

Animals in science



Phasing out the rabbit
pyrogenicity test –
challenges and
opportunities

*EDQM-EPAA Symposium:
Pyrogen testing 2.0
25-26 February 2026*

The Three Rs

➤ **REDUCE**



➤ **REPLACE**



➤ **REFINE**



Replacement, a legal requirement



Photo by Vincent Pelletier - Pexels

Article 13 of the Directive:

Animal use can no longer be authorised^{*)} if another method, not entailing the use of animals, is recognized under the legislation of the Union.

^{) unless product specific validation fails}*

2023 Conference - Outcomes



- *Willingness to eliminate unnecessary use of RPT*
- *Industry and contract laboratories shared experiences designing, validating, and successfully implementing different MAT assays*
- *Identification of many unresolved challenges*
 - Lack of expertise and training opportunities
 - Associated costs
 - Availability of monocyte cell sources
 - Limited number of regulatory texts recognising MAT
 - Lack of global harmonisation

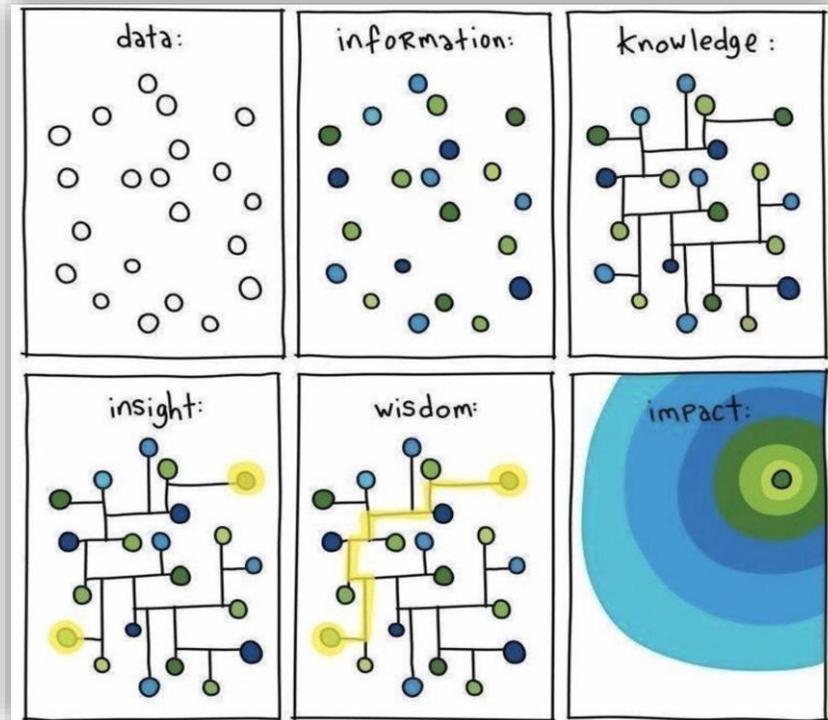
2026 Conference - Objectives



How successfully have we tackled the identified issues?

- *Progress in the RPT phase-out strategy in Europe*
- *Challenges and solutions with the transition to MAT*
- *Status of the bacterial endotoxins test, BET*
- *Collaboration to align regulatory frameworks globally*

Transparency to help drive progress



How many uses of rabbits for pyrogenicity?

