The fundamental principles of 3R on the example of the abnormal toxicity test (ATT) - Drivers for recent changes implemented by WHO and EDQM

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May 2019, Moscow

European Federation of Pharmaceutical Industries and Associations
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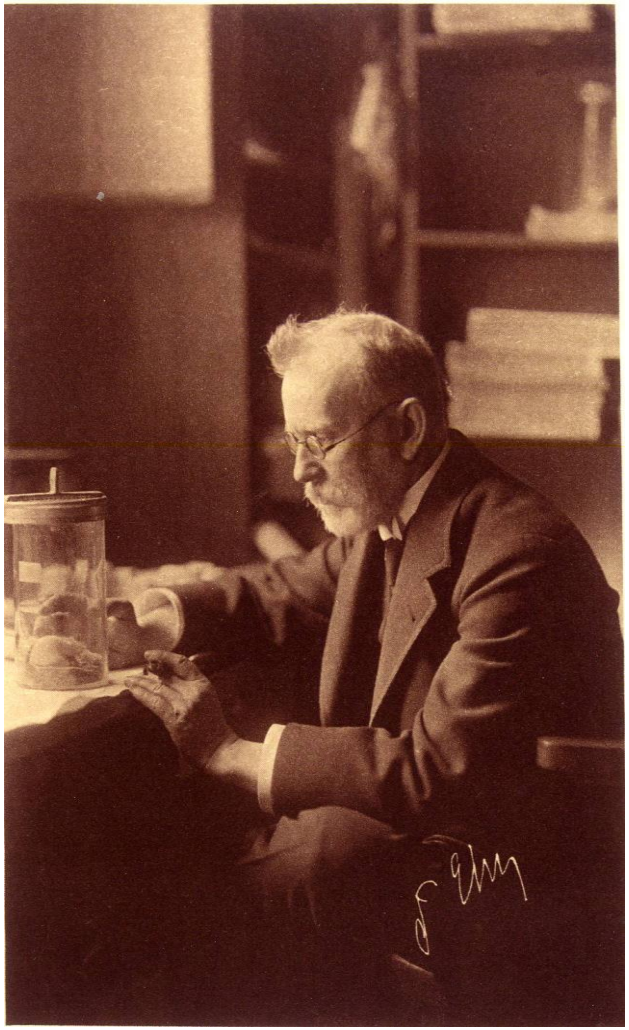
Early Days Around The Time of Birth of the ATT

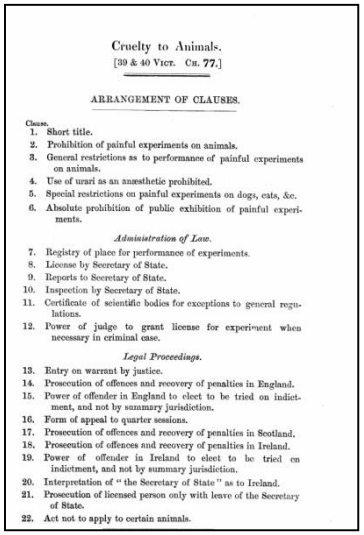
Emil von Behring performing animal tests; Berlin, 1889

Around 1910: Paul Ehrlich examining mice



From the History of 3Rs

1876, UK



Cruelty to Animals.
[39 & 40 Vict. Ch. 77.]

ARRANGEMENT OF CLAUSES.

Clause.
1. Short title.
2. Prohibition of painful experiments on animals.
3. General restrictions as to performance of painful experiments on animals.
4. Use of urari as an anæsthetic prohibited.
5. Special restrictions on painful experiments on dogs, cats, &c.
6. Absolute prohibition of public exhibition of painful experiments.

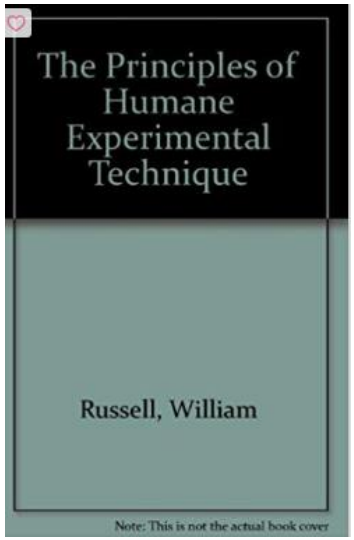
Administration of Law.

