Bundesinstitut für Impfstoffe
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Abnormal Toxicity Testing – Origin, propagation and abolition in Europe

Dr. Klaus Cussler

Symposium on Global Harmonization
of Vaccine Testing Requirements
Rome – March 19, September 2019

ATT – What are we talking about?

Until recently you found the requirement for ABNORMAL TOXICITY TESTING in the General Part of the Ph.Eur. under Section 2.6.9. This were general safety tests for injectable medicines to be performed in mice and guinea pigs.

Similar, but not identical tests are/had been listed in legal requirements for human biologicals around the world, e.g.

- General Safety Test in the US-CFR
- Innocuity test in the WHO-Requirements

Comparable requirements exist for veterinary biologicals in some regions; e.g. Mouse safety test and Guinea pig safety test in the USDA 9CFR.

2.6.9. ABNORMAL TOXICITY

GENERAL TEST

Inject intravenously into each of 5 healthy mice, weighing 17 g to 24 g, the quantity of the substance to be examined prescribed in the monograph, dissolved in 0.5 mL of *water for injections R* or of a 9 g/L sterile solution of *sodium chloride R*. Inject the solution over a period of 15 s to 30 s, unless otherwise prescribed.

The substance passes the test if none of the mice die within 24 h or within such time as is specified in the individual monograph. If more than one animal dies the preparation fails the test. If one of the animals dies, repeat the test. The substance passes the test if none of the animals in the 2nd group die within the time interval specified.

CAVE!

The Ph.Eur. lists two different tests under ABNORMAL TOXICITY:

- The GENERAL TEST
- The test for IMMUNOSERA AND VACCINES ad us. hum.

IMMUNOSERA AND VACCINES FOR HUMAN USE

Unless otherwise prescribed, inject intraperitoneally 1 human dose but not more than 1.0 mL into each of 5 healthy mice, weighing 17 g to 24 g. The human dose is that stated on the label of the preparation to be examined or on the accompanying leaflet. Observe the animals for 7 days.

The preparation passes the test if none of the animals shows signs of ill health. If more than one animal dies, the preparation fails the test. If one of the animals dies or shows signs of ill health, repeat the test. The preparation passes the test if none of the animals in the 2nd group die or shows signs of ill health in the time interval specified.

The test must also be carried out on 2 healthy guinea-pigs weighing 250 g to 400 g. Inject intraperitoneally into each animal 1 human dose but not more than 5.0 mL. The human dose is that stated on the label of the preparation to be examined or on the accompanying leaflet. Observe the animals for 7 days.

The preparation passes the test if none of the animals shows signs of ill health. If more than one animal dies the preparation fails the test. If one of the animals dies or shows signs of ill health, repeat the test. The preparation passes the test if none of the animals in the 2nd group die or shows signs of ill health in the time interval specified.

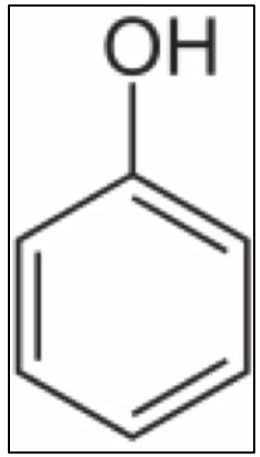
The origin of the ATT

The ATT for biological products nowadays includes two separate animal safety tests: the mouse test and the guinea pig (gp) test.

Both tests developed independently and were introduced for specific purposes around 1900.

The first governmental regulations related to safety tests in mice and gp originate from Germany.

The origin of the ATT



The German government introduced specific regulations for diphtheria sera already in 1894.

A serum sample was considered as “safe” if it

- is entirely clear and free from major precipitation,
- does not contain any bacterial impurities,
- does not contain more than 0.5% phenol

(Ehrlich, 1896: Berliner Klinische Wochenschrift, 441)

- [is free from toxins, in particular tetanus toxin.] **(Otto, 1906)**

Preservation of Antisera

Phenol and cresol were considered to be the most appropriate preservatives at the time:

- The content had to be restricted due to the toxicity of phenolic compounds
- but needed to be effective (against glanders)

→ A limit of 0.5% phenol (0.4% tricresol) was requested.