➤ ***ALURES statistical database***

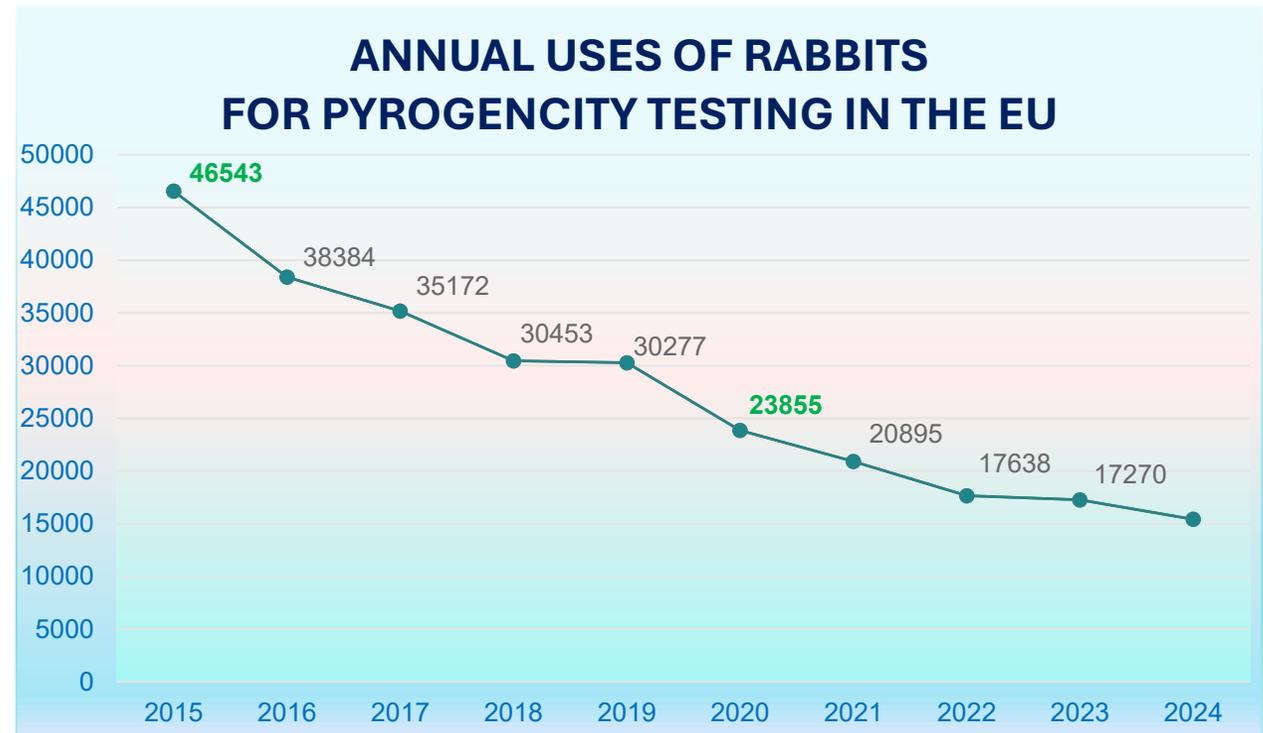
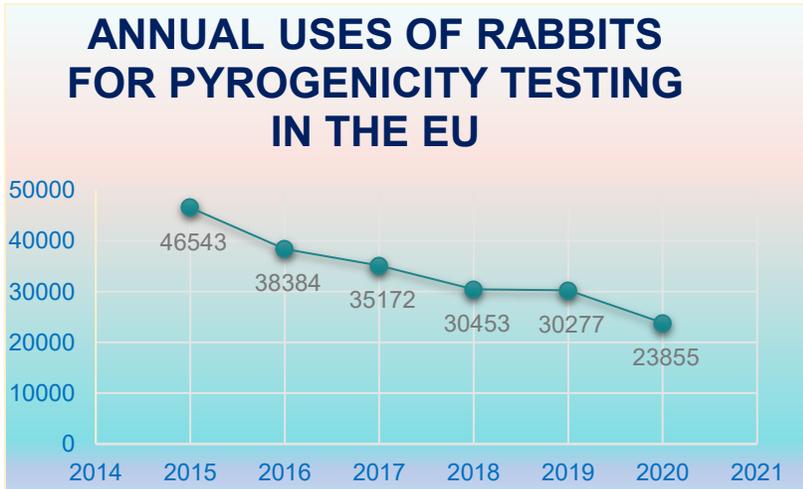
Can we find out more about the reasons?