7. Registry of place for performance of experiments.
8. License by Secretary of State.
9. Reports to Secretary of State.
10. Inspection by Secretary of State.
11. Certificate of scientific bodies for exceptions to general regulations.
12. Power of judge to grant license for experiment when necessary in criminal case.

Legal Proceedings.

13. Entry on warrant by justice.
14. Prosecution of offences and recovery of penalties in England.
15. Power of offender in England to elect to be tried on indictment, and not by summary jurisdiction.
16. Form of appeal to quarter sessions.
17. Prosecution of offences and recovery of penalties in Scotland.
18. Prosecution of offences and recovery of penalties in Ireland.
19. Power of offender in Ireland to elect to be tried on indictment, and not by summary jurisdiction.
20. Interpretation of "the Secretary of State" as to Ireland.
21. Prosecution of licensed person only with leave of the Secretary of State.
22. Act not to apply to certain animals.

1959



➔introduced and defined the terms

- ***Replacement***
- ***Reduction***
- ***Refinement***

which subsequently have become known as ‘alternatives’ or ‘alternative methods’ for minimizing the potential for animal pain and distress in biomedical research.

1986 Council of Europe

DIRECTORATE OF LEGAL ADVICE
AND PUBLIC INTERNATIONAL LAW
TREATY OFFICE

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

November 2018

EUROPEAN CONVENTION FOR THE PROTECTION OF VERTEBRATE ANIMALS USED FOR EXPERIMENTAL AND OTHER SCIENTIFIC PURPOSES of 18 March 1986

(ETS No. 123, entered into force on 1 January 1991)

Subject: **Accession by States which are not member States of the Council of Europe**

I. Participation in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes is not exclusively limited to member States of the Council of Europe and to the European Union.

The Convention is also open for accession by other non-member States, provided that they have been formally invited to accede by the Committee of Ministers of the Council of Europe. The relevant provision of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes - Article 34, paragraph 1 - reads as follows:

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3Rs – the EMA perspective*

3Rs principles

The 3Rs stand for:

- **replacing** the use of animals with non-animal methods where possible;
- **reducing** the number of animals used to a minimum while still obtaining scientifically valid results;
- **refining** practices to minimise the stress and improve the welfare of study animals used for regulatory purposes.

EU adopted a directive on the protection of animals used for scientific purposes (2010/63/EU38 – replaces first directive 86/609/EEC)

"….These principles encourage alternatives to the use of animals in the testing of medicines while safeguarding scientific quality and improving animal welfare where the use of animals cannot be avoided"

➔ **The European Medicines Agency (EMA) supports the implementation of the so-called 3Rs principles for the ethical use of animals in medicine testing across the European Union (EU).**

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HTTPS://WWW.EMA.EUROPA.EU/EN/HUMAN-REGULATORY/RESEARCH-DEVELOPMENT/ETHICAL-USE-ANIMALS-MEDICINE-TESTING#RECOMMENDATION

3Rs – the EDQM* perspective

3Rs principles

The 3Rs stand for:

- **replacing** the use of animals with non-animal methods where possible;
- **reducing** the number of animals used to a minimum while still obtaining scientifically valid results;
- **refining** practices to minimise the stress and improve the welfare of study animals used for regulatory purposes.

In addition to the traditional 3Rs, the Ph. Eur. Commission has employed 'Removal', a fourth 'R', as a strategy to end the unnecessary use of animals. This involves the removal of the need for regular performance of an animal test that, after scientific scrutiny, has proved to be no longer relevant and can be deleted without replacement with another test.**

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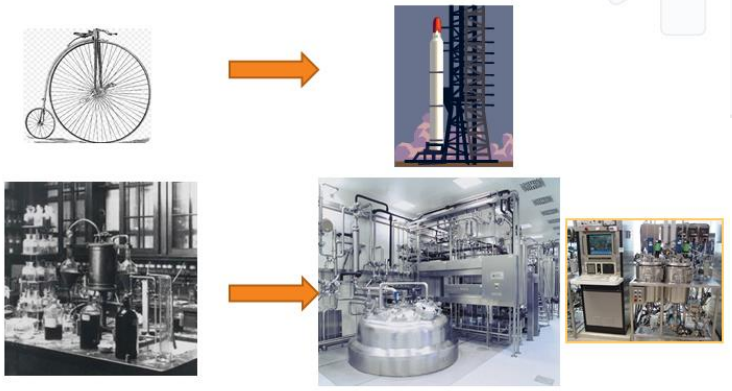
HTTPS://WWW.EMA.EUROPA.EU/EN/HUMAN-REGULATORY/RESEARCH-DEVELOPMENT/ETHICAL-USE-ANIMALS-MEDICINE-TESTING#RECOMMENDATION

