Approval number of the Central Authority for Food and Consumer Product Safety (NVWA) 1.2 Name of the licensed establishment. 1.3 Title of the project. 2. Categories <ul style="list-style-type: none"> Basic research Translational or applied research Regulatory use or routine production Research into environmental protection in the interest of human or animal health or welfare Research aimed at preserving the species subjected to procedures Higher education or training Forensic research Maintenance of colonies of genetically altered animals not used in other animal procedures 3. General description of the project <ol style="list-style-type: none"> 3.1. Background (motivation, background and context) 3.2. Purpose (main objective) 3.3. Relevance (scientific and/or social relevance) 3.4. Research strategy <ol style="list-style-type: none"> 3.4.1 Overview of the overall design of the project (strategy). 3.4.2 Basic outline and the type(s) of animal procedures that will be performed. 3.4.3 Describe the coherence between the different components and steps of the project. 3.4.4 List the different types of animal procedures. 	<ol style="list-style-type: none"> 1. General information <ol style="list-style-type: none"> 1.1 Approval number of the Central Authority for Food and Consumer Product Safety (NVWA) 1.2 Name of the licensed establishment. 1.3 Serial number and name of animal procedure 2. Description of animal procedures <ol style="list-style-type: none"> A. Experimental approach and primary outcome parameters B. The animals (species, origin, numbers, life stages) C. Re-use D. Replacement, reduction and refinement E. Repetition F. Accommodation and care G. Location where the animal procedures are performed H. Pain and pain relief I. Other aspects compromising the welfare of the animals J. Humane endpoints K. Classification of severity of procedures L. Method of killing

Sections with most relevant information on
 disease or indication
 motivation of choice

Fig. 1: General outline of project application form in the Netherlands¹

Sections with most relevant information on the disease or indication are marked yellow and sections with most relevant information on the motivation of choice are marked orange.

The data consists of the full-text of project applications for animal procedures issued from 2017 to 2019 by the Dutch Central Authority for Scientific Procedures on Animals (CCD) to Utrecht University, University Medical Center Utrecht, Radboud University Nijmegen and Radboud University Medical Center Nijmegen, the Netherlands. Only these Dutch universities and university hospitals, 4 institutions, voluntarily published the full-text of their approved project applications for animal procedures on their institutional website for transparency reasons. Project applications were in either English or Dutch. In the documents, some information was undisclosed for privacy reasons of employees or protection of intellectual property as regulated via the Dutch Public Access to Government Information Act ('Wet openbaarheid van bestuur' or 'WOB') (Binnenlandse Zaken en Koninkrijksrelaties, 2018), which regulates the disclosure of information by the Dutch government.

We evaluated approved project applications for human unmet medical needs in the domain of translational or applied research. From these project applications the following information (Table 1) was extracted:

Tab. 1: Parameters for data extraction from approved project application forms for translational and applied research

Parameter	Description	Example
modelID	Unique animal model number, consisting of the application number and a serial number; one application can encompass up to five different animal models	#20198365_01; mouse model of food allergy
licenseID	Approved project application number as assigned by the Central Authority (CCD)	#20198365
project_title	The title of the project application as reported in the Non-Technical Summary (NTS); in local language (Dutch)	Preventie van voedselallergie door middel van omega-3 algenolie
project_domain	The purpose of the project application (as defined in Directive 2010/63/EU - Article 5B)	Translational or applied research
project_target	Project (primarily) aims to solve an unmet medical need for humans or non-humans	Unmet medical needs for humans
model_title	The title of the animal model as described in the project application Main Text section 3.4.4 and Appendix section 1.3	#20197585_05; Treatment efficacy studies in MPTP induced PD mouse model
model_language	Language in which the project application is written	English (ENG) or Dutch (NLD)
species	Species used in the animal model	Dog, guinea pig, mouse, pig, etc.
spec_loc	Section in the project application where information on species is described	Non-Technical Summary (NTS), Main Text or Appendix of the approved project application
dis_area	Disease area of the animal model, according to the Human Disorders classification in the Directive 2010/63/EU Implementing decision (European Parliament, 2012)	Cancer, Infectious Disorders, Cardiovascular Disorders, Nervous and Mental Disorders, Respiratory Disorders, Immune Disorders, etc.
dis_loc	Section in the project application where information on disease area is described	Non-Technical Summary (NTS), Main Text or Appendix of the approved project application
model_class	Animal model class; how the (disease) symptoms are obtained in the animal model, according to Hau's classification (Hau, 2008)	Spontaneous, (experimentally) induced, genetically modified, naïve or healthy (non-induced)
class_loc	Section in the project application where information on animal model class is described	Non-Technical Summary (NTS), Main Text or Appendix of the approved project application
intervention_type	Type of the intervention to be used in the animal model	Small molecule, biological, gene- or cell therapy, herbal product, vaccine, medical device or diagnostic tool
intervention_name	Description of the intervention	Wound cover, antibody, drug, etc.
intervention_loc	Section in the project application where information on the intervention is described	Non-Technical Summary (NTS), Main Text or Appendix of the approved project application
model_choice	Explanation for the choice of the animal model	#20197585_04; availability of the model, prior studies and similar disease pathology/symptoms
choice_section	Section in the project application where information on choice of a specific animal model is described	Non-Technical Summary (NTS), Main Text or Appendix of the approved project application
species_choice	Explanation for the choice of a specific species (Appendix, section 2B) <i>"Provide information on species. Justify your choice with respect to the objectives of this particular type of animal procedure."</i>	#20197585_01; PINK1 -/- male rats show a progressive phenotype, on behavioral, pathological and neurochemical measurements
spcchc_loc	Section in the project application where information on choice of species is described	Non-Technical Summary (NTS), Main Text or Appendix of the approved project application
outcome_choice	Explanation for the choice of the primary and secondary outcomes (Appendix, section 2A) <i>"Describe the primary and secondary outcome parameters. Justify the choice with respect to the purpose of the project. For scientific research, these may include clinical parameters and/or experimental data."</i>	#20198365_01: ear thickness following intradermal challenge with the allergen in the ear as measure for the allergic reaction (skin response), serum IgE and mast cell (mMCP-1) levels.
outcome_loc	Section in the project application where information on choice of outcomes is described	Non-Technical Summary (NTS), Main Text or Appendix of the approved project application
replace_choice	Justification for replacement of the animal model. (Appendix, section 2D) <i>"Describe which other options have been taken into consideration and explain why these options were not considered applicable for this project. Explain why the objectives of this project cannot be achieved: without the use of animals; ..."</i>	#20197585_02; It is not possible to obtain the required pharmacokinetic information solely on the basis of <i>in vitro</i> or <i>in silico</i> studies
replace_loc	Section in the project application where information on replacement is described	Appendix of the approved project application
reduce_choice	Justification for reduction of the number of animals. (Appendix, section 2D)	#20197585_02: We are using the absolute minimum number of animals necessary, still enabling reliable results.

