

Joint EDQM-EPAA Event

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

14-15 February 2023



Joint EDQM-EPAA Event

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

Opening Session



Animals in science



Phasing out the rabbit
pyrogenicity test –
challenges and
opportunities

EDQM-EPAA Pyrogenicity Event
14-16 February 2023
Susanna Louhimies

Controversial views on animal use in research and testing



- *Animals recognised as sentient beings in the EU*
- *Ethical concerns*
- *Animal welfare concerns*
- *Sensitive and often an emotional topic*

Unique legislation laying out a Union objective

*“...this Directive represents an important step towards achieving the **final goal of full replacement** of procedures on live animals for scientific and educational purposes **as soon as it is scientifically possible to do so**”*



Photo by Maneesha Mohan - Unsplash

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14-16 February 2023



Impatience is growing

2021 EP Resolution:

- Draw up an EU-wide action plan to drive **active phase-out of animal use in research and testing**

EU Citizens' Initiative "Save cruelty free cosmetics" (2022)

- Commit to a legislative proposal plotting **a roadmap to phase-out all animal testing in the EU**



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Legal framework in the EU – Directive 2010/63/EU



- *Legislation to protect animals used in science since 1986*
- *Fully revised in 2010 – amended in 2019 to further improve transparency*
 - *Level playing field for industry and academia*
 - *Improved transparency and enforcement*
 - *Implementation of the Three Rs is a legal obligation*

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The Three Rs

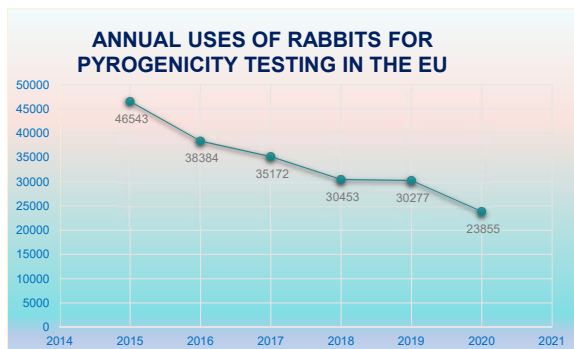
- **REDUCE**
- **REPLACE**
- **REFINE**



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Total RPT use in the EU



- MAT test adopted in the European Pharmacopeia in 2009
- Directive 2010/63/EU took effect in 2013

➤ In 2015, almost 50K uses of rabbits in RPT persist

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14-16 February 2023

Including estimated 2020 data (publication in February 2023)
https://ec.europa.eu/environment/chemicals/lab_animals/alures_en.htm



Replacement a legal requirement

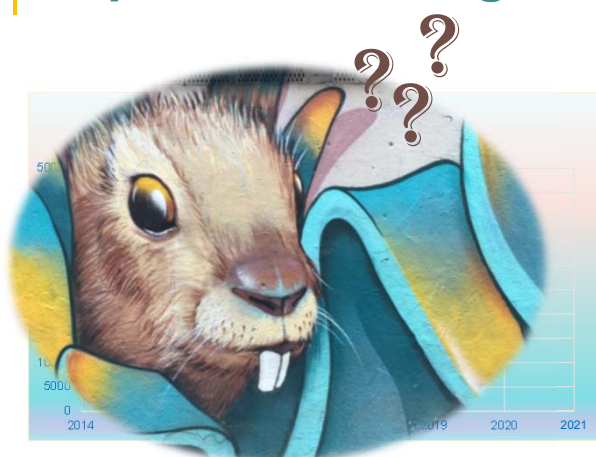


Photo by Vincent Pelletier - Pixels

- 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.**

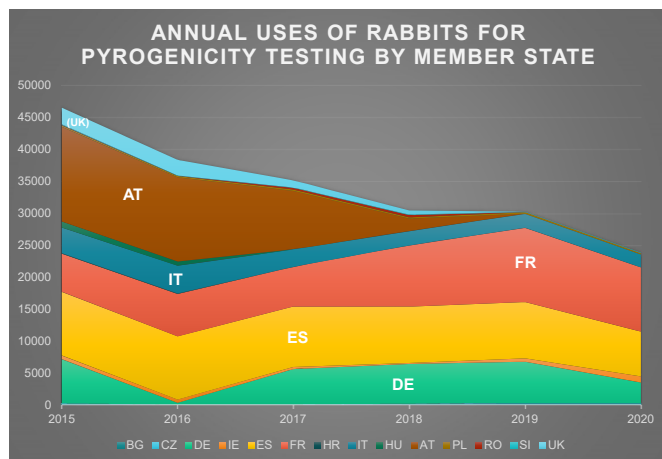
^{*)} unless product specific validation fails

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14-16 February 2023

Including estimated 2020 data (publication in February 2023)
https://ec.europa.eu/environment/chemicals/lab_animals/alures_en.htm



RPT use in the EU by MS



- 2015: 14 MS reported RPT
- 2020: down to 10 MS of which 93,5% uses by 4 MS
- 2021 indications: total RPT use significantly higher than in 2020
- **Success stories:** AT from the highest RPT use in 2015 to no further use since 2019

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Including estimated 2020 data (publication in February 2023)
https://ec.europa.eu/environment/chemicals/lab_animals/alures_en.htm



Learning from others: an example of an approach by a MS 1/2



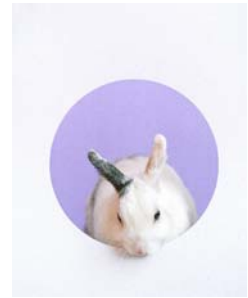
1. New project applications and monitoring of on-going projects

- New project application to include a detailed justification of why RPT is required
- CROs required to have robust system for the implementation of Replacement and appropriate scrutiny to assess requests for RPT (product property information and efforts made to validate an alternative)
- Only when alternative is confirmed unfeasible, can a CRP progress to RPT
- Limited duration of project authorisations with periodic updates on progress to developing and validating alternatives; timelines for regulatory submission

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Learning from others: an example of an approach by a MS 2/2



2. *Inspection process*

- Compliance reviewed during inspections
- CROs internal processes for compliance
- Review of RPT records to verify that only those products that do not have regulatory accepted non-animal alternative available are tested

3. *Refinement*

- Group housing
- Use of enriched floor pens

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Current efforts at EU level



Photo by FeedLooma - Pixabay

- *RPT use discussed regularly with MS*
 - meetings of MS authorities twice a year
 - bilaterally
- *Annual reporting changed to require explanation of justifications for animal use where alternatives are available (MS narratives from 2021 data onward)*
- *EPAA-EDQM project on RPT*

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Conclusions and next steps



*Between 2015 - 2020, RPT use **decreased by 49%** - downward trend **not yet confirmed** – a general decrease in animal use in 2020*

- *What issues slow down the transition to alternatives?*
- *What needs to happen to replace all RPT in EU?*
- *What can be learnt from the success stories?*

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Thank you for your attention!

