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Short paper

Integrating 3Rs approaches in WHO guidelines for the batch release testing of biologicals

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ABSTRACT

Animal testing has long been integral to the development of biologicals, including vaccines. The use of animals can provide important information on potential toxicity, insights into their mechanism of action, pharmacokinetics and dynamics, physiologic distribution, and potency. However, the use of these same methods is often adopted into the post-licensure phase of the product life cycle for the monitoring of product qualities, such as potency or safety, as part of their routine batch release. The UK National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) and the World Health Organization (WHO) are collaborating on a project to review animal-based testing methods described in WHO manuals, guidelines and recommendations for biologicals to identify where updates can lead to a more harmonised adoption of 3Rs principles (i.e. Replacement, Reduction, and Refinement of animal tests) in batch release testing requirements. An international working group consisting of more than 30 representatives from pharmaceutical and biotechnology companies, national control laboratories and regulatory bodies is performing this review. This project aims to address concerns about inconsistencies in the guidance for the scientifically justified use of animal methods required for the post-licensure quality control and batch release testing of biologicals, and the near absence of recommendations for the application of 3Rs principles within the relevant guidelines. Improved adoption of 3Rs principles and non-animal testing strategies will help to reduce the delays and costs associated with product release testing and help support faster access to products by the global communities who need them most urgently.

1. Background

Animals are used extensively in the development, production and quality control of biologicals 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 [1]. 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 (see Box 1) 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 (see Box 2) to improve implementation of the 3Rs in quality control and batch release testing of biological products and support the adoption of non-animal approaches of equivalent or superior scientific relevance.

Although there are ethical issues and political pressures surrounding animal testing, there are also significant scientific, regulatory, and economic concerns for their use in this area:

1.1. Scientific issues

From a scientific perspective, the important issues regarding the use of animals for quality control and batch release testing of biologicals include the inherent variability and poor robustness of many *in vivo* assays, the relevance of an animal response to the human condition, and that some tests still in use may not have been validated to the strict requirements expected of quality control methods today. The relevance of an animal batch release test to safety and efficacy in humans may be questionable as inter-species differences in biological responses are

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Box 1
The 3Rs

The 3Rs concept [2] has become a standard in legislation and guidelines concerning animal experimentation in many countries. Replacement involves the substitution of an *in vivo* assay with relevant non-animal-based methods. These substitutions may be with mammalian cell lines or cultured tissues, immunological methods (e.g. ELISA), proteome analysis, physicochemical techniques, molecular biology, or even mathematical modelling of existing data sets. Reduction concerns minimising the number of animals used to achieve the goals of a study by using appropriately designed and analysed animal experiments that are robust and reproducible. Refinement is advancing animal welfare by exploiting the latest *in vivo* technologies and animal welfare science to reduce to the minimum, pain, suffering, distress or lasting harm animals experience across their lifetime. Examples include the use of non-lethal endpoints for the potency testing of rabies vaccines or the use of immunochemical methods to determine serum titre responses to vaccination instead of an immunisation-challenge approach.

Box 2
The NC3Rs

The NC3Rs is an independent scientific organization. It supports the UK science base by driving and funding innovation and technological developments that replace or reduce the need for animals in research and testing and lead to improvements in welfare where animals continue to be used. The Centre promotes robust and ethical scientific practice through collaborating with research funders, academia, industry, regulators, and animal welfare organisations, both in the United Kingdom and internationally. The NC3Rs has achieved success in the field of regulatory testing through a number of initiatives including the adoption of the Fixed Concentration Procedure (FCP) in acute inhalation studies of chemicals (OECD TG 433)[REF- https://www.nc3rs.org.uk/adoption-fixed-concentration-procedure-acute-inhalation-studies] and removal of the requirement for conventional single dose rodent acute toxicity testing prior to first-in-human studies from the international pharmaceutical guidelines, ICH M3 [REF - https://www.nc3rs.org.uk/single-dose-acute-toxicity-studies]. The NC3Rs has also supported the development and adoption of non-animal alternative approaches in biologicals development and testing, including in vitro biochemical and biological assays to replace the histamine sensitisation test for pertussis vaccines [REF -https://www.nc3rs.org.uk/development-alternatives-histamine-sensitisation-test-pertussis-vaccines-vitro-biochemical-and and [10]].

common. In addition, the level of variability inherent in most animal methods can make them insufficient for use in ensuring the production of a consistent product between batches. Far more appropriate for supporting the consistency approach [3] for the routine release of biologicals would be to utilise more relevant, product-specific non-animal assays with less variability and more readily transferrable protocols.

The substitution of *in vivo* methods with non-animal assays for the quality control and batch release testing of biologicals is already being introduced and recognised by some regulatory authorities [4]. For example, the monocyte activation test as a substitution to the rabbit pyrogenicity test, and the CHO cell clustering assay for monitoring residual pertussis toxin as a substitution to the mouse histamine sensitisation test are now included in the European Pharmacopeia (Ph. Eur. 2.6.30 [5]; Ph. Eur 2.6.33 [6], respectively).