	<i>"Describe which other options have been taken into consideration and explain why these options were not considered applicable for this project. Explain why the objectives of this project cannot be achieved: ... ; using another experimental design that requires less animals; ..."</i>	
reduce_loc	Section in the project application where information on reduction is described	Appendix of the approved project application
refine_choice	Justification for refinement of the animal model's procedures. (Appendix, section 2D) <i>"Describe which other options have been taken into consideration and explain why these options were not considered applicable for this project. Explain why the objectives of this project cannot be achieved: ... ; using another experimental design that brings less distress or harm to the animals."</i>	#20197585_02; To minimize the animals discomfort in the subsequent efficacy studies, we are aiming on the administration route with the lowest discomfort.
refine_loc	Section in the project application where information on refinement is described	Appendix of the approved project application

2.2 Thematic Content Analysis of the project application forms

The data collection of the parameters listed in Table 1 was obtained by qualitative content analysis. In brief, this method consists of data collection in a structured way, by extracting the data from the identified sources and grouping these parameters into more general, broader categories. (Elo and Kyngas, 2008) This method consists of data collection in a structured way, by extracting data from identified sources and grouping these parameters into more general, broader categories. This is a way of manually coding information, in which a code is a part of a phrase or words representing a category. We investigated the consistency in argumentation within project applications by comparing the justification of the choice of the animal model to the choice of species or outcome. We used color coding to show the number of project applications using similar argumentation (darker colors indicating high number of project applications and lighter indicating low number of project applications).

To summarize the data, we used R version 3.6.1 and R studio (The R Foundation of Statistical Computing). The scripts are provided in supplementary file S1⁴, paragraph 1.

3 Results

In total, 110 project applications were available for evaluation; 69 from Utrecht University and University Medical Center Utrecht, 41 from Radboud University and Radboud University Medical Center Nijmegen. These project applications consisted of 150 animal models for the purpose of translational or applied research. Of these, a total of 125 animal models were used for human clinical purpose. The project applications were written in English (75%) or Dutch (25%). Raw data are provided in supplementary file S2⁵.

3.1 Distribution of animal models across disease area and animal model class

The mouse, rat and guinea pig were the predominant rodent species (61, 33 and 5 animal models, respectively). Pig and sheep were the predominant non-rodent species (12 and 5 animal models, respectively). Mice and rats were used across disease areas while other species seemed to be used in specific disease areas, such as pigs in diseases of the circulatory system, guinea pigs in diseases of the ear and mastoid process, and sheep for diseases of the musculoskeletal system (see Figure 2). Raw data are provided in Table S1⁴.

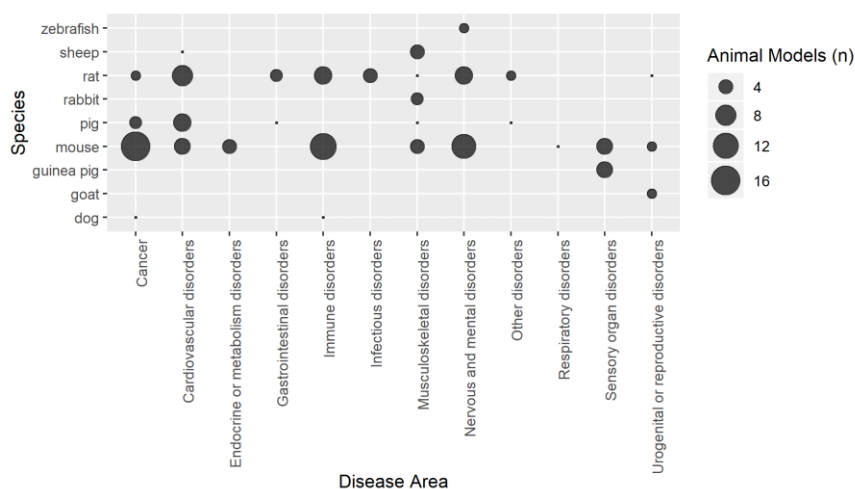


Fig. 2: Distribution of animal models across disease areas and species

Disease area classification was according to the Human Disorders classification in the Directive 2010/63/EU Implementing decision (European Parliament, 2012). The bubble size represents the number of animal models with a specific species in a specific disease area.

