





The RAT list: five animal tests that could end immediately in the UK

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Cruelty Free International

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solely to end animal
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International Council on Animal Protection in OECD Programmes

The RAT list: a tool for highlighting areas of animal use ready for replacement

What the RAT list is

How we use the RAT list

Future plans for the RAT list

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RAT list = Replace Animal Tests list

- Five animal tests that are still conducted despite having valid, accepted replacements
- Regulatory, mostly for “safety”
- Use an estimated 80,000 animals each year in the UK alone

crueltyfreeinternational.org/replace-animal-tests-uk-rat-list



UK RAT list tests

1

**Skin
irritation**

#TheRatList
Replace Animal Tests



2

**Skin
sensitisation**

#TheRatList
Replace Animal Tests



3

**Botulinum toxin
(Botox) batch potency**

#TheRatList
Replace Animal Tests



4

**Antibody
production**

#TheRatList
Replace Animal Tests



5

**Veterinary
vaccines batch safety**

#TheRatList
Replace Animal Tests



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UK RAT list tests

1

Skin irritation

#TheRatList
Replace Animal Tests



Species: Rabbits

Number of tests in the UK: 18

Product test is used for: Medical devices

Animal method: A test substance is applied to a 6 cm area of a rabbit's shaved skin and held in place with a bandage for four hours. The rabbit is then examined for signs of skin damage for 14 days. If there are no signs of irritation, two more rabbits are used in a confirmatory test. Rabbits are singly housed and can suffer from painful skin reactions and rashes.

Replacement method: Reconstituted human skin models can be used to predict skin irritation in humans with more accuracy than the rabbit test.

UK RAT list tests

2

Skin sensitisation

#TheRatList
Replace Animal Tests



Species: Mice

Number of tests in the UK: 357

Product test is used for: Chemicals, foods and food contact materials

Animal method: A substance is applied to the ears of mice every day for three days. The mice are then killed three days later, and their ears are dissected.

Replacement method: Several chemical-, computer- and cell-based tests can be used in combinations known as “defined approaches”, formalised in a guideline in 2021. These tests include the direct peptide reactivity assay (DPRA), which measures the binding of a substance to proteins, the keratinocyte assay and the human Cell Line Activation Test (h-CLAT) based on human immune cells.

UK RAT list tests

3

**Botulinum toxin
(Botox) batch potency**

#TheRatList
Replace Animal Tests



Species: Mice

Number of tests in the UK: 56,804

Product test is used for: Human medicines

Animal method: Mice are injected into their abdomens with botulinum toxin and, over the following three days, become increasingly paralysed. Without intervention, mice in the higher dose groups stop being able to breathe and suffocate to death.

Approximately 50% of the mice die per test, and survivors are killed at the end of the test.

Replacement method: The major botox manufacturers have developed a cell-based test to replace the animal batch test for their products.

UK RAT list tests

4

**Antibody
production**

#TheRatList
Replace Animal Tests



Species: Mice, rabbits, sheep, goats, others

Number of tests in the UK: 27,263

Product test is used for: N/A

Animal method: Animals are injected with a substance that stimulates the immune system to produce specific antibodies before their blood, spleen cells or abdominal fluid are taken to harvest the desired antibodies. The animals often suffer from harmful side effects and are killed when they are no longer useful.

Replacement method: Phage display technology can be used to produce a wide range of antibodies that are better quality and more stable, relevant and consistent than those produced in animals. They can also be much faster and cheaper to produce.

UK RAT list tests

5

**Veterinary
vaccines batch safety**

#TheRatList
Replace Animal Tests



Species: Unknown

Number of tests in the UK: Unknown

Product test is used for: Veterinary medicines

Animal method: In the target and laboratory animal batch safety tests, animals are injected with two doses of an inactivated vaccine, or ten doses of a live vaccine, and then observed for at least two weeks. Animals can suffer painful reactions on the rare occasion the batch is contaminated, and as a result of large injection volumes relative to the animal's size (young animals are often used).

Replacement method: Improvements in manufacturing processes have made animal batch tests redundant. Analytical tools and cell-based tests help to establish a "product profile" for each vaccine batch, which is used as part of a "consistency approach" to ensure that each batch is consistent with previously tested batches.

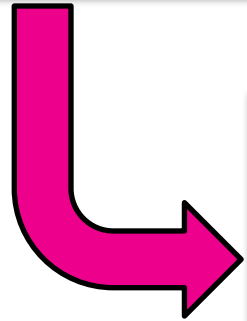
What's the significance of the RAT list?

ELIZABETH II

Animals (Scientific Procedures) Act 1986

1986 CHAPTER 14

If the principle of replacement was properly implemented, the RAT list would not exist!