1.2. Regulatory issues

From a global regulatory perspective, the wider adoption of 3Rs methods related to animal testing for the licensure and post-licensure release of biologicals has been hampered due to poor harmonisation of regulations and guidelines pertaining to their safety, efficacy, and/or potency testing requirements [4,7]. For example, testing requirements and/or specifications for batch approval can differ significantly between national regulatory authorities for common products from different manufacturers as well as for a single manufacturer's product released in multiple countries. In addition, protocol requirements for the same animal assay can have differences between regions. These differences, such as animal strain, housing conditions, timing, controls, or references, can significantly impact an assay outcome and determine pass/fail criteria.

As a biological may be registered in dozens of countries, this results in either duplication of animal testing or partial implementation of 3Rs approaches for products that are distributed worldwide. Unfortunately, this discourages and slows the development and implementation of innovative testing approaches. The adoption of 3Rs approaches has been a legal requirement in Europe since 1986 (former Directive 86/609/EEC; current Directive 2010/63/EU) and other jurisdictions are moving towards similar legal requirements or regulatory implementation; however, the lack of harmonisation between countries can act as a barrier to industry with regard to implementation of 3Rs principles. The adoption of harmonised non-animal methods, elimination of the corresponding animal tests, and promotion of a consistent testing approach for the quality control of biologicals offers a good opportunity to ease the regulatory burdens across countries or regulatory regions.

1.3. Economic issues

From an economic perspective, *in vivo* tests are expensive, time consuming, and human resource intensive [8]. Animal housing and husbandry costs alone can be very high, and the tests themselves may have long durations which can significantly increase the time to batch release. Vaccine potency tests, for example, can require several weeks or months. The high variability of *in vivo* responses increases the risk of rejection of product batches which may actually be safe and efficacious, thereby leading to re-testing, possible investigations into out-of-specifications results, and further delays to market release and/or product shortages. Additional delays and costs can be caused if the National Control Laboratories (NCLs; responsible for post-approval, pre-market independent batch release testing), do not conduct their batch release testing concurrently with the manufacturer, or results differ between the two.

Despite the increased costs and timelines due to the use of animal testing, it adds little or no value to the quality or safety of a product manufactured under current Good Manufacturing Practices (cGMP) [9].

1.4. NC3Rs WHO 3Rs project

The global influence and reputation of the WHO ideally places the organization to encourage the integration of 3Rs principles into the pre-

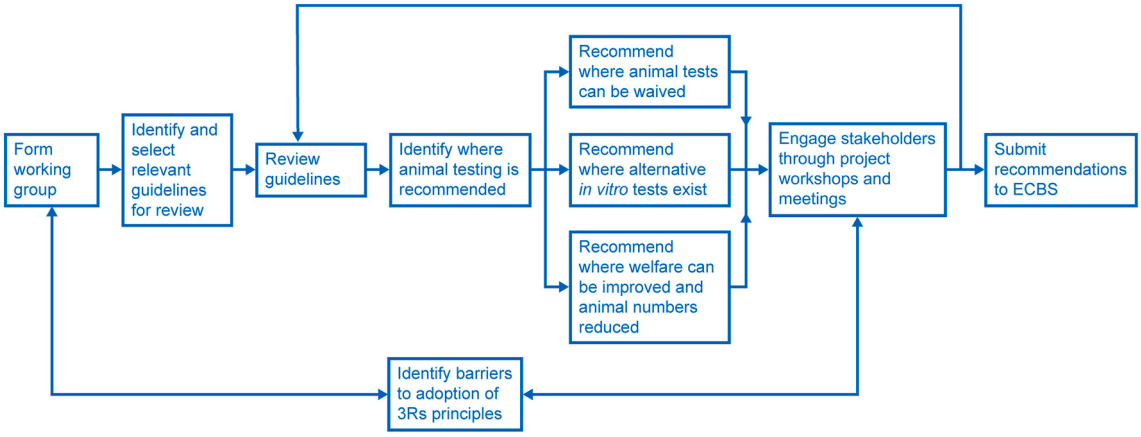

Fig. 1. Flow chart of phase one of the project.

and post-licensure quality control requirements for biologicals and to promote a harmonised adoption of non-animal methods. The WHO guidelines and recommendations on biologicals are considered by most regulatory authorities and manufacturers. However, the extent to which animal use is required within the collection of WHO guidelines and recommendations for biologicals has never been reviewed. There are gaps where non-animal technologies have been validated and approved within some regulatory jurisdictions but are not yet included in the recommendations, or non-animal methods that are in the recommendations but not implemented by some regulatory authorities. For example, the WHO Expert Committee on Biological Standardization (ECBS) agreed at their annual meeting in 2018 to discontinue the inclusion of the general safety (innocuity) test (also known as the abnormal toxicity test) in routine batch release testing requirements from WHO guidelines, recommendations and other documents for biological products [11]. This is in line with the United States Food and Drug Administration and the European Pharmacopeia who had previously deleted this test from their requirements. Whilst this is an important step towards regulatory convergence at the global level, many WHO guidelines and recommendations still do not reflect the deletion of the innocuity test and it is unclear whether some regulatory authorities still receive submissions with, or require data from, this test. Also, in contrast to the European Pharmacopeia (Ph. Eur 5.2.14 [12]), the WHO does not currently provide guidance on the implementation of 3Rs principles for use in the batch release and quality control testing of biologicals, or for the substitution of existing *in vivo* assays with animal-free methods.

