New regulations covering the use of laboratory animals

The new directive of the European Parliament and of the Council on the protection of animals used for scientific purposes was adopted on 22nd September 2010. The new legislation was subsequently implemented into the German Animal Welfare Act, which came into force on 1st February 2013. On 7th June 2013, the German Bundesrat also adopted an animal welfare/laboratory animal regulation drafted by the BMELV. These regulations have implications for companies and researchers alike. In recent years, a growing number of alternative methods to animal testing have been developed. Dr. Nina Hasiwa, CEO of AtaX-Advice – Alternatives to animal Xperiments based in Konstanz, is an expert in this area. In an interview with Anna Weiß for BIOPRO, Dr. Hasiwa talks about the impact and possible future prospects of the new legislation and the monocyte activation test (also known as in-vitro pyrogen test (IPT) or human whole blood pyrogen test) as one such alternative method to the use of animals.

Dr. Hasiwa, what is your view on the impact so far of the new EU legislation on research, chemical manufacturers and animals and what do you think will happen in the future?

The new European Laboratory Animals Directive has no major effect on chemical companies. However, researchers who use animals for experiments will now have to work harder to obtain permission to use animals in experiments. In addition, the new legislation imposes severe restrictions on the use of animals, large apes in particular. These animals are now better protected. In my opinion, the most significant progress made by the new regulations is the greater financial and societal support given to the development and recognition of alternative methods.

This is very important in the sense that only well-developed, validated in-vitro testing methods that come up with more meaningful information than animal experiments will make industry, research and legislation adopt a different stance. Every legal step taken is progress because it makes alternatives to animal testing more acceptable and more likely to receive higher levels of funding. The tighter the rules on animal testing, the sooner industry and research will be willing to use innovative, animal-free testing methods. Therefore, many researchers have already shifted their focus to the development and validation of animal-free testing methods.

Why do many pharmaceutical companies still use animal experiments despite the fact that some alternative methods would be cheaper and hence more cost-efficient for the companies?

Dr. Nina Hasiwa, CEO of AtaX-Advice – Alternatives to animal Xperiments, is a specialist on alternative methods to animal experiments.

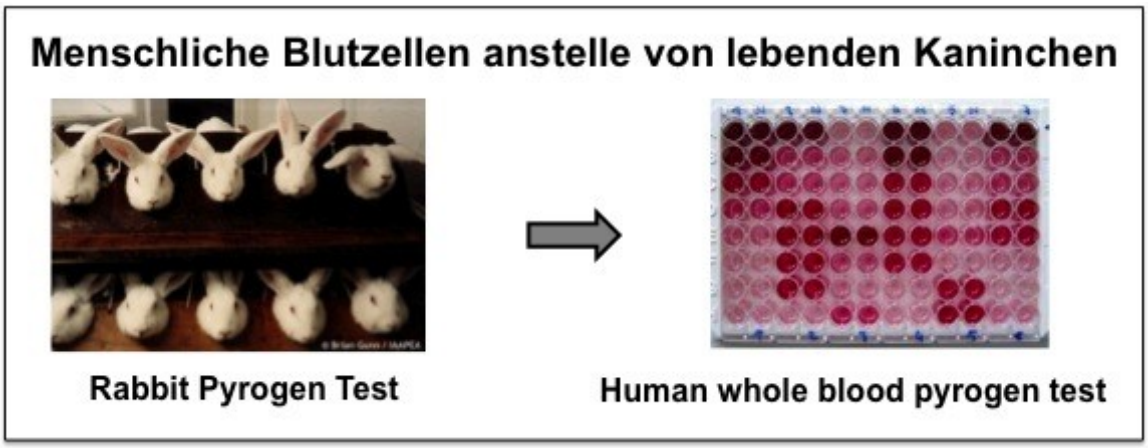
The problem for pharmaceutical companies is that drug development is costly and extremely time-consuming. Incorrect decisions can ruin a company. Therefore, pharmaceutical companies tend to rely on traditional methods which lead to data that compare well with data from previous experiments.

Does this mean that the pharmaceutical industry is not interested in alternative methods to animal testing?

Not at all. The pharmaceutical industry in particular has been looking for alternative methods for many years. Many pharmaceutical companies work with other companies in seeking to actively develop in-vitro methods for the prediction of a compound's toxic effect in humans. They present these methods at international conferences, thereby making an active contribution to the advancement of alternative, animal-free test methods.

The cooperation with CAAT-Europe (Center for Alternatives to Animal Testing at the University of Konstanz) is a good example of this kind of development. CAAT-Europe brings together industry and academics to address the needs of human-relevant methods; representatives of the pharmaceutical industry enrich the expert workshops offered by CAAT-Europe, present their research and are willing to fund alternative in-vitro methods. CAAT, along with the legislative authorities and the industry (in particular the pharmaceutical, chemical and cosmetics industries), have made a considerable contribution to reaching the goal of developing reliable test methods without the use of animals.

