Review of animal testing requirements in WHO guidelines and recommendations for biologics: a proposal to implement 3Rs principles

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The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and World Health Organization (WHO) have formed a new partnership to review the animal testing requirements described in WHO guidance documents for biologics to identify opportunities for the integration of the 3Rs. The aim is to enable vaccines manufacturers and regulators to apply the latest non-animal testing approaches and strategies to support faster access to cheaper vaccines by the global communities who need them most urgently. This poster presentation will introduce the project, highlighting why it has been established, its scope and how interested parties can get involved.

Introduction

Animals are used extensively in the development, production and quality control of biological products such as vaccines, cytokines, enzymes, and hormones. It has been estimated that more than 10 million animals a year are used worldwide for these purposes. The use of such a large number of animals puts a significant financial burden on manufacturers and national control laboratories, is time and resource intensive, and the methods themselves can cause significant pain and distress to the animals. The 3Rs principles are increasingly being applied to support more humane and scientifically robust animal research and as a framework for the proper scientific justification on the choice of testing methods adopted in many fields of research and testing across the biosciences. Here, we describe an initiative between the WHO and the NC3Rs to improve implementation of the 3Rs in quality control and batch release testing of biologicals and support the adoption of more scientifically relevant non-animal approaches.

NC3Rs WHO 3Rs project

In 2019 a project was presented to WHO Expert Committee on Biological Standardization (ECBS) to propose a systematic review of WHO written standards for the animal testing requirements and procedures recommended for use in the post-licensure quality control and batch release of biologicals. The review would determine how much animal testing is currently included within these documents and to what extent relevant 3Rs strategies are included and what opportunities are available for greater adoption of the 3Rs. ECBS approved the project (World Health Organization. Expert Committee on Biological Standardization, Seventieth report. WHO Technical Report Series. 2020; 1024: Section 2.2.2) and work began in June 2020.

The project will be conducted over two stages. The first stage would be led by the NC3Rs, co-funded by the NC3Rs and the Bill and Melinda Gates Foundation [Grant number 005622] and facilitated by an international Working Group including WHO staff and members from NRAs, NCLs, manufacturers, and other interested organisations. The objectives of this first stage of the project are to:

1. Review the extent to which animal testing is included in current WHO recommendations for biologicals and to identify opportunities to increase adoption of the 3Rs principles including application of alternative methods that have already been validated and approved elsewhere.
2. Engage with organisations that produce, regulate and test biologicals to identify opportunities and barriers to better integration of 3Rs.
3. Produce comprehensive recommendations for presentation to the ECBS to enable harmonised 3Rs practices for post-licensed products to be established.

The second stage of the project would be an implementation phase coordinated by WHO and dependent on the recommendations from NC3Rs. This stage should take two to three years to complete and is likely to include amendments to existing WHO guidelines and/or a separate guidance document to support the wider acceptance of 3Rs principles into vaccine and biologic products control and batch release testing.

