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Use of non-human primate disease models

An Opinion on non-human primates testing in Europe

Michelle M. Epstein[1], Theo Vermeire[2,*,‡]

[1]Medical University of Vienna, Department of Dermatology, Experimental Allergy Laboratory, Währinger Gürtel 18-20, 4P9.02, 1090, Vienna, Austria
[2]National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands

The Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) responded to a mandate from the European Commission on 'The need for non-human primates in biomedical research, production and testing of products and devices'. An overview of this Opinion is presented. The Opinion focuses on the approaches aimed at the replacement, reduction and refinement (3Rs) of the use of non-human primates in scientific experimentation in the areas of 1) development and safety testing of pharmaceuticals and medical devices, 2) treatment and prevention of infectious diseases, 3) neuroscience, 4) ophthalmology and 5) (xeno)transplantation. While it is not possible to predict how long it will be before the use of NHPs in Europe are phased-out, the Opinion summarizes the research gaps and provides recommendations such as alternative methods, training, improvement of techniques and protocols, sharing of knowledge and removal of barriers. Finally, research needs are given.

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Introduction

European law requires the European Commission (EC) to review the "animal protection Directive" (Directive 2010/63/EU, [1]) paying specific attention to the use of non-human primates (NHPs) and any advances which might reduce their use or render it obsolete. Therefore, the EC has requested the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) to review and update its 2009 Opinion [2] on the need for NHPs in biomedical research, production and testing of products and devices [3]. The previous Opinion in 2009 [2] stated that animals should only be used in medical research when it is unavoidable and validated alternative methods are not available and NHPs are essential for scientific progress in critical areas of disease, biology, research and safety testing. As a consequence of the 2009 SCHER Opinion, Directive 2010/63/EU included regular reviews of the topic. The 2017 SCHEER Opinion [4] responds to 6 issues in the

*Corresponding author: T. Vermeire (theo.vermeire@rivm.nl)
‡ The authors are rapporteur and chair, respectively, of the Working Group (WG) on Non-Human Primates (NHPs) Testing of SCHEER, one of the non-food Scientific Committees of the European Commission, http://ec.europa.eu/health/scientific_committees/index_en.htm. Other WG members include: Romina Aron Badin (Atomic Energy Commission, France), Paul Andrew Flecknell (Newcastle University, UK), Michelle Hudson-Shore (Fund for the Replacement of Animals in Medical Experiments (FRAME), UK), David Jones (Safety Working Party, European Medicines Agency (EMA), UK), Jan Langermans (Foundation Biomedical Primate Research Centre, The Netherlands), Mark Prescott (National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), UK), Alain Simonnard (Institut National de Recherche et Sécurité, France).

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Box 1. The issues from the mandate [3].

The areas of research (fundamental, translational and applied) and testing of products and devices in which non-human primates continue to be used today.
The currently available possibilities by type of research or testing to replace their use either with methods not entailing the use of animals or by using other species of animals including those genetically altered.
The opportunities for the reduction and refinement of their use in areas where no replacement can be foreseen in medium or long term as per the principles of the Three Rs.
Identification of specific research areas where effort should be made to advance replacement, reduction and refinement of the use of non-human primates in scientific procedures.
The scientific viewpoint on when their use would no longer be necessary, considering the type of research and areas of testing with a view to the establishment of a specific phasing-out time-table where possible.
Potential implications for biomedical research (e.g., immune based diseases, neurodegenerative disorders, infectious diseases and serious diseases) should the use of NHPs be banned in the EU.

mandate (see Box 1) and focuses on the 3Rs – 'Replacement, Reduction and Refinement principle' [5] for studies with NHPs. Additionally, it addresses issues that hinder the introduction of alternative approaches for NHP use in scientific research along with recommendations on how to advance 3Rs, with particular emphasis on improving 1) training, 2) techniques, 3) protocols, 4) sharing of knowledge, 5) the removal of barriers and 6) knowledge on research needs.

Methods

Call for information

An initial literature search was undertaken by the SCHEER in July 2016 to identify key publications for the 2017 Opinion [6] and the SCHEER WG included additional relevant publications in areas of their expertise. A call for information was published on 8 June 2016 for collecting papers on new 3Rs technologies for the use of NHPs in biomedical research, production and testing of products and devices. Nineteen submissions contained more than 100 papers from individual scientists, research institutions, professional societies, pharmaceutical industry and animal protection organisations.

Stakeholder involvement

A public consultation on the 2017 SCHEER Opinion was opened on the Scientific Committees website from 10 February to 26 March 2017 which resulted in 190 contributors from academia, researchers, Member States, Non-Governmental Organisations and industry and a total of 318 comments addressing multiple issues. A public hearing on 14 March 2017 in which 19 organisations participated followed the public consultation. The comments and responses from the public consultation are available at the SCHEER website [7].

Results

Here, we summarise the main findings and conclusions of the 2017 SCHEER Opinion. The Opinion includes extensive scientific rationale, recommendations and pertinent literature (SCHEER, 2017) [4].