How should this requirement be controlled?

The Mouse Test

The mouse test was first performed in Germany in the governmental control institute of Paul Ehrlich.

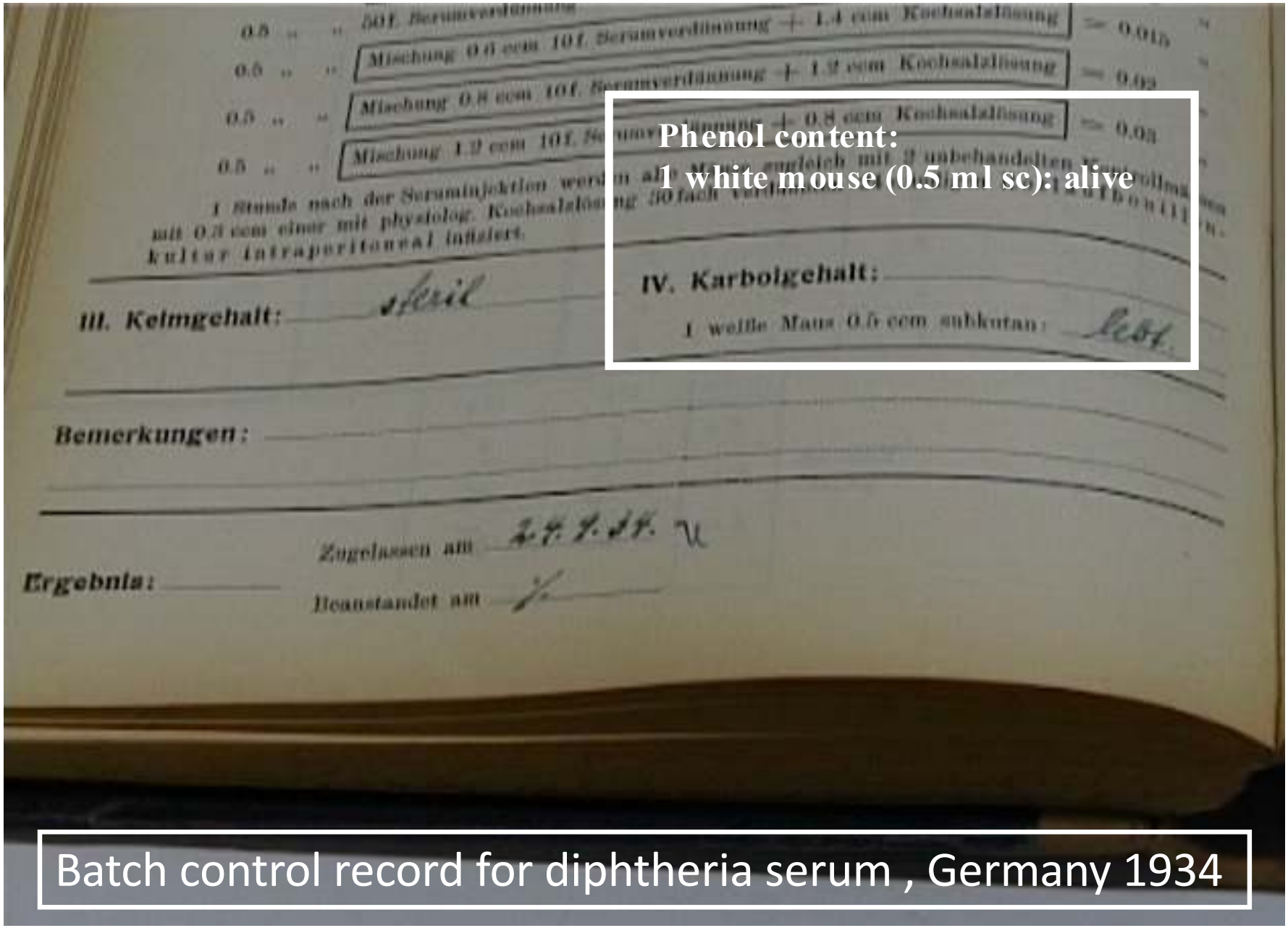
Here we see him inspecting laboratory mice; Frankfurt, around 1905

