National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

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

Dr Elliot Lilley – Programme Manager

Pioneering Better Science

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

NC 3Rs

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

NC 3Rs

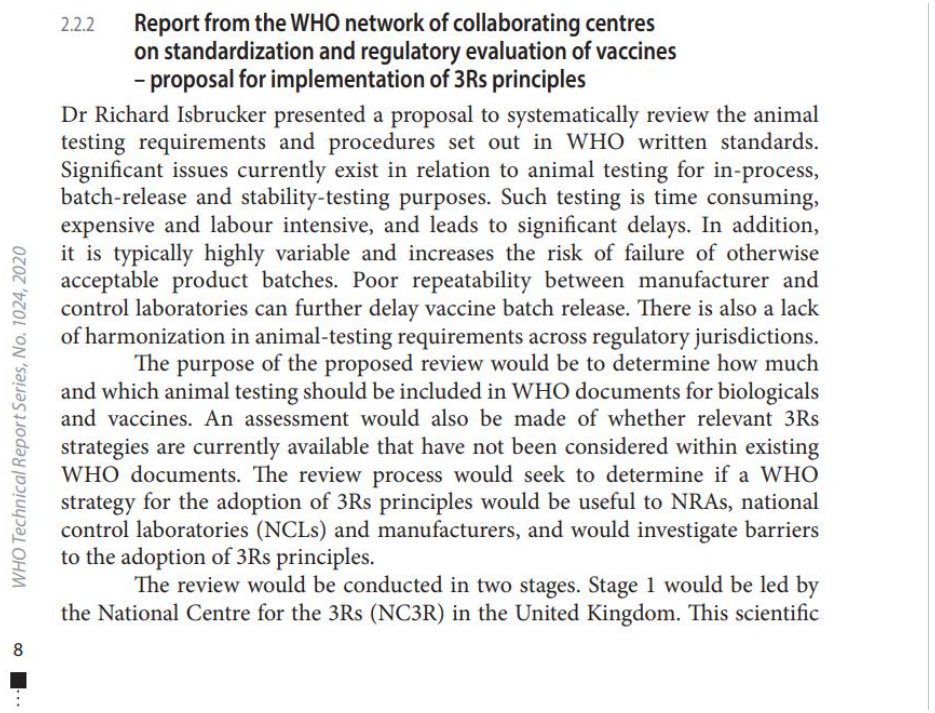
The project

- 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?

NC 3Rs

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.).



2.2.2 **Report from the WHO network of collaborating centres on standardization and regulatory evaluation of vaccines – proposal for implementation of 3Rs principles**

Dr Richard Isbrucker presented a proposal to systematically review the animal testing requirements and procedures set out in WHO written standards. Significant issues currently exist in relation to animal testing for in-process, batch-release and stability-testing purposes. Such testing is time consuming, expensive and labour intensive, and leads to significant delays. In addition, it is typically highly variable and increases the risk of failure of otherwise acceptable product batches. Poor repeatability between manufacturer and control laboratories can further delay vaccine batch release. There is also a lack of harmonization in animal-testing requirements across regulatory jurisdictions.

The purpose of the proposed review would be to determine how much and which animal testing should be included in WHO documents for biologicals and vaccines. An assessment would also be made of whether relevant 3Rs strategies are currently available that have not been considered within existing WHO documents. The review process would seek to determine if a WHO strategy for the adoption of 3Rs principles would be useful to NRAs, national control laboratories (NCLs) and manufacturers, and would investigate barriers to the adoption of 3Rs principles.

The review would be conducted in two stages. Stage 1 would be led by the National Centre for the 3Rs (NC3R) in the United Kingdom. This scientific