I wish you a productive and successful event!

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



Joint EDQM-EPAA Event

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

Petra Doerr
Director, EDQM, Council of Europe

1. The future of pyrogenicity testing

14 FEBRUARY 2023

- 09:00-10:30 – Opening session
- 11:10-12:00 – In-depth exploration of the monocyte-activation test (MAT)
- 13:30-15:00 – MAT (cont.)
- 15:30-17:00 – Pulling the rabbit out of the hat: Industry perspectives

15 FEBRUARY 2023

- 09:00-10:30 – Pulling the rabbit out of the hat: Industry perspectives (cont.)
- 11:00-12:00 – Regulatory Session: So what will rabbit-free pyrogen testing look like in Europe? How about the rest of the world?
- 13:30-17:30 – Regulatory Session (cont.)

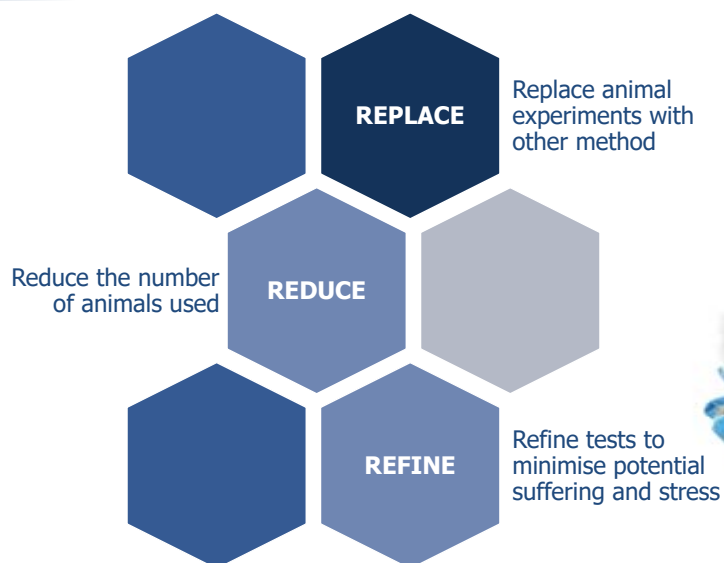
16 FEBRUARY 2023 (morning only)

EDQM-EPAA MAT Training Session

Hands on experience, case studies, troubleshooting with technicians from different laboratories

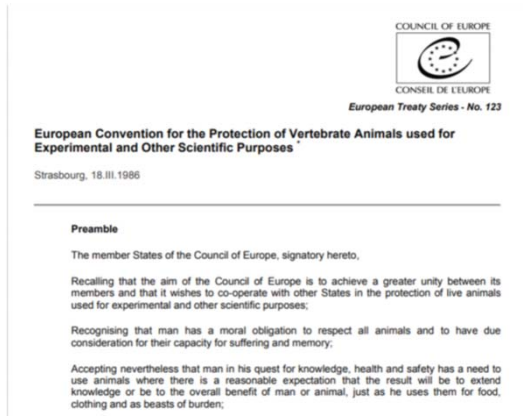
- 09:00-09:10 – Welcome and Opening
- 09:10-09:50 – Qualification of Peripheral Blood Mononuclear Cells (PBMCs)
- 09:50-10:20 – Freezing and thawing of PBMCs
- 10:40-12:00 – Cell handling in the MAT assay
- 12:15-12:30 – Readout options
- 12:30-13:00 – Round table on the technical topic: Regulatory Acceptance
- 13:00-13:10 – Closure and Goodbye

2. The 3R principles



3. Roots of the initiative

- 1986 – Council of Europe’s European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes
- 1998 – Ratification by the EU



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

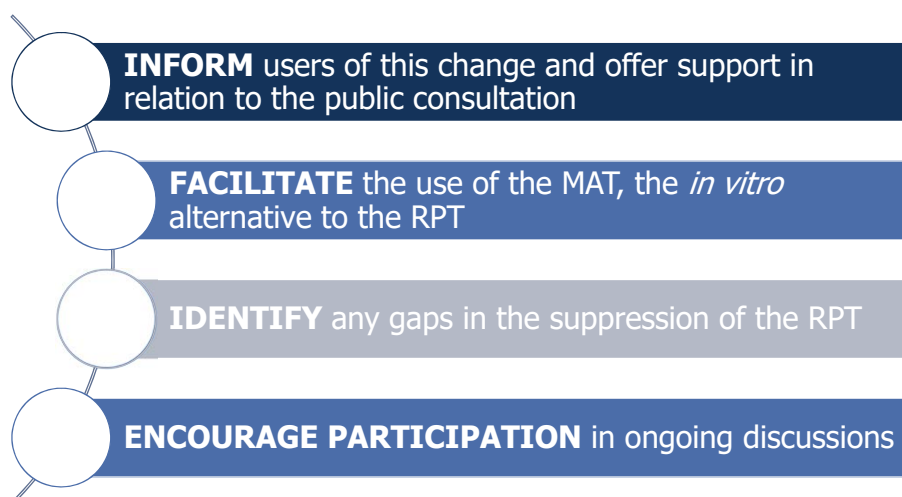
**60 texts in *Pharmeuropa* 35.1
for consultation
as of 1 January 2023**



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4. Objectives of this joint event



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HARMONISATION OF THE THREE RS IN BIOLOGICALS: STRIKING THE RIGHT NOTE

**Dr. Katrin Schutte, European Commission
Dr. Shahjahan Shaid, GSK
co-chairs of the EPAA Biologicals project team**

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CONTENTS

- 1. The EPAA Partnership**
- 2. The EPAA Biologicals Team and Project background**
- 3. 1st focus area Safety testing of vaccines**
- 4. 2nd focus area Pyrogenicity testing**
- 5. Pyrogen detection methods**
- 6. Lack of international harmonisation**
- 7. Benefits of the MAT-assay**

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European Partnership for Alternative Approaches to Animal Testing (EPAA) in 2022



Collaboration between the European Commission and Industry stakeholders from 8 sectors (est. 2005)

Vision: The replacement, reduction and refinement (3Rs) of animal use for meeting regulatory requirements through better and more predictive science.

