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

Dr Elliot Lilley – Programme Manager
Aims and objectives of the project

- A new partnership between NC3Rs and the WHO
- A scientifically-driven review of animal testing requirements described in WHO guidance documents for biologics and vaccines
- To identify evidence-based opportunities for the integration of the 3Rs
- To support vaccines manufacturers, regulators and control laboratories in applying the latest non-animal testing approaches and 3Rs strategies
- To support faster access to cheaper vaccines
Animal use in biologics development and testing

- Animals are used extensively in the quality control and lot release testing of biologicals.

- There are significant issues with this, including:
  - Large numbers of animals are used.
  - Potential to cause considerable pain and suffering.
  - Expensive and labour intensive.
  - Time consuming and a cause of significant delays.
  - A high degree of variability and risk of failure of otherwise acceptable product batches.
  - Often poor repeatability between manufacturer and control laboratories.
  - Lack of harmonisation in assay requirements.
A timely opportunity

- The WHO is mandated to “establish and stimulate the establishment of international standards for biological, pharmaceutical and similar products”

- A systematic review of established WHO guidelines for 3Rs purposes has never been done

- There is a global movement across sectors to embed the 3Rs in regulatory guidance and provide direction in implementing their integration

- Some progress has been made in biologics testing, but the process is slow and piecemeal
The project

- To review the animal testing requirements described in WHO guidance documents for biologics and vaccines to identify opportunities for the integration of the 3Rs.
  - What is the extent of animal testing included and are there alternative methods that should be included in the recommendations?
  - Would a WHO guideline for the adoption of 3Rs principles into the quality control and lot release of licensed vaccines be useful for harmonisation of non-animal methods and for guidance to WHO member states?
  - What are the barriers that are hindering the adoption of 3Rs principles?
Formally endorsed by WHO

The project has been endorsed by the WHO Expert Committee on Biological Standardization (ECBS) (World Health Organization. Expert Committee on Biological Standardization, Seventieth report. WHO Technical Report Series. 2020; 1024: Section 2.2.2.).
**Project process**

**Stage 1 – NC3Rs**
- Review and recommendations
- Formation of an expert working group
- Review of WHO Guidelines
- Recommendations for integration of 3Rs principles
- Identify barriers for adoption
- Stakeholder engagement workshops

Estimated timeline: 3 years

**Stage 2 – WHO**
- Drafting & implementation
- WHO working group
- Drafting a response
- Putting the recommendations into practice
- Implementation workshops

Review is submitted to ECBS for their endorsement to proceed to Stage 2

Estimated timeline: 2-3 years
Why the NC3Rs is leading stage 1

- Established in 2004 by the UK Government
- Research funder plus in-house programs
- Works across the biosciences with industry, academia, regulators & funders
- Remit includes any area of animal use for research purposes
- 30 staff between London and our regional posts
- Budget ~ £10 million p.a.
- Reviewed every five years
- [www.nc3rs.org.uk](http://www.nc3rs.org.uk)
Why the NC3Rs is leading stage 1

- Can provide staff and partial funding
- Independent from WHO
- A track record in delivering advances in, and acceptance, of the 3Rs
- Funding *in vitro* model development for vaccine manufacture, quality control and batch release testing
- Addressing scepticism and inertia in the uptake of new models
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- Addressing scepticism and inertia in the uptake of new models
- A track record in supporting regulatory change to deliver 3Rs impacts

**PharmaTimes**

Acute toxicity test no longer needed, industry review finds

By Peter Mansfield

A drug safety test that uses thousands of laboratory mice and rats each year in Europe is no longer necessary, a review involving 15 pharmaceutical companies and contract research organisations has found.

Published in the journal *Regulatory Toxicology and Pharmacology*, the review co-ordinated by the UK-based National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3RS) concluded that information gained from the single-dose acute toxicity test, usually conducted in...
# The project scope

## In scope
- Review of WHO written / physical standards relevant to biologics & vaccines regulation
- All 3Rs (not just replacement)
- Methods used in the post-licensure control of biologics and vaccines
- Identification of barriers towards adopting 3Rs strategies in the quality control and lot release of biologics and vaccines
- Development of scope and process for stage 2

## Out of scope
- Development or validation of 3Rs methods
- Documents not publicly accessible
- Animal methods not related to regulation of biologics or vaccines
- Non-constructive criticisms of WHO
- Ethical review of the use of animals
- Drafting of revisions to *in vivo* approaches in existing guidelines
- Animal testing or methods used in the development of biologics or vaccines

*Note: The table is intended to provide a clear overview of what is included in the project scope and what is not.
Our approach