➤ ***MS narratives for statistical data***

➤ ***Non-technical project summaries (NTS)***



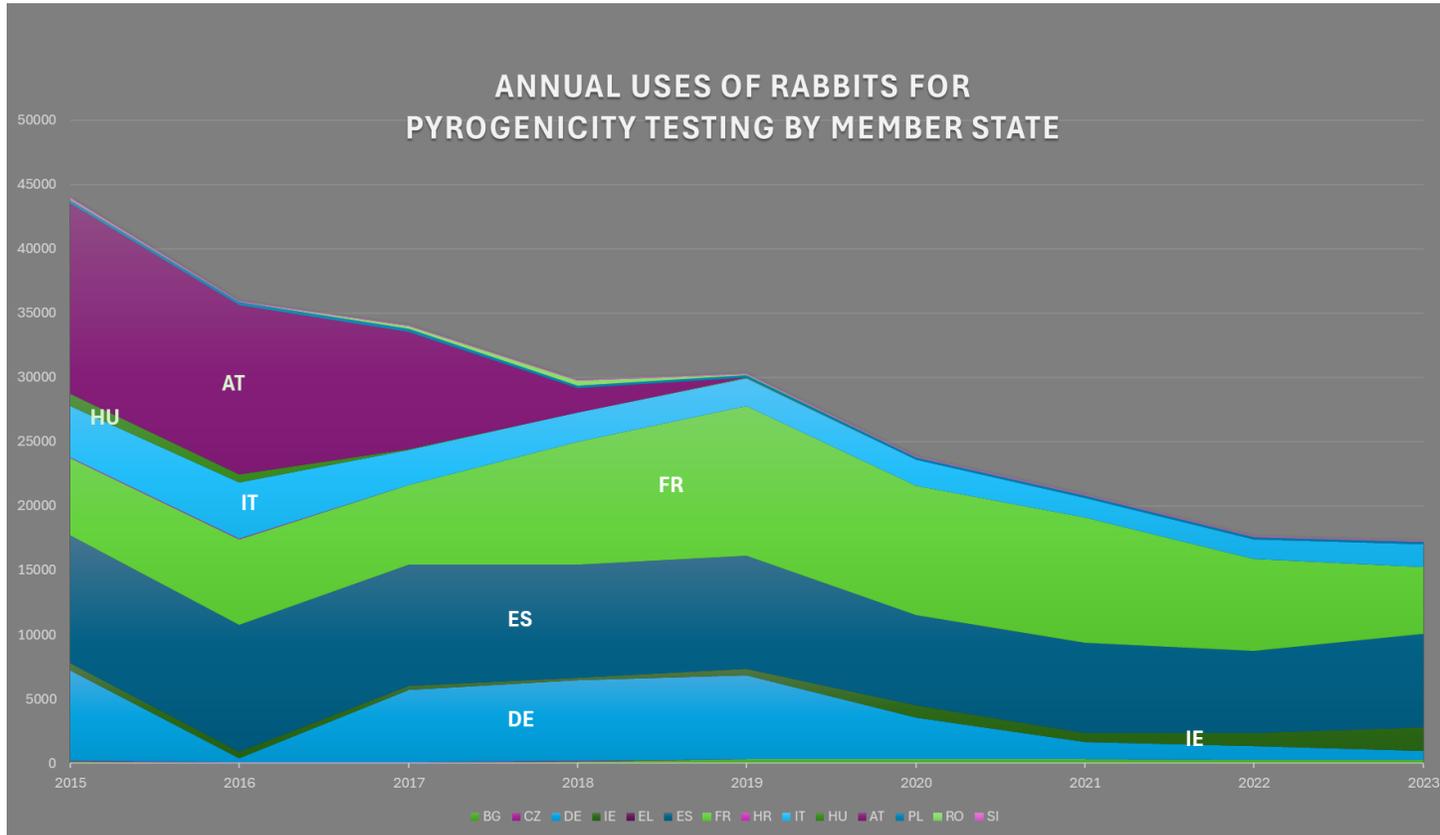
Total RPT use in the EU



- 2015-2020 (5 years): > **49%**
- 2020-2024 (4 years): > **35%**



RPT use in the EU by MS



Selected countries

| | AT | HU | IT | FR | ES | IE | DE | Total | Year-on-year decrease |
|------|-------|-----|------|-------|------|------|------|-------|-----------------------|
| 2015 | 14794 | 952 | 4007 | 5971 | 9960 | 570 | 6992 | 43246 | |
| 2016 | 13157 | 646 | 4352 | 6639 | 9878 | 506 | 347 | 35525 | -21.7% |
| 2017 | 9125 | 29 | 2717 | 6191 | 9472 | 312 | 5591 | 33437 | -6.2% |
| 2018 | 1923 | 20 | 2243 | 9578 | 8802 | 145 | 6291 | 29002 | -15.3% |
| 2019 | 0 | 37 | 2177 | 11597 | 8827 | 525 | 6457 | 29620 | +2.1% |
| 2020 | 0 | 14 | 2025 | 10041 | 7005 | 943 | 3223 | 23251 | -27.4% |
| 2021 | 0 | 0 | 1531 | 9711 | 7055 | 657 | 1371 | 20325 | -14.4% |
| 2022 | 0 | 0 | 1518 | 7145 | 6429 | 966 | 1078 | 17136 | -18.6% |
| 2023 | 0 | 0 | 1784 | 5161 | 7294 | 1834 | 695 | 16768 | -2.2% |
| 2024 | | | | | | | | | -12.0% |