The Mouse Test

Mice are very sensitive to phenol:

- with 0.5% phenol in 0.5 ml of serum s.c. they start trembling and shaking
- more than 0.5% result in convulsions and death

→ The laboratory mouse was used as a biological test tube (already in 1895; Throm, 1995)



Batch control record for diphtheria serum , Germany 1934

The Mouse Test

This mouse safety test for diphtheria serum was maintained for tetanus serum and lateron also for the first bacterial vaccines (typhoid and cholera).

→ The mouse test became a standard test for all biologicals preserved with phenol.

The Mouse Test

The first inactivated whole cell vaccines, namely cholera vaccine and typhoid vaccine were also preserved with phenol with the same concentration used for antisera. For that reason the mouse test also became a requirement for the quality control of vaccines. After the 2nd World War this *in vivo* phenol test was still in use and found introduction into international WHO requirements, as documented for the WHO REQUIREMENTS FOR BIOLOGICAL SUBSTANCES. 4. Cholera Vaccine. WHO TRS No. 179, 1959:

> 5.3.2 *Test for abnormal toxicity of phenol*
>
> If phenol has been used in the preparation of the vaccine, three or more white mice weighing 15-20 g shall be injected subcutaneously with 0.5 ml of vaccine. If after injection one or more of the mice are observed to react with tremor and spasms persisting for more than 30 minutes, the test shall be repeated in twice the number of mice. If the reactions occur again in the second test the vaccine shall be discarded.

The Mouse Test

In 1943 a “colour test for phenolic compounds” was established (Emerson, 1943).

→The analytical test was adapted to measure phenol derivatives in medicines (Pfeiffer & Manns, 1957).

→**From todays point of view (3Rs) this would have been the time to replace the mouse safety test.**

→**The original purpose of the mouse test sank into oblivion.**

→The mouse test was continued and became part of the ATT

The Guineapig Test

Emil von Behring performs animal tests in his laboratory;
Berlin, 1889

The Guineapig Test

The St. Louis Republic., November 02, 1901

"Jim", a former milk wagon horse in St. Louis, died on October 2, 1901.
Jim had shown signs that he had contracted tetanus and was euthanized.

Jim was used to produce serum containing diphtheria antitoxin. Jim produced over 7.5 US gallons of diphtheria antitoxin in his career.
The horse had been bled shortly before contracting the disease.

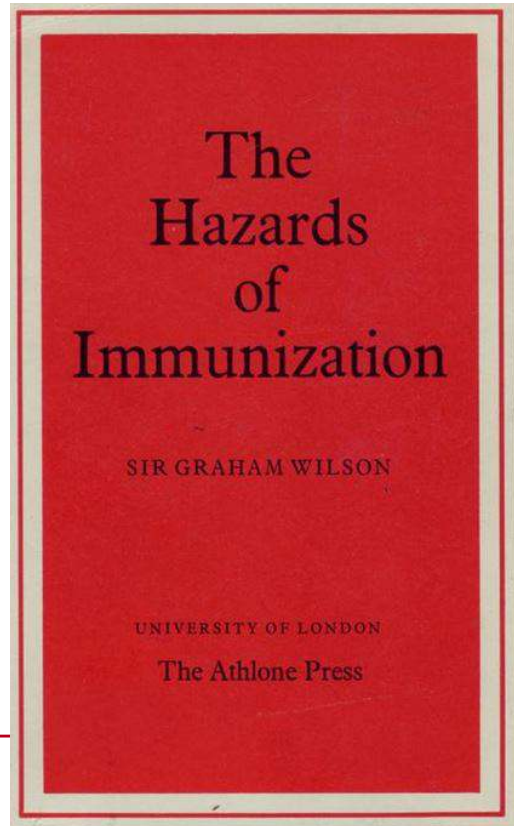
The Guineapig Test

These failures in oversight led to the distribution of antitoxin that caused the death of 12 children. This incident, and a similar one involving contaminated smallpox vaccine, led to the Biologics Control Act of 1902 and the ensuing tragedy and reaction, thus established a precedent for the regulation of biologics, leading to the 1906 formation of the US FDA.

