The RAT list: a tool to identify animal use replacement opportunities

Laura Alvarez
Deputy Director of Science & Regulatory Affairs
Laura.Alvarez@crueltyfreeinternational.org

Why we created the RAT list

It may be commonly assumed that animal tests are not used, or very rarely, in cases where non-animal methods are available.

The reality is that such tests can persist, and even increase, long after suitable non-animal replacements have become available.

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Replace Animal Tests: our RAT list

The RAT list highlights ten animal tests that are still conducted despite having available non-animal replacements. We use this as a tool to track trends across sectors and regions, raise awareness and identify areas where there is near-term opportunity for replacement.

If these 10 tests were removed, approximately 1.2 million animals could be spared every year in Europe alone!

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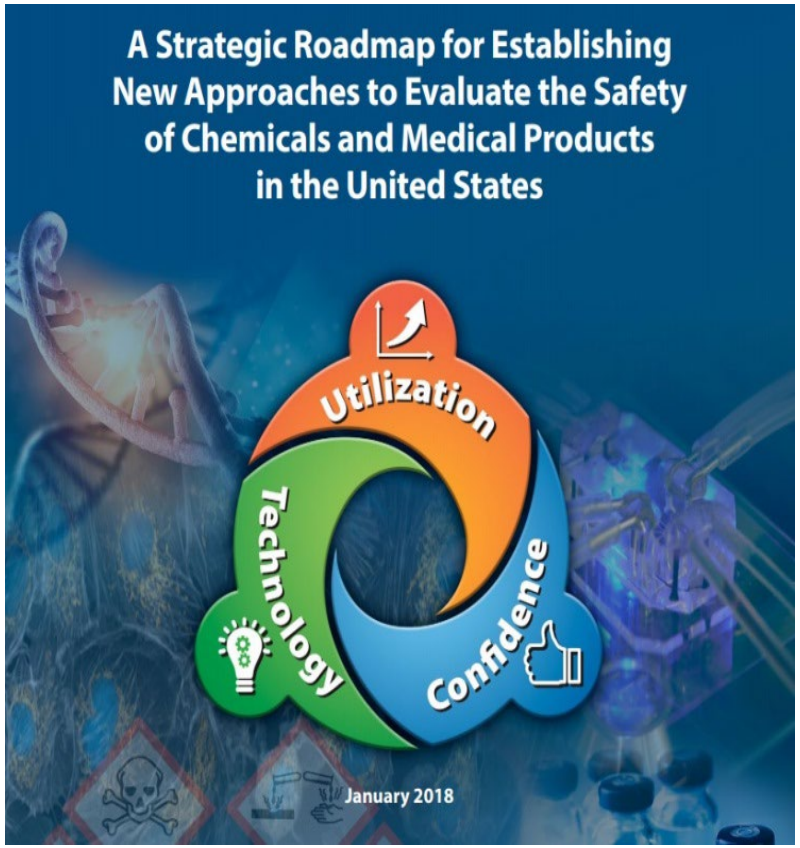
ICCVAM Strategic Roadmap

Multi-agency strategy for toxicity testing to improve human relevance and reduce the use of animals



Goal 3: Encourage the adoption and use of new methods and approaches by federal agencies and regulated industries.

- Provide clear language regarding the acceptance of NAMs
- Collaborate with international partners to facilitate global harmonization and regulatory acceptance
- Explore processes to incentivize and promote the use of NAMs
- Identify appropriate metrics for prioritizing activities, monitoring progress, and measuring success.

Roadmap to Reducing Animal Testing in Preclinical Safety Studies

Executive Summary

This roadmap outlines a strategic, stepwise approach for FDA to reduce animal testing in preclinical safety studies with scientifically validated new approach methodologies (NAMs), such as organ-on-a-chip systems, computational modeling, and advanced *in vitro* assays. By partnering with federal agencies like NIH and VA through ICCVAM, FDA can accelerate the validation and adoption of these human-relevant methods, improving predictive accuracy while reducing animal use. This transition will enhance public health by streamlining drug development and ensuring safer therapies reach patients faster, while positioning FDA as a global leader in modern regulatory science and innovation.