- Regular stakeholder engagement, throughout the project.
- Change the *emphasis* in WHO guidelines to *promote* adoption of non-animal alternatives.
- Animal tests will only be recommended for deletion with a sound scientific basis.
- General 3Rs guidance will be drafted to promote the scientific benefits of non-animal alternatives, optimised experimental design and high standards of animal welfare.
Phase one flow chart

Form working group
Identify and select relevant guidelines for review
Review guidelines
Identify where animal testing is recommended
Recommend where animal tests can be waived
Recommend where alternative *in vitro* tests exist
Recommend where welfare can be improved and animal numbers reduced
Engage stakeholders through project workshops and meetings
Submit recommendations to ECBS
Identify barriers to adoption of 3Rs principles
Current status/timeline

- Proposal presented to ECBS and approved October 2019
- Gates funding awarded June 2020
- First meetings of the working group held June/July 2020
- Survey of the working group members August 2020
- Second meetings of the working group held November 2020
- Review process started in January 2021 finished end of April 2021
- Third meetings of the working group held June 2021
- Manufacturers survey finalised and distribution started in July 2021
- NCL/NRA survey in development
- Regional stakeholder workshops planned for late 2021/early 2022
Guideline reviews

- 69 reviews added
- 472 lines in the database
- 350 ‘in scope’ (animal test for batch/lot release testing*)
- Of these 207 have suggested alternatives
GST/ATT/Innocuity

3.13 Deletion of the innocuity/abnormal toxicity test for biological products

The Committee was reminded that the innocuity test (also referred to as the abnormal toxicity test or general safety test) is a quality control test carried out on the final product for the purpose of licensing or lot release. Developed in the early 1900s, the test was intended to ensure the safe and consistent production of serum products and later became a general safety test to detect extraneous contaminants in all biological products. The test has historically been included in WHO Recommendations and Guidelines from the onset and in national pharmacopoeias worldwide.

In recent years, however, the value of the test has been called into question – both from the perspective of regulatory science and in the context of the principles of animal use. The Committee was reminded that one of the main outcomes of a 2015 conference of the International Alliance for Biological Standardization (IABS) was the elimination of the innocuity test from the 2016 edition of the WHO Biological Standardization manual. "the Committee recommended the immediate discontinuation of the inclusion of the innocuity test in all future WHO Recommendations, Guidelines and manuals for biological products published in the Technical Report Series. The Committee further recommended that the inclusion of this test in previously published WHO Technical Report Series documents be disregarded."

In the Review, the GST was mentioned 38 times, only 3 guidelines stated that this was no longer required.
Examples of 3Rs language

- Subsequent activities were undertaken aimed at providing greater flexibility in procedures, reducing the number of animals used and refining endpoints without prejudice to the principle of expressing vaccine potency.....

- 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.

- 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.

- More than any other system used for testing, animals have to be handled and maintained appropriately to generate accurate, reliable, and reproducible results. It is essential to be aware of all the factors that may affect the biological functions of the test animals, and thus interfere with the outcome of a potency, safety or toxicity test.

- In some jurisdictions, legislation requires the application of the 3Rs principles (Replacement, Reduction and Refinement of animal experiments) during product development in order to reduce animal suffering. In particular, studies with non-human primates should be avoided if possible. In vivo animal studies should be considered only when it is expected that such studies would provide relevant additional information. In general, the additional value of in vivo nonclinical studies for the demonstration of comparability of SBP and RBP is questionable when previous physicochemical, structural and in vitro functional tests have demonstrated their similarity.

- 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’

- Manufacturers are encouraged to avoid the use of materials of animal origin wherever possible.

- It is the ethical responsibility of the manufacturer to use only the minimum number of experimental animals to measure the efficacy of an antivenom.

- The development of in vitro methods validated for replacing animal experiments is strongly encouraged.

- To avoid the unnecessary use of monkeys, virus seed lots should be prepared in large quantities.

- Equines are the most commonly used for production of hyperimmune plasma in antivenom production and have specific physiological and psychological requirements for good health and the minimization of pain and distress. Manufacturers must recognize these needs and structure their use of animals to ensure that their social, physical and environmental needs are appropriately met.
Pyrogenicity / endotoxin testing

Each final lot should be tested for pyrogenic substances. The test procedures should be approved by the national regulatory authority.

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.

A test that has been found to be suitable for the current vaccine involves injection into the ear vein of rabbits….

The endotoxin content of each lot of purified Vi polysaccharide should be determined and shown to be within limits agreed with the NRA. Suitable in vitro methods include the Limulus amoebocyte lysate (LAL) test.