The Guineapig Test

A similar incident (18 tetanus cases/13 fatalities) occurred in Italy at the same time after treatment of children with diphtheria serum from the Serotherapeutic Institute of Milan.

→ A gp test was introduced as biological indicator test for tetanus and other bacterial toxins (Germany, 1901) and later on in other countries.



The Guineapig Test

- This test was introduced for the control of diphtheria serum but was consecutively applied to other sera and later on also for vaccines.
- This test was used in Germany without modifications until 1935.

The Guineapig Test

In the UK, the Extra Pharmacopoeia (22nd edition, 1941) lists a gp test for all forms of diphtheria prophylactics “to ensure freedom from toxicity which consists in injecting 5.0 ml into each of five healthy guinea-pigs and in seeing that none die within 6 days”.

Origin of the ATT

- In the 1940th when governmental regulations in Germany were revised the new guidelines for vaccines mentioned for the first time a safety test which consisted of the gp test and the mouse test.

→The basic outline for the ATT was created

→The combination of two totally independent safety tests became a general safety test.

Propagation of the ATT

- The ATT as such was mentioned for the first time in 1951 when the WHO started to develop internationally accepted guidance.
- In the following years the test was introduced in nearly all general testing requirements for immunological and biological medicines around the globe, both in the human and in the veterinary field.

Propagation of the ATT

Test for freedom from abnormal toxicity

(Appendix 34 of the First Edition of the International Pharmacopoeia, 1951)

Both the following tests are applied:

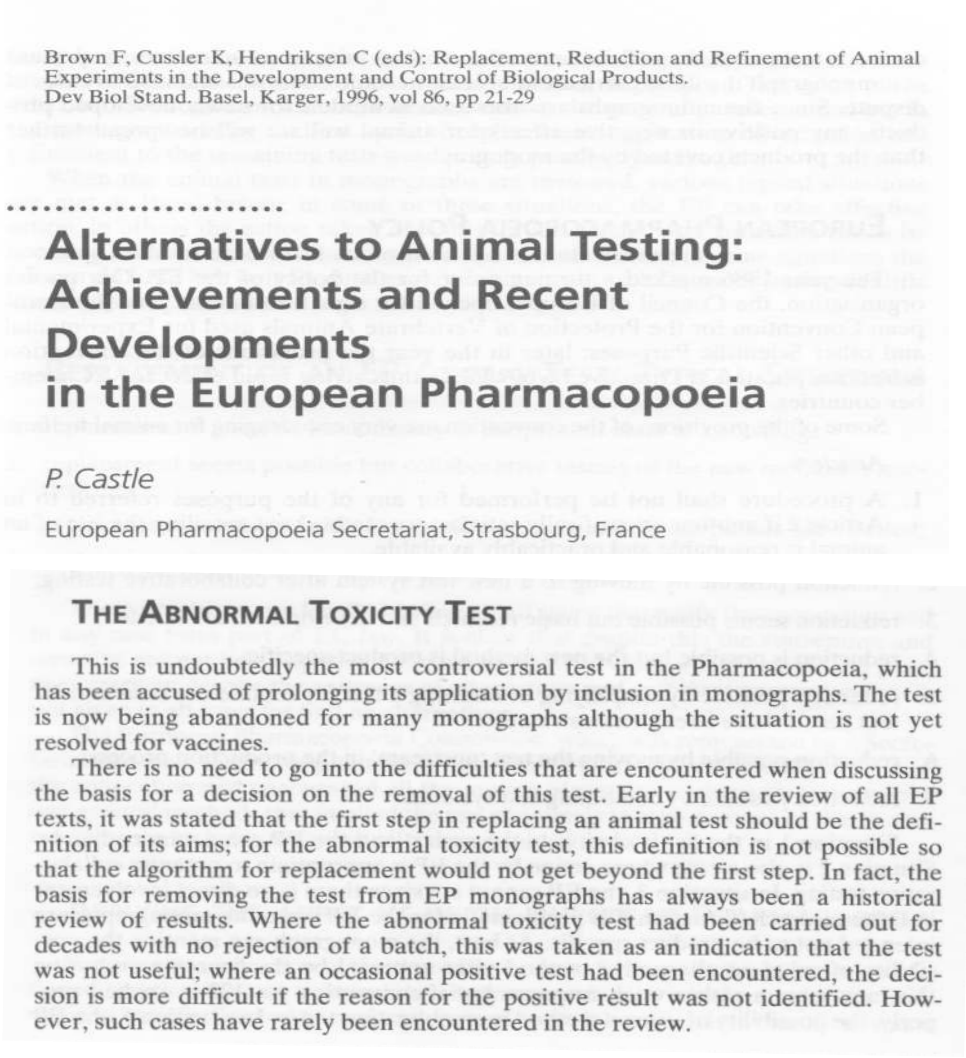
- Inject 0.5 ml under the skin of a healthy mouse weighing about 20 g; neither serious symptoms, nor death, ensue within six days.
- Inject 5.0 ml under the skin or into the peritoneal cavity of a healthy guinea-pig weighing 250-400 g; neither serious symptoms, nor death, ensue within six days.
- [The ATT included also a rabbit test which became later the pyrogen test]