Ways to immediately reduce animal testing

- What has already been tested internationally that doesn't require additional data?
- What regulations have worked in Europe and other parts of the world to reduce animal testing?
- What non-animal tests are already widely accepted?
- Use ELSA to identify nams already accepted by FDA
- Incentivize meeting early about reducing animal testing and incorporating nams; avoid animal data if possible
- FDA can widely publicize use cases where animal testing reduced/nams data are accepted

WORKSHOP | IN PERSON

FDA-NIH Workshop: Reducing Animal Testir

JULY 7, 2025

Examples of immediate actions FDA can take (Identified in collaboration with NIH)

- **Rabies vaccine batch release testing.** Adopt ELISA assays to measure antigen content that have been validated in Europe & replace animal testing
- **Shellfish**. Replace the mouse bioassay to identify toxins in shellfish intended for human consumption with well-established non-animal methods that can better protect human health.
- **Leptospirosis vaccine potency testing**. Non-animal ELISA available from the USDA, but only four of six manufacturers have adopted its use. Encourage widespread and exclusive use.
- **Protein quality testing.** Replace rat digestibility test that has long been required for protein content claims with validated in vitro assays (e.g. pH-drop and pH-stat methods).
- **Pyrogen testing for parenteral products.** Replace rabbit pyrogen test and horseshoe crab-blood based endotoxin tests with in vitro Monocyte Activation Test and recombinant Bacterial Endotoxin Test methods.
- **Skin irritation testing.** Adopt ISO 10993-23 standard on skin irritation testing that gives preference to in vitro methods (e.g. for evaluating medical devices).

What's in the RAT list

- **How many animals are used** – annual figures (EU data) to show scale and help prioritise
- **What the tests involve** – description of both the animal test and non-animal test
- **Regulatory drivers** – which sector (e.g. chemicals, medicines) and which region (e.g. EU vs non-EU)
- **Where the test stands globally** – is the alternative accepted in some regions (e.g. EU) but not others (e.g. US)?
- **What's stopping change** – key barriers to NAMs uptake e.g. guidance needs, additional validation work, training and awareness

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Why do these tests persist?

- Lack of regulatory enforcement and guidance
- Need for a defined approach
- Product specific validation required
- Availability of the non-animal method
- Lack of confidence in the non-animal replacement
- Lack of global harmonization

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Using the RAT list

e.g. to compare uptake of non-animal methods in EU vs US

Test	Progress in the EU	Progress in the US	Main Barrier(s)
	Since 2016, REACH legislation no longer requires the animal tests. 2015 EMA GL on local tolerance testing includes option to use in vitro tests. Animal tests still widely used for medical devices and plant protection products (PPP).	Since 2020 FDA accepts alternatives for screening purposes but prefers guinea pig tests over LLNA. Guinea pig tests required for medical devices. Since 2018 EPA accepts alternatives for single chemicals, not mixtures.	• Validation needed for medical devices • Lack of regulatory enforcement and guidance • Lack of global harmonization
	MAT introduced in Ph. Eur. in 2009 and strongly encouraged as a replacement in 2016. rFC method introduced in 2016 and given its own chapter in 2020. In 2021, a five-year plan was announced: RPT to be deleted from Ph. Eur. by July 2025.	FDA issued guidance in 2012 stating that the MAT or rFC can be used after product-specific validation. MAT is under MDDT review. Provision to use in vitro tests included in 2017 update to USP.	• Product specific validation required • Lack of regulatory enforcement and guidance • Cost and training associated with non-animal methods
	In 2020, ECVAM recommended that companies switch to the 'phage display' alternative method. Uptake low in some countries (e.g., France still using ascites method).	Events hosted by NICEATM and ICCVAM to discuss the advantages of moving away from animal-based antibodies, but no formal initiative or regulatory requirement.	• Availability of the alternative method • Lack of confidence in the non-animal replacement • Lack of regulatory enforcement