3Rs Mission:

- Promote development & acceptance
- Foster cross-sector knowledge-sharing
- Increase international collaboration
- Facilitate stakeholder dialogue

38 Companies (including 1 SME)



5 DG's of the EC



DG GROW
DG ENV
DG SANTE
DG JRC
DG RTD

Including Partner Agencies



Mirror Group (Advisory body)

Emily Molvor (Chair), Dr Tuula Heinonen, Dr Christiane Hohensee, Dr Helena Kandarova, Sirpa Pietikainen (MEP), Prof. Vera Rogiers
(Thank you to Ana Barros, Belén Pintado, Prof. Horst Spielmann & Marina Pereira)

8 Sectoral Associations



EPAA website: https://ec.europa.eu/growth/sectors/chemicals/epaa_en

E-mail: GROW-EPAA@ec.europa.eu

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EPAA BIOLOGICALS PROJECT TEAM MEMBERS

- | | |
|--|------------------|
| <ul style="list-style-type: none"> • GSK • European Commission (DG Environment) • Sanofi Vaccines • ZOETIS • Novo Nordisk • EFPIA (Pharmaceutical Industry Association) • Animal Health Europe (Industry Association) • EDQM (EU Directorate for the Quality of Medicines, Council of Europe) • VACCINES EUROPE (EU vaccines producers) • Humane Society International | <p>Co-chairs</p> |
|--|------------------|

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BIOLOGICALS PROJECT BACKGROUND

- **Biologicals** (vaccines, hormones, immunoglobulins, blood products) are manufactured by biological processes of inherent variability and require a strict quality control strategy to secure consistent quality from batch to batch.
- Required **safety and efficacy (potency control) tests** (in vivo/in vitro) stated in monographs of relevant pharmacopoeias.
- Differences in test requirements and protocols between countries still give rise to unnecessary repetition of testing.
- Article 13 of **EU Directive 2010/63** asks that a procedure using animals is not carried out if a non-animal method for obtaining the same result is recognized under EU legislation.

Global harmonization of 3Rs in biologicals is an EPAA priority within its focus on international convergence of testing requirements.

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FIRST FOCUS AREA: VACCINES TESTING

Deletion/waiving of **general safety tests** (abnormal toxicity test (ATT) or GST, TABST)) for **human vaccines** at WHO level / **veterinary vaccines** at VICH level and from national regulatory requirements.

Rationale: Since introduction of Good Manufacturing Practice and the use of adequate/stringent Quality Control measures, the relevance of the ATT has become highly questionable.

EPAA successes:

- International workshop on deletion of these tests in **2015** and publication: <http://www.sciencedirect.com/science/article/pii/S1045105617300647>
- ATT deleted from 49 monographs of the **European Pharmacopoeia** since 2019
- **OIE** allowed waiving of TABST based on EPAA request as part of the 2018 OIE *Terrestrial Manual*
- **WHO** recommendation in 2018 for immediate discontinuation of inclusion of the ATT/GST test in all WHO documents on vaccines and biologicals published in the Technical Report Series (including WHO Recommendations, Guidelines and manuals).

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5. New focus area: pyrogenicity testing

- The **in-vivo Rabbit Pyrogen Test** is still conducted for Biological products despite alternative assays being available since long
- **Lack of international harmonisation** of testing requirements suspected here as well
- *In vitro* alternatives **BET** (Bacterial Endotoxin Test) and **MAT** (Monocyte Activation Test) assays are in Ph.Eur.
- **Can EPAA facilitate** the implementation of *in vitro* alternatives to the Rabbit Pyrogen Test?
- **Biologicals team conducted a survey mid 2018** on users' experience with in vivo /in vitro tests for pyrogens



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6. Pyrogenicity survey 2018 : top-line results

Responses from 28 companies & testing institutes:

- 17 use the RPT in vivo assay
- 12 say MAT is applicable to their products in principle
- 22 say BET is applicable to their products in principle

Responses from 5 Member States:

- 3 MS say in-vivo assay is not conducted
- 1 MS: accepts in-vivo assay when pyrogenicity of substances other than bacterial endotoxins have to be tested
- 1 MS: use of the in-vivo assay has gone down, follow-up with each user to help apply alternative method

Products that the in-vivo assay is still used for:

- Blood products - Vaccines - Antibiotics - Excipients of pharma products - Medical devices



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6. Pyrogenicity survey: top-line results 2

Main reasons given for still conducting the in-vivo assay:

- In-vivo test used for detection of non-microbial pyrogens
- BET not a full replacement assay, does not detect all pyrogens (only certain bacterial endotoxins)
- Technical difficulties with the in-vitro assays (endotoxin masking and with product specific validation)
- Legal requirement in other jurisdictions (China, Japan, US)
- Long time for variation approval of changing to in-vitro test
- Cost (especially MAT)*



* not a legally valid reason not to use alternative!

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In-vivo test used for detection of non-microbial pyrogens

Rabbit Pyrogen Test will be removed in the EP by 2025. Substitution with non-animal technologies is mandatory in EU (Directive 2010/63)



Pyrogens detection

MAT: EP 2.6.30

- Recognizes pyrogens with a high sensitivity.
- Established in the PEP
- Mentioned in the Ch.P

RPT: EP 2.6.8

- Measure of the rabbit body temperature after injection of the product.
- Former gold standard.

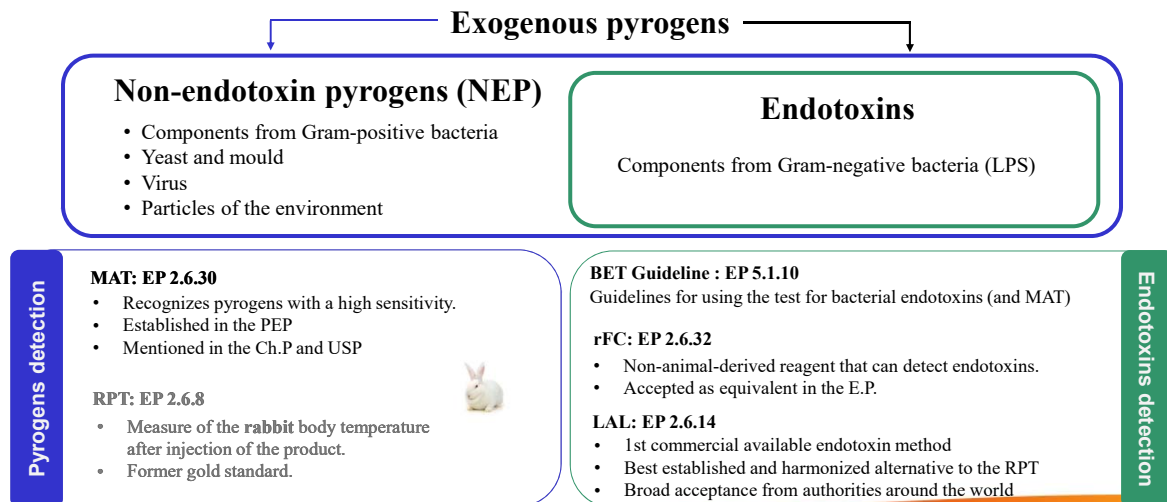


RPT will be a method not described in the EP. European manufacturers will have to ensure patients safety regarding Non-endotoxin pyrogens by relying on the MAT.