⁴ doi:10.14573/altex.2003301s1

⁵ doi:10.14573/altex.2003301s2

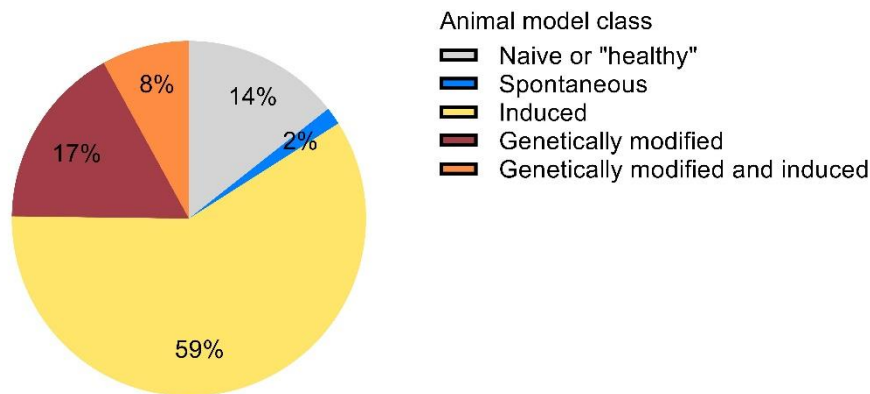


Fig. 3: Proportion of animal model in different animal model classes
Animal model classification, i.e. how the (disease) symptoms are obtained in the animal model, according to Hau's classification (Hau, 2008).

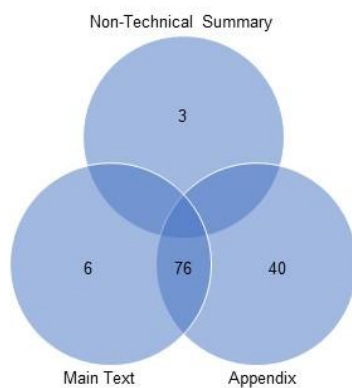


Fig. 4: Justification of animal model choice across different sections of the project applications

Different sections of the project application are: the Non-Technical Summary, Main Text and the Appendix describing the animal model showed. Data (numbers) is the number of animal models in a specific section of the project application, where the explanation for the choice of the animal model was given. The data in the cross-sections indicate justification in more than one section of the same project application.

Animal models were classified according to the classification of Hau. (Hau, 2008) In 84% of the animal models, disease phenomena were induced chemically, biologically, physically or by genetic modification, in 2% the disease phenomena developed spontaneously, while in 14% of the animal models the interventions were tested in healthy animals (Figure 3). Raw data are provided in supplementary file S2⁵.

The following intervention types were tested in the animal models we studied: diagnostic tools (24%), small molecular compounds (23%), medical devices (21%), biologicals (19%), gene- and cell-based therapies (18%), herbal products (2%) and undisclosed (3%). The intervention type 'diagnostic tools' were the interventions used to diagnose or detect disease state (e.g. MRI, PET/SPECT, ultrasound, radiolabeled antibody, etc.). The intervention type 'medical device' were interventions which were able to facilitate the cure of the disease (e.g. ultrasound to remove plaques, wound-sealant, joint distraction, etc.). Whether the interventions were novel or existing, used to test a theory or compared to other interventions was not assessed.

3.2 Selection of animal models, species and outcome measures

The project application form (Figure 1) did not contain a specific section in which the applicant should justify the choice of a specific animal model. The section of the project application where the explanation of animal model choice was given, was found across the Non-Technical Summary, Main Text, the Appendixes and in both Main Text and Appendixes, see (Figure 4).

Thematic content analysis of the full text of the project application forms, i.e. the Non-Technical Summary, Main Text and the Appendix describing the animal model showed that animal models were mainly chosen because of the availability of the model (79%); i.e. the animal model existed, was used or described earlier; the availability of expertise (62%); similar disease pathology/symptoms (59%) (Figure 5). Specific examples are presented in Table 2. Raw data are provided in supplementary file S2⁵.

Within individual project applications, we evaluated the correlation between the choice of a specific animal model (in any section of the project application) in relation to the justification of the choice of a specific species (in section Appendix 2B of the project application) or outcome (in section Appendix 2A of the project application). In individual project applications, similar explanations were given for the choice of a specific animal model as well as the choice of species (Figure 6) or the choice of outcome (Figure 7). Only one animal model referred to a disease-specific guideline for the choice of outcome measures. Specific examples of applicants' explanations are shown in Table 3 (species) and Table 4 (outcomes). Raw data are provided in Table S2⁴ (model vs species) and Table S3⁴ (model vs outcome).

3.3 Implementation of 3Rs in project application forms

Filing a project application for animal procedures does not discharge applicants from the obligation to describe the implementation of the 3Rs principles replacement, reduction and refinement. The applicants justified their choices in unspecific (general) phrasing (Figure 8). Specific examples are shown in Table 5.

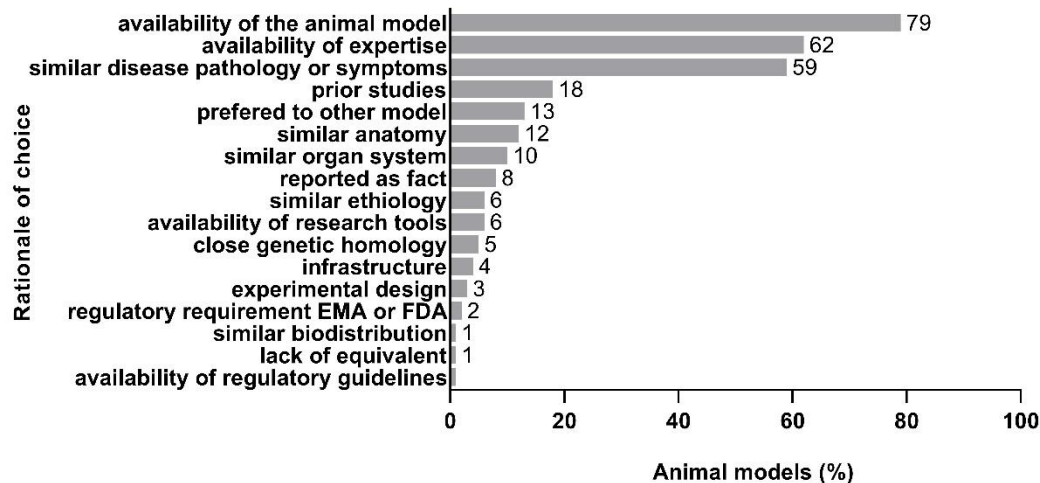


Fig. 5: Justification for choice of a specific animal model in project applications

Applicant's justification for the choice of a specific animal model, grouped per indicated keywords (y-axis). Fraction (%) of animal models with a specific justification, an individual animal model could have used more than one of the indicated keywords. Specific examples are presented in Table 2. Raw data are provided in supplementary file S2⁵.