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

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

Stage 2 – WHO

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

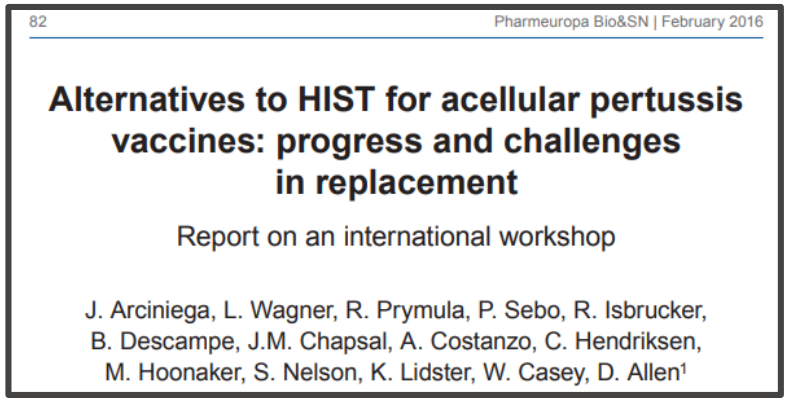
Estimated timeline: 2-3 years

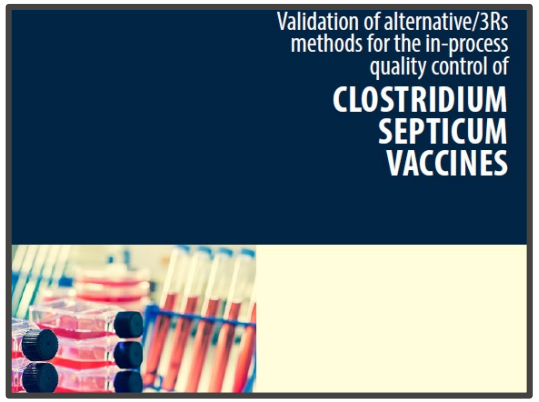
Why the NC3Rs is leading stage 1

- 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

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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Acute toxicity test no longer needed, industry review finds

9th January 2008

by Peter Mansell

A drug safety test that uses thousands of laboratory mice and rats each year in Europe is no longer necessary, a review involving 18 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


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NC3Rs refines animal acute inhalation testing

International effort defines 'evident toxicity' to improve animal welfare

17 December 2015 / Asia Pacific, Europe, North America, Risk assessment

A global initiative to refine acute animal inhalation testing has defined "evident toxicity" as an endpoint, in a draft test protocol. This could remove the final barrier to the adoption of a new, more humane *in vivo* test guideline.

The work, led by the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), has used