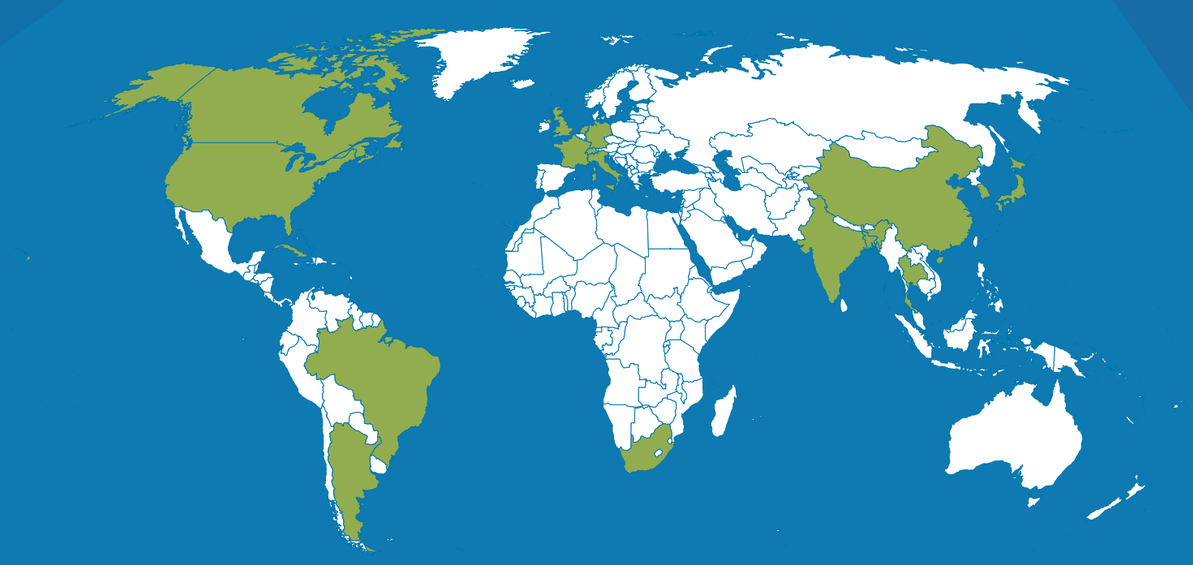

Figure 1. The countries represented on the working group (green).

Regulatory Agencies	Manufacturers	National Control Laboratories	Others
▪ MHRA ▪ FDA ▪ South Africa National Control Laboratory ▪ EDQM, France ▪ Health Canada ▪ ANMAT, Argentina	▪ GSK ▪ Janssen ▪ Merck ▪ Sanofi ▪ Serum Institute India ▪ IFPMA, DCVMN ▪ Finlay Institute, Cuba	▪ NIBSC, UK ▪ Paul Ehrlich Institute, Germany ▪ National Institute of Infectious Diseases, Japan ▪ National Institutes for Food & Drug Control, China ▪ Ministry of Public Health, Thailand ▪ RIVM, Netherlands ▪ National Control Laboratory Network	▪ WHO ▪ Seoul National University, South Korea ▪ European Commission Joint Research Centre ▪ IABS ▪ Expert Committee on Biological Standardization ▪ African Academy of Sciences

Figure 2. The organisations represented on the working group.

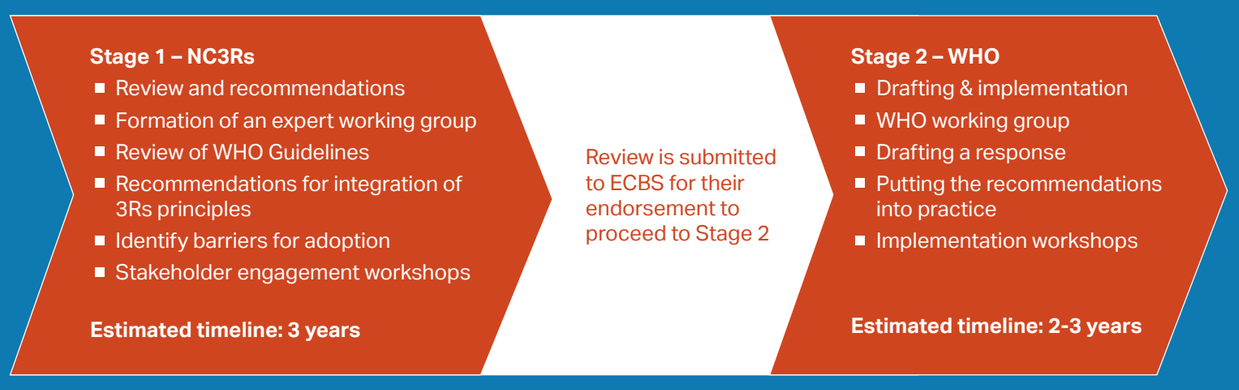

Figure 3. The stages of the project.