Tab. 2: Justification for the applicants' choice of a specific animal model

Applicant's justification for the choice of a specific animal model, grouped per indicated keywords of which some examples are given.

Explanation	Example
availability of the animal model	"The procedures we are planning to use have shown to be successful in goats as published by ..." #20173344_01 "The mouse was selected because immunodeficient strains are available that allow growth of human tumor xenografts. Mice are commonly used" #20174286_02
similar disease pathology or symptoms	"Full thickness skin defects are used to mimic typical wounds seen in the clinic." #20171825_01 "Animal with closest genetic homology to humans... There are various methods to induce the most important aspects to consider are neuropathological and electroencephalographic features that should be replicated in mice as that seen in human patients. Model characterized for its aspects of ... closeness to human ... cell morphology and animal behavior." #20184646_02 "The brains of mice and rats have relatively conserved neuroanatomy compared with human brains, including Involvement of other organs and systems including the enteric system, the olfactory system, and the innate and adaptive immune systems, can be easily studied in mouse and rat model of PO. They include acute toxin models, such as Some toxin models are well established and have been widely used to test treatment of motor symptoms, but they do not replicate the progressive nature seen in human PD. Genetic models overcome some drawbacks of acute models and can recapitulate specific features of PD such as the progressive nature, however it is unlikely that one model can mimic all features of human PO. Although one model is currently not able to recapitulate PD as seen in humans, it is important to target and investigate different aspects of this complex neurodegenerative disease by using multiple models, it allows us to model the PD phenotype better." #20197585_05
availability of expertise	"In our department, we performed already extensive research on selected targets and treatments, and we have over 30 years of experience with mouse models for Furthermore, we have all equipment and tools available to analyze the therapeutic effects of these therapies in our animal models, and we have fruitful collaborations with...." #20173164_02 "We have an excellent expertise in the field of ...The ... model, described in this application is established and validated. We are well trained in the procedures involved in the study. The study will be performed in collaboration with a research group in, ensuring knowledge transfer." #20184568_01

Implementation of the 3Rs was achieved for replacement (Figure 8A) by the usage of prior studies (54%), mainly *in vivo*, *in vitro* or *ex vivo* prior studies. Reduction (Figure 8B) was achieved by obtaining the optimal experimental design of the experiments (59%), largely due to an optimal combination of readouts and substantiated statistics (54%), mainly through statistical power calculation. Refinement (Figure 8C) was achieved by reducing the degree of discomfort (85%) and using the best available methods (42%). The reduction of discomfort was mainly achieved by providing the best pre- and post-procedure care, and the use of best available methods was achieved due to the applicants' expertise, or access to expertise via collaborations.

Main reasons why applicants thought or assessed the full replacement of the animal model with alternative (non-live animal) approaches insufficient were the requirements of (Figure 9): the complex interaction between cells, tissue and environment (47%), an intact and functional (organ) system (30%), biodistribution (15%), or the only way to obtain the information or the only way to obtain the material (10%), and the use of the lowest possible species in class (4%). Raw data are provided in supplementary file S2⁵.

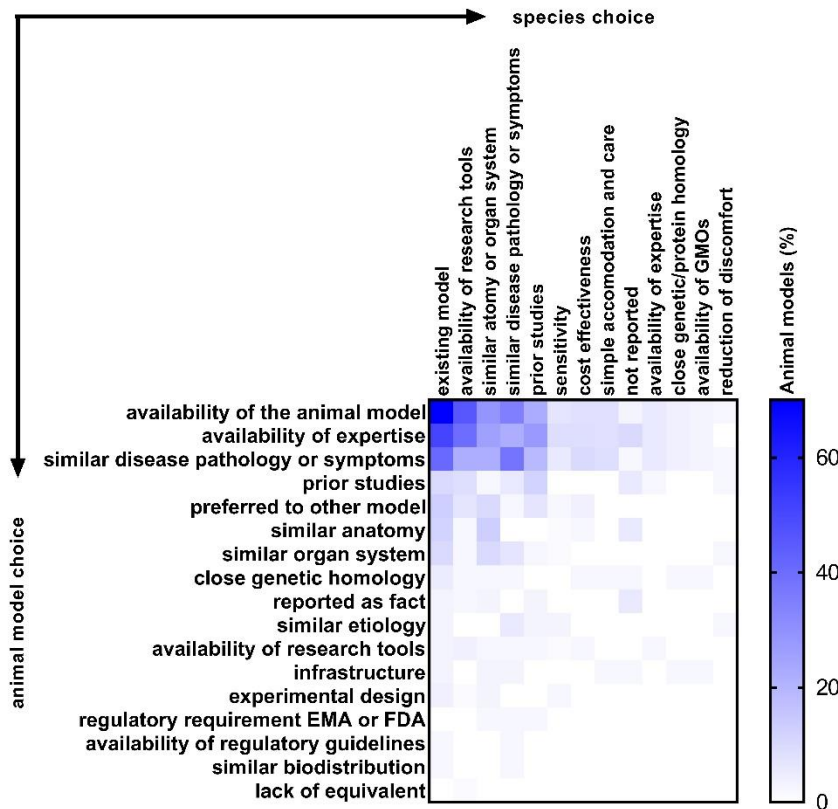


Fig. 6: Correlation between animal model- and species choice in individual project applications

Any information given by the applicant for the choice of a specific animal model (rows) and the choice of a specific species (columns), within the same project application, grouped per indicated keywords. The gradient is the fraction (%) of animal models with an identified correlation.