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

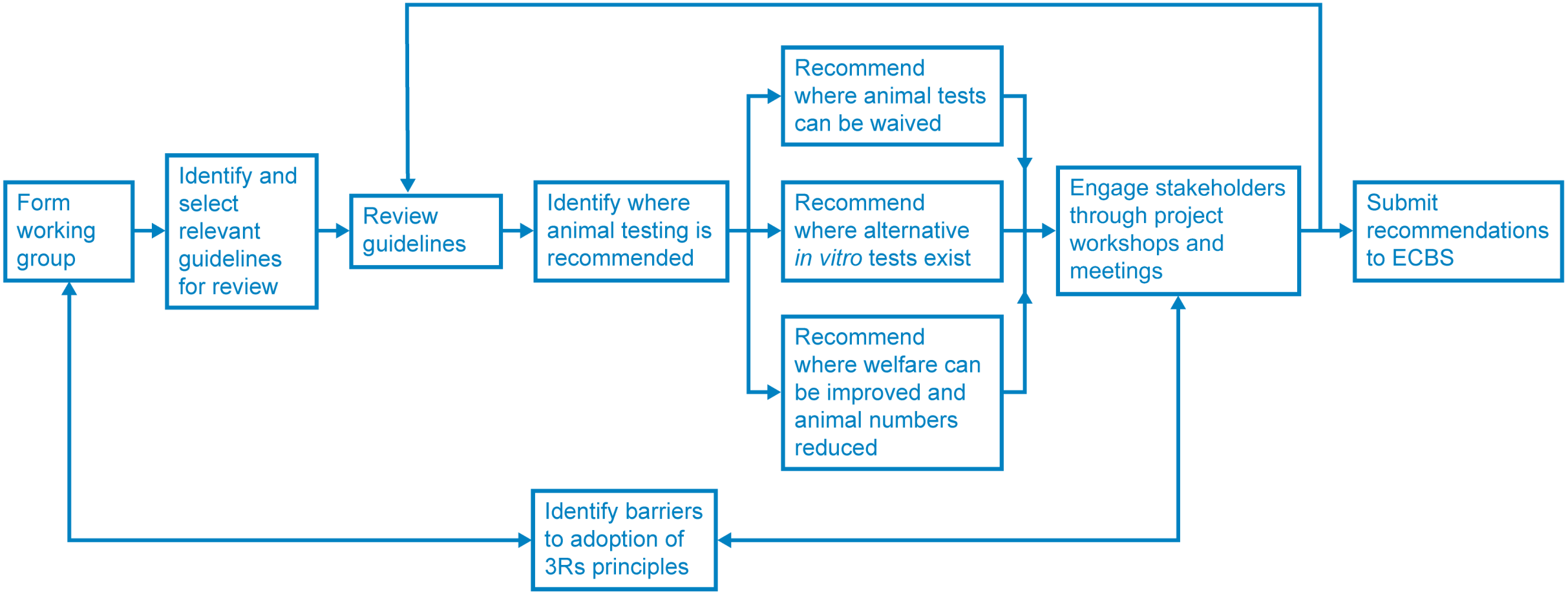
Out of scope

- 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

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.**

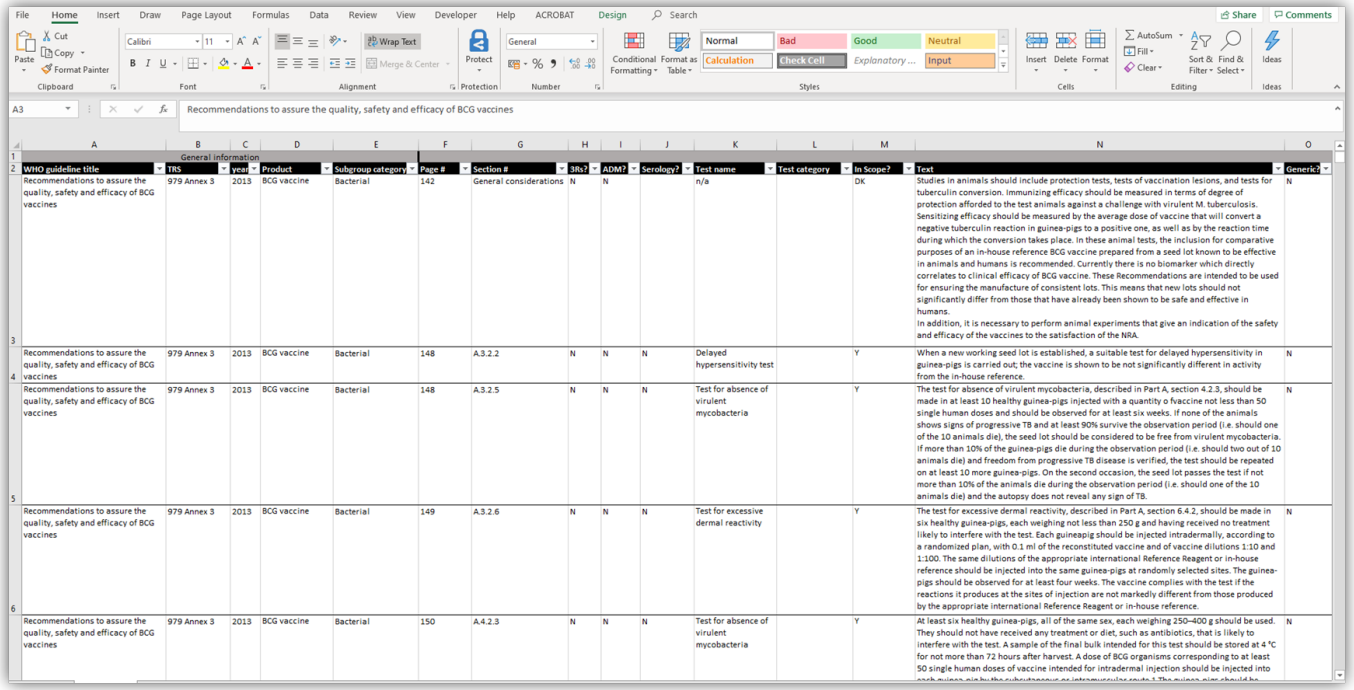
Phase one flow chart



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



Recommendations to assure the quality, safety and efficacy of BCG vaccines

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1		General information													
2	WHO guideline title	TRS	year	Product	Subgroup category	Page #	Section #	3Rs?	ADM?	Serology?	Test name	Test category	In Scope?	Text	Generic?
3	Recommendations to assure the quality, safety and efficacy of BCG vaccines	979 Annex 3	2013	BCG vaccine	Bacterial	142	General considerations	N	N		n/a		DK	Studies in animals should include protection tests, tests of vaccination lesions, and tests for tuberculin conversion. Immunizing efficacy should be measured in terms of degree of protection afforded to the test animals against a challenge with virulent M. tuberculosis. Sensitizing efficacy should be measured by the average dose of vaccine that will convert a negative tuberculin reaction in guinea-pigs to a positive one, as well as by the reaction time during which the conversion takes place. In these animal tests, the inclusion for comparative purposes of an in-house reference BCG vaccine prepared from a seed lot known to be effective in animals and humans is recommended. Currently there is no biomarker which directly correlates to clinical efficacy of BCG vaccine. These Recommendations are intended to be used for ensuring the manufacture of consistent lots. This means that new lots should not significantly differ from those that have already been shown to be safe and effective in humans. In addition, it is necessary to perform animal experiments that give an indication of the safety and efficacy of the vaccines to the satisfaction of the NRA.	N
4	Recommendations to assure the quality, safety and efficacy of BCG vaccines	979 Annex 3	2013	BCG vaccine	Bacterial	148	A.3.2.2	N	N	N	Delayed hypersensitivity test		Y	When a new working seed lot is established, a suitable test for delayed hypersensitivity in guinea-pigs is carried out; the vaccine is shown to be not significantly different in activity from the in-house reference.	N
5	Recommendations to assure the quality, safety and efficacy of BCG vaccines	979 Annex 3	2013	BCG vaccine	Bacterial	148	A.3.2.5	N	N	N	Test for absence of virulent mycobacteria		Y	The test for absence of virulent mycobacteria, described in Part A, section 4.2.3, should be made in at least 10 healthy guinea-pigs injected with a quantity o fvaccine not less than 50 single human doses and should be observed for at least