Guideline review

- 69 WHO guidelines identified as relevant to review.
- Reviewed by working group with a focus on animal tests for quality control/batch/lot release and 3Rs language.
- 472 individual items found during review, 267 of these are 'in scope' animal tests. Of these, 179 have suggested non-animal alternatives or identified 3Rs approaches are available.
- The review also highlighted some significant discrepancies and inconsistencies in language relating to specific tests and the 3Rs (Figure 4).

3Rs language	Pyrogenicity/endotoxin testing language
For ethical reasons, it is desirable to apply the 3Rs concept of "Replace Reduce Refine" to minimize the use of animals in research, and consideration should be given to the use of appropriate *in vitro* alternative methods for safety evaluation.	A test that has been found to be suitable for the current vaccine involves injection into the ear vein of rabbits...
WHO has promoted the replacement of animals for experimental purposes, both for ethical reasons and in the interests of progressive improvement in product safety and quality.	Each final lot should be tested for pyrogenic substances. The test procedures should be approved by the national regulatory authority.
...attention should be paid to the care and handling of laboratory animals to minimize effects of environment and nutrition and to maximize efficacy in their use, particularly in the quality control of bacterial vaccines. Animals should be bred and maintained in such a way that the maximum possible standardization and reproducibility are obtained.	The vaccine in the final container should be tested for pyrogenic activity by intravenous injection into rabbits or by a Limulus amoebocyte lysate (LAL) test, which should be validated for this purpose.
Any scientist carrying out bioassays using animals should be aware of the 3Rs, as described by Russell and Burch (1959). Thus, *in vivo* bioassays should only be used if scientifically valid *in vitro* or other techniques are not available. Refinement should be introduced as far as possible in *in vivo* bioassays. For example, several of the assays described here employ 'humane endpoints'.	The endotoxin content of the final product should be determined using a suitable in vitro assay such as a LAL test. When required, the monocyte activation test (MAT) or rabbit pyrogenicity test may be used for monitoring potential pyrogenic activity subject to the agreement of the NRA.

Figure 4. Examples of language related to the 3Rs and pyrogenicity/endotoxin testing in current WHO guidelines.

Surveys

We have produced a survey for biologicals manufacturers to gather global information on the opportunities and barriers for implementing the 3Rs in quality control, batch and lot release testing of biologicals. Understanding how these factors differ between regions will be critical in developing recommendations that can be supported by manufacturers globally.

If you are a manufacturer of biological products at any scale anywhere in the world, we want to hear about the products you manufacture, the approaches you use for quality control and batch release testing, and the challenges you face in adopting non-animal and 3Rs approaches. The survey is open until mid-September and can be found at www.nc3rs.org.uk/WHOproject.

A second survey for National regulatory Authorities (NRA) and National Control Laboratories (NCL) will be circulated later in 2021. If you would like to receive either the manufacturer or regulatory survey, or can help distribute it, please contact elliot.lilley@nc3rs.org.uk.

Stakeholder engagement

We will be hosting a series of regional workshops to focus on region-specific challenges to implementing non-animal and 3Rs approaches in quality control and batch release testing so that these can be considered as we develop our recommendations to ECBS. Regions covered are Europe, Africa, Asia & Oceania, Latin & South America, North America & Canada.

These workshops are one of the key mechanisms to understand what the impact will be by region of integrating non-animal and 3Rs approaches more widely in WHO guidelines, so please participate and make sure your voice is heard. To keep informed of workshop dates, sign up for the NC3Rs newsletter at www.nc3rs.org.uk/register.

Get involved

Please contact elliot.lilley@nc3rs.org.uk if you would like to know more about this project or support it by:

- Participating in or helping to develop the regional workshops to meet the needs of your region.
- Completing or disseminating the surveys.
- Offering your expertise in a specific assay or product to support the guideline review and drafting of recommendations.

www.nc3rs.org.uk/WHOproject