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].

Fédération Européenne d'Associations et d'Industries Pharmaceutiques
Leopold Plaza Building Rue du Trône 108 Boîte 1 B-1050 Bruxelles
T +32 2 626 25 55 F +32 2 626 25 66 www.efpia.eu
TVA BE.418.762.559

Historical Test Application

Licensing procedures were not in place in the early 1900's, and analytical techniques not available to appropriately detect phenol levels in serum products. Therefore, mice - as a susceptible species - were used for the detection of potentially toxic phenol levels. The test with guinea pig was introduced around 1900 as a biological indicator for the presence of tetanus toxin in antiserum preparations[6,7].

Accordingly, the test dates from an era when production processes and QC for biological products were poorly established. In spite of significant evolution of analytical techniques and instrumentation as well as advanced process understanding and validation approaches, this biological test remains, and evolved from an analytical test to become an additional safety test intended to detect product/process contaminants to avoid batch-to-batch differences in quality.

Scientific Expert Agreement: Unreliability of Abnormal Toxicity Testing

There is no evidence that ATT is useful to predict or control harmful batches. Publications by the Paul Ehrlich Institute provide evidence that the test does not serve its purpose, and does not add any further information already obtained from the QC release testing under GMP[8]. A retrospective analysis of several thousand test results conducted for vaccines revealed that there were no true positive results[4,9,10].

Scientifically, there is no rationale as to why an animal test for batch release would be more appropriate than analytical procedures to detect contamination. Other methodologies, such as those outlined in the table overleaf, are far better suited and have a clear scientific rationale, since the relationship between the measured endpoint and the causing contamination (e.g. bacterial endotoxin) is well understood and established.

The outcome of the ATT is also unreliable, as it does not fulfill the international validation criteria. It is non-specific, as many factors other than contaminants can influence the result (e.g. the body weight as well as species and strain of the animals). Identical batches tested in different laboratories have produced significantly different test results. Positive results never showed a correlation to the product quality and contamination respectively[10]. Additionally, misinterpretation of responses caused by the active ingredients itself, or its formulation components, may lead to false positive results, for example, since administered concentrations are unrealistically high compared to the human situation[11]. Depending on the test design, the administered dose is purely based on volume. Thus, the full human dose may be administered to guinea pigs of 250 g to 400 g body weight[1]. In this case, assuming a human body weight of 60 kg, a guinea pig would receive 150-fold the human dose. A mouse of 20 g would receive 3000-fold the human dose.

EFPIA member companies' examples of false positive test results:

- A response caused by benzyl alcohol, which is used as formulation component for a recombinant protein.
- A response caused by high sugar content in an oral pediatric vaccine, when administered according to a national pharmacopoeia by intravenous injection.

Taking these aspects into account, it has been recommended by various scientific experts that ATT be removed from all pharmacopoeias world-wide[10,11,12,13,14].

Control of Contaminants

A set of measures are nowadays available to detect and control different types of contaminants. These include:

- Extended product characterization during process development and process validation,
- Manufacture according to Good Manufacturing Practices (GMP),
- Routine QC release testing, that verifies batch-to-batch consistency and that a specific batch has been manufactured according to the previously validated process.

The following table lists several contaminants, which may be considered for e.g. parenteral preparations:

Type of contaminant	**Measure to verify the absence of contaminants in a product batch (Examples)**
microbiological	- bioburden test (in-process control) - sterility test
pyrogen[a]	validation of depyrogenization (as part of the process validation)
endotoxin	bacterial endotoxins (Limulus Amebocyte Lysate, LAL) test
residual contaminants[b]	- extended product characterization - process validation - manufacture under GMP - QC during batch release to confirm batch-to-batch consistency

[a] the formerly used rabbit pyrogens test has been replaced by the bacterial endotoxins test in numerous EP monographs[9]

[b] the formerly used abnormal toxicity test has been deleted based on historical review in numerous EP monographs[9,15]

Modern Product Development Ensures Comprehensive Process Understanding and Well-Characterized Products

State-of-the-art manufacturing is highly regulated and controlled. The pharmaceutical industry has established appropriate control for manufacturing through substantially advanced process understanding, in-process controls, validation of the manufacturing process and release testing complying with international GMP standards.

During formulation and process development, many studies are conducted with different formulation components (incl. preservatives) to investigate degradation profiles, product compatibility with various materials/surfaces and leachables[16,17,18,19]. Pharmaceutical compounds are tested extensively with regards to their safety/toxicity profile in *in vitro* assays and animals models as well as in clinical trials in accordance with international (e.g. ICH guidelines) and national guidelines. Only when a positive benefit/risk assessment could be demonstrated, the marketing authorisation is granted by the relevant health authorities.

Today, pharmaceutical manufacturers produce highly-developed medicines with well-defined purity and safety characteristics. Risk of contamination is extremely low, if a manufacturer complies with GMP rules (e.g., globally recognized regulations[20,21,22,23]) and if consistency in production is guaranteed[24,25]. Abnormal product contamination is extremely unlikely if the validated manufacturing process is followed.