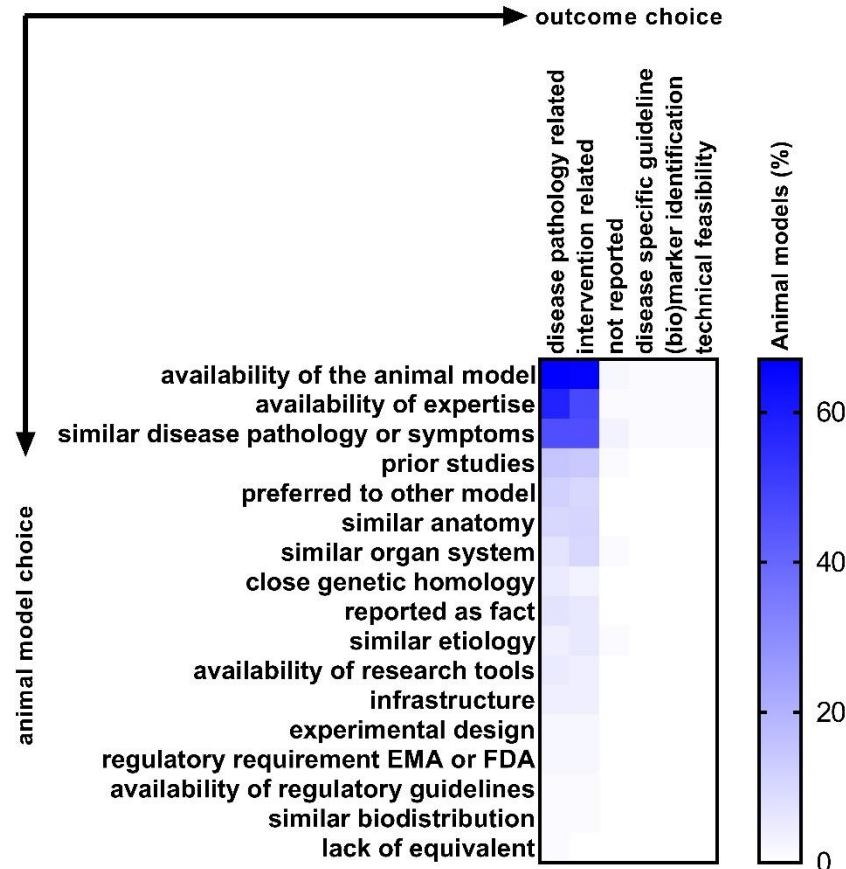


Fig. 7: Correlation between animal model and outcome choice in individual project applications

Any information given by the applicant for the choice of a specific animal model (rows) and the choice of a specific outcome (columns), within the same project application, grouped per indicated keywords. The gradient is the fraction (%) of animal models with an identified correlation.

Tab. 3: Examples of applicants' explanation for the choice of a specific species

Applicant's justification for the choice of a specific species, as reported in project application - Appendix, section 2B, grouped per indicated keywords of which some examples are given.

Explanation	Example
availability of the animal model	"The canine species is considered to be a suitable model to study intrinsic cartilage repair." #20173964_01 "We will carry out the future tests in the models as we have validated them in the past." #20198365_01
availability of research tools	"Standard human diagnostic and interventional devices and imaging equipment can be used. The relative size of the model allows accurate visualization." #20173424_01 "Rat as a model system are chosen since the size and loading capacity of the microspheres limits the needles size and injection volume. Whereas in a murine knee joint only 6-10ul can be injected, the rat joint enables to inject 60-100ul with a larger needle." #20185824_01
similar anatomy or organ system	"The pig has similar anatomy and (cardio)vascular physiology to humans and is the preferred model for cardiology procedures." #20173424_01 "The sheep model is an excellent animal model for orthopedic research...similar bone composition and bone sizes as humans.." #20186348_01
similar disease pathology or symptoms	"Like humans, dogs suffer for OA and there are well described and validated canine models for OA, including the Groove model employed for this project. The dog serves as a preclinical model for humans, what it serves also the veterinary patient." #20173964_01 "Two transgenic DMI mouse models are available: DMSXL, which have symptoms of skeletal muscle and of the central nervous system. These mice are the only model with clear behavioral defects related to the CNS involvement. The transgene contains the human DMPK locus with an expanded repeat, which very closely mimics the situation in DMI patients. HSALR mice, which have a strong phenotype, but exclusively in skeletal muscle. These mice are best suited for EMG measurements of myotonia. The transgene contains an expanded repeat like in DMI, but in the context of the human skeletal actin gene instead of the DMPK gene." #20186204_04

Tab. 4: Examples of applicants' explanation for the choice of a specific outcome measure

Applicant's justification for the choice of a specific outcome, as reported in project application - Appendix, section 2A, grouped per indicated keywords of which some examples are given.

Explanation	Example
disease pathology related	"Tail cuff pressure and blood urea will also be measured at multiple time points after scaffold implantation. At the end-point, under full anesthesia, kidney function will be assessed and intra-arterial catheters will enable us to measure the drop in pressure over the scaffold while a flow probe will enable u to measure flow through the scaffold." #20173344_02 "Primary outcome parameters will be histology (joint assessment), micro-CT and/or imaging (VHH visualization). Other outcome parameters may include: blood components (cells, inflammation markers, drug concentration), synovial fluid components, GAG distribution, RNA expression, AvVFtest and dynamic weight bearing of hind paws (pressure plate measurements)." #20185184_01 "A primary outcome can be based on the mapping of labeled neurons and projections, for example the average distance from the injection site of all labeled neurons projecting to the brain area of interested. Or morphological characteristics of dendrites or axonal tracks." #20197585_04
Intervention related	"Detection of cracks, fractures and/or material deformation of the implanted CPC, by in vivo imaging procedures; analysis of the bone apposition and formation in the region of interest by histomorphometric procedures; determination of the flexural strength and toughness of the explanted hemi-mandibles by means of a tensile bench set-up." #20173828_01 "The primary outcome parameter is biodistribution, as measured by fluorescence intensity or other methods such as hybridization-ligation." #20186204_02
disease specific guideline	The combination of these parameters together provides insight into the tissue sensitivity of the plaque, as described in the guidelines of the American Heart Association (PMID: 7648691). #20198024_02

