

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)



European Pharmacopoeia strategy for phasing out the Rabbit Pyrogen Test

National Contact Point (NCP) meeting 1-2 October 2024

EDQM

The Rabbit Pyrogen Test



- Principle: rectal measurement of the rise in body temperature in rabbits following IV injection of the substance to be examined
- Historical test, can detect endotoxin and non-endotoxin pyrogens

But: not quantitative, low sensitivity, no controls, animal-based (method variability, animal welfare...), method not harmonised across regions



The test still consumes a large number of animals

- EU: ~50,000 in 2015, decreased by half between 2015 and 2020¹
- Many more outside Europe (~400,000/year globally²)

¹ Publications Office of the European Union. ALURES statistical EU database, https://ec.europa.eu/environment/chemicals/lab_animals/alures_en.htm (2023)

² Hartung et al., 2016

New Ph. Eur. Pyrogenicity strategy

1971



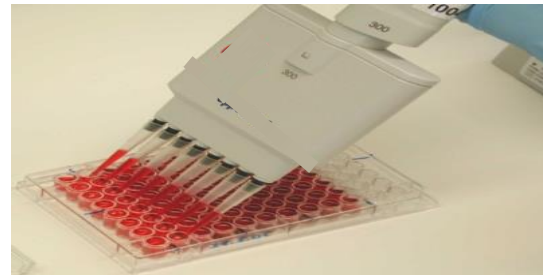
Pyrogens (2.6.8)

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.



Proposal



- New chapter **5.1.13 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

Public consultation in Pharmeuropa 35.1

New Ph. Eur. Pyrogenicity strategy

→ Published on Pharmedropa webpage: <https://go.edqm.eu/NewPyrogenicityStrategy>



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**Strategy for removing or replacing the rabbit
pyrogen test:
New pyrogenicity strategy of the European
Pharmacopoeia Commission
September 2022**

2.6.8. Pyrogens in the Ph. Eur.



General monographs (3)

- Substances for pharmaceutical use (2034)
- Radiopharmaceutical preparations (0125)
- Immunoserum for human use, animal (0084)

BET WP

G14

G15

Dosage form monographs (3)

- Parenteral preparations (0520)
- Preparations for irrigation (1116)
- Intravesical preparations (2811)

G12



Pyrogens (2.6.8)
(Rabbit pyrogen test)
60 Ph. Eur. texts

Individual monographs (50)

DIA WP

CRB WP

G6B

G15

G7

G9

G10D

G12

- large-volume parenterals (4)
- blood products (17)
- vaccines for human use (17)
- antibiotics (8)
- other chemical substances (4)
- solutions for organ preservation

General chapters (3)

Plastics

- Sterile plastic containers for human blood and blood components (3.3.4)
- Sets for the transfusion of blood and blood components (3.3.7)