Areas of research

NHPs are now mainly used in 1) the development and safety testing of pharmaceuticals and medical devices, 2) treatment and prevention of infectious diseases and 3) neuroscience with fewer NHPs also used in 4) ophthalmology and 5) (xeno) transplantation. Notably, NHPs remain essential for assessing the safety of some classes of drugs and medical devices and, along with routine production, this constitutes approximately 75% of all NHP use. In 2014, a total of 8898 uses of NHPs (based on experimental procedures and include first use as well as any subsequent reuse of the NHPs) were publicly reported by European Member States (For more information on EU Member State reports: see Ref. [8]).

The 3 Rs

The replacement of NHPs occurs when NHPs are no longer the relevant species for a specific scientific question or study because alternative methods are available. However, because one model may not fully mimic all aspects of human disease, replacement is not always possible (OECD [9] and ECVAM [10]). Consequently, an integrated strategy using *in silico*, *in vitro*, *ex vivo* and *in vivo* methodology, clinical research and a weight-of-evidence approach based on the identification of Adverse Outcome Pathways and through the integration of Mode of Action, biokinetics and biodynamics is necessary [11]. Notably, such recent developments with multiple approaches in biomedical research are trending towards improving the selection of the most promising candidates for new therapies before further assessment *in vivo*. A recent example is a cell culture technique to study drugs against dormant stages of specific malaria species that substantially reduced the number of NHP experiments [12,13]. Box 2 illustrates more examples of currently available replacement possibilities. Additionally, novel *in vitro* disease models, e.g., *in silico* modelling, –omics, microfluidic chambers/chips, cell culture techniques, artificial whole-body models, patient-derived-induced pluripotent stem cells (iPS cells), or organoids may eventually replace or reduce the use of NHPs. Notably, substitution of NHPs with rodents or other laboratory animal species is not 'replacement' as defined by Russell and Burch in 1959 [5]. However, this is ethically desirable if the available evidence indicates that the non-primate species is a valid model but likely to suffer less harm.

Researchers are encouraged to increase the yield of data per animal and experimental session – without increased suffering – and to share data and tissues with other researchers and to publish negative/null results. Approaches to reduction

often focus on principles of good experimental design, and better interpretation and reporting of studies, helping to improve the quality and reproducibility of animal experiments [14]. Greater efforts are needed to assess the degree of pain and distress experienced by NHPs to ensure the implementation of refinements especially because animals free from pain and suffering yield better quality and more reproducible data [15]. In neuroscience, advance in brain imaging technologies and non-invasive electrophysiological methods have furthered efforts to refine NHP investigations for example by integrating structural and functional MRI with transient inactivation of targeted brain regions [16]. For studies examining the effects of lesions or other interventions within or between groups of animals, it is essential to use factors such as the magnitude of the studied parameters to reduce sample sizes. There are strong scientific and business drivers for the 3Rs, which are increasingly leading to changes in practice in both industry and academia [17]. Examples of currently available possibilities for reduction and refinement are in Box 2.

Research gaps

There is an urgent need to conduct systematic reviews, where possible, and meta-analysis of all areas of NHP use to 1) potentially reduce the number of NHPs used, 2) assign resources to identify the suitability of current models, and 3) assess their contribution to scientific knowledge. These reviews and analyses will provide evidence for more 'targeted uses' of NHPs, which is relevant for scientists, animal ethics committees and funding institutions. Emphasis should also be on ensuring proper reporting of NHP studies, efficient knowledge transfer, focusing NHP research in centres of excellence, and the development of harmonised training courses. Continued work is necessary to develop improved means of assessing pain, suffering and distress in NHPs, including the psychological impact of their use in research. Additionally, harm-benefit assessments require more scientific knowledge about the welfare impact of husbandry and procedures including after applying refinement strategies. Examples of efforts needed in specific research areas are in Box 3.

Timetable for complete replacement of NHPs

Several factors determine the timeline for the complete replacement of NHP use (see Box 4), which include a broad spectrum of positive and negative incentives for NHP use and thus, makes it challenging to predict a timetable for full replacement for each of the main NHP research areas.

Implications of an immediate total ban of NHP use on biomedical research

Recognising the high levels of public concern about NHP studies, regulatory authorities in some world regions have also adopted ethical limits or boundaries on NHP use. In the EU, for instance, Great Apes and wild-caught NHPs should not be used, and re-use is not allowed after a severe procedure. However, the close phylogenetic relationship of NHPs with humans makes them the best available animal models for addressing particular research questions. Therefore, there is consensus within certain sections of the scientific community that, where alternatives do not exist, appropriate use of NHPs remains essential in specific areas of biomedical and

Box 2. Examples of advancing replacement, reduction and refinement.