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.

Each final lot should be tested for pyrogenic substances, if appropriate. Tests for bacterial endotoxin (for example, the limulus amoebocyte lysate (LAL) test) should be performed. However, if there is interference in the test – for example, because of the addition of an immunostimulant such as 3-O-desacyl-4'-monophosphoryl lipid A – a test for pyrogens should be performed. The classical rabbit pyrogen test should now be replaced by a validated monocyte-activation test approved by the NRA.
Focus groups

Several thematic test categories emerged from the review:

- Potency/immunogenicity testing
- Pyrogenicity/endotoxin testing
- Neurovirulence testing
- Adventitious agent testing
- Specific toxicity testing

We have established focus groups to evaluate the potential for adoption of 3Rs principles
<table>
<thead>
<tr>
<th>National Regulatory Agencies</th>
<th>Manufacturers</th>
<th>National Control Laboratories</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHRA</td>
<td>GSK</td>
<td>NIBSC, UK</td>
<td>WHO</td>
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<tr>
<td>FDA</td>
<td>Janssen</td>
<td>Paul Ehrlich Institute, Germany</td>
<td>Seoul National University, S. Korea</td>
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<tr>
<td>South Africa National Control Laboratory</td>
<td>Merck</td>
<td>National Institute of Infectious Diseases, Japan</td>
<td>Eur Commission Joint Research Centre</td>
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<td>Sanofi</td>
<td>National Institutes for Food &amp; Drug Control, China</td>
<td>IABS</td>
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<td>Health Canada</td>
<td>Serum Institute India</td>
<td>Ministry of Public Health, Thailand</td>
<td>Expert Committee on Biological Standardization</td>
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<td>ANMAT, Argentina</td>
<td>IFPMA, DCVMN</td>
<td>RIVM, Netherlands</td>
<td>African Academy of Sciences</td>
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<td></td>
<td>Finlay Institute, Cuba</td>
<td>National Control Laboratory Network</td>
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How you can get involved

- Surveys
  - dissemination/completion
Manufacturers survey

The survey is available for download on our website:

https://nc3rs.org.uk/review-animal-use-requirements-who-biologics-guidelines
How you can get involved

- Surveys
  - dissemination/completion
- Regional stakeholder workshops
  - Local organising committee/delegate
# Regional stakeholder workshops

- To engage, connect and understand
- **Regions:**
  - N America/Canada,
  - Latin/S America,
  - Europe
  - Africa
  - Asia/Oceania
- Supported by local organising committee, but need help from the WG to identify this
- Draft agenda as starting point
- To be hosted during 2021 – Q1 2022

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Description</th>
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<tbody>
<tr>
<td>5 mins</td>
<td>Welcome and introduction</td>
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<tr>
<td>10 mins</td>
<td>Aims and objectives for the meeting</td>
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<td></td>
<td>Delivered by a local host to make it clear the focus is on a regional perspective</td>
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<td></td>
<td>Scene setting</td>
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<tr>
<td>15 mins</td>
<td>Introduction to NC3Rs project</td>
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<tr>
<td>30 mins</td>
<td>Local regulator and manufacturer perspective</td>
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<td>To give their perspectives on animal use in QC and batch release testing and the opportunities/challenges for implementing 3Rs approaches.</td>
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<td>20 mins</td>
<td>BREAK</td>
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<td>3Rs models/approaches state of the art</td>
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<tr>
<td>30 mins</td>
<td>3x flash talks (10 mins each) from (ideally) local manufacturers/scientists/regulators on the development and application of 3Rs approaches.</td>
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<tr>
<td>10 mins</td>
<td>Introduction to the breakout sessions</td>
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<tr>
<td>45 mins</td>
<td>BREAK</td>
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<td></td>
<td>Breakout sessions</td>
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<tr>
<td>50 mins</td>
<td>Breakout discussion session</td>
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<td>Will include 3 sessions focussing regionally on (i) barriers to 3Rs approaches, (ii) opportunities for the 3Rs, and (iii) current state of the art in the region.</td>
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<tr>
<td>30 mins</td>
<td>Feedback session</td>
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<tr>
<td>10 mins</td>
<td>Wrap up</td>
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</tbody>
</table>
How you can get involved

- Surveys
  - Dissemination/completion
- Regional stakeholder workshops
  - Local organising committee/delegate
- Join the working group
  - Please email Elliot to discuss
Thank you!

For more information

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🏠 www.nc3rs.org.uk
🐦 @NC3Rs

Keep in touch

Our monthly newsletter provides the latest updates from the NC3Rs, including funding calls and events www.nc3rs.org.uk/register