G16

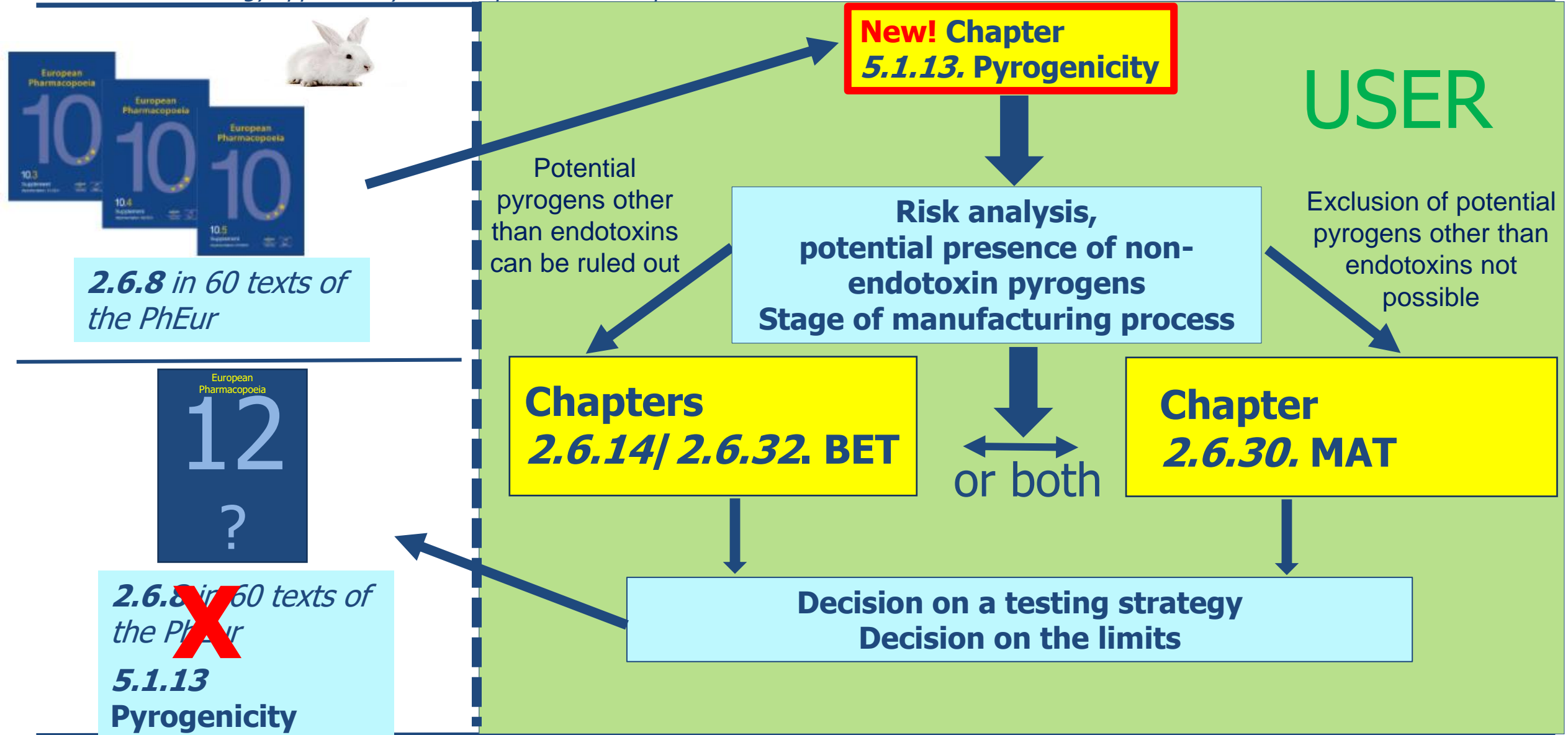
Vaccines for human use

- Carrier proteins for the production of conjugated polysaccharide vaccines for human use (5.2.11)

G15

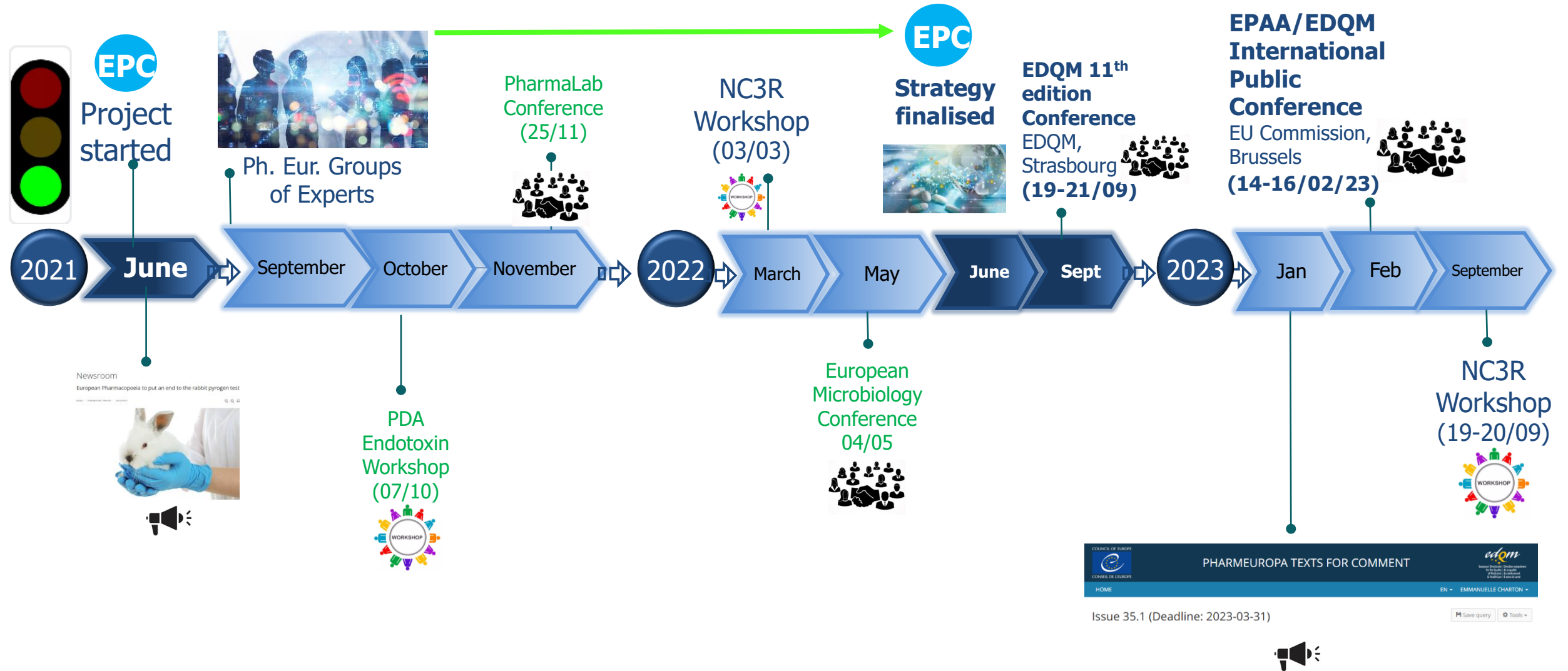
Replacement of chapter 2.6.8: proposed strategy