4 Discussion

We assessed project applications for animal procedures in the Netherlands. In the domain of translational and applied research, we found that the applicant's justification of animal model selection was based mainly on the availability of the model, the availability of (or access to) expertise and/or the presence of disease-related symptoms (Figure 5). While the ultimate goal of any project application is to develop new treatment options for a specific disease, the choice of animal model outcomes (Figure 7) was not necessarily driven by diagnostic or prognostic outcome markers or regulatory guidelines, as published by EMA or FDA. In this regard, Langhof and colleagues recently published a summary of available clinical guidelines per drug class (Langhof et al., 2018), which could give more focus on relevant outcome markers for pre-clinical research.

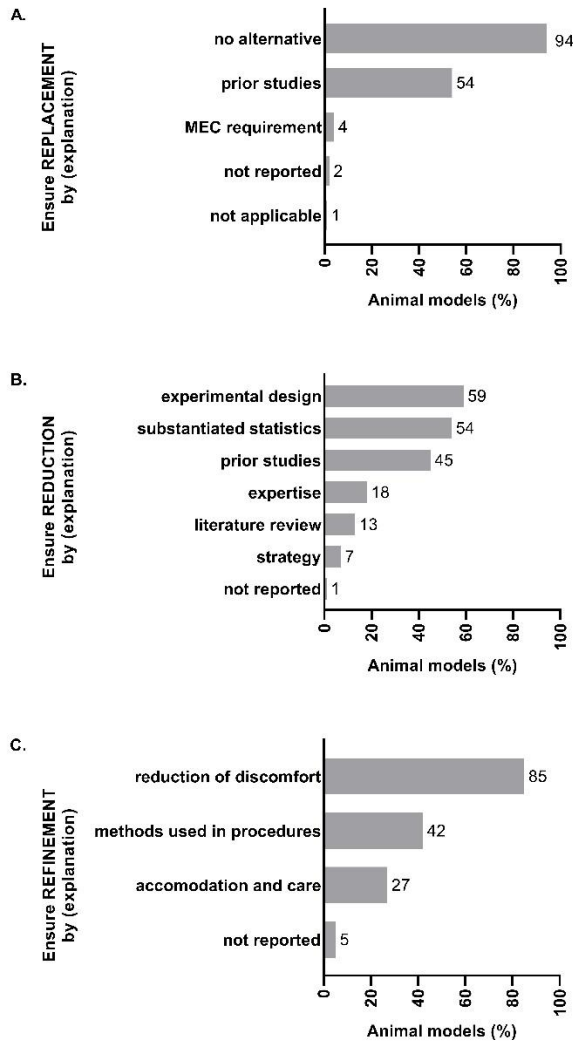


Fig. 8: Foreseen activities to implement replacement, reduction and refinement

Thematic content analysis of any information given by the applicant in the project application appendix, section 2D, of other options which have been taken into consideration with regard to replacement (A), reduction (B) and refinement (C) of the animal model, grouped per indicated keywords, an individual animal model could have used more than one of the indicated keywords. Data (numbers) is the fraction (%) of animal models with a specific explanation.

Tab. 5: Examples of applicants' explanation for implementation of the 3R principles: replacement, reduction and refinement

Applicant's justification for the implementation as reported in project application - Appendix, section 2D grouped per indicated keywords of which some examples are given. Numbers are the fraction of animal models with a specific explanation.

3R principle	Explanation	Examples
replacement	<ul style="list-style-type: none"> no alternative - complex interaction between environmental factors, cells or tissue (47%) - intact and functional (organ) system (30%) - kinetics or biodistribution (15%) - obtain required information or material (10%) - lowest possible species (4%) prior studies - <i>in silico</i>, <i>in vitro</i> or <i>ex vivo</i> pre-screening (54%) - literature review (2%) - <i>in vivo</i> (pilot) studies (1%) MEC requirement - allowing clinical trial (4%) not reported (2%) not applicable (1%) - obtain required information or material (1%) 	<p>"There are no ex-vivo systems available that can mimic complex interactions between immune cells, brain cells and fatigue in a rheumatic disease such as" #20174167_01</p> <p>"Replacement not possible since the entire peripheral hearing organ must be intact and functional in and including the connection to the root stem through the auditory nerve." #20174315_04</p> <p>"Any chelate will first be characterized extensively in vitro. Only chelates and methods with promising characteristics will be applied in animals to study their in vivo characteristics." #20173885_03</p> <p>"And before a new therapy can enter clinical evaluation pre-clinical in vivo testing is required." #20174288_01</p>