six weeks. If none of the animals shows signs of progressive TB and at least 90% survive the observation period (i.e. should one of the 10 animals die), the seed lot should be considered to be free from virulent mycobacteria. If more than 10% of the guinea-pigs die during the observation period (i.e. should two out of 10 animals die) and freedom from progressive TB disease is verified, the test should be repeated on at least 10 more guinea-pigs. On the second occasion, the seed lot passes the test if not more than 10% of the animals die during the observation period (i.e. should one of the 10 animals die) and the autopsy does not reveal any sign of TB.	N
6	Recommendations to assure the quality, safety and efficacy of BCG vaccines	979 Annex 3	2013	BCG vaccine	Bacterial	149	A.3.2.6	N	N	N	Test for excessive dermal reactivity		Y	The test for excessive dermal reactivity, described in Part A, section 6.4.2, should be made in six healthy guinea-pigs, each weighing not less than 250 g and having received no treatment likely to interfere with the test. Each guineapig should be injected intradermally, according to a randomized plan, with 0.1 ml of the reconstituted vaccine and of vaccine dilutions 1:10 and 1:100. The same dilutions of the appropriate international Reference Reagent or in-house reference should be injected into the same guinea-pigs at randomly selected sites. The guinea-pigs should be observed for at least four weeks. The vaccine complies with the test if the reactions it produces at the sites of injection are not markedly different from those produced by the appropriate international Reference Reagent or in-house reference.	N
	Recommendations to assure the quality, safety and efficacy of BCG vaccines	979 Annex 3	2013	BCG vaccine	Bacterial	150	A.4.2.3	N	N	N	Test for absence of virulent mycobacteria		Y	At least six healthy guinea-pigs, all of the same sex, each weighing 250–400 g should be used. They should not have received any treatment or diet, such as antibiotics, that is likely to interfere with the test. A sample of the final bulk intended for this test should be stored at 4 °C for not more than 72 hours after harvest. A dose of BCG organisms corresponding to at least 50 single human doses of vaccine intended for intradermal injection should be injected into	N

- 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

3.1.3 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

"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

NC 3Rs

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

…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.

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.

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’

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.