Consolidated strategy approved by the European Pharmacopoeia Commission in June 2022



- *"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 general 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."*

Communication to stakeholders



EPAA/EDQM International Public Conference



To mark the first official milestone of the strategy, i.e. the publication of revised Ph. Eur. texts omitting the RPT in Pharmeuropa 35.1 (January 2023)

Date: 14-16 February 2023

Venue: European Commission premises, Brussels



**The future of pyrogenicity testing:
phasing out the rabbit pyrogen test**



14 to 16 February 2023

Joint EDQM-EPAA event



EPAA/EDQM International Public Conference



Hosted by the European Commission in Brussels

250 participants from Industry, Academia, Regulatory Authorities (worldwide), WHO, Pharmacopoeias (European, United States, Japan, Brazil, China, India), National Control Laboratories, MAT kit manufacturers and developers, service providers

Take home messages:

- **In Europe**, stakeholders are showing great enthusiasm towards the Ph. Eur. strategy aimed at phasing out the Rabbit Pyrogen Test (RPT)
- **Outside Europe**, the strategy is generally seen positively, however, alternative methods such as MAT are not described in detail nor even mentioned in most Pharmacopoeias, where the RPT is still required in monographs. The journey towards complete removal might therefore take longer
- **International convergence** toward the same goal is important
- **Implementing the MAT** has been facilitated greatly in the last years by the standardisation of reagents and the increase in available kits
- **The time has come to switch from *in vivo* RPT to *in vitro***



Slides, video and training resources: <https://www.edqm.eu/en/-/joint-edqm-epaa-event-the-future-of-pyrogenicity-testing-phasing-out-the-rabbit-pyrogen-test>



Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

The future of pyrogenicity testing: Phasing out the rabbit pyrogen test. A meeting report

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ABSTRACT



















The rabbit pyrogen test (RPT) was the benchmark for pyrogenicity testing, but scientific advancements have provided innovative and humane methods, such as the *in vitro* monocyte-activation test (MAT). However, transitioning from the RPT to the MAT has been challenging. The European Directorate for the Quality of Medicines & HealthCare, the Council of Europe, and the European Partnership for Alternative Approaches to Animal Testing jointly hosted an international conference entitled "The future of pyrogenicity testing: phasing out the rabbit pyrogen test". The conference aimed to show how the European Pharmacopoeia intends to remove the RPT from its texts by 2026, facilitate the use of MAT, and identify gaps in the suppression of RPT. The events contributed to a better understanding of the barriers to RPT replacement and acceptance of *in vitro* alternatives.


→ Link to article:

<https://www.sciencedirect.com/science/article/pii/S1045105623000404>

Suppression of the Rabbit Pyrogen Test - Timelines



WHAT	WHO	WHEN		
		Public enquiry	Adoption by EPC	Implementation date
 Elaboration of new chapter on Pyrogenicity (5.1.13) (and revision of chapter 5.1.10)	BET WP	 	 	
REVISION <i>Gen. monograph 2034</i>	BET WP			
<i>Gen. monograph 0520</i>	G12			
All other Ph. Eur. texts*	GoEs/WPs			
 Pyrogens (2.6.8)		Proposed suppression of chapter 2.6.8: 180 th EPC Session (Nov 2024) →  		



(*) Nearly 60 texts revised omitting the rabbit pyrogen test

Thank you for your attention



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