reduction	<p>experimental design</p> <ul style="list-style-type: none"> - optimal combination of readouts (43%) - use of one or both sexes (9%) - essential treatments groups (6%) - method of induction (3%) - method of readout (3%) <p>substantiated statistics</p> <ul style="list-style-type: none"> - statistical power calculation (30%) - use of statistical methods (13%) - sample size calculation (7%) - consult (bio)statistician (5%) <p>prior studies</p> <ul style="list-style-type: none"> - <i>in vivo</i> (pilot) studies (28%) - <i>in silico</i>, <i>in vitro</i> or <i>ex vivo</i> pre-screening (20%) - optimal combination of readouts (1%) - relevant information (1%) <p>expertise</p> <ul style="list-style-type: none"> - experience with model or methodology (16%) - expert collaborations (3%) <p>literature review</p> <ul style="list-style-type: none"> - relevant information (8%) - optimal combination of readouts (4%) - build on existing literature (1%) <p>strategy</p> <ul style="list-style-type: none"> - go/no-go strategy (7%) not reported (1%) 	<p>"As many readout parameters as possible will be combined within one animal without increasing the discomfort. Furthermore, our strategy, will guarantee a careful decision process before continuing with the next step in the research plan." #20173164_02</p> <p>"Finally,, a power calculation will be performed ... if necessary, experienced pre-clinical researchers and biostatisticians will be consulted ..." #20173828_01</p> <p>"In addition, only the that demonstrate to have the most favorable <i>in vitro</i> performance ... will be selected for the animal experiments..." #20173828_01</p> <p>"Our technicians will be trained by experts in the field...resulting in a steep learning curve...Our laboratory has a long history of ... in rodents." #20184985_01</p> <p>"Our technicians will be trained by experts in the field...resulting in a steep learning curve...Our laboratory has a long history of ... in rodents." #20184985_01</p>
refinement	<p>reduction of discomfort</p> <ul style="list-style-type: none"> - (pre and post) operative care (58%) - expertise (21%) - duration of the study (4%) - equipment (4%) - route of administration (4%) - prior (pilot) studies (2%) - terminal anesthesia (2%) - best reagents and protocols (1%) - experimental design (1%) <p>methods used in procedures</p> <ul style="list-style-type: none"> - expertise (28%) - best reagents and protocols (8%) - prior (pilot) studies (6%) - experimental design (6%) - lowest possible species (2%) - equipment (1%) <p>accommodation and care</p> <ul style="list-style-type: none"> - social housing (15%) - cage enrichment (14%) - expertise (3%) not reported (5%) 	<p>"During surgical procedures, anesthesia an analgesia is applied to reduce the discomfort...pain control...softened food after the operation...state-of-the-art facility... specialized in housing of ... animals." #20185825_01</p> <p>"We try to minimize discomfort and inefficient use as much as possible." #20186348_05</p> <p>Only experienced personnel will carry out ... several techniques have been initialized and optimized within our department." #20184785_01</p> <p>"Most of the used <i>in vivo</i> models have already been optimized and routinely used in the lab.... We have expertise in this field of research...Since literature showed that this is sufficient to induce .." #20186349_01</p> <p>"Where possible, animals will be housed socially. If animals were to be housed individually, it would be for a maximum of 5 weeks" #20173846_01</p> <p>"To reduce anxiety, mice will be housed in groups and not individually." #20173885_05</p>

Next, filing a project application for animal experiments does not discharge the researcher from implementing the 3Rs principles, replacement, reduction and refinement in these animal models. The applicants must demonstrate⁶ that aspects of the 3Rs principles have been considered and implemented where possible (Figure 8) in accordance with Directive 2010/63/EU, Article 13 (European Parliament, 2010). However, the phrasing in the project applications on how this was achieved was overall unspecific, and did not meet specific, measurable, achievable or timely (SMART) criteria. Therefore, it was not possible to ascertain that the applicants were compliant with the Directive. For example, applicants proposed to perform a literature research, but none of the applications included information on databases (e.g. PubMed, Web of Science,

⁶ <https://www.ncadierproevenbeleid.nl/dierproeven-en-3V-methoden/themas/vervanging-vermindering-en-verfijning> (Accessed 24/02/2020)

EMBASE) or a description of the search string used, inclusion or exclusion criteria, etc., nor the results of these searches were reported and discussed. Also, applicants intended only to proceed with their interventions in animal studies, with promising *in vitro* candidates, as well as the use of experts and best reagents and protocols, but did not include any qualification criteria for these attributes.

Interestingly, the applicant's gave explanations for not being able to (fully) replace the animal experiment with a non-life animal alternative model. Explanation were the requirement of the model to comprise of complexity or the requirement of an intact system in order to answer the research question (Figure 9). This suggests that either researchers are not aware of appropriate non-live animal alternative models, non-live animal alternative models are currently not good enough to be able to fully replace the animal models, or that not all animal models are eligible for full replacement at all. When looking at the total number of animal experiments in the Netherlands, we did not see a strong reduction of the total number of animal experiments in the Netherlands the last three years, despite the strong governmental endorsement of development of animal free innovations (Nederlandse Voedsel- en Warenautoriteit, 2018;⁷).

Overall, in all project applications it remained unclear whether the selected animal model was the model with the highest likelihood of predicting the clinical outcome. A clear justification and scientific discussion on "why" and based on "what" specific animal models or outcomes are chosen was often absent, suggesting the applicants selected their animal model based on a "trust me" (tradition) rather than a "show me" approach. These tradition-driven approach in animal model selection, is described by Innovation scientist Kooijman as 'lock-in', i.e. that the use of animals to predict events in humans, are 'locked-in' because they are embedded in a well-aligned set of institutions, such as regulations, norms and values, that are taken for granted and are normatively endorsed. (Kooijman, 2013) This 'lock-in' slows down innovations in this area.

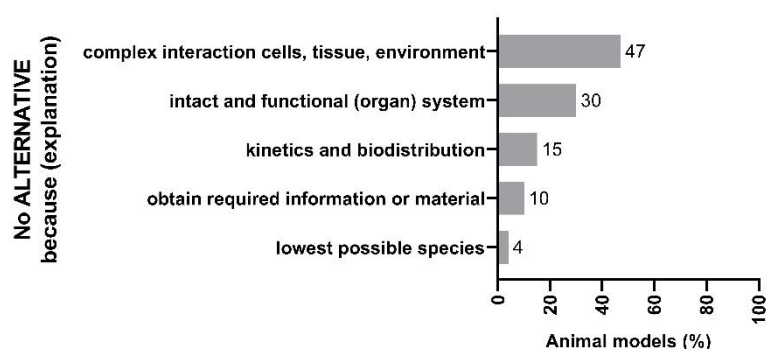


Fig. 9: Justification of the applicants' inability to replace the animal model with a non-live animal alternative model
Applicant's justification, given in project application Appendix section 2D, of other options which have been taken into consideration with regard to replacement of the animal model, grouped per indicated keywords. Fraction (%) of animal models with a specific explanation.