** FROM HTTPS://WWW.EDQM.EU/SITES/DEFAULT/FILES/ARTICLE-PHEUR-REPLACEMENT-REDUCTION-REFINEMENT-MAY2018.PDF

Some considerations regarding ATT#

- Developed as analytical “assay” in the early 1900’s - nowadays adequate and reliable analytical tools available
- ATT does not comply with international assay validation requirements (e.g., specificity, reproducibility)
- False positive test results may lead to delayed supply of medicines to patients

Time has moved on...



Fermenter

Common Technical Document (CTD)
Good Laboratory Practice (GLP)
Good Clinical Practice (GCP)
Good Manufacturing Practice (GMP)
Good Distribution Practice (GDP)

- Modern pharmaceutical manufacturers
  - extensive product characterization during development
  - thorough control of manufacturing process & appropriate quality control (QC) in place.
- General lack of value & scientific rationale for ATT as QC test

Garbe *et al.* (**2014**) Historical Data Analyses and Scientific Knowledge Suggest Complete Removal of the Abnormal Toxicity Test as a Quality Control Test. *J. Pharm. Sci.* 103.

A Russian translation of this review is published in “Drug Development & Registration”, http://pharmjournal-world.com; “Разработка и регистрация лекарственных средств”, http://pharmjournal.ru

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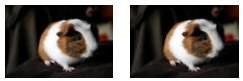
Substantial number of animals used for ATT

* If test performed , the number of animals affected:

per experiment ➔ several thousand per year (species: Mouse)



per experiment ➔several thousand (species: Guinea pig)

Table 2. Retrospective Analysis of Abnormal Toxicity Tests of Vaccine Products, Test Results, and Number of Animals[12]

Number of Tests	Analyzed Preparations	Used Mice	Used Guinea Pigs	Batch Rejections
5896	416	30193	12420	0

➔ Substantial number of animals used for a test with unproven and questionable suitability to detect contaminants and increase product safety*

* KRAEMER B, NAGEL M, DUCHOW K, SCHWANIG M, CUSSLER K. 1996. IS THE ABNORMAL TOXICITY TEST STILL RELEVANT FOR THE SAFETY OF VACCINES, SERA AND IMMUNOGLOBULINS? ALTEX 13(1):7–16.[7]

Recent developments - Regulatory Authorities/ pharmacopoeias have removed or are removing the ATT

Health Authority	Position
The European Partnership for Alternative Approaches to Animal Testing Summary	• International Workshop by EPAA on **September 2015** • Around 45 regulators and industry representatives from 15 countries, e.g. authority representatives included WHO, EMA, US FDA, Health Canada, Netherlands, India, Brazil, Germany, Mexico, Japan • Agreed to actively **encourage deletion of ATT** from all relevant legal requirements and guidance documents, i.e. pharmacopoeia, monographs, WHO recommendations, and World Organization for Animal Health guidelines
U.S. FOOD & DRUG ADMINISTRATION	• **July 2015,** FDA amended the biologics regulations by **removing the general safety test** (GST) requirements for biological products.
edqm	• **July 2018**- the European Pharmacopoeia Commission has **removed the requirements** for a test for abnormal toxicity from 49 monographs as well as the General Chapter 2.6.8. of the European Pharmacopoeia (Ph. Eur.).
	• **October 2018** - The Expert Committee for Biological Standards (ECBS) recommends **immediate discontinuation of the inclusion of the innocuity test in all future WHO documents on vaccines and other biologicals** published in the Technical Report Series (including WHO Recommendations, Guidelines and manuals).
国家药品监督管理局 National Medical Products Administration	• **November 2018** – Chinese Pharmacopoeia evaluated import testing results and local release results of mAb and recombinant protein in the last 5 years. At a NIFDC meeting, ChP announced revision of ATT in general chapter of rDNA biotechnology and mAb, ATT will be not mandatory requirement for both category products, public commenting expected soon

