

Langen, 14 December 2020

STATEMENT

PYROGEN TESTING ON RABBITS

The Paul-Ehrlich-Institut welcomes in principle the switch from animal testing to non-animal test systems. However, the quality and informative value of the tests must permit this replacement, because the quality and safety of the medicinal products must not be jeopardised.

Analytical and Safety Tests in the Pharmacopoeia

In pharmacopoeias, various analytical and safety tests are mandatory for medicinal products and their dosage forms. According to Section 55 German Medicines Act (Arzneimittelgesetz, AMG), a pharmacopoeia is a collection of recognised pharmaceutical rules published by the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM) in agreement with the Paul-Ehrlich-Institut and the Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL). These are decided upon by the European Pharmacopoeia Commission, the German Pharmacopoeia Commission or the German Homeopathic Pharmacopoeia Commission.

If these tests are not performed and passed, a medicinal product will not receive a marketing authorisation or subsequent batch releases.

Pyrogen Testing

The safety tests also include testing for pyrogens (Rabbit Pyrogen Test, RPT), which is carried out on rabbits. The purpose of this is to prevent serious to life-threatening side effects caused by pyrogenic impurities in medicines.

In recent years, about 6000-7000 rabbits per year have been used in the pyrogen testing in Germany. This is a notifiable animal experiment, which is authorised by the responsible state authorities for a period which has to be extended every five years.



Since 2010, the monocyte activation test (MAT) has been laid down in the European Pharmacopoeia, which represents a validated animal-free substitute for pyrogen testing. With the help of human blood cells, the MAT simulates the fever reaction in the test tube (in vitro). Since around 2015, several MAT kits have been available from different suppliers. Pharmaceutical companies can thus switch from RPT to MAT as soon as the suitability of MAT for the medicines concerned has been experimentally confirmed and approved by regulatory authorities. The process of validation with the aim of approval can take months to years, depending on the product.

The pyrogen test continues to be a method of the European Pharmacopoeia. Based on the EU Directive 2010/63 ("Protection of Experimental Animals") and its implementation into German law in 2013, the legal basis for the re-approval of the rabbit pyrogen testing (RPT) has become stricter. A new authorisation can only be granted in the exceptional case that valid alternative methods such as MAT or bacterial endotoxin tests (BET) prove to be unsuitable. The applicant has to prove this experimentally. However, no binding transition period was specified. In 2016, the Paul-Ehrlich-Institut held an international MAT workshop to explain the urgency of the methodological transition to all stakeholders involved.

Changeover from RPT to Replacement Methods

At the end of 2018, the competent state authority rejected a blanket extension of the pyrogen test for the largest German pyrogen tester for another five years. Almost 100 per cent of pyrogen tests in Germany are carried out at this contract tester.

The drug manufacturers concerned had to submit a plan for each individual product to describe the planned switch to the BET or the MAT. Based on these plans, the authority issued temporary individual approvals (< five years) for the pyrogen testing in order not to jeopardise patient supply with medicinal products. In coordination with the German Federal Ministry of Health (Bundesgesundheitsministerium, BMG) and the BfArM, the Paul-Ehrlich-Institut has accompanied this process. The first notifications of changes have been submitted to the Paul-Ehrlich-Institut. A reduction in the number of rabbits can be expected soon. The aim is to largely replace the pyrogen test with the MAT or the BET. However, there might be some medicinal products which, due to certain properties, cannot be tested in the BET or MAT, but only in the RPT. For the

medicinal products concerned, it is the responsibility of the respective medicinal product manufacturers to demonstrate the necessity of the pyrogen testing.

Alternative Method Bacterial Endotoxin Test (BET) or Limulus Amebocyte Lysate (LAL)

The pyrogen test has been replaced for decades by the specific test for bacterial endotoxin (strongest known pyrogen) for many products. This test, known as the bacterial endotoxin test (BET) or Limulus amoebocyte lysate (LAL), is not an animal test. However, the main component is obtained from the haemolymph of horseshoe crabs. The main source is *Limulus polyphemus* (east coast of the USA), whose population is monitored and regulated. The animals (approx. 500,000 animals p.a.) largely survive the collection of a partial quantity of blood. The mortality of horseshoe crabs caused by the entire collection procedure (capture, transport, blood collection, transport back to the capture site) is estimated by BET producers at three to five percent and by government agencies at 15 percent. The three Asian species are threatened with extinction and, like *Limulus*, are on the endangered species list. While the BET test is not the reason for the threat to the animals, the limited animal resource may jeopardise drug safety. Moreover, the threat to this species is in the focus of discussions as the number of BET tests is continuously increasing globally.

The European Pharmacopoeia has responded to the situation by adding recombinant bacterial endotoxin tests to the pharmacopoeia in July 2020 (2.6.32. Test for bacterial endotoxins using recombinant factor C). The amendment will enter into force on 1 January 2021. No endangered wild animal population is required to produce these recombinant variants.

With the inclusion of the monocyte activation test MAT in the European Pharmacopoeia in 2010 (2.6.30 Monocyte Activation Tests) and in 2020 (2.6.32 Test for bacterial endotoxins using recombinant factor C), the course was set for advanced and sustainable pyrogen and endotoxin testing.

For many decades, the Paul-Ehrlich-Institut has been actively and successfully involved in the development of animal test replacement methods and will continue to work intensively on this in the future.