Abolishing the ATT in Europe

When alternatives to animal testing (3Rs) became a topic in the area of biologicals, the ATT was at once in the focus.

- However, there was no consensus for a deletion of the ATT.
- Many regulators and QC people stressed the fact that the test has been „successfully“ used over decades.
- The tests were considered to be non-severe, and the number of animals used was low.

Abolishing the ATT in Europe



Brown F, Cussler K, Hendriksen C (eds): Replacement, Reduction and Refinement of Animal Experiments in the Development and Control of Biological Products.
Dev Biol Stand. Basel, Karger, 1996, vol 86, pp 21-29

Alternatives to Animal Testing: Achievements and Recent Developments in the European Pharmacopoeia

P. Castle

European Pharmacopoeia Secretariat, Strasbourg, France

The Abnormal Toxicity Test

This is undoubtedly the most controversial test in the Pharmacopoeia, which has been accused of prolonging its application by inclusion in monographs. The test is now being abandoned for many monographs although the situation is not yet resolved for vaccines.

There is no need to go into the difficulties that are encountered when discussing the basis for a decision on the removal of this test. Early in the review of all EP texts, it was stated that the first step in replacing an animal test should be the definition of its aims; for the abnormal toxicity test, this definition is not possible so that the algorithm for replacement would not get beyond the first step. In fact, the basis for removing the test from EP monographs has always been a historical review of results. Where the abnormal toxicity test had been carried out for decades with no rejection of a batch, this was taken as an indication that the test was not useful; where an occasional positive test had been encountered, the decision is more difficult if the reason for the positive result was not identified. However, such cases have rarely been encountered in the review.

- **The ATT „is undoubtedly the most controversial test in the Pharmacopoeia“.**
- **A defition of the aim of the ATT is not possible.**
- **The basis for removing the test from Ph.Eur. Monographs has always been a historical review of the results.**

The PEI inventory

To provide facts and figures the PEI performed a survey in Germany about the ATT.

- Supported by the German Ministry for Education and Research
- 1994 – 1995
- Human and veterinary sera and vaccines

- Evaluation of test performance and test results
- Industry data (via questionaire) and PEI data

The PEI inventory

- 4367 ATTs for 159 different products using
  - more than 19,000 mice and
  - more than 8,700 guinea pigs
- 1.1 % of ATTs needed a repeat test
- All batches passed the test
- However, due to inherent toxicity of certain vaccines (whole cell pertussis, cholera and typhoid vaccine are mentioned) test modifications were noted.