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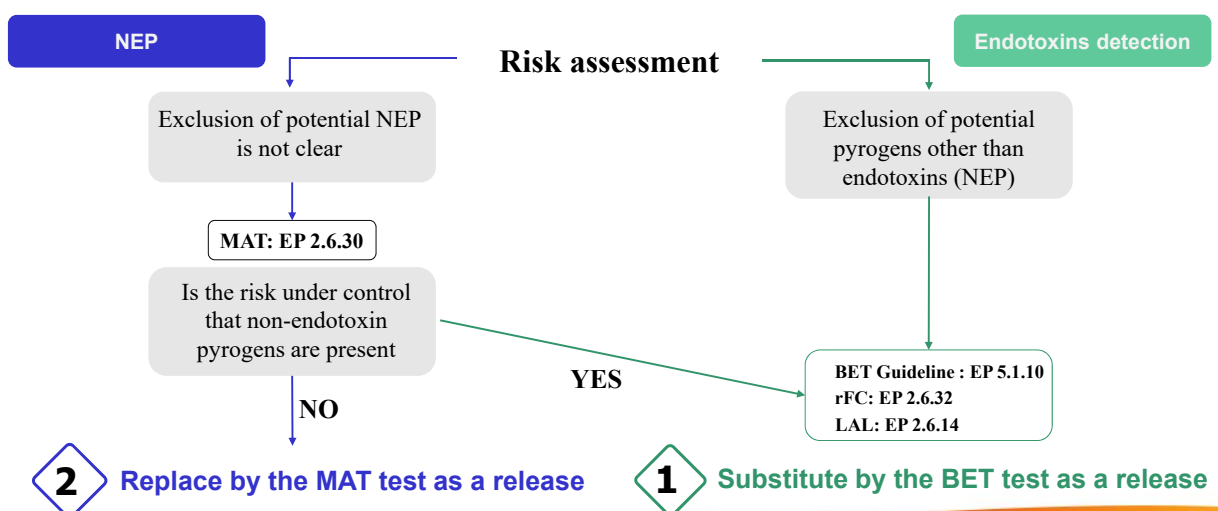
BET not a full replacement assay, does not detect all pyrogens
(only certain bacterial endotoxins)



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Pyrogen detection method with a risk-based approach



2022 EDQM Conference

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Technical difficulties with the in-vitro assays (endotoxin masking) and with product specific validation

- MAT, BET, RPT differ due to their biochemical reaction in sensitivity, specificity and readout. Is a full comparison useful or even needed?
- E.g. they are differently affected by matrix effects from e.g. adjuvants. Effects can occur in MAT, BET, rFC that are not detectable in the RPT. This can impact method performance.
- Those matrix interference and endotoxin masking as well as cytotoxic effect require attention in the method validation.
- Expertise and product adaptation of the in vitro methods are required to overcome those constraints.

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Legal requirement in other jurisdictions (China, Japan, US)

Today the pyrogen methods are not aligned between the Compendia's. If this trend continues, there will be no "common ground" ?

	Non-endotoxin pyrogens			Endotoxin pyrogens		
	RPT	MAT	Other methods	LAL	rFC	rLAL
E.P	Not foreseen	Sole method	Not mentioned	Foreseen	Equivalent method	To be determined
WHO	Foreseen	Proposed*	Not mentioned	Foreseen	Proposed*	To be determined
USP	Foreseen	Not mentioned	Not mentioned	Foreseen	To be determined	To be determined
China	Foreseen	Supplemental**	In discussion	Foreseen	Available	To be determined
Korea	Foreseen	Not mentioned	In discussion	Foreseen	Expected	To be determined
Japan	Foreseen	Guideline**	Not mentioned	Foreseen	Alternative method	To be determined
India	Foreseen	Available	Not mentioned	Foreseen	Alternative method	To be determined
Brazil	Foreseen	Foreseen	Not mentioned	Foreseen	Expected	To be determined

*NC3r led project

** mentioned in guidelines

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Long time for variation approval of changing to in-vitro test

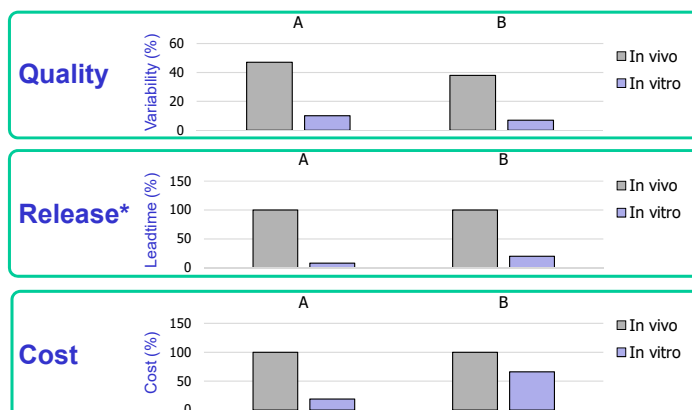
- The non-alignment likely triggers prolonged approval times due to Questions when replacement methods are submitted.
- Extensive parallel testing of RPT, BET and MAT are requested. Shifting timelines to fade out RPT.
- A comparison between the three methods is challenging due to their analytical target profiles and read outs
- **How can we move away from considering the RPT as the gold standard outside of Europe?**

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Cost of Replacing the RPT by MAT

In vitro replacement does not jeopardize quality, it improves it with more reliable analytics.



Activity Based Costing to consider overhead and indirect cost.

Improve availability of Vaccines :

- Faster release (repeats, OOS, etc.)
- Reduced stock planning
- Avoidance of write offs
- Shortened test time
- Robust assay: less deviations and repeats

Reduce Touch time:

- In vitro is less labor intense
- Allows automation
- Streamlined due to robust assay

Reduced costs of goods e.g. assay reagents

Capacity of Scale

Reduced costs for National Control

Lower infrastructure maintenance

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Scope of the workshop

- The different experts will address the mentioned concerns and share strategies to overcome them.
- Several Health authorities will present their perception on the future of pyrogen testing
- A half day training will allow deep dive on technical procedures and questions **with the in vitro MAT method.**

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The logo for EPAA (European Pyrogenicity Association) is a stylized, handwritten-style script in white, set against a dark orange background.

Thanks for your attention!

- **EPAA website:**
http://ec.europa.eu/growth/sectors/chemicals/epaa_en

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The logo for EPAA (European Pyrogenicity Association) is a stylized, handwritten-style script in white, set against a dark orange background.