Improved assessments for non-rodent species as replacements.
Regulatory guidance stressing the importance of using of alternative methods in pharmaceutical safety assessments.
New approaches with controlled human challenge models for infectious diseases to replace NHP studies.
In vitro and *in silico* models for diseases, e.g., liver injury, idiosyncratic adverse drug reactions, etc.
New clinical imaging technology instead of cognitive neuroscience studies with NHPs.
Replacement should be ensured when NHPs are no longer necessary in specific areas
More knowledge and experience with techniques for safety assessment of chemicals and drugs, which do not require animals to, in particular, reduce the number of NHPs used.
Development of clinical pain protocols providing early clinical proof of concept for phase I clinical trials with drugs, such as analgesics.
The use of microdosing in exploratory in clinical drug trials.
Potential reduction or replacement of NHPs for preclinical testing with new technology including patient-derived induced pluripotent stem cells (iPSCs).
Use of transgenic animals, e.g., mice or zebrafish can replace NHPs.
Use of homologous proteins in rodents instead of NHPs.
Use of new imaging technology in animals can reduce the number of animals required in experiments.
New technological advances to refine surgery and other invasive procedures in areas such as neuroscience.
Refinement of anaesthetic and analgesic protocols to speed recuperation for surgical and imaging procedures.
Refine imaging techniques by developing non-invasive imaging methods.
The use of refined food and fluid control protocols.
The use of novel wireless technology will allow for refinement of current experimental approaches.

Box 3. Research needs for replacement of NHPs.

For drug safety testing, in particular, more knowledge is needed from new technologies including, molecular biology, signalling pathways, modelling and bioinformatics, integrated testing strategies (e.g., Adverse Outcome Pathways (AOPs)) leading to human diseases.
New models are needed for reproductive toxicity and approaches for biopharmaceutical safety.
More information is needed on NHP relevance and limitations for infectious diseases and therapeutics.
Develop new techniques using non-NHP animal models e.g., humanised mice and –omics and organoïds technology.
Advances are needed in noninvasive imaging technology in neuroscience to replace awake, behaving electrophysiology studies using NHPs.
For replacement in vision research it is essential to increase understanding of the eye, improve techniques in organ culturing, *in vitro* and *in silico* modelling.

Box 4. Factors influencing the timetable for replacement of NHPs.

Adequate resources for research for suitable alternatives to NHPs and making them fit for purpose.
Lack of harmonisation of regulations and guidelines within and across sectors within the EU.
Need for NHPs as the sole, relevant model for addressing emergent and re-emergent infectious diseases.
WHO supports NHP models for biosimilars because of their high tolerance for human proteins.
Difficulty moving to new alternative methods where less data are available.
Progress in the formal validation of alternative test methods within the regulatory arena and in reducing the timescale and bureaucracy associated with this process.
The risk aversive nature of society makes it difficult to move away from familiar methods.

biological research and for the safety assessment of pharmaceuticals. As long as sufficiently validated alternatives are not available, a total ban would make progress in critical research and safety studies impossible, at least in Europe. Because animal welfare standards for laboratory NHPs are on average higher in many European countries than in other parts of the world, it is possible that NHP research done outside of Europe would likely result in a net decrease in animal welfare. The loss of NHP research in Europe might also impact the quality of 1) European scientific research, 2) public health, 3) accessibility of treatments developed under different standards and 4) local economy. Because a total ban is not yet feasible, it is essential when communicating with the public about NHP use that the scientific community provides 1) accurate descriptions of advantages and limitations of the research, 2) realistic claims about potential outputs and impacts and 3) accurate and sufficient information about harm to the animals.

Barriers

In the 2017 SCHEER Opinion, the barriers to NHP alternatives apply to animal use more generally but amplified due to the strong ethical and social concerns surrounding NHP experimentation. Barriers limit the alternative scientific methods described in this Opinion and increase the uncertainty of how to translate the findings from such models and build up the necessary reference knowledge base. In particular, legislative barriers [18] refer to the lack of regulatory harmonisation within and across sectors and the condition that an alternative method for regulatory purposes must be scientifically valid, justified and accepted. Moreover, there is a lack of resources for developing alternatives to NHP models. Additionally, the potential to replace primates includes scientific data but also is strongly related to and reliant upon dynamics such as competition, the reputation of the scientists and entrenchment and policy that creates polarisation [19,20].

SCHEER Opinion highlights

The use of NHPs remains necessary for particular types of research. When their use is required – to be determined on a case-to-case basis and only if no viable alternatives are available – it is essential to adopt the highest standards of NHP housing and husbandry and to follow best practice in the conduct and refinement of scientific procedures. The wide spectrum of positive and negative incentives for NHP use makes it difficult to predict a timetable for a complete replacement for each of the main research areas. To fully apply the 3Rs and maximise the benefits, there is a need to ensure that as new knowledge, technologies and approaches emerge there are timely assessment and evolution of research strategies, study designs, scientific procedures and husbandry. As long as a total ban is not feasible, when communicating about NHP use with the public, the scientific community should provide an accurate description of the benefits, harms to animals and limitations of such research, and be realistic about the potential outputs and impacts.

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