→Conclusion: Deletion of the ATT

→Initiation of a Request for Revision

The PEI inventory

ELSEVIER

PII: S0264-410X(97)00074-1

Vaccine, Vol. 15, No. 10, pp. 1047–1048, 1997

Printed in Great Britain
0264–410X/97 $17+0.00

Comment

Elimination of abnormal toxicity test for sera and certain vaccines in the European Pharmacopoeia

M. Schwanig*†, Margit Nagel*, Karin Duchow*
and Beate Krämer*

The PEI inventory

Request for Revision to delete the ATT from the *Ph.Eur.*
(November 1995 via German Pharmacopoeia Commission)

Results:

- Complete deletion accepted for Veterinary products
- Different approach for human products:
  - Complete deletion only accepted for
    - sera and immunoglobulins
    - all DPT vaccines
  - For all other products:
    - Deletion as a routine batch release test, but
    - ATT remains to be part of the production section

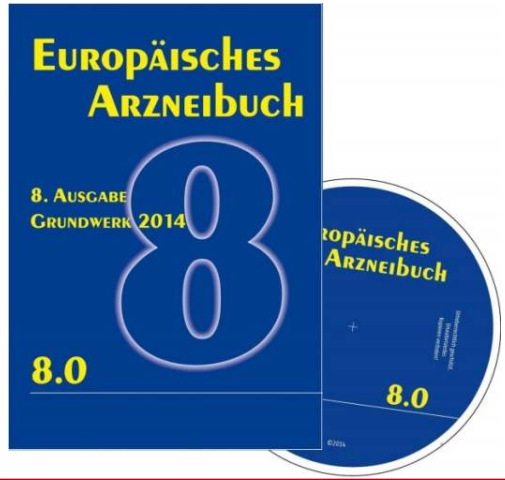
→ ATT was still listed as a requirement in the *Ph.Eur.*

The „interim“ status of the ATT in the Ph.Eur.

Although the ATT had been deleted as a generel batch safety test from the *Ph.Eur.*, the test was still performed

- as a requirement for product development in Europe.
- as a batch test due to legal requirements in third countries.

Several countries which started to develop a pharmacopoeia „copied“ the ATT.



The „interim“ status of the ATT in the Ph.Eur.

- The ATT project has been very successful concerning products marketed only within Europe.
- However, not much changed for products marketed outside Europe.

➢ **Increasing problem for industry**

→ European Partnership for Alternative Approaches to Animal Testing (EPAA)

Biologicals 48 (2017) 55–65

Contents lists available at ScienceDirect

Biologicals

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

Modern science for better quality control of medicinal products "Towards global harmonization of 3Rs in biologicals": The report of an EPAA workshop