- 2015 - 2024: from 14 to 9 MS reporting RPT
- In 2024, overall estimated reduction in the entire EU since 2015 > 67%



Member State narratives



In 2023, such cases were identified in six instances. Explanations point to inconsistencies with other legislation or the specific nature of the tested product as the

authorisation. A significant decrease of 28% was observed in 2023, with 5,161 uses compared to 7,145 in 2022. However, two areas of concern remain. The first has to do with vaccines produced in Europe and exported to third countries that maintain the RPT obligation. The second concerns the use of this test for medical devices that are outside the scope of the European Pharmacopoeia.

legislation of the Union. However, the use of these methods for certain classes of medicinal products (e.g., some blood/protein-based medicines) poses challenges. Therefore, in order to meet the necessary regulatory safety requirements for release of certain medicinal products onto the market there is currently no alternative to the use of the rabbit pyrogen test. However, sponsor companies are engaged in validating non-animal alternative tests and gaining regulatory acceptance for these.

Non-technical project summaries (NTS)



- *Objectives of the project*
- *Expected **benefits***
- *Predicted **harms** (numbers, species, severities)*
- *A demonstration of **compliance** with the requirement of **Replacement**, *Reduction and Refinement**

N.B. MS can waive the requirement for NTS if project contains only 'mild' procedures.

Non-technical project summaries



...This is due to the molecular make-up of this medicine, which causes the MAT and BET options to give untrustworthy results which are not reliable enough to guarantee the medicine is safe for use, meaning the safety of treated horses could be compromised. Therefore, to guarantee safety and to ensure that this medicine is still available, a European medicines regulator has given short term permission to continue the use of the RPT specifically for this drug substance until an alternative method can be developed. There have been ongoing efforts to establish the alternative test for this product over the previous years, however it has proven to be very difficult, and as yet ultimately unsuccessful. However, efforts are ongoing to develop an animal-free assay for this product, ...

The animal organism acts as a complete and complex living biological system that cannot be completely replaced by alternative methods. For this test for the evaluation of pyrogenic impurities, there is an alternative method, **the endotoxin detection test by the LAL method (Limulus Amebocyte Lysate)**, but the **LAL test only detects the febrile reaction produced by endotoxins**. The "*in vivo*" test on rabbits detects both bacterial endotoxins (including endotoxins adsorbed on organic or inorganic surfaces) and non-endotoxin pyrogens (including non-endotoxin pyrogens adsorbed on organic or inorganic surfaces).

Non-endotoxin pyrogens:

- endotoxin-associated proteins;
- peptidoglycans;
- muramyl peptides (MDP and peptidoglycan subunits synergistic with endotoxins);
- porins;
- bacterial surface proteins;
- bacterial DNA;
- lipoteichoic acid and other components of the Gram-positive bacterial wall;
- nonmicrobiological contaminants (cytokines, media, cells, degradation products);
- superantigens;
- exotoxins;
- lipoarabinomannan (from mycobacteria);
- fungal components (mannans, glucans, mannoproteins);
- parasite components (phosphoinositol);
- viruses;
- solid materials (medical devices, plastic)

The LAL method has not been validated for non-endotoxin pyrogens

(2023)



Statistics and NTS



- *Factual data on remaining RPT uses*
- **Scientific obstacles**
- **Technical hurdles**
- **Regulatory misalignment**
 - Regulatory areas (e.g., biologicals, medicinal devices)
 - Regulatory countries and regions
- *Reveal lack of awareness*



Ready for a deep-dive?



Lack of expertise and training opportunities
Associated costs
Availability of monocyte cell sources
Limited number of regulatory texts recognising MAT
Lack of global harmonisation

- *What progress have we made?*
- *What insights have we gained?*
- *Are more bold steps needed to replace all RPT worldwide?*
- *How to elevate collaboration and unite all players?*



Thank you for your attention!