2A Principles of replacement, reduction and refinement

- (1) The Secretary of State must exercise his or her functions under this Act with a view to ensuring compliance with the principles of replacement, reduction and refinement.
- (2) For the purposes of this Act—
 - (a) the principle of replacement is the principle that, wherever possible, a scientifically satisfactory method or testing strategy not entailing the use of protected animals must be used instead of a regulated procedure;

“Reasons... for slow uptake of 3Rs advances included... Lack of published data on how results using replacement technologies compare to established animal models and concerns about acceptance... by the regulator”



The role of review
and regulatory
approvals processes
for animal research
in supporting
implementation
of the 3Rs

A report by Dr Frances Rawle,
commissioned by the NC3Rs

February 2023

“Replacement does not seem to be covered well by any of the review processes”

“AWERBs and ASRU inspectors rarely suggest use of replacements”

The RAT list: a tool for highlighting areas of animal use ready for replacement

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How we use the RAT list – understanding the problem

- For each test:
 - How many (where possible)
 - Why i.e. legislative requirement (chemicals, medicines etc.) and origin (UK vs. non-UK)
- Main reasons:
 - Lack of global harmonisation
 - Unclear regulation or guideline
 - Lack of enforcement
 - Lack of sector- or product-specific validation



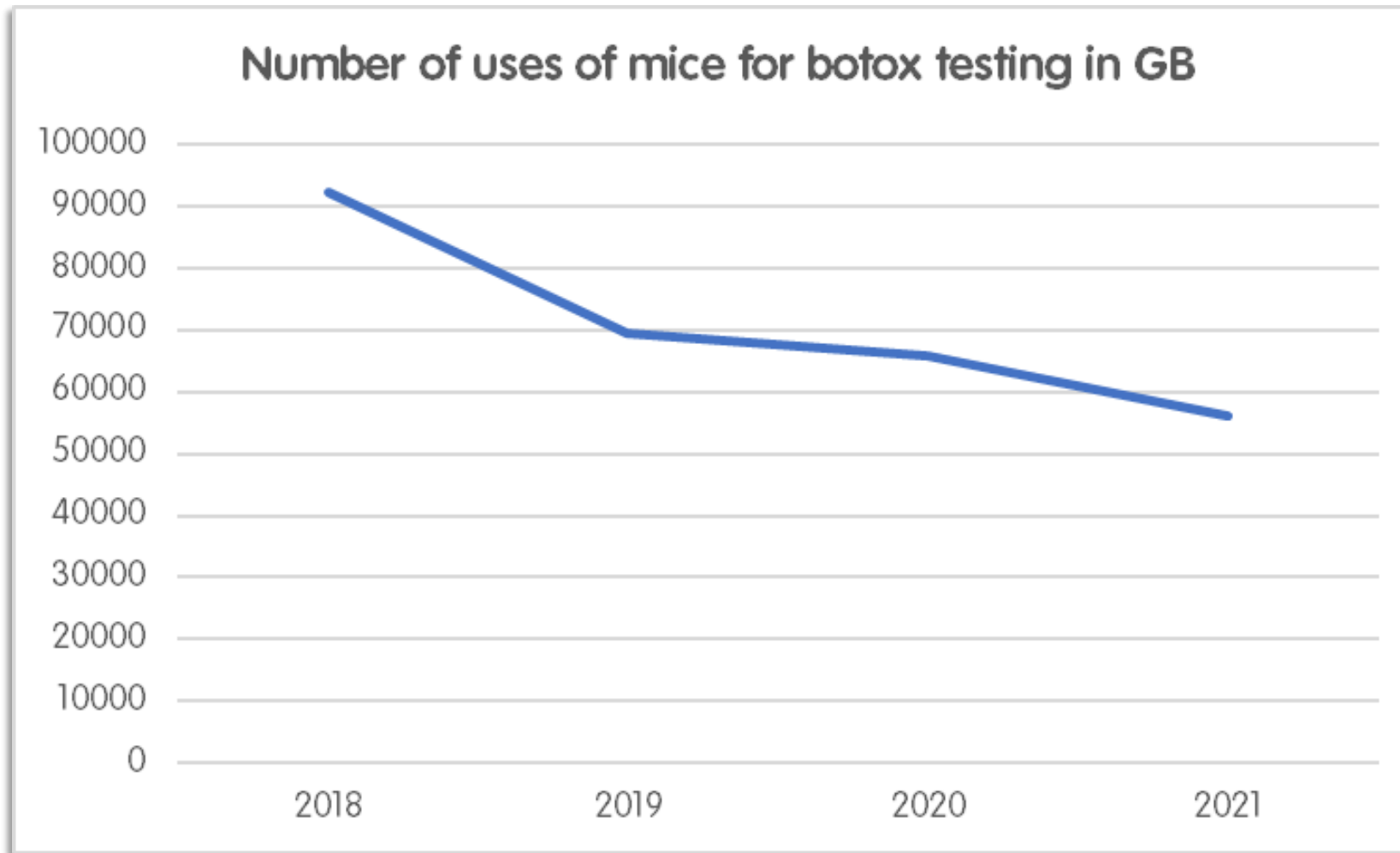
How we use the RAT list – understanding botox testing