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



Pulling the rabbit out of the hat: how the European Pharmacopoeia is tackling the rabbit pyrogen test of the European Pharmacopoeia



Dr. Emmanuelle Charton
Head of DivB
European Pharmacopoeia Department

The European Pharmacopoeia



European Pharmacopoeia 11.0

IBUPROFEN
Bupropionem

CC(=O)C1=CC=C(C=C1)C2=CC=CC=C2C3=C1C(=C(C=C3)O)C

IDENTIFICATION
A. Melting point (2.2.16): 75 °C to 78 °C.
B. Ultraviolet and visible absorption spectrophotometry (2.2.25).
Test solution: Dissolve 50.0 mg in a 4 g/L solution of sodium hydroxide R and dilute to 100.0 mL with the same solvent.
Spectral range: 240–300 nm, using a spectrophotometer with a band width of 1.0 nm and a scan speed of not more than 50 nm/min.
Absorption maxima: at 264 nm and 272 nm.
Shoulder: at 258 nm.
Absorbance ratio:
– A_{264}/A_{272} = 1.20 to 1.30;
– A_{264}/A_{258} = 1.00 to 1.10.
C. Infrared absorption spectrophotometry (2.2.26).
Comparison: diphenyl CRS.
D. Thin layer chromatography (2.2.27).
Test solution: Dissolve 50 mg of the substance to be examined in methylene chloride R and dilute to 10 mL with the same solvent.
Reference solution: Dissolve 50 mg of ibuprofen CRS in methylene chloride R and dilute to 10 mL with the same solvent.
Plate: TLC silica gel plate R.
Mobile phase: methylene chloride R, ethyl acetate R, hexane R (5:24:75 V/V/V).
Application: 1 µL.
Development: over a path of 10 cm.
Drying: at 100 °C for 10 min.
Detection: lightly spray with a 10 g/L solution of potassium permanganate R and heat at 200 °C for 30 min; examine in ultraviolet light at 365 nm.
Result: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS
Solution S: Dissolve 2.0 g in methanol R and dilute to 20 mL with the same solvent.
Appearance of solution: Solution S is clear (2.2.1) and colourless (2.2.2, Method 2).
Optical rotation (2.2.7): –0.87° to +0.07°.
Dissolve 0.50 g in methanol R and dilute to 20.0 mL with the same solvent.
Related substances: Liquid chromatography (2.2.29).
Test solution: Dissolve 20 mg of the substance to be examined in 2 mL of acetonitrile R and dilute to 10.0 mL with mobile phase A.
Reference solution (a): Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.
Reference solution (b): Dilute 1.0 mL of diphenyl impurity B CRS to 10.0 mL with acetonitrile R (solution A). Dissolve 20 mg of diphenyl CRS in 2 mL of acetonitrile R, add 1.0 mL of solution A and dilute to 10.0 mL with mobile phase A.
Reference solution (c): Dissolve the contents of a vial of diphenyl for peak identification CRS in acetonitrile R. Add 10 µL of acetonitrile R and dilute to 1 mL with mobile phase A.
Column:
– size: ≈ 6.5 m, ϕ = 4.6 mm;
– stationary phase: end-capped n-undecylsilyl amorphous porous silica gel for chromatography R (3 µm).
Mobile phase:
– mobile phase A: mix 0.5 volumes of phosphoric acid R, 340 volumes of acetonitrile R1 and 660 volumes of water for chromatography R, allow to equilibrate and dilute to 1000 volumes with water for chromatography R;
– mobile phase B: acetonitrile R2.
Time table:

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 – 10	100	0
10 – 15	100	0
15 – 20	0	100

Flow rate: 1 mL/min.
Detection: spectrophotometer at 214 nm.
Injection: 50 µL.
Identification of impurities: use the chromatogram supplied with diphenyl for peak identification CRS and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A1 and A2.
Relative retention times with reference to diphenyl retention time: about 12 min; impurity 1: about 12; impurity 2: about 12; impurity A: about 12.
System suitability: reference solution (b):
– peak to valley ratio: minimum 1.5, where H_p = height above the baseline of the peak due to impurity B, and H_v = height above the baseline of the baseline of the valley separating this peak from the peak due to diphenyl. If necessary, adjust the concentration of acetonitrile in mobile phase A.
Limits:
– impurities A, 1, 2: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent);
– unspecified impurities: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).
See the information section on general monographs (lower pages).

Example of quality standard on Ibuprofen

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What is the European Pharmacopoeia?

- A compilation of > **2800** documentary standards for the quality control of medicines
- Binding in the **39** signatory member states **and in the EU**
- Used as a reference world-wide, including in 31 observer countries, from all continents
- Plays a major role in protection of public health
- Facilitates the free movement of medicinal products in Europe and beyond

Covering

- All stages of the life-cycle of a medicine from development through to production and market surveillance
- All components used during the production process ... from raw materials, intermediates or synthesis to medicinal products

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Members & Observers

European
Pharmacopoeia:

39 member states
and the EU
(Albania in February 2020);

31 observers
(Ethiopia in Nov 2022)



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New Pyrogenicity strategy

1971



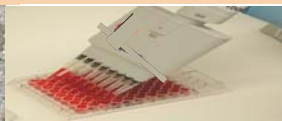
Pyrogens (2.6.8)

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 5.13 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

Pharmeuropa
35.1

6



New Pyrogenicity strategy

- <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

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2.6.8. Pyrogens in the Ph. Eur.



General monographs (3)

- Substances for pharmaceutical use (2034) **BET WP**
- Radiopharmaceutical preparations (0125) **G14**
- Immunoserum for human use, animal (0084) **G15**

Dosage form monographs (3)

- Parenteral preparations (0520)
- Preparations for irrigation (1116) **G12**
- Intravesical preparations (2811)



60 texts

Plastics

- Sterile plastic containers for human blood and blood components (3.3.4) **G16**
- Sets for the transfusion of blood and blood components (3.3.7)

Vaccines for human use

- Carrier proteins for the production of conjugated polysaccharide vaccines for human use (5.2.11) **G15**

General chapters (3)

Individual monographs (50)

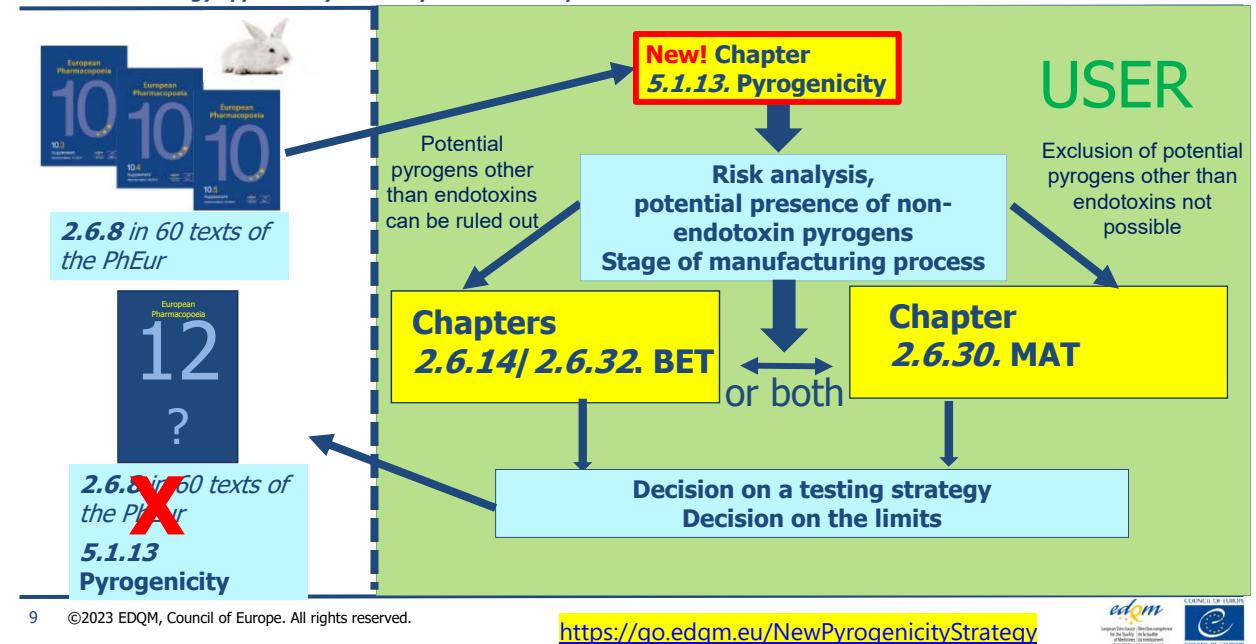
- solutions (4) **G12** **DIA WP**
- blood products (17) **G6B**
- vaccines for human use (17) **G15**
- antibiotics (8) **G7**
- other chemical substances (4) **G10D** **G9** **CRB WP**