Deletion of ATT from Pharm. Eur. – a stepwise approach

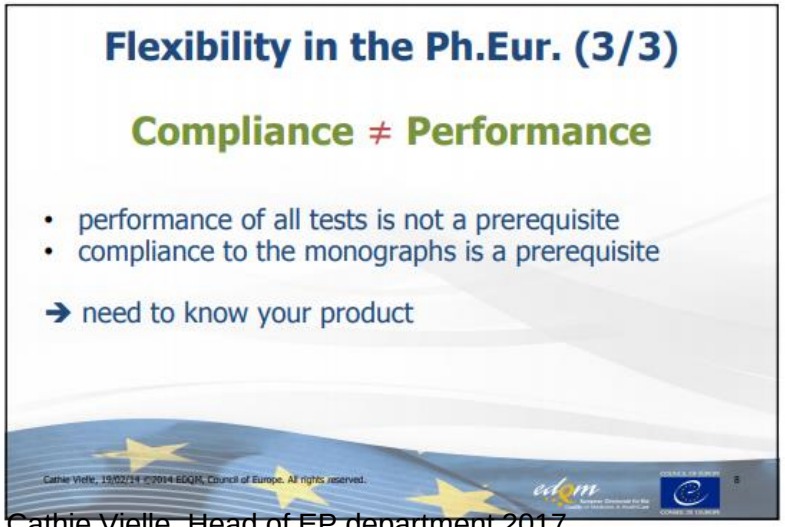
*

Review of historical batch data in 1999

➔removal of ATT from test sections of over 80 monographs

➔Statement in the production section that manufacturing method should be validated in such a way that the test would comply if tested

➔Deletion of test for routine batch release



Extracted from Cathie Vielle, Head of EP department 2017
General concepts in the Pharm. Eur.: theory and rationale

Deletion of ATT from Pharm. Eur. in 2018

edqm — European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE — CONSEIL DE L'EUROPE

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.

The scientific validity and rationale of the test for abnormal toxicity, which is carried out on animals, has been the subject of debate for some time in Europe. It was originally developed to detect external contaminants in biological products, but over time the introduction of Good Manufacturing Practices and the use of appropriate and stringent quality control measures have rendered its use less necessary. Current scientific evidence suggests that, in light of such debatable relevance, the omission of the test for abnormal toxicity would not compromise the safety of biological medicines.

The Ph. Eur. Commission had already removed this test from routine testing in 1998. The P Eur. Commission then decided to embark on the complete removal of the test for abnorm toxicity from its monographs, which cover areas including vaccines and immunosera for hum use, biotherapeutics, allergens, antibiotics, antimycotics and plastic containers. A detai evaluation was subsequently conducted for each monograph concerned before the decision suppress the test was taken.

The Ph. Eur. Commission remains fully committed to the reduction of animal use wherever possible in pharmacopoeial testing, in accordance with the *European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes*. The decision to suppress the test for abnormal toxicity at the 159th session of the Ph. Eur. Commission is a strong illustration of this commitment.

The suppression of the test for abnormal toxicity will be reflected in Supplement 9.6 European Pharmacopoeia and become effective on 01 January 2019.

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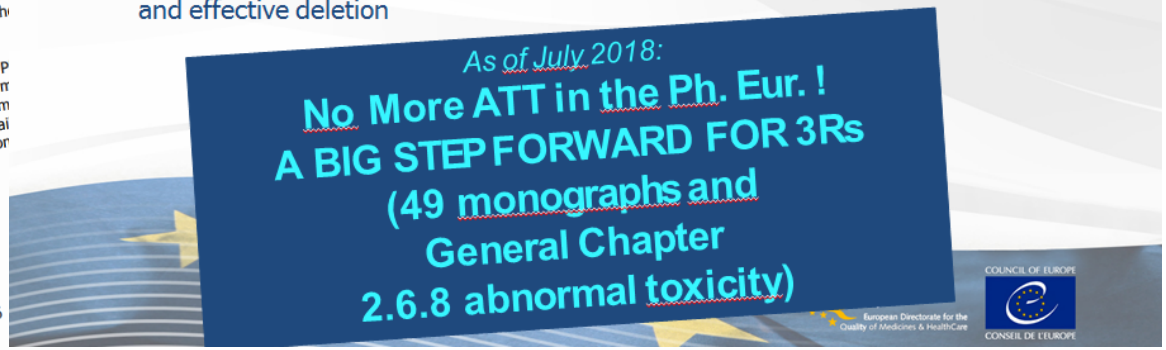
Converging Regulatory Agreement *