In 2019 a project was proposed to ECBS to conduct 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 [13]. The purpose of the review would be to determine how much and which animal testing should be included within these documents and whether relevant 3Rs strategies are currently available that have not been considered within them. The review process was also proposed to determine if a WHO strategy for the adoption of 3Rs principles would be useful for national regulatory authorities (NRAs), NCLs and manufacturers, and evaluate barriers to the adoption of 3Rs principles.

The project is being conducted over two stages. The first stage (Fig. 1) is 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 (see appendix 1). 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 which 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.

Table 1
The scope of stage one of the project.

In scope	Not in scope
The review of all WHO standards relevant to the regulation or control of human biologicals under the purview of ECBS,	**The development or validation of 3Rs methods.**
Review to identify animal tests currently recommended for the post-licensure control of biologicals.	**The review of confidential documents, or any guidelines, recommendations, or other documents which are not within the public domain.**
Current best-practice in the 3Rs will be identified and recommended, this will include improvements to animal welfare and experimental design as well as non-animal alternatives.	**The review of animal testing included in within WHO documents that are outside of the purview of the ECBS (e.g. international pharmacopeia).**
The identification of possible barriers towards adopting or implementing 3Rs strategies in the quality control and lot release of biologicals.	**The evaluation of non-WHO standards, guidelines, or regulations, outside of the purposes for providing examples and/or suggestions.**
The development of scope and process for Stage 2.	**The ethical review of the use of animals in the control of biologicals.**
	The drafting of revisions to the animal-based methods in existing vaccine-specific guidelines and recommendations.
	The review of animal testing or methods used in the development (pre-licensure) phase of biologicals development.
	The out of context criticism of WHO, its member states, or their regulatory processes.

The scope of this first stage, which is due to finish in Q4 2023, is summarised in Table 1.

The second stage of the project is an implementation phase coordinated by WHO and dependent on the recommendations from NC3Rs. This stage will 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 control and batch release testing of vaccine and biological products.

This two-stage process will help to reduce any perceptions or risk of bias if WHO were to review its own documents, and to better ensure a proper inclusion of 3Rs principles for which WHO has limited expertise.

2. Concluding remarks

There is a global movement towards more widespread adoption of 3Rs principles within national and international regulations across the biosciences. This movement stems not only from ethical and public concerns for animal welfare, but also from very strong scientific, regulatory, and economic rationales. There already exist several projects to support 3Rs principles focused on specific tests within biologicals quality control and batch release testing. It is not the intention of the current project to replicate or duplicate these important efforts, but rather to build on them by providing a vehicle for the integration of the methods being developed/advocated for by others within WHO guidelines and drive significant change on a global scale. Providing the WHO with an appropriate path forward to integrating 3Rs strategies will encourage harmonised testing recommendations globally and support vaccines manufacturers, regulators and control laboratories to improve the quality and efficiency of batch release testing of biologicals with a reduced emphasis on the use of animals.

Appendix 1. Organisations represented on the working group

African Academy of Sciences.

Developing Countries Vaccines Manufacturers Network.

European Directorate for the Quality of Medicines & HealthCare, France.

Finlay Vaccine Institute, Cuba.

FIOCRUZ/INCQS – BraCVAM, Brazil.

French National Agency of Medicine and Health Products Safety, France.

GlaxoSmithKline.

Health Canada, Canada.

Incepta Vaccine Ltd, Bangladesh.

Institute of Biological Products, Ministry of Public Health, Thailand.

Integrated Laboratory Systems, LLC.

International Alliance for Biological Standardization.

International Federation of Pharmaceutical Manufacturers & Associations.

Janssen Vaccines and Prevention.

Japanese National Institute of Infectious Diseases, Japan.

Joint Research Centre, European Commission, Italy.

Merck.

National Administration of Drugs, Foods and Medical Devices, Argentina.

National Centre for the Replacement, Refinement and Reduction of Animals in Research, UK.

National Institute for Biological Standards and Control, UK.

National Institutes for Food and Drug Controls, China.

National Institute for Public Health and the Environment, The Netherlands.

Paul-Ehrlich-Institut, Germany.

Sanofi Pasteur.

Seoul National University, South Korea.

Serum Institute of India, India.

US Food and Drug Administration, USA.

World Health Organization.

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