Suggested new activities for ICCVAM

We recommend that ICCVAM establishes the following workgroups:

Topic	Suggested WG activities
Batch testing for biologicals	• Support phase-out of TABST, LABST and ATT • Address implementation gaps in VICH waivers • Support harmonized regulatory acceptance of NAMs through WHO, EMA collaboration • Identify priority areas (e.g. leptospirosis, rabies) for full transition to in vitro methods
Skin sensitization	• Reactivate skin sensitization workgroup • Promote harmonized guidance on use of OECD-defined approaches • Address remaining validation needs (e.g. medical devices)
Botulinum toxin potency testing	• Conduct survey of manufacturers and regulators to identify barriers to CBA adoption • Promote harmonized use of CBA across all production stages and support phase-out of LD50 test
Antibody production	• Publish formal recommendation on use of non-animal-derived antibodies • Organize workshops/training webinars to tackle misconceptions and highlight scientific benefits • Develop national resource listing suppliers of non-animal antibodies.
Pyrogenicity testing	• Support deletion of rabbit pyrogen test from legal requirements • Conduct nationwide survey to scope use of RPT (e.g. EPAA survey in EU) • Organize workshops/training webinars to tackle misconceptions and encourage use of non-animal methods.
Marine biotoxin testing	• Conduct nationwide survey on use of mouse bioassay and barriers to alternatives • Organize workshop to support broader uptake of NAMs and key challenges

Political Momentum: UK and EU Parliaments

We presented our RAT list to MPs and MEPs at roundtable events in UK and European Parliaments

<u>Key points raised:</u>

- The need for greater engagement and input from industry on these tests
- Importance of cross-sector as well as global harmonisation
- Political support for increased funding to address remaining barriers

There is clear political support to end the use of animals in RAT List tests

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

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Laura Rego Alvarez, Cruelty Free International, London, UK

Introduction

It may be commonly assumed that those tests on animals which have non-animal replacements available are no longer conducted, or at least rarely. The reality is that such tests can persist, and even increase, long after the adoption of suitable alternative methods.

To draw attention to this issue, we created the Replace Animal Tests (RAT) list to highlight ten regulatory tests using animals that are still conducted despite having validated non-animal replacements. The total number of animals still being used in these tests could be 1.4 million in Europe alone, based on statistics from 2020.