Let's crack this together!



More information at:

<https://ec.europa.eu/animals-in-science>

The views expressed in this presentation are solely those of the presenter and do not reflect the official view of the European Commission.

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European Directorate for the Quality of Medicines & HealthCare Council of Europe



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for the Quality
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Direction européenne
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COUNCIL OF EUROPE



CONSEIL DE L'EUROPE

Opening session European Pharmacopoeia update

**Dr. Emmanuelle Charton, Head of Division B, European Pharmacopoeia
Department**

EDQM, Council of Europe

Wednesday, 25 February 2026



Flashback, Brussels 2023

1971



Pyrogens (2.6.8)

1987



BET (2.6.14)

2010



MAT (2.6.30)

2020



BET using rFC (2.6.32)



The RPT continues to be widely performed



Experts of the Ph. Eur.



The European Partnership for Alternative Approaches to Animal Testing



Proposal:

Pharmeuropa
35.1

New chapter 5.13 Pyrogenicity
Deletion of the rabbit pyrogen test from **60 Ph. Eur. texts** by 2025 and suppression of chapter 2.6.8 from the Ph. Eur. by 2026



we did it!

Suppression of Rabbit Pyrogen Test: Major Milestone Achieved!



<https://www.edqm.eu/en/-/ph.-eur.-bids-adieu-to-rabbit-pyrogen-test-in-its-monographs>

 **This concerns 57 texts**

Non animal pyrogenicity approaches instead (BET, MAT)
5.1.13 Pyrogenicity

- ★ the use of the RPT is **no longer** required in any text of the Ph. Eur.
- ★ Implementation date : **1 July 2025**
- ★ The chapter itself has been removed from the Ph. Eur. on 1 January 2026
- ★ A major achievement for animal welfare and the advancement of modern in vitro approaches!

Deletion of the Rabbit Pyrogen test: aftermath of June 2024

An achievement applauded by stakeholders!

“Finally we are there ! European Pharmacopoeia is the first regulatory body to take this courageous decision.”

“So happy to hear that advances are being made in this area. Hoping to see other regions and nations follow suit.”

“Finally- so good to hear”



“Congratulations, finally after a more than 40 years journey!”

“Sincere gratitude to the scientists and regulators who made this possible!”

“Hoping that this change will spread globally”

“Great achievement!”

“Fantastic news”

“More than good news! Time has come to replace RPT with the MAT!”

European Directorate for the Quality of Medicines & HealthCare

★ Contributes to **public health and access to good quality medicines and healthcare in Europe**



Council of Europe

Council of Europe

- ★ Established in **1949**
- ★ **46 member states**
- ★ Based in **Strasbourg**
- ★ Founded on three main values:
**human rights, democracy
and the rule of law**



**European Convention for the
Protection of Vertebrate Animals
used for Experimental and other
Scientific Purposes (ETS No. 123)**
March 1986