- 56,084* tests on mice in 2021
- Animal test: mice injected with botulinum toxin to measure its potency – expected outcome is that mice suffer and die
- Cell-based assay (replacement method) approved since 2012

*Batch potency tests on mice to meet requirements of human medicines legislation, assumed to all be for botox



How we use the RAT list – understanding botox testing



How we use the RAT list – understanding botox testing

Question



Luke Pollard
Labour
Plymouth, Sutton and Devonport

 Commons

To ask the Secretary of State for the Home Department, pursuant to the Answer of 22 March 2022 to Question 121942, on Animal Experiments, of the 65,888 botulinum tests carried out, what product was being tested in those tests; and for what reason a validated cell-based assay was not used for those tests.

[^ Hide full question](#)

Answer



Damian Hinds
Conservative
East Hampshire

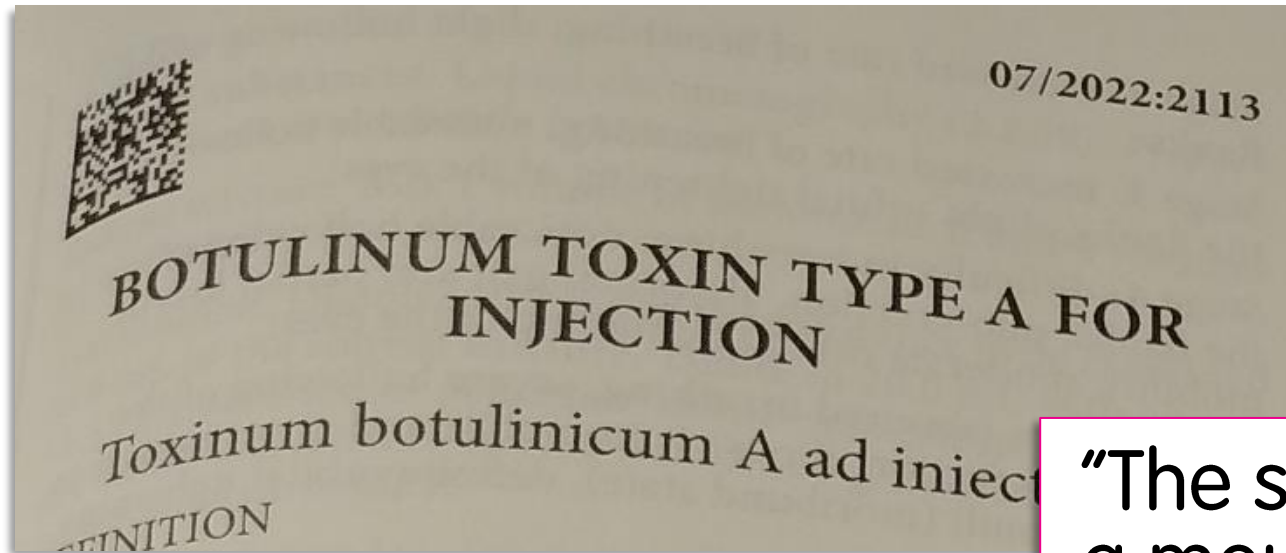
 Commons

Answered on
30 March 2022
Pursuant to answer 121942 the substance being tested in 65,888 procedures was Botulinum Toxin.
Animal testing was authorised because there was not a validated non-animal alternative acceptable to the relevant regulator to meet these legal requirements.

100% of assumed botox tests
in 2021 were conducted to
meet EU requirements

Relevant regulator =
European Medicines Agency?

How we use the RAT list – understanding botox testing



“The specific activity is confirmed in a mouse model of toxicity or, in the interests of animal welfare, by an *in vitro* method (e.g. a cell-based assay) validated with respect to the LD₅₀ assay”

How we use the RAT list – understanding botox testing

Why is the animal test still taking place?

- ~~Lack of global harmonisation~~
- Lack of enforcement
- Lack of sector or product-specific validation
- ~~Unclear regulation or guideline~~



How we use the RAT list – educating and lobbying

- On Cruelty Free International website to educate public
- Used internally to inform and direct campaigns
- Used to support lobbying activities
 - Example: EU RAT list presented to Member States' National Contact Points for Directive 2010/63/EU in 2021



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- Continue to grow and develop the RAT list based on animal use figures and scientific progress
 - Remove tests that are no longer conducted
 - Keep an eye on what's becoming replaceable e.g. fish acute toxicity test
- Continue to use RAT list to highlight, track, educate and lobby



Thank you for your attention



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Further reading:

- UK RAT list:
<http://crueltyfreeinternational.org/replace-animal-tests-uk-rat-list>
- NC3Rs report: <http://nc3rs.org.uk/role-review-and-regulatory-approvals-processes-animal-research-supporting-implementation-3rs-2023>
- Botox testing paper:
<https://www.altex.org/index.php/altex/article/view/1138>