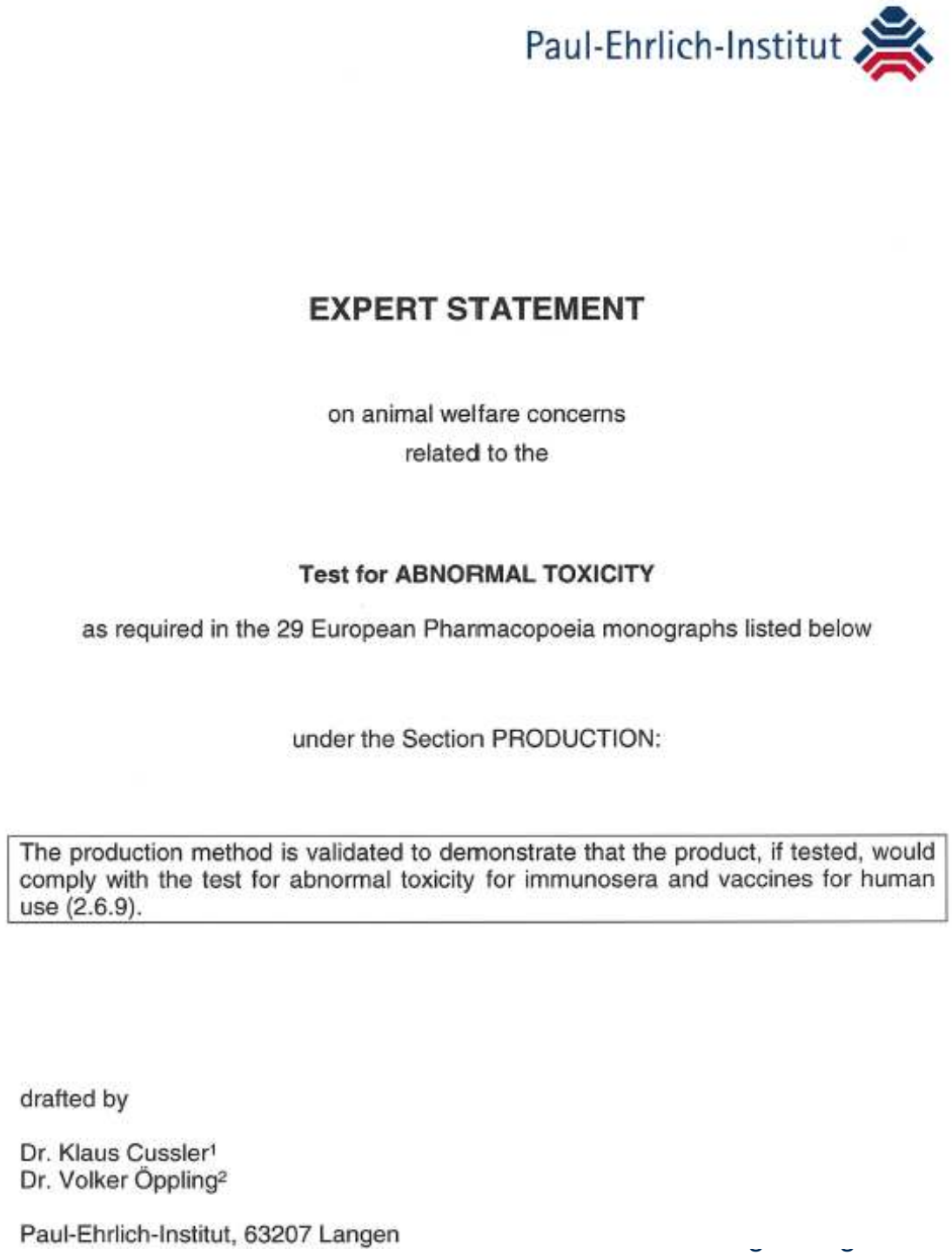
ABSTRACT

As regards the safety testing of biologicals, the workshop participants agreed to actively encourage the deletion of abnormal toxicity tests and target animal batch safety tests from all relevant legal requirements and guidance documents (country-specific guidelines, pharmacopoeia monographs, WHO recommendations).

Mandated by the EPAA expert statements were prepared to substantiate the various *Requests for Revision* concerning 49 different monographs:

- 36 human vaccines
- 2 botulinum monos
- 4 antibiotic monos
- Allergens
- 2 antimycotics
- 2 aprotinin monos
- Protamine sulfate
- Streptokinase

+ General monographs



Paul-Ehrlich-Institut

EXPERT STATEMENT

on animal welfare concerns
related to the

Test for ABNORMAL TOXICITY

as required in the 29 European Pharmacopoeia monographs listed below

under the Section PRODUCTION:

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (2.6.9).

drafted by

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08 December 2017, Strasbourg, France

Suppression of the Test for Abnormal Toxicity from the European Pharmacopoeia

During its 159th plenary session, held in Strasbourg on 21-22 November 2017, the European Pharmacopoeia Commission endorsed the complete suppression of the test for abnormal toxicity from the European Pharmacopoeia (Ph. Eur.).