**Convention on the Elaboration
of a European Pharmacopoeia
(ETS No. 050)**
July 1964

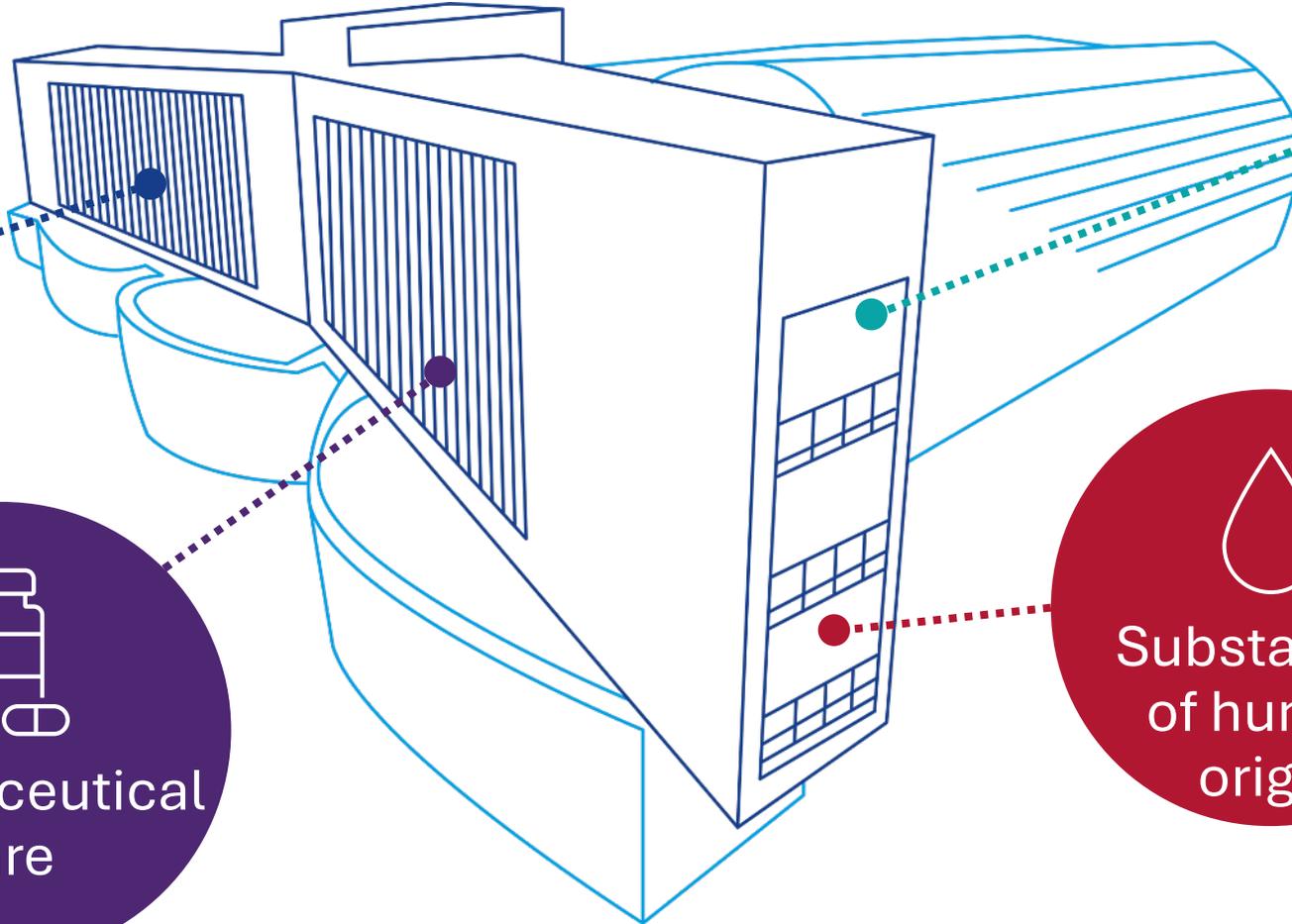


4. Sustainability

We will ensure a sustainable EDQM by future-proofing our operations and activities.

Medicinal Products

Pharmaceutical care



Substances of human origin

Consumer health

European Pharmacopoeia: Documentary and reference standards



Legally binding in the **40 signatory parties** of the Ph. Eur. Convention and used as a reference **worldwide**; **33 observers** from all continents

