The Scientific Relevance of the ATT – Today and from a historical Perspective

Dr. Klaus Cussler
Scientific relevance of the ATT

• ATT – What are we talking about?
• The roots of the ATT
  – The mouse safety test
  – The guinea pig safety test
  – Combination of both safety test
• The ATT- a 3R problem
• The PEI inventory
• The current status of the ATT/GST/Innocuity Test
• Conclusion
ATT – What are we talking about?

In the General Part of the Ph.Eur. under Section 2.6.9 you find the requirement for ABNORMAL TOXICITY. Here we find general safety tests for biological products to be performed in mice and guinea pigs.

Similar, but not identical tests are listed in all legal requirements around the world, e.g.
- General Safety Test in the US-CFR
- Innocuity test in the WHO-Requirements
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.
## Abnormal Toxicity Test – test design in different countries (examples)

<table>
<thead>
<tr>
<th>Scope</th>
<th>European Pharmacopoeia</th>
<th>United States(^a)</th>
<th>WHO</th>
<th>Russian Pharmacopoeia</th>
<th>Chinese Pharmacopoeia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank Control</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Blank control</td>
</tr>
<tr>
<td>Animal Quantity</td>
<td>5 mice</td>
<td>≥ 5 mice</td>
<td>5 mice</td>
<td>5 mice</td>
<td>5 mice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 2 guinea pigs</td>
<td>2 guinea pigs</td>
<td></td>
<td>2 guinea pigs</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>17-24</td>
<td>&lt; 22 (m)</td>
<td>17-22 (m)</td>
<td>19-21</td>
<td>18-22 (m)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 400 (gp)</td>
<td>250-350 (gp)</td>
<td></td>
<td>250-350 (gp)</td>
</tr>
<tr>
<td>Dose/ Administration Volume</td>
<td>1 human dose ≤ 1.0 ml</td>
<td>≤ 0.5 ml (m) ≤ 5.0 ml (gp)</td>
<td>1 human dose ≤ 1.0 ml (m) ≤ 1.0 ml (gp)</td>
<td>0.5 ml</td>
<td>0.5 ml (m) 5.0 ml (gp)</td>
</tr>
<tr>
<td>Injection Route</td>
<td>i.v.</td>
<td>i.p.</td>
<td>i.p.</td>
<td>i.v.</td>
<td>i.p.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or following the approved route of product administration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation Time</td>
<td>24 hours</td>
<td>48 hours</td>
<td>48 hours</td>
<td>48 hours</td>
<td>7 days</td>
</tr>
<tr>
<td>Acceptance Criteria</td>
<td>No animal dies within 24 hours or within such time as specified in the individual monograph.</td>
<td>No animal dies or exhibits any response which is not specific for or expected from the product and which may indicate a difference in its quality. No loss of body weight.</td>
<td>No animal dies within at least 7 days or shows significant signs of toxicity</td>
<td>No animal dies within the specified follow-up period.</td>
<td>All animals remain healthy and survive the observation period, without any abnormal reaction, and with increase of body weight by the end of observation period</td>
</tr>
<tr>
<td>Re-Test(s) Number/ Description</td>
<td>1 If one animal dies, repeat the test.</td>
<td>2 If the initial test/ first repeat test fails, a repeat test may be conducted.</td>
<td>no</td>
<td>1 If an animal dies, repeat experiment with 5 mice (20 ± 0.5 g).</td>
<td>1 If the test fails, it may be repeated once with 10 mice/4 guinea pigs.</td>
</tr>
</tbody>
</table>


\(^b\) Exemptions: therapeutic DNA plasmid products, therapeutic synthetic peptide products of 40 or fewer amino acids, monoclonal antibody products for in vivo use, or therapeutic recombinant DNA-derived products.

i.p., intraperitoneal
i.v., intravenous
m, mice
gp, guinea pigs
The roots of the ATT

Session 1: 3Rs alternatives and safety testing

ABNORMAL TOXICITY TESTING – ROOTS AND EVOLUTION OF FIRST ANIMAL SAFETY TESTS FOR BIOLOGICALS

Klaus Cussler
The roots of the ATT

The German government introduced specific regulations for diphtheria sera 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,
- is free from toxins, in particular tetanus toxin.

(Otto, 1906)
The German government introduced specific regulations for diphtheria sera in 1894.

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(Otto, 1906)
Preservation of Antisera

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

• The content had to be restricted due to the toxicity
• but needed to be effective (against glanders)
→ A limit of 0.5% phenol (0.4% tricresol) was requested.