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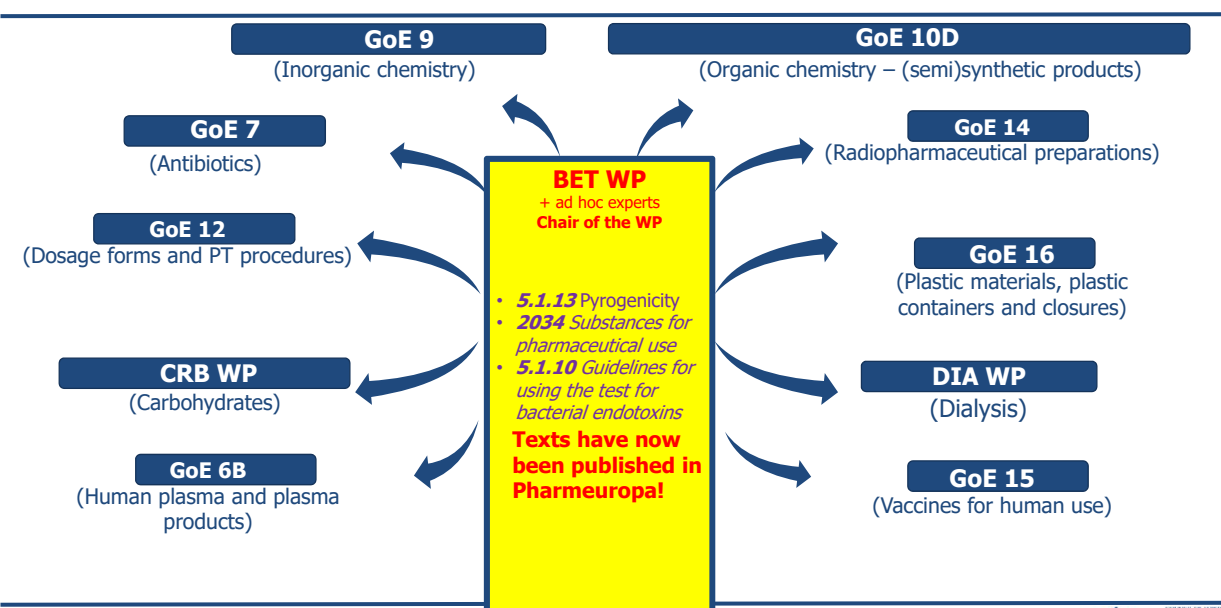


Replacement of chapter 2.6.8: *proposed strategy*

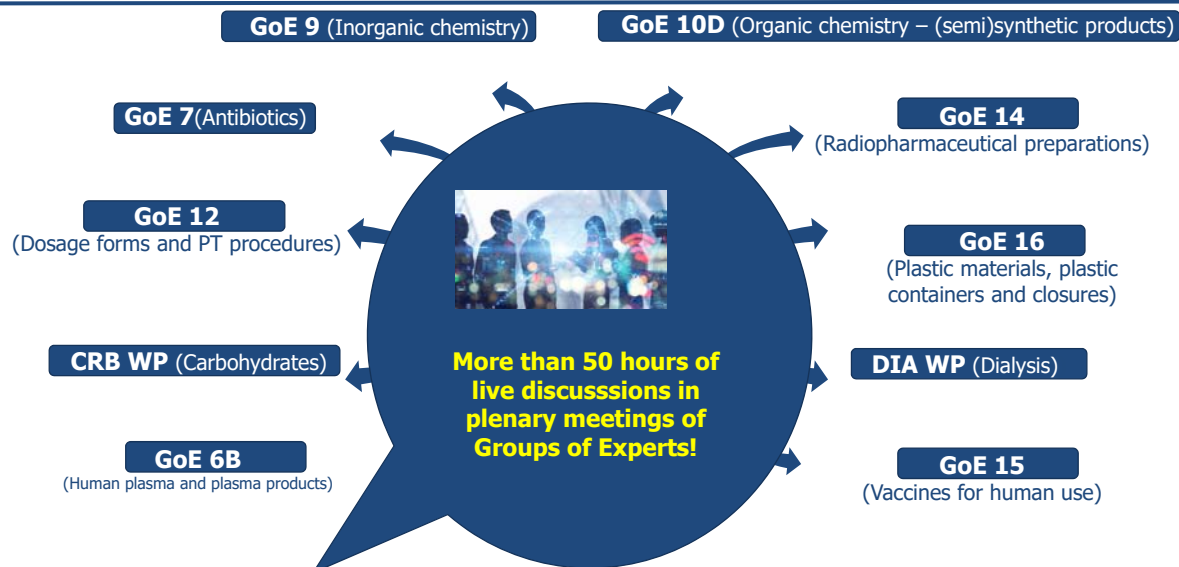
Consolidated strategy approved by the European Pharmacopoeia Commission in June 2022



Activities and actions at the level of GoEs and WPs



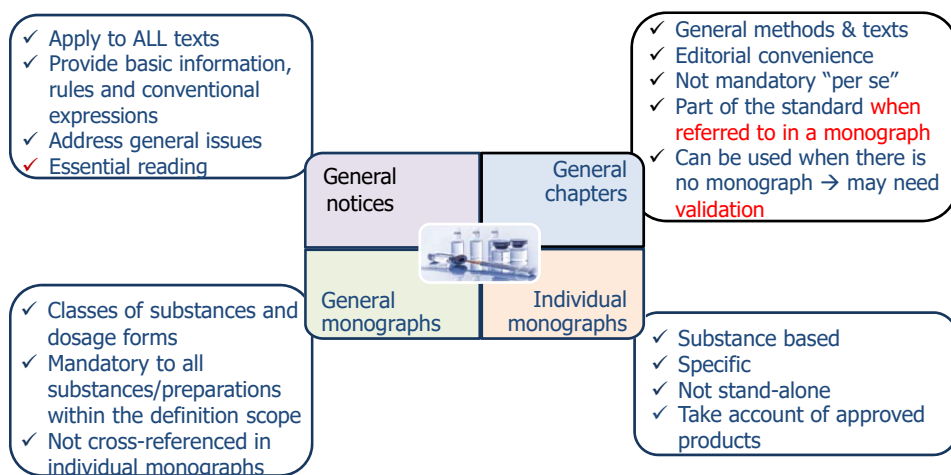
Activities and actions at the level of GoEs and WPs