About **2 900 documentary standards** for the quality control of medicines

About **3 200 reference standards** shipped to **127 countries**



European Pharmacopoeia Commission and its **experts' groups**

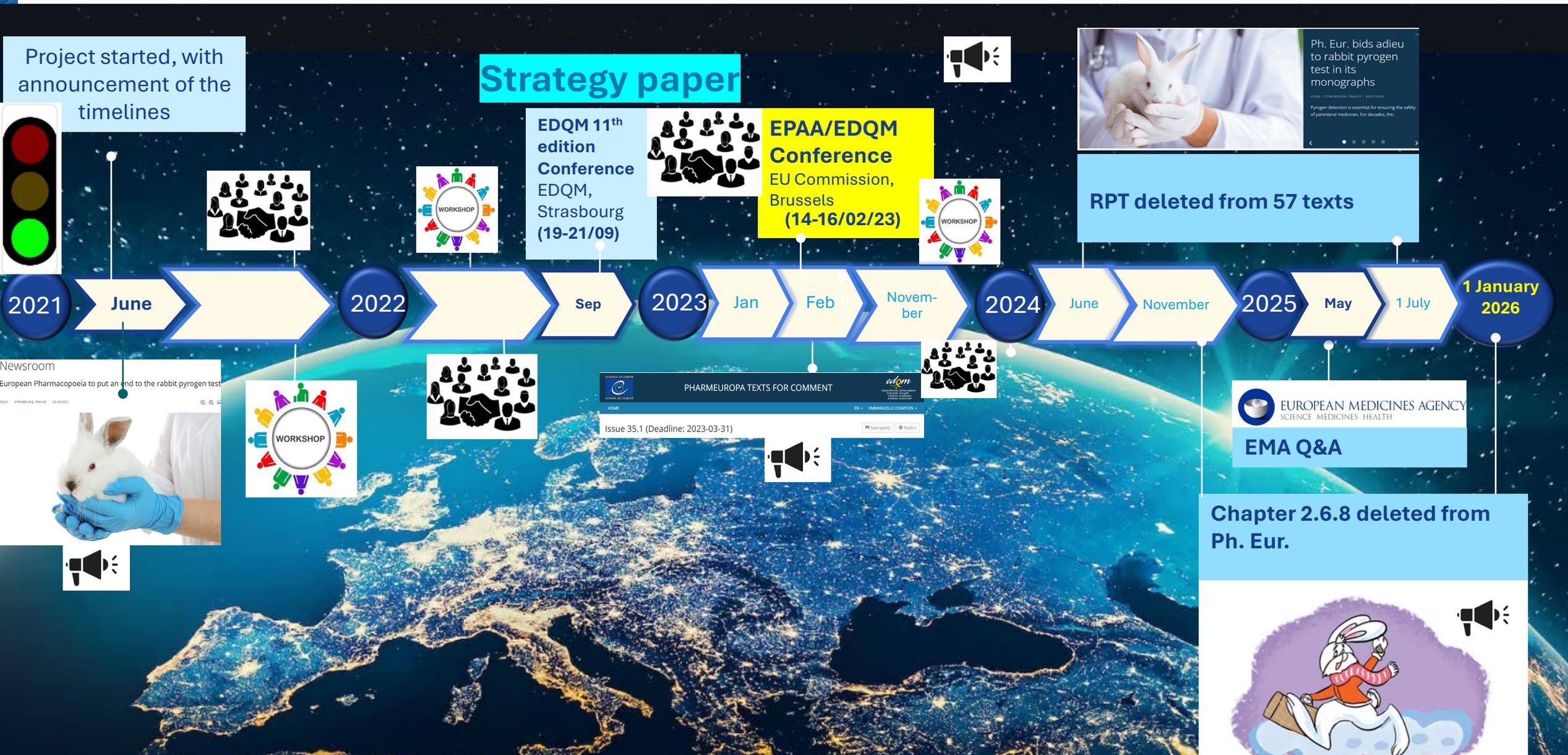


PUBLIC HEALTH IMPACT

- Ensures quality and safety of medicinal products
- Facilitates their free movement in Europe and beyond