How should this requirement be controlled?
Paul Ehrlich inspecting laboratory mice, around 1910
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)
The Mouse Test

This safety test for diphtheria serum was maintained for tetanus serum and lateron for the first bacterial vaccines (typhoid and cholera) which were also preserved with phenols.

► The mouse test became a standard test (to check the preservative content)
The Mouse Test

- In 1943 a “colour test for phenolic compounds” was established which was then adapted to measure phenol derivatives in medicines.

→ From today's point of view this would have been the time to replace the mouse safety test.
Emil von Behring performs animal tests in his laboratory; Berlin, 1889
The Guinea-Pig Test

- A batch of diphtheria antiserum had been contaminated with tetanus toxin in St. Louis, US (1901)
  - twenty children became ill with tetanus
  - fourteen children died
- A similar incidents (contamination with tetanus spores) occurred in Italy at the same time (Dec. 1900). More than 18 cases of tetanus with 13 fatalities were reported after treatment of children with diphtheria serum from the Serotherapeutic Institute of Milan.

→ A guinea-pig test was introduced as biological indicator test for extraneous clostridial toxins (tetanus) in Germany (in 1901) and thereafter in many other countries.
The Guinea-pig Test

- This test was introduced specifically for the control of diphtheria serum at the time but was consecutively applied to other sera and later on also for vaccines.
- This testing procedure was used in Germany without modifications until 1935.
Abnormal Toxicity Test (ATT)

- In the 1940\textsuperscript{th} when governmental regulations in Germany were developed for several vaccines the revised guidelines mentioned a test for the first time which consisted of the guinea pig test and the mouse test.

→ The basic outline for the ATT was created

The combination of two formerly independent specific safety tests became a general safety test.
The ATT as such was mentioned for the first time when the WHO started to develop internationally accepted guidance after WW II.

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.
Abnormal Toxicity Test (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]
The ATT- a 3R problem

When alternatives to animal testing became a topic in the area of biologicals, the ATT was one of the first tests in the focus. However, as the purpose of the test(s) was not clearly defined, it became clear that the 3Rs could not be applied.

The only way forward would have been a deletion of the test(s). But there was no consensus for such a big step. Many regulators and QC people stressed the fact that the test has been „successfully“ used over decades. The tests were considered to be not severe, and the number of animals used was relatively low.
To provide facts and figures the PEI decided to perform a survey about the ATT about the usefulness of the animal tests.

- 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 questionnaire) 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
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)

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

→ ATT is still listed as a requirement in the *Ph.Eur.*
The current status of the ATT/GST/Innocuity Test

Although the ATT has been deleted as a general batch safety test from the *Ph.Eur.*, the test is still performed

- as a requirement for product development in Europe.
- as a batch test due to legal requirements in non-European countries.
Abnormal Toxicity for Vaccines for Human Use

PRODUCTION
GENERAL PROVISIONS

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.
The current status of the ATT/GST/Innocuity Test

Global situation:
Heterogenous picture, ranging from a routine testing requirement to a complete revocation of the test.
Many countries/regulatory requirements seem to allow exemptions from routine testing.
Conclusions

The mouse safety test and the guinea pig 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

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 revocation of this testing requirement at a global level.
# Batch release for human vaccines in Germany

<table>
<thead>
<tr>
<th>Batch Release</th>
<th>Human Vaccines</th>
<th>Guinea pigs</th>
<th>Mice</th>
</tr>
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<tbody>
<tr>
<td>PEI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>3,809</td>
<td>7,618</td>
<td>19,045</td>
</tr>
<tr>
<td>2013</td>
<td>4,235</td>
<td>8,470</td>
<td>21,175</td>
</tr>
<tr>
<td>2012</td>
<td>3,043</td>
<td>6,086</td>
<td>15,215</td>
</tr>
<tr>
<td>2011</td>
<td>2,701</td>
<td>5,402</td>
<td>13,505</td>
</tr>
<tr>
<td>2010</td>
<td>1,735</td>
<td>3,470</td>
<td>8,675</td>
</tr>
<tr>
<td>annual average</td>
<td>3,105</td>
<td>6,210</td>
<td>15,523</td>
</tr>
<tr>
<td>Total</td>
<td><strong>15,523</strong></td>
<td><strong>31,046</strong></td>
<td><strong>77,615</strong></td>
</tr>
</tbody>
</table>
Thank you for your attention!

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