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Substances for pharmaceutical use (2034)

EUROPEAN PHARMACOPOEIA 11.0

Substances for pharmaceutical use

Related substances. Unless otherwise prescribed or justified and authorised, organic impurities in active substances are to be reported, identified wherever possible, and qualified as indicated in Table 2034.-1 or in Table 2034.-2 for peptides obtained by chemical synthesis.

Table 2034.-1. – Reporting, identification and qualification of organic impurities in active substances

Use	Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Veterinary use only	Not applicable	> 0.10 per cent	> 0.20 per cent	> 0.50 per cent

Table 2034.-2. – Reporting, identification and qualification of

microbial contamination. Depending on the nature of the substance and its intended use, different acceptance criteria may be justified.

Sterility (2.6.1). If intended for use in the manufacture of sterile dosage forms without a further appropriate sterilisation procedure, or if offered as sterile grade, the substance for pharmaceutical use complies with the test for sterility.

Bacterial endotoxins (2.6.14). The substance for pharmaceutical use complies with the test for bacterial endotoxins if it is offered as a bacterial endotoxin-free grade or if it is intended for use in the manufacture of parenteral preparations or preparations for injection without a further appropriate procedure. The limit, when the test for bacterial endotoxins is determined in accordance with the test for bacterial endotoxins, is determined in accordance with the test for bacterial endotoxins.

Pyrogens (2.6.8). The test for pyrogens is specified rather than the test for bacterial endotoxins. If the substance for pharmaceutical use is offered as pyrogen-free grade, it complies with the test for pyrogens. The test method for pyrogens is specified in the test for pyrogens. The test method for pyrogens is specified in the test for pyrogens.

Pyrogenicity
(5.1.13)



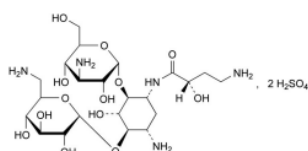
Individual monographs on substances for pharmaceutical use



01/2019:1290
corrected 10.0

AMIKACIN SULFATE

Amikacini sulfas



$C_{22}H_{42}N_8O_{13}S_2$
[39831-55-5]

M_r 782

DEFINITION

6-O-(3-Amino-3-deoxy- α -D-glucopyranosyl)-4-O-(6-amino-6-deoxy- α -D-glucopyranosyl)-1-N-[(2S)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine sulfate.

Antimicrobial substance obtained from kanamycin A.

Semi-synthetic product derived from a fermentation product.

Content: 96.5 per cent to 102.0 per cent (dried substance).

Loss on drying (2.2.32): maximum 13.0 per cent, determined on 0.500 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa for 3 h.

Pyrogens (2.6.8). If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the test for pyrogens, it complies with the test for pyrogens. The test for pyrogens is specified in the test for pyrogens. The test for pyrogens is specified in the test for pyrogens.

ASSAY

mobile phase and dilute to 10.0 mL with the mobile phase.

Column:

- size: $l = 0.25$ m, $\varnothing = 4.6$ mm;
- stationary phase: end-capped octadecylsilyl silica gel for chromatography R (5 μ m);
- temperature: 40 °C.

The new requirements of
general monograph 2034 apply

Parenteral preparations (0520)

Parenteral preparations



07/2021:0520

PARENTERAL PREPARATIONS

Pyrogenicity (5.1.13)

necessarily apply
immunological
various species
veterinary use
the preparation is

DEFINITION

Parenteral preparations are sterile preparations intended for administration into the human or animal body. They may be administered by injection, infusion or implantation.

They are liquid, semi-solid or solid preparations containing one or more active substances in a suitable vehicle. Liquid preparations for injection or infusion are solutions, colloidal dispersions, emulsions or suspensions.

Sterility (2.6.1). Parenteral preparations comply with the test.

Bacterial endotoxins - pyrogens. Parenteral preparations for human use, if applicable after reconstitution or dilution, comply with the test for bacterial endotoxins (2.6.14), where justified and authorised, with the test for pyrogen (2.6.15). Recommendations for limits for bacterial endotoxins are given in general in the limits of the monographs.

Where the label states "Not for injection" or "For oral use only," the preparation complies with the test for bacterial endotoxins or the test for pyrogenicity (see USP <85>) or with the test for pyrogenicity (see USP <86>).

Parenteral preparations for primary use are for intramuscular or intravenous use for bacterial endotoxin (pyrogen) or with the following components (2.6.8) when the total volume to be injected in a single dose is 15 mL or more and is equivalent to a dose of 0.2 mL or more per kilogram of body mass.

STORAGE

In a sterile, airtight, tamper-evident container.



Plasma-derived products

HUMAN VON WILLEBRAND FACTOR

Factor humanus von Willebrandi

DEFINITION

Pyrogenicity (5113)

Pyrogenicity (5.1.13)

This monograph applies to preparations formulated according to the human von Willebrand factor activity.

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of human von Willebrand factor per millilitre.

Sterility (2.6.1). It complies with the test.

Pyrogens (2.6.14) or Bacterial endotoxins (2.6.14). It complies with the test methods or, preferably, where justified and authorized, validated methods as the test for bacterial endotoxins.

For the pyrogen test, a volume equivalent to 10 IU of human von Willebrand factor was used.

Where the titer is less than 0.6, the preparation is to be examined for endotoxin. The endotoxin per International Unit of human von Willebrand factor.