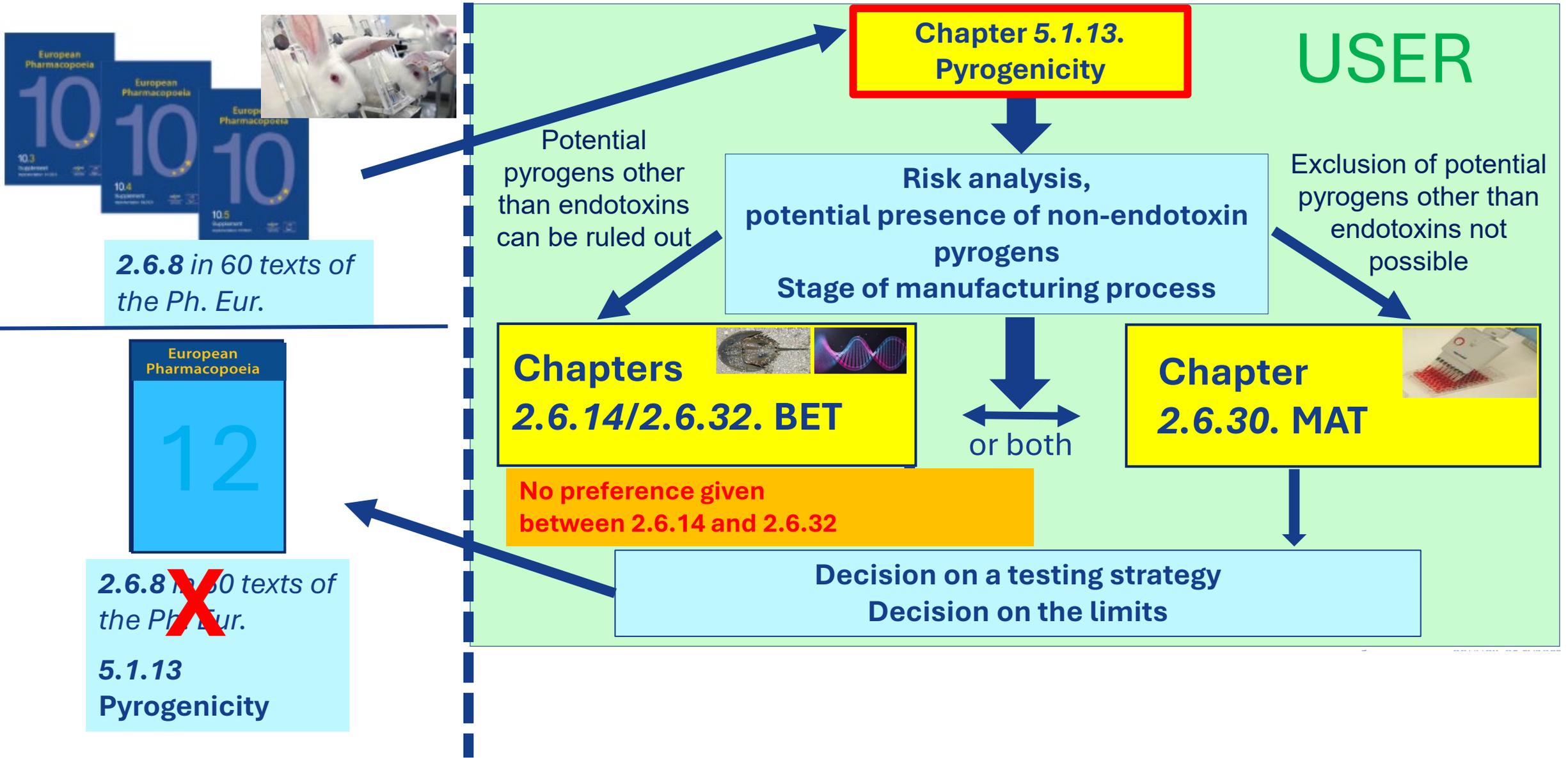


Phasing out the Rabbit Pyrogen Test - Communication to stakeholders



Strategy for the replacement of chapter 2.6.8

Consolidated strategy approved by the EPC in June 2022



Briefing notes for all texts (Pharmeuropa, Januray 2023)

“It should be noted that the exercise will ultimately lead to the suppression of general chapter 2.6.8 from the Ph. Eur. Manufacturers still using the rabbit pyrogen test are strongly encouraged to take the necessary steps to proceed with its replacement by a suitable in vitro alternative (e.g. the monocyte-activation test), in line with the new requirements of this monograph.”

“Importantly, the revision of this text does not call into question strategies involving the test for bacterial endotoxins that are already used by manufacturers to control the pyrogenicity of their products and have been authorised by the competent authority, nor is it intended to prompt a retrospective assessment of pyrogenicity.”

What we would like to hear from you today

- How is the implementation of the Ph. Eur. pyrogenicity strategy progressing across regions and stakeholders?
- What measurable progress has been made in the phasing-out initiative?
- What scientific, regulatory, or practical hurdles remain?
- How can we collectively address these challenges and support stakeholders?
- How close are we to a worldwide, harmonised discontinuation of the Rabbit Pyrogen Test?

EDQM Symposium: Microbiology on the Move

13-15 October 2026, EDQM, Strasbourg

<https://www.edqm.eu/en/-/symposium-microbio>



Internationally Harmonised chapters

- ✓ 2.6.1 Sterility
- ✓ 2.6.12/2.6.13 Non-sterile products

Rapid methods

Thank you for your attention!

More information

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 <https://go.edqm.eu/Newsletter>

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