	Progress in the EU	No. of EU tests	Progress in the US	Progress internationally
1 Skin irritation	Since 2016 EU REACH no longer requires the animal test by default. 2015 EMA GL on local tolerance testing includes option to use in vitro tests.	4,070	FDA no longer recommends standalone skin irritation studies but leaves decision with manufacturers. Waivers accepted by EPA since 2012.	ISO 10993-23 published in 2021 to include in vitro tests for medical devices. OECD published IATA in 2014. ICH M3(R2) does not require standalone studies.
2 Eye irritation	Since 2016 EU REACH no longer requires the animal test by default. 2015 EMA GL on local tolerance testing includes option to use in vitro tests.	491	CDER recommends non-animal tests for reformulated topical drug products and accepts waivers for other products. Waivers accepted by EPA since 2012.	ISO 10993-23 published in 2021 to include in vitro tests for medical devices. OECD published defined approaches (TG 467) in 2022. ICH M3(R2) does not require standalone studies.
3 Skin sensitisation	Since 2016 EU REACH no longer requires the animal test by default. 2015 EMA GL on local tolerance testing includes option to use in vitro tests.	38,024	Since 2020 FDA accepts non-animal tests for screening but prefers guinea pig tests over LLNA. Since 2018 EPA accepts non-animal tests for single chemicals, not mixtures.	2023 ISO guidance on validation of non-animal test methods for medical devices. Can only be used as screening methods as per ISO 10993-10. ICH M3(R2) does not require standalone studies.
4 Pyrogenicity	MAT and rFC introduced in Ph. Eur. in 2009 and 2016, respectively. In 2021, 5-year plan announced to delete rabbit test from EU Ph.	24,139	FDA issued guidance in 2012 stating that the MAT or rFC can be used after product-specific validation. Provision to use in vitro tests included in 2017 update to USP.	ISO 10993-1 (2018) gives preference to in vitro methods when they yield equivalent information to rabbit test. ICH Q4B, Annex 14 states LAL can be used interchangeably in ICH regions.
5 Botulinum toxin test	Cell-based method (CBA) included in Ph. Eur. since 2012 (mouse test remains default). Three manufacturers received EU approval in 2011-2018.	273,955	Between 2011 and 2019 three major manufacturers have had the CBA approved by the FDA. Mouse test still the default test accepted by FDA.	Other manufacturers appearing on the market (e.g., from South Korea and China) may or may not be using the CBA.
6 Antibody production	In 2020, ECVAM recommended that companies switch to the 'phage display' alternative method.	1 million	Events have been held by NICEATM and ICCVAM to discuss the advantages of moving away from animal-based antibodies.	The same issues of awareness of the new technology and lack of pressure to switch over likely to exist all over the world.
7 Leptospira vaccine potency test	Ph. Eur. updated in 2015 to include option to waive hamster test based on 'consistency of production'.	3,642	In 2013 USDA published guidance for obtaining exemption to hamster test. 60% of companies thought to have transitioned to the ELISA.	Not known if other drug regulators around the world will accept the ELISA and if this is a reason for the continued use of hamsters.
8 Veterinary vaccines batch test	LABST deleted from the Ph. Eur. in 1997 and TABST deleted in 2012, except for three vet vaccines.	720	TABST and LABST waivers have been accepted in US as per VICH guidelines since 2013 and 2019, respectively.	Waivers are accepted in Japan and Canada. Potential for deletion/waivers under discussion in India and Brazil. TABST/LABST still required for other markets (e.g., China, South Korea, Russia).
9 Abnormal toxicity	Test completely deleted from the Ph. Eur. in 2019.	2,810	No longer required since 2015 but companies must proactively request for test to be removed from their product licenses.	Test discontinued by the WHO in 2018. No longer required in Brazil, Canada, South Korea and India. Potential for deletion/waivers under discussion in China, Japan, Russia and others.
10 Shellfish toxins	In 2014 mouse test (MBA) was removed from EU regulation as reference method for detecting DST and in 2019, PST.	25,884	In 2014, Maine became first state to receive FDA approval to use the HPLC method. MBA not recommended in US FDA NSSP guide for AST and DST but is still listed for PST.	MBA no longer routinely used in Canada, Australia, the United Kingdom, Ireland or New Zealand. Still recommended in Codex Alimentarius as method for routine control.

Why do these tests persist?

The main issues with achieving complete replacement of these ten tests are:

- Lack of global harmonization
- Lack of monitoring and enforcement
- Need for defined approaches
- Product specific validation required
- Availability of the non-animal alternative
- Lack of confidence in the non-animal alternative

Conclusion

The RAT list is a useful tool to highlight animal tests that are long overdue for replacement, track trends across sectors and regions, educate the public and lobby the relevant regulatory authorities to complete the replacement process once and for all, for both scientific and ethical reasons.

For more information contact: Laura.Alvarez@CrueltyFreeInternational.org

Poster presentation at WC12, Canada

2023

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Session 143

Sailing the Seas of Validation: Challenges Encountered to Chart the Course Ahead

Track: Human Health - Policies

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- Abstract 435: The RAT list: tracking replaceable animal tests and identifying systemic barriers to non-animal methods

  Laura Alvarez, *Cruelty Free International*

Laura.Alvarez@crueltyfreeinternational.org