Limits for BET maintained,
Endotoxin Equivalents(5.1.13)

Vaccines for human use

Monograph/chapter	Requirement for RPT
Hepatitis B-containing vaccines	- Hep B (1056) - DT-Hep B* (2062) - DTaP-Hep B* (1933)
3-O-Desacyl-4'-monophosphoryl lipid A (MPL) (2537)	- RPT on an intermediate
Haemophilus influenza type b-containing vaccines	- Hib (1219) - DTaP-Hib (1932) - DTaP-IPV-Hib (2065) - DTwP-IPV-Hib* (2066) - DTaP-IPV-Hep B-Hib (2067) - Hib-Men C (2622)
Meningococcal vaccines	- Men PS vaccine (0250) - Men C conjugate vaccine (2112) - Men A, C, W135, Y conjugate vaccine (3066)
Pneumococcal vaccines	- Pneumococcal polysaccharide vaccine (0966)
	- Pneumococcal conjugate vaccine (2150)
Rabies vaccine (0216)	- RPT on the final lot in case non-endotoxin pyrogens are present
Tick-borne encephalitis vaccine (1375)	- RPT on the final lot
Carrier proteins for the production of conjugated vaccines (5.2.11)	- RPT for <i>N. meningitidis</i> outer membrane protein complex (OMP)

+ Revise general monograph *Vaccines for human use* (0153)

*monographs will be suppressed from the Ph. Eur. as of July 2023 (Supplement 11.2)



General monograph *Vaccines for human use* (0153)

NOTE ON THE GENERAL MONOGRAPH

Pyrogenicity. The section on Bacterial endotoxins in the Tests part of the monograph has been replaced with a new section on Pyrogenicity, referring to new general chapter 5.1.13 Pyrogenicity which provides guidance for selection and implementation of a suitable test for pyrogenicity (test for bacterial endotoxins or monocyte-activation test).

In addition, a statement has been introduced under General provisions in the Production part of the monograph to stress the need to characterise pyrogenicity during development studies and whenever revalidation is necessary.

This revision of general monograph 0153 is part of a broader exercise affecting multiple Ph. Eur. texts and aiming at the complete suppression of the rabbit pyrogen test from the Ph. Eur.

As part of this exercise, the following texts have been published in the same issue of *Pharmeuropa*: 1) new general chapter 5.1.13 Pyrogenicity; 2) monographs on individual vaccines for human use that were revised to delete the reference to the rabbit pyrogen test. The revised individual monographs no longer contain any mention of the rabbit pyrogen test, and, as a result, the requirements of general monograph 0153 for General provisions and Tests will apply.



Importantly, the revision of the monograph does not call into question established manufacturers' strategies to control the pyrogenicity of their products using the test for bacterial endotoxins that were authorised by the competent authority, and is not intended to prompt a retrospective assessment on pyrogenicity.

PRODUCTION

General provisions. The production method for a given product must have been shown to yield consistently batches comparable with the batch of proven clinical efficacy, immunogenicity and safety in man.

Product specifications including in-process testing should be set. Specific requirements for production including in-process testing are included in individual monographs. Where justified and authorised, certain tests may be omitted where it can be demonstrated, for example by validation studies, that the production process consistently ensures compliance with the test.

Unless otherwise justified and authorised, vaccines are produced using a seed-lot system. The methods of preparation are designed to maintain adequate immunogenic properties, to render the preparation harmless and to prevent contamination with extraneous agents.

Pyrogenicity is characterised during development studies and controlled whenever revalidation is necessary. Guidance for selection of a suitable pyrogenicity test is given in general chapter 5.1.13.

TESTS

Vaccines comply with the tests prescribed in individual monographs including, where applicable, the following:

Bacterial endotoxins. Unless otherwise justified and authorised, a test for bacterial endotoxins is carried out on the final product. Where no limit is specified in the individual monograph, the content of bacterial endotoxins determined by a suitable method (2.6.14) is less than the limit approved for the particular product.

Pyrogenicity. The vaccine complies with a suitable test for pyrogenicity. Guidance for selection of a test is given in general chapter 5.1.13. Where no limit is specified in the individual monograph, it complies with the limit approved for the particular product.

NOTES ON THE TEXTS

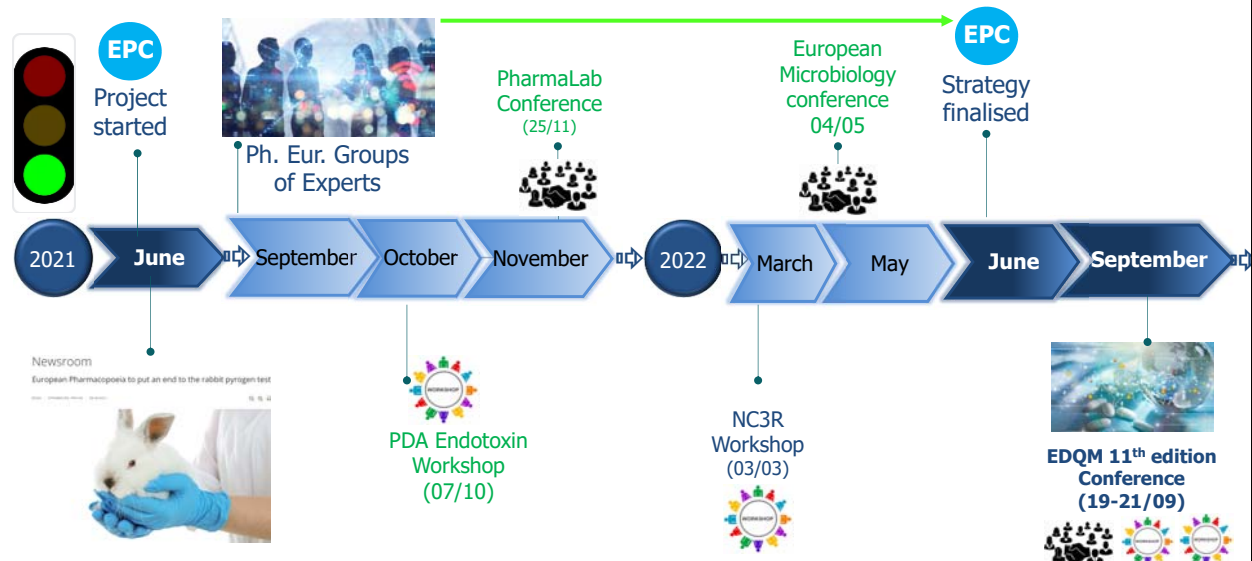
- "It should be noted that the exercise will ultimately lead to the suppression of general chapter 2.6.8 from the Ph. Eur. Manufacturers still using the rabbit pyrogen test are strongly encouraged to take the necessary steps to proceed with its replacement by a suitable in vitro alternative (e.g. the 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."

Timelines



WHAT	WHO	WHEN	
		Publication in PhPa	Envisaged implementation date
Elaboration of Pyrogenicity (5.1.1.3) (together with revision of 5.1.10)	BET WP		
REVISION			
2.6.30	BET WP		
2034	BET WP		
0520	G12 with BET WP support		
remaining texts	GoE/WP with BET WP support		
DELETE Pyrogens (2.6.8)			

Communication to stakeholders



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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

Today!

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Acknowledgements

- The BET Working Party and its Chair, Dr. Ingo Spreitzer
- All the experts in Groups of Experts and Working Parties (6, 6B, 7, 9, 10D, 12, 14, 15, 16, CRB, DIA) and their respective Chairs
- All EDQM European Pharmacopoeia Department staff members who worked on the revised and new texts, with particular thanks to Dr Gwenaël Ciréface who co-ordinated the exercise

Thank you for your attention



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