How long does it usually take to validate new methods like the pyrogen test as new standards?



Pyrogen testing is used by drug manufacturers to see if a drug causes inflammatory reactions. It is based on an immunological reaction in human whole blood.

It takes many years to develop and validate a new test system, which greatly improves the quality of the test. The in-vitro pyrogen test, which was developed at the University of Konstanz, is an excellent example that shows how long it takes to develop such a test. Pyrogen testing is used to see whether a drug or medicinal product contains toxic substances that might cause fever. The test was published in 1995, validated by ECVAM (European Centre for the Validation of Alternative Methods) in 2005 and included in the European Pharmacopoeia as a replacement for the rabbit pyrogen test in 2009/2010. Despite all this, the test is still not applied on a large scale.

Why do you think this is?

The monocyte activation test, as the in-vitro pyrogen test is also known, was validated and extensively tested for contamination with Gram-negative bacteria. However, contaminations with Gram-positive bacteria, yeasts and fungi, for example were not included in the validation. This is the reason why the rabbit pyrogen test is often used in addition to the in-vitro test. Rabbits have a similar pyrogen tolerance to humans. The presence of toxins is determined by injecting rabbits with a sterile solution of the substance under investigation and observing the response in their body temperature.

What are the differences between the pyrogen test and the Limulus amoebocyte lysate (LAL) test that is standard in the pharmaceutical industry?

The in-vitro pyrogen test involves mixing human whole blood with the substance under investigation. The mixture is incubated and the release of proinflammatory cytokines provides information about the level of inflammation.

The principle of the LAL test is based on a different mechanism from the pyrogen test and uses haemolymph, the arthropod (e.g. Limulus polyphemus, horseshoe crab) equivalent to mammalian blood, for testing drugs for the presence of contaminants. The drug to be tested is mixed with Limulus haemolymph, which reacts with contaminants, Gram-negative bacteria (e.g. rod-shaped bacteria) for example, and causes clots to form. Gram-negative bacteria are bacteria that are surrounded by a thin cell wall. However, Limulus haemolymph also coagulates in the presence of substances that are not toxic for humans. Another disadvantage of the LAL test is that clots often do not form in the presence of substances that are harmful for humans. Many pyrogens remain undetected, which in my opinion makes the LAL test rather unreliable.

Does this mean that the LAL test is not as reliable as it could be?

In the early days of pyrogen testing, the LAL test was definitely an excellent alternative to the rabbit pyrogen test. It was the first test that allowed the quantification of pyrogenic contaminations. But since the LAL test is not fully predictive for humans, it is rather unreliable. The solution lies in the use of the in-vitro pyrogen test. The advantage of the in-vitro pyrogen test is that it reacts to anything that causes an immunological reaction, i.e. a reaction to an antigen, in human whole blood. This test is therefore far more reliable than the LAL test. In addition, the in-vitro pyrogen test detects the entire range of potential contaminations, something that is not possible with the LAL test.

Do you think that animals will no longer be used for testing in about 10 to 20 years' time?

I see the future of methods to replace animal experiments as rather positive. The more money and time spent on the development of new and innovative methods, the earlier we will have in-vitro tests that are able to predict the effect of substances in humans. And these tests will also be far superior to animal tests. It is impossible to predict the point when no more animal experiments will be carried out, but I am sure that each new development is bringing us closer to our goal.

Legal regulations:

Directive 2010/63/EU of the European Parliament and of the Council, revising Directive 86/609/EEC on the protection of animals used for scientific purposes, was adopted on 22nd September 2010 and came into force in November 2012. The aim of the new directive is to strengthen legislation, and improve the welfare of those animals that still need to be used and to create standardised conditions for the protection of laboratory animals used in research and industry throughout Europe. The new directive is specifically aimed at firmly anchoring the principle of the three Rs, to replace, reduce and refine the use of animals, in EU legislation. The EU member states had until November 2012 to implement Directive 2010/63/EU into their respective national laws before it took full effect on 1st January 2013. As far as Germany is

concerned, in December 2012, the German Bundestag adopted the German Federal Ministry of Nutrition, Agriculture and Consumer Protection's (BMELV) draft amendment to the German Animal Welfare Act, which was passed by the German Bundesrat on 1st February 2013. On 7th June 2013, the German Bundesrat also adopted an animal welfare/laboratory animal regulation drafted by the BMELV. In addition to the principles stipulated in the Animal Welfare Act, the latter also contains detailed guidelines regarding the protection of laboratory animals, including strict requirements on animal keeping, breeding and use.
(Sources:
https://www.gesundheitsindustrie-bw.dewww.bundesrat.de/nn_8396/DE/service/thema-aktuell/13/20130201-Tierschutznovelle.html
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Animal experiments: alternatives need to be found urgently