As part of this exercise, 49 monographs revised to remove the test for abnormal toxicity were adopted by the Commission; notably, these included 36 monographs on vaccines for human use. In addition, as the general chapter Abnormal Toxicity (2.6.9) will no longer be referenced in any monograph, it will subsequently be rendered obsolete and will also be deleted from the Ph. Eur.

Effective on 01 January 2019

Conclusions

The mouse safety test and the gp safety test (known as ATT/GST/Innocuity test) were established around 1900 with a clear rationale at the time.
In the meantime alternative tests exist which make both tests superfluous.

→ Today there is no scientific reason to continue these safety tests.

Conclusions

Within the last 20 years since the deletion of the ATT as a routine batch safety test more than 50,000 batches of human vaccines and more than 40,000 batches of veterinary vaccines have been released in Germany **without any noticeable problems**.

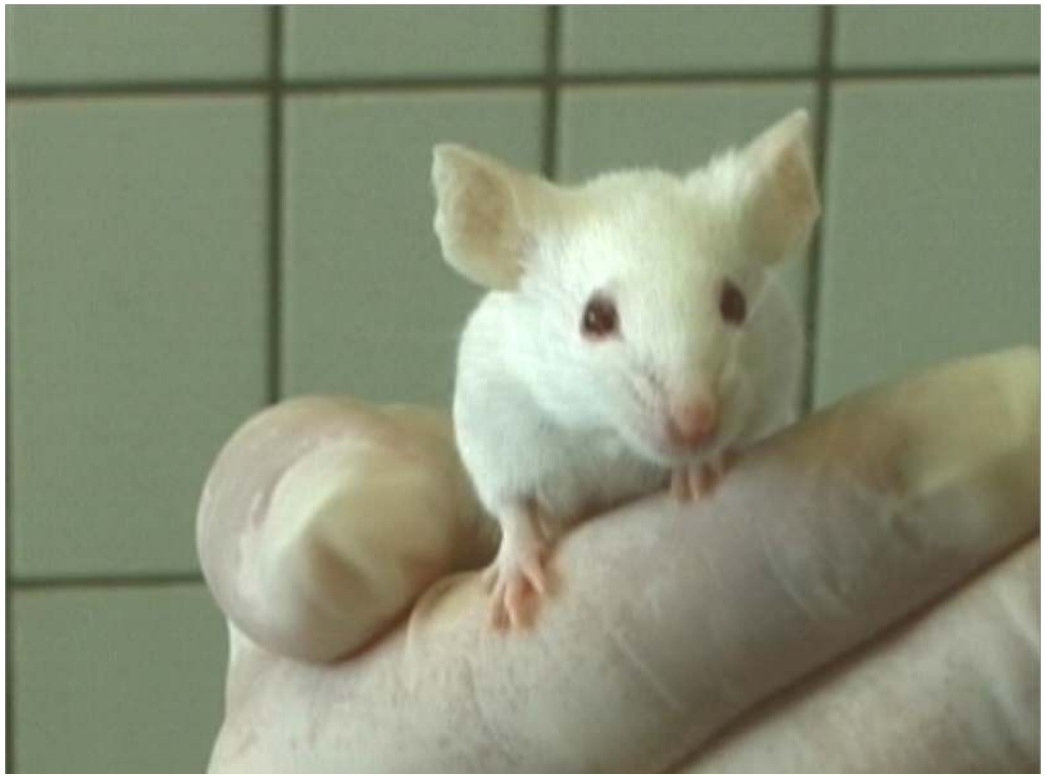
→ It´s high time for a revokation of this testing requirement at global level.

Current Situation

- The FDA removed the requirement for a General Safety Test for biologics in the 21 CFR 610.11.
- The Ph.Eur. Commission deleted the ATT from all Ph.Eur. monographs.
- The WHO Expert Committee on Biological Standardization decided in November 2018 the immediate discontinuation of the inclusion of the innocuity test in all future WHO documents on vaccines and other biologicals.

However, mouse and gp safety tests are still required in many pharmacopoeias and regulations around the world.

Thank you for your attention!



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