- Discussed the ATT issue in depth (including Workshop 2016) attended by participants from several EU Member States, Brazil, Canada, China, India, Japan, Mexico and the USA (European Partnership for Alternative Approaches to Animal Testing (EPAA))
- Conclusion: the ATT lacks scientific relevance and its omission does not compromise the safety of biologics. *Consensus to strive for deletion of the ATT from the regulatory requirements*
- Deletion of ATT should be addressed at a global level
  - A harmonised approach by all regulators across the globe is important for a real and effective deletion



* SLIDES PRESENTED BY E. CHARTON(EDQM) AT EFPIA/ AIPM EEU WORKSHOP IN BRUSSELS, APRIL 2018

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Rationale by WHO ECBS

"…**Current manufacturing processes**, which **include the implementation of Good Manufacturing Practices (GMP) and comprehensive quality control measures** (including in-process controls), were considered to be **more appropriate than the innocuity test** in assuring the quality and safety of vaccines and other biological products.

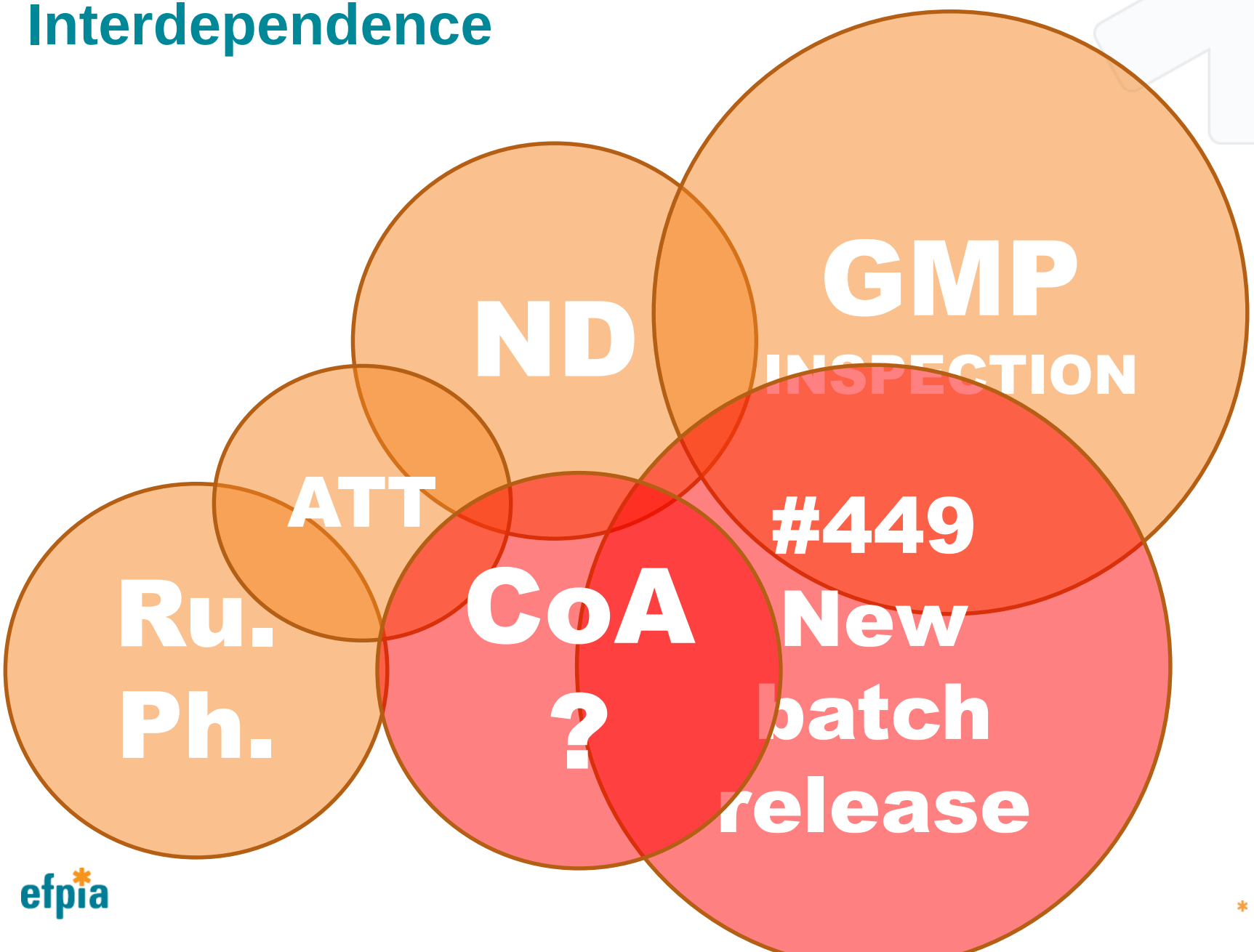
The Committee reviewed the historical inclusion of the innocuity test in the documents published in the WHO Technical Report Series and concluded that its **complete omission would not compromise the quality and safety of vaccines and other biological products**.