NC 3Rs

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

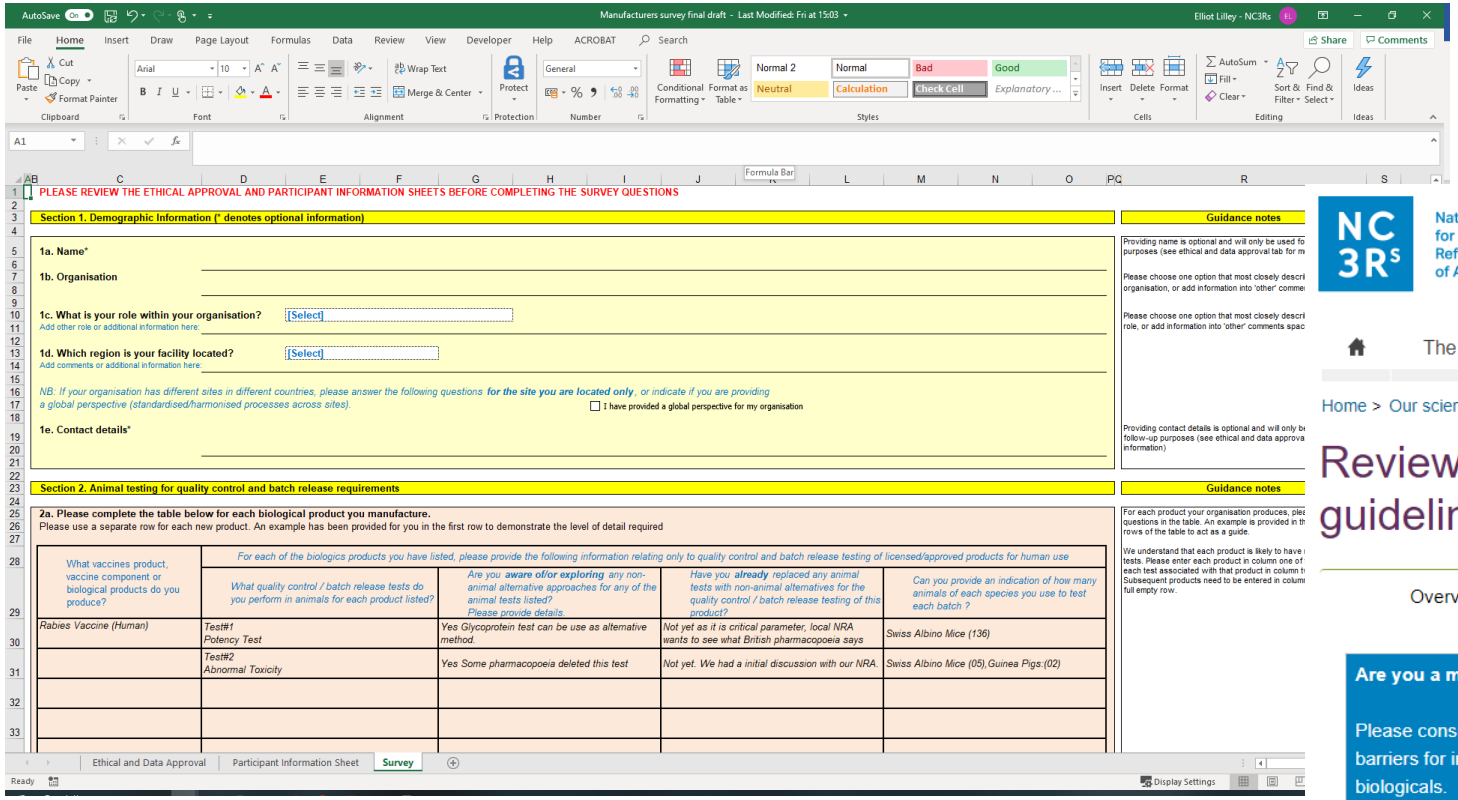
Engaging relevant expertise

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

How you can get involved

- Surveys
  - dissemination/completion

Manufacturers survey



PLEASE REVIEW THE ETHICAL APPROVAL AND PARTICIPANT INFORMATION SHEETS BEFORE COMPLETING THE SURVEY QUESTIONS

Section 1. Demographic Information (* denotes optional information) — Guidance notes

1a. Name*

1b. Organisation

1c. What is your role within your organisation? [Select]
Add other role or additional information here:

1d. Which region is your facility located? [Select]
Add comments or additional information here:

*NB: If your organisation has different sites in different countries, please answer the following questions **for the site you are located only**, or indicate if you are providing a global perspective (standardised/harmonised processes across sites).*