4.1 Recommendations for applicants

This reported lack of detailed information and tradition-driven way of working, i.e. "I am an expert" or "I used this model before", is not unknown in the field of animal experimentation. Herrmann and Flecknell also showed this tradition-driven way or working for the regimens used for anesthetics and analgesia, as well as humane endpoints (Herrmann and Flecknell, 2018, 2019).

To improve the value of animal models in drug development several steps could be taken. First, a clear and scientific justification should be made for the appropriateness of the animal model, in relation to other models for the same indication, as well as their expected predictive value for human outcome. A standardized methodology, like the Framework to Identify Models of Disease (FIMD), is a way to facilitate this process. (Ferreira et al., 2019). FIMD addresses the following aspects of animal models in a standardized way: what is known of the biology of the human disease, how is this reflected in the animal model, is the disease target similar, and is the target similarly regulated in the animal compared to humans, why are particular parameters measures, how similar and relevant are they for the human disease, what is the effect of known human effective and ineffective interventions, etc. Second, to improve the quality of animal studies. This could be achieved by the mandatory use of study design protocols, preregistration tools and correct reporting. (Smith et al., 2018; Macleod and Mohan, 2019). The scientific community is increasingly aware of the need for more explicit and transparent reporting of animal research in scientific publications, leading to the publication of reporting standards such as the ARRIVE reporting guidelines (Kilkenny et al., 2010). Unfortunately, although the use of the ARRIVE guidelines is endorsed by many scientific journals, the reporting quality of animal studies has not improved (Leung et al., 2018) neither did the level of reproducibility of animal studies (Freedman et al., 2015). At least correct reporting should be checked by journals before publication. Third, an increase transparency of animal research for scientific purposes is needed. There is a public perception that animal research is secretive, and thus not transparent enough. (Pound and Blaug, 2016; Leaman et al., 2014). Although part of the data obtained from animal studies are available via publications of the results from animal studies in scientific journals, most of data generated by industry is not published. Due to the implementation of Directive 2010/63/EU, the Non-Technical Summary of project applications of all license holders (public and private) is published online in all member states of the European Union, which provides some insights into animal research in the European Union. The full publication of project applications, as done by Utrecht University, University Medical Center Utrecht, Radboud University Nijmegen and Radboud University Medical Center Nijmegen, is a leading example of higher transparency, "show me" research.

Besides increased transparency, full publication of project applications may also decrease repetition of studies and create a platform for peer-review which will improve the study results, i.e. their reliability. To facilitate this, the Central

⁷ <https://www.ncadierproevenbeleid.nl/adviezen-ncad/documenten/rapport/2016/12/15/ncad-advies-transitie-naar-proefdiervrij-onderzoek> (Accessed 24/02/2020)

Authority (CCD) in the Netherlands, stated in her annual plan for 2020⁸: “Although the Non-Technical Summary of the issued projects provides insight into the types of research that are taking place in the Netherlands, there is also a call from society for further insight into the projects. The CCD therefore strongly supports and encourages active and full disclosure of projects by the institutes themselves.”

4.2 Limitations of our study

Our data includes project applications for the use of animal experiments for scientific purposes. These project applications were filed because the applicants were not able to (fully) replace the animal model using a non-live animal alternative model (Figure 8). It is unclear how many applications were not filed, because it was possible to fully replace the animal model with a non-live alternative model.

Our data does not reflect the total of translational and applied research in the Netherlands, since only project application forms of 4 out of 80 institutes with an active license to perform animal experiments in the Netherlands were available for assessment. However, personal communication with representatives from different Institutional Animal Welfare Bodies and Animal Ethical Committees in the Netherlands, both industry and academia, suggest similar outcomes across the country.

4.3 Perspectives for researchers, regulatory bodies and funding agencies

The road from “trust me” toward “show me” is not only made by transparent reporting, i.e. the full-text publication of the project applications. The road from “trust me” toward “show me” also needs more specific description, including the expected level of detail, on “how” (method), and “what” (deliveries) is expected from the applicant as justification for the animal model selection.

We suggest the following improvements for the current project application form: (1) add the disease area in human beings or animals for which prevention, diagnosis or treatment is intended; (2) add why a specific animal model is chosen over others; (3) add clinically relevant diagnostic or prognostic markers, and when available EMA/FDA/EFSA suggested markers to show efficacy of interventions.

We strongly recommend researchers to scientifically justify their choice of animal model as the best model to predict for clinical outcome. Ethical committees and the Central Authority should demand specific and standardized argumentations. Funding agencies should refrain from funding projects when it is unclear that the chosen animal models are the best models to predict clinical outcome.

In summary, the current choice of a specific animal model in project applications for the use of animals in scientific applications seems to be based on tradition rather than its potential to predict the clinical outcome. A specific and standardized substantiation for the choice of an animal model will lead to more robust science, better prediction of drug efficacy, and more responsible use of animals in drug development.

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⁸ <https://www.centralecommissiedierproeven.nl/documenten/vergaderstukken/2020/1/jaarplan/jaarplan2020>

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Conflict of interest

All authors have completed the ICMJE uniform disclosure form⁹ and declare: GSF reports personal fees from Merck KGaA and Curare Consulting B.V. outside of the submitted work. DVG reports personal fees from Nutricia Research B.V. and Merck Sharp & Dohme outside of the submitted work. None of the other authors has any conflicts of interest.

Author's contributions

Conception and design (DVG, GF and PvM), acquisition, analysis and interpretation of data (DVG), drafting the article (DVG) and revising it critically for important intellectual content (DVG, GF, WB, HS, EM and PvM) and final approval of the version to be published agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (all authors).

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⁹ www.icmje.org/coi_disclosure.pdf