Therefore, the Committee **recommends the discontinuation** of the inclusion of the innocuity test **in all future WHO Recommendations, Guidelines and manuals for biological products** published in the Technical Report Series, and that a clear indication be made in its report that the inclusion of this test in previously published WHO Technical Report Series documents be disregarded."

ECBS_Executive_Summary_final_20_NOV_2018.IK.pdf

RUSSIAN AND EURASIAN PHARMACOPOEIAL REQUIREMENTS BEYOND INTERNATIONAL STANDARDS - EXAMPLE OF ABNORMAL TOXICITY TESTING

Interdependence



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Divergence in national regulations may create uncertainty about compliance and delay patient supply with drugs esp. with new batch release procedure (FL #449)

- **ILLUSTRATIVE CASE STUDY**
- The normative document for a product includes four tests, including the abnormal toxicity test, that are not currently required in Module 3 by international standards (e.g. Ph. Eur.)
- The Russian MOH requests these tests be performed according to the Russian Pharmacopeia.
  - Historically these requirements were established for the tests/control only on the territory of RF.
- EU manufacturing site confirms the product quality on mentioned parameters by GMP validated manufacturing process, in-process control, some alternative methods etc.
- But often it is considered as an observation during GMP inspection and products cannot be released to a country.
- Current approach - Third party contractor lab engaged to perform toxicity testing in Russia
- **==> country specific tests will not be included on Certificate of Analysis (CoA) provided by the manufacturer!!**

EFPIA position

- With todays GxPs, elimination of the ATT from all Pharmacopoeias and regulatory requirements worldwide is safe for patients
  - No alternative assays or replacement for ATT are needed as adequate manufacturing and quality control measures are already in place.
- With the implementation of the new batch release procedure (RU/FL #449) in November 2019 the ATT should not be required as part of the Certificate of Analysis provided by the manufacturer
- EFPIA members encourage alignment of Russian and EAEU pharmacopoeial standards with international standards and practices

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Большое Спасибо!

Thank You Very Much For Your Attention!

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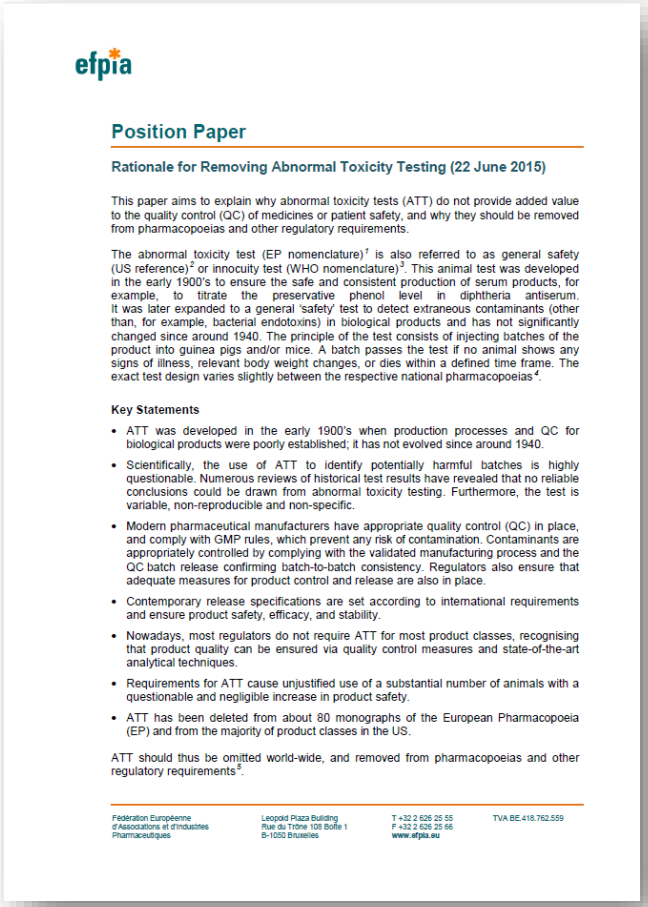
Abnormal Toxicity Test
Additional Readings