☐ I have provided a global perspective for my organisation

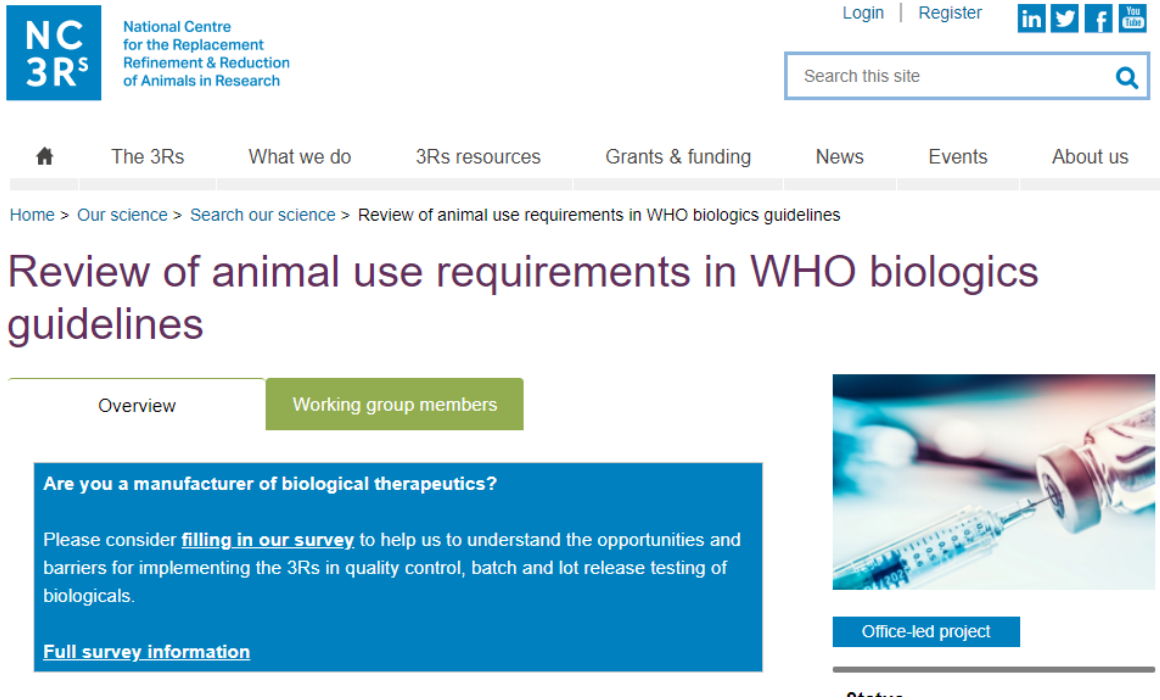
1e. Contact details*

Section 2. Animal testing for quality control and batch release requirements — Guidance notes

2a. Please complete the table below for each biological product you manufacture.
Please use a separate row for each new product. An example has been provided for you in the first row to demonstrate the level of detail required

What vaccines product, vaccine component or biological products do you produce?	For each of the biologics products you have listed, please provide the following information relating only to quality control and batch release testing of licensed/approved products for human use			
	What quality control / batch release tests do you perform in animals for each product listed?	Are you **aware of/or exploring** any non-animal alternative approaches for any of the animal tests listed? Please provide details.	Have you **already** replaced any animal tests with non-animal alternatives for the quality control / batch release testing of this product?	Can you provide an indication of how many animals of each species you use to test each batch ?
Rabies Vaccine (Human)	Test#1 Potency Test	Yes Glycoprotein test can be use as alternative method.	Not yet as it is critical parameter, local NRA wants to see what British pharmacopoeia says	Swiss Albino Mice (136)
	Test#2 Abnormal Toxicity	Yes Some pharmacopoeia deleted this test	Not yet. We had a initial discussion with our NRA.	Swiss Albino Mice (05),Guinea Pigs:(02)

Ethical and Data Approval | Participant Information Sheet | Survey



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Review of animal use requirements in WHO biologics guidelines

Overview | Working group members

Are you a manufacturer of biological therapeutics?

Please consider **filling in our survey** to help us to understand the opportunities and barriers for implementing the 3Rs in quality control, batch and lot release testing of biologicals.

Full survey information

Office-led project

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

5 mins	Welcome and introduction
10 mins	Aims and objectives for the meeting *Delivered by a local host to make it clear the focus is on a regional perspective*
Scene setting	
15 mins	Introduction to NC3Rs project
30 mins	Local regulator and manufacturer perspective *To give their perspectives on animal use in QC and batch release testing and the opportunities/challenges for implementing 3Rs approaches.*
20 mins	BREAK
3Rs models/approaches state of the art	
30 mins	3x flash talks (10 mins each) from (*ideally*) local manufacturers/scientists/regulators on the development and application of 3Rs approaches.
10 mins	Introduction to the breakout sessions
45 mins	BREAK
Breakout sessions	
50 mins	Breakout discussion session 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.
30 mins	Feedback session
10 mins	Wrap up

How you can get involved

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

National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

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

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