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Fewer tests on animals and safer drugs: new EU tests save 200,000 rabbits per year

New, groundbreaking methods of drug testing to replace animals with safe alternatives, saving up to 200,000 rabbits per year, were unveiled today in Brussels by European Research Commissioner Philippe Busquin. The set of six tests detects potential fever-causing agents (pyrogens) in drugs, by using human blood cells instead of rabbits. The new tests have been developed by a EU-supported research team, involving national control laboratories, test developers, and companies. The tests are being validated by the Commission. They are already being used in over 200 laboratories across the world. Thanks to these alternative methods rabbits will no longer be needed to test the presence of pyrogens in parenteral (non oral) drugs.

"The use of animals to test drugs is unfortunately necessary to safeguard human health," said European Research Commissioner Philippe Busquin. "But we can reduce, replace and refine animal testing, with EU-sponsored research leading the way at world level. The EU's validation of these new testing methods will encourage their broad take-up by industry, ensure drug safety and quality, and reduce the use of animal research. This is an example of the European Research Area in action, developing an environment in which scientific results can be rapidly exploited and transformed into products and processes that improve quality of life, increase competitiveness and benefit animal welfare."

The safety and potency of commercially available medicines and vaccines must be guaranteed. Innovative research, funded and validated by the Commission, aims to replace existing animal-based test methods for fever-causing agents (pyrogens) in parenteral drugs with a new generation of in vitro tests that are more accurate, quicker and more cost-effective.

Blood cells replace rabbits

Understanding of human immunology has advanced rapidly in the past 20 years. Work on human fever reaction and development of test systems for fever mediator molecules, combined with improved cell biology techniques, now enables the innovative use of human cells as biosensors for pyrogens (fever-causing agents). The EU study[1] set out to compare and harmonise six in vitro assays to develop a "state-of-the-art" method for inclusion into the European Pharmacopoeia - which sets the requirements for the quality control of drugs in Europe - thus improving consumer safety.

The EU role

[1] Cell factory project: *Comparison and validation of novel pyrogen tests based on the human fever reaction, with a view to the ultimate replacement of the rabbit pyrogen test and the Limulus assay* (QLK3-1999-00811)

The research project funded by the Commission under the EU Fifth Research Framework Programme (1998-2002) brought together the best teams from academia, industry and regulatory bodies. The Commission's Joint Research Centre (the "ECVAM" facility, or "European Centre for Validation of Alternative Methods") played a major role in the project through provision of scientific and technical advice on the design of the validation study, application of good laboratory practice procedures and distribution and coding of test material.

Industry and regulators jump on board

Interest from both regulatory authorities and industry is very high, with many contributions coming from outside the project consortium that included national control laboratories, test developers, a major pharmaceutical company and a producer of diagnostic kits. For example, the European Pharmacopoeia has set up an international expert group to draft a general method on these new tests. In fact, the tests are already in use in about 200 laboratories worldwide, with great success.

Further take-up and new applications

The Commission will take responsibility for further application of this multidisciplinary, international validation study, including an intended patent. This will encourage successful transfer of the tests and help open new fields for pyrogen testing, such as cellular therapies, medical devices and pollution control in the work place.

Reducing, replacing or refining animal experimentation

Drug quality control is a trans-national matter, which is standardised and regulated in Europe at EU level, thus requiring international collaborative efforts. The European Commission ensures full support for applications to reduce, replace or refine animal experimentation as required by the 1986 Council Directive[2]. This aim is echoed by the European Pharmacopoeia. The "Three Rs" provide a strategy to minimise animal use, without compromising the quality of the scientific work being done.

ECVAM's role is to co-ordinate international validation studies, act as a focal point for the exchange of information, to set up and maintain a database on alternative methods, and to promote dialogue among legislators.

Background: pyrogen and non-oral drugs

Parenteral drugs are commonly employed throughout Europe for treating a variety of illnesses. Ensuring the safety of such widely used drugs requires strict monitoring and control against any possible pyrogenic contamination on a batch-by-batch basis. The most important pyrogen is endotoxin, a constituent of the cell wall of gram-negative bacteria that can generate endogenous fever mediators by white blood cells, particularly monocytes and macrophages.

Rabbits or…

In the rabbit pyrogen test, the test substance is injected into rabbits and any subsequent change in body temperature recorded. A significant rise in temperature indicates the presence of pyrogens. While it has served drug safety control for more than 50 years, it fails for important new therapies such as cellular products or species-specific agents.

[2] *Novel in-vitro testing as alternatives to animal testing*; Council Directive 86/609/EEC

… horseshoe crabs?

Until now, the only in vitro alternative available is the LAL test, based on coagulation of blood from the horseshoe crab (Limulus polyphemus). However the LAL test detects only one class of pyrogens – endotoxins from gram-negative bacteria – leaving patients at risk from "non-endotoxin" pyrogens such as gram-positive toxins, viruses and fungi. It is also subject to interference by various non-pyrogenic substances. And, as it is based on the defence system of an arthropod, it cannot provide results perfectly relevant to humans.

No – human blood cells!

Six alternative cellular assays have therefore been developed to replace the animal rabbit pyrogen test and close the safety gap presented by use of the LAL test in controlling parenterals. All these test systems are based upon the response of human leukocytes (principally monocytes), which release inflammatory mediators (endogenous pyrogens) in response to pyrogenic contamination (exogenous pyrogens).

Quicker, more accurate and more effective

The new tests have several advantages compared with the rabbit test: they are less laborious, cheaper and more sensitive. Results of the validation study suggest that testing on animals can be completely replaced. In contrast to the LAL, the new assays are not restricted to endotoxins from gram-negative bacteria but detect all classes of pyrogens and reflect the potency of different endotoxins in mammals, without suffering interference from endotoxin-binding components in blood products. A commercial kit version for one of the assays has already been developed and standardised, and pre-tested cryopreserved (frozen) blood as a versatile test reagent containing the blood cells as biosensors is under development.

For further information please visit:

http://ecvam.jrc.it/index.htm

http://europa.eu.int/comm/research/quality-of-life/cell-factory/volume1/projects/qlk3-1999-00811_en.html