EFPIA Position Paper
"Rationale for Removing Abnormal Toxicity Testing"



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Position Paper

Rationale for Removing Abnormal Toxicity Testing (22 June 2015)

This paper aims to explain why abnormal toxicity tests (ATT) do not provide added value to the quality control (QC) of medicines or patient safety, and why they should be removed from pharmacopoeias and other regulatory requirements.

The abnormal toxicity test (EP nomenclature)[1] is also referred to as general safety (US reference)[2] or innocuity test (WHO nomenclature)[3]. This animal test was developed in the early 1900's to ensure the safe and consistent production of serum products, for example, to titrate the preservative phenol level in diphtheria antiserum. It was later expanded to a general 'safety' test to detect extraneous contaminants (other than, for example, bacterial endotoxins) in biological products and has not significantly changed since around 1940. The principle of the test consists of injecting batches of the product into guinea pigs and/or mice. A batch passes the test if no animal shows any signs of illness, relevant body weight changes, or dies within a defined time frame. The exact test design varies slightly between the respective national pharmacopoeias[4].

Key Statements

- ATT was developed in the early 1900's when production processes and QC for biological products were poorly established; it has not evolved since around 1940.
- Scientifically, the use of ATT to identify potentially harmful batches is highly questionable. Numerous reviews of historical test results have revealed that no reliable conclusions could be drawn from abnormal toxicity testing. Furthermore, the test is variable, non-reproducible and non-specific.
- Modern pharmaceutical manufacturers have appropriate quality control (QC) in place, and comply with GMP rules, which prevent any risk of contamination. Contaminants are appropriately controlled by complying with the validated manufacturing process and the QC batch release confirming batch-to-batch consistency. Regulators also ensure that adequate measures for product control and release are also in place.
- Contemporary release specifications are set according to international requirements and ensure product safety, efficacy, and stability.
- Nowadays, most regulators do not require ATT for most product classes, recognising that product quality can be ensured via quality control measures and state-of-the-art analytical techniques.
- Requirements for ATT cause unjustified use of a substantial number of animals with a questionable and negligible increase in product safety.
- ATT has been deleted from about 80 monographs of the European Pharmacopoeia (EP) and from the majority of product classes in the US.

ATT should thus be omitted world-wide, and removed from pharmacopoeias and other regulatory requirements[5].

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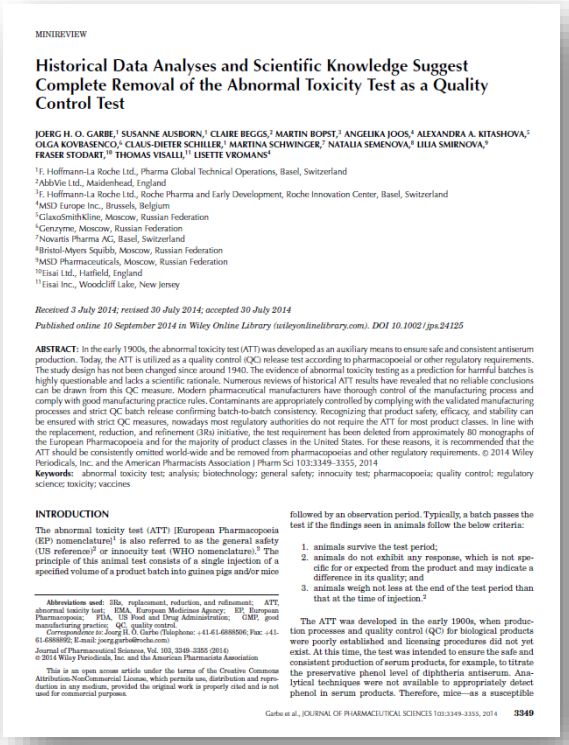
Abnormal Toxicity Test
Additional Readings

Scientific Review

Garbe, J.H.O., S. Ausborn, C. Beggs, M. Bopst, A. Joos, A.A. Kitashova, O. Kovbasenco, C.D. Schiller, M. Schwinger, N. Semenova, L. Smirnova, F. Stodart,
T. Visalli, L. Vromans (**2014**) Historical Data Analyses and Scientific Knowledge Suggest Complete Removal of the Abnormal Toxicity Test as a Quality Control Test. *J. Pharm. Sci.* 103.