Appropriate analytical methods (e.g. mass spectrometry applications) are capable of detecting contamination and ensure batch-to-batch consistency. Advanced product testing is applied for extended product characterization as well as for release testing.

Release Specifications are Set According to International Requirements and Ensure Product Safety, Efficacy & Stability

Multinational manufacturers supply innovative medicines globally. Thus, a batch is usually released for use in the global market. Accordingly, all countries get the same high quality drug. In line with international regulations, ATT is not part of the release specifications for these globally marketed products. For example, EMA and US FDA approved specifications for commercial drug products do not require ATT as part of the QC release analysis for the majority of product classes. However, a batch already released for EU and/or US would have to be tested for abnormal toxicity in other countries, for example, China[26] and the Russian Federation[27], to be released for the local market. To the best of our knowledge, no batches meeting EMA or US FDA approved specifications, delivered a positive ATT result in both of these countries (apart from false positive test results, as aforementioned)[4,9,10].

Health Authorities' and International Perspective

European Pharmacopoeia (EP) generally does not require ATT in the monographs for "Parenteral Preparations"[28], "Monoclonal Antibodies for Human Use"[29] or "Products of Recombinant DNA Technology"[30]. Numerous reviews of test results revealed that no additional value could be concluded from abnormal toxicity testing. As a consequence and in accordance with the *European Convention on the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes*, the test has been deleted from about 80 monographs for biotechnological products, blood products, antibiotics and vaccines based on the review of historical data[9,15]. In remaining 50 monographs, the test was kept as non-mandatory, acknowledging that "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)". With this, the ATT is in fact no longer required by the EP and could be eliminated consequentely. Such elimination is correspondingly strongly recommended by several publications[4,12,31,32,33,34].

The US Code of Federal Regulations Title 21 requires general safety testing be done for biological products (21 CFR, Part 610[2].11). However, FDA realized that *"after more than a decade of experience with these products, we found that we could evaluate many aspects of a biological product's safety, purity, or potency with tests other than those prescribed in part 610"*. Thus, the FDA amended the biologics regulations regarding general biological products standards by adding an administrative procedure for obtaining exemptions from the general safety test requirements[35]: 21 CFR, Part 601.2[36] specifies that the test is exempted as a requirement for license applications for biological products (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). In August 2014 the FDA proposed to amend the biologics regulations by removing the general safety test (GST) requirements for biological products. FDA is recommending this action because the existing codified GST regulations are duplicative of requirements that are also specified in biologics licenses, or are no longer necessary or appropriate to help ensure the safety, purity, and potency of licensed biological products[37].

In 2002, the WHO Expert Committee on Biological Harmonisation *"noted that, in one region of the world, the abnormal toxicity test had been deleted for most products. This was linked to the implementation of, and compliance with, good manufacturing practices and, where this occurred, there was abundant evidence that the abnormal toxicity test did not provide additional assurances of the quality of the product"* [38].

Patient Supply

The test for abnormal toxicity is non-specific and may interfere with well-known formulation components. The test design is not harmonized and varies between national pharmacopoeias. Accordingly, the test response of the same batch is unpredictable and may give contradictory results.

Due to the unreliability of the abnormal toxicity testing, false positive results may occur and delay batch release and therefore patient access to life-saving medicines.

Animal Welfare

The substantial number of laboratory animals used for this test cannot be justified in view of its unproven and questionable suitability to detect contaminants and increase the product safety[11]. As a consequence, the test was reviewed for replacement, reduction and refinement (3Rs) in the scope of the *European Convention on the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes*. As aforementioned, the test has been consequently deleted from numerous EP monographs[9,15].

In this context, the EU adopted a new directive on the protection of animals used for scientific purposes (2010/63/EU[39]). The directive plays a significant role in minimizing the number of animals used in experiments and the EDQM continues to push forward the implementation of 3Rs alternatives in the future[40].

Conclusion

Based on the rationale provided in this paper and in line with the scientific knowledge and regulatory trends outlined herein, EFPIA considers it is fully justified to completely eliminate abnormal toxicity testing from pharmacopoeias and other regulatory requirements.

This would be in agreement with animal welfare concerns (e.g. 3Rs initiatives) and would contribute to a continuous patient supply to life-saving medicines and a sustainable reduction of valuable governmental resources utilized in conducting this test on every batch.

Additional Reading

The following publication provides a comprehensive review of the ATT, explains its original purpose and historical evolution. It outlines relevant requirements and scientific publications in relation to the test:

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.[5]

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

Й.Х.О. Гарбе, С. Озборн, К. Беггс, М. Бопст, А. Йос, А.А. Киташова, О.М. Ковбасенко, К.-Д. Шиллер, М. Швингер, Н.Ю. Семенова, Л.А. Смирнова, Ф. Стодарт, Т. Визалли, Л. Вроманс (**2015**) Исключение теста на аномальную токсичность в качестве теста контроля качества: исторический анализ данных и научные знания. Разработка и регистрация лекарственных средств 2015 № 2 (11).

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