MINIREVIEW

Historical Data Analyses and Scientific Knowledge Suggest Complete Removal of the Abnormal Toxicity Test as a Quality Control Test

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ABSTRACT: In the early 1900s, the abnormal toxicity test (ATT) was developed as an auxiliary means to ensure safe and consistent antiserum production. Today, the ATT is utilized as a quality control (QC) release test according to pharmacopoeial or other regulatory requirements. The study design has not been changed since around 1940. The evidence of abnormal toxicity testing as a prediction for harmful batches is highly questionable and lacks a scientific rationale. Numerous reviews of historical ATT results have revealed that no reliable conclusions can be drawn from this QC measure. Modern pharmaceutical manufacturers have thorough control of the manufacturing process and comply with good manufacturing practice rules. Contaminants are appropriately controlled by complying with the validated manufacturing processes and strict QC batch release confirming batch-to-batch consistency. Recognizing that product safety, efficacy, and stability can be ensured with strict QC measures, nowadays most regulatory authorities do not require the ATT for most product classes. In line with the replacement, reduction, and refinement (3Rs) initiative, the test requirement has been deleted from approximately 80 monographs of the European Pharmacopoeia and for the majority of product classes in the United States. For these reasons, it is recommended that the ATT should be consistently omitted world-wide and be removed from pharmacopoeias and other regulatory requirements. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3349–3355, 2014

Keywords: abnormal toxicity test; analysis; biotechnology; general safety; innocuity test; pharmacopoeia; quality control; regulatory science; toxicity; vaccines

INTRODUCTION

The abnormal toxicity test (ATT) [European Pharmacopoeia (EP) nomenclature][1] is also referred to as the general safety (US reference)[2] or innocuity test (WHO nomenclature).[3] The principle of this animal test consists of a single injection of a specified volume of a product batch into guinea pigs and/or mice followed by an observation period. Typically, a batch passes the test if the findings seen in animals follow the below criteria:

1. animals survive the test period;
2. animals do not exhibit any response, which is not specific for or expected from the product and may indicate a difference in its quality; and
3. animals weigh not less at the end of the test period than that at the time of injection.[2]

The ATT was developed in the early 1900s, when production processes and quality control (QC) for biological products were poorly established and licensing procedures did not yet exist. At this time, the test was intended to ensure the safe and consistent production of serum products, for example, to titrate the preservative phenol level of diphtheria antiserum. Analytical techniques were not available to appropriately detect phenol in serum products. Therefore, mice—as a susceptible

Abbreviations used: 3Rs, replacement, reduction, and refinement; ATT, abnormal toxicity test; EMA, European Medicines Agency; EP, European Pharmacopoeia; FDA, US Food and Drug Administration; GMP, good manufacturing practice; QC, quality control.

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Journal of Pharmaceutical Sciences, Vol. 103, 3349–3355 (2014)

A Russian translation of this review is published in
“Drug Development & Registration”, http://pharmjournal-world.com/
“Разработка и регистрация лекарственных средств”, http://pharmjournal.ru.

Drug development and control of contaminants in a sterile product today

- **Extended product characterization** during process development and process validation
  - Investigation of **degradation profiles**, product **compatibility with various materials/surfaces**
- **Advanced process understanding**, in-process controls, **validation** of the manufacturing process and **release testing** complying with international standards
- Determination of **safety/toxicity profile in *in vitro* assays and animals models** as well as in **clinical trials** before marketing authorization in accordance with international and national guidelines

How do we control contaminants today?

Examples

Type of contaminant	Measure to verify the absence of contaminants in a product batch (Examples)
Microbiological	• Bioburden test (in process control) • Sterility
Pyrogen	• Validation of depyrogenization (as part of the process validation)
Endotoxin	• Bacterial endotoxins (Limulus Amebocyte Lysate, LAL) test
Residual contaminants	• Extended product characterization • Manufacturing process validation • Batchwise QC testing to confirm batch to batch consistency

Today, reliable and validated tests for different potential contaminants are available