EPAA INTERNATIONAL WORKSHOP REPORT

Modern science for better quality control of medicinal products

"Towards global harmonisation of 3Rs in biologicals"

15-16 September 2015

International Workshop organised by the EPAA: Modern science for better quality control of medicinal products "Towards global harmonisation of 3Rs in biologicals"

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Executive summary

The European Partnership for Alternative Approaches to Animal Testing (EPAA), a public private collaboration between the European Union (EU) Commission, European trade associations, and companies from seven industry sectors, convened an international workshop *Modern science for better quality control of medicinal products: Towards global harmonisation of 3Rs in biologicals* that took place on 15 and 16 September 2015 in Egmond aan Zee, The Netherlands. The 45 invited participants represented EU and international organisations and institutions, national regulators from EU member states and non-EU countries as well as human and veterinary vaccines manufacturers and industry federations. The workshop participants were invited to make concrete recommendations to overcome existing barriers to the global deletion of abnormal toxicity tests (ATTs), or general safety tests (GSTs), from all human biologicals requirements and of GSTs and target animal batch safety tests (TABSTs) from veterinary vaccines requirements. Further, they were asked to identify means to facilitate the global regulatory acceptance of specific non-animal methods that are available for the potency testing of human diphtheria and tetanus vaccines and veterinary swine erysipelas vaccines.

In regard to safety tests, workshop participants agreed to actively encourage deletion of ATTs, GSTs and TABSTs from all relevant legal requirements and guidance documents, such as pharmacopoeia monographs, World Health Organisation recommendations, and World Organisation for Animal Health guidelines. In respect to vaccine potency tests, international convergence on the scientific principles of the use of appropriately validated *in vitro* assays in place of *in vivo* methods was identified as overarching goal. In pursuing this goal, it was considered essential to include key regulators and manufacturers early on in the corresponding discussions. As an outcome of such discussions, collaborative studies to advance new assays should be initiated as appropriate. Establishing requirements for new assays was addressed as a possible means to unify regulatory approaches in different jurisdictions. Manufacturers and responsible groups, e.g., at the European Directorate for the Quality of Medicines and Health Care of the Council of Europe or the European Medicines Agency were invited to consider leadership for such international collaboration.

1 Introduction

Established in 2005, the European Partnership for Alternative Approaches to Animal Testing (EPAA) is a public private collaboration between the European Union (EU) Commission, European trade associations, and companies from seven industry sectors. The partners are committed to pooling knowledge and resources to accelerate the development, validation and acceptance of alternative approaches to animal use in regulatory testing. The overall aim of activities is the replacement, reduction and refinement (3Rs) of animal use in regulatory testing. First defined by Russell and Burch (1959), the 3Rs principle has been implemented in *Directive 2010/63/EU on the protection of animals used for scientific purposes* (EP and Council of the EU, 2010).

In 2013, the EPAA launched the project *Harmonisation of 3Rs in Biologicals.* Biologicals include a wide variety of products, such as hormones, immunoglobulins, blood products and vaccines. Biologicals are generally more complex products relative to their small molecule counterparts and this is often reflected in the manufacturing processes for biologic drugs. Therefore, strict quality control (QC) strategies are employed to ensure consistent batch-to-batch quality of marketed human and veterinary vaccines. These QC strategies encompass validated production processes and analytical techniques that may include animal tests or non-animal *in vitro* methods and approaches. The regulatory requirements for the QC safety and potency batch release testing of vaccines and other biologicals are typically incorporated in pharmacopoeia monographs and guidelines. However, jurisdictions often differ in their legal requirements for batch release testing, just as they may differ in their requirements for marketing authorisation. In some jurisdictions, specific animal tests for the QC of vaccines and other biologicals have been deleted or replaced by non-animal approaches, whereas, the same animal tests may still be required in other jurisdictions. Such regional regulatory differences may lead to the unnecessary continuance of scientifically unsupported animal testing if a product is intended for several international markets. Therefore, these regional regulatory differences merit evaluation to determine whether scientific evidence may facilitate the global regulatory acceptance of available non-animal methods or the deletion of no longer relevant tests. This may bring additional gains in resources and in timely patient access to medicines.

Against this background, the EPAA convened an international workshop *Modern science for better quality control of medicinal products: Towards global harmonisation of 3Rs in biologicals,* organised by **Katrin Schutte** (EU Commission, Directorate General for the Environment, Belgium) and **Anna Szczepanska** (European Federation of Pharmaceutical Industries and Associations (EFPIA), Belgium), joint leaders of the EPAA Biologicals project team. The workshop focused mainly on vaccines, while addressing other biologicals as well, and took place on 15 and 16

September 2015 in Egmond aan Zee, The Netherlands. This report presents its outcome and the recommendations spelled out by the workshop participants.

The 45 invited participants of the workshop represented the World Health Organisation (WHO), the World Organisation for Animal Health (OIE; Office International des Epizooties), the European Directorate for the Quality of Medicines and Health Care (EDQM of the Council of Europe), the EU Commission and the European Medicines Agency (EMA), national regulators from different EU member states, Brazil, Canada, China, India, Japan, Mexico, and the USA as well as different human and veterinary vaccines manufacturers and industry federations. The goals of the workshop encompassed a scientific evaluation of specific animal tests used for the QC of vaccines and other biologicals. The workshop aimed at addressing whether their deletion or replacement by non-animal methods, as applicable, may improve the scientific reliability of safety and potency testing. This included an exploration of the reasons for prevailing interregional differences in QC testing requirements. The workshop participants were invited to make concrete recommendations to overcome existing barriers to the possible global deletion of no longer relevant batch safety tests and global regulatory acceptance of specific non-animal methods identified in concrete areas.

Katrin Schutte (EU Commission, Directorate General for the Environment, Belgium, *Setting the scene)* opened the workshop. Subsequently, two introductory presentations served to exemplify challenges in meeting the scientific and legal obligation to implement the 3Rs principle in QC testing (**Catrina Stirling** (Zoetis, United Kingdom), *3Rs in product development and QC of biologicals: What do science and technology offer today?* and **Iwona Wilk-Zasadna** (GSK, Switzerland), *Regulatory harmonisation of 3Rs in QC testing requirements for biological products from the viewpoint of users).* These challenges fundamentally differ between general safety testing on the one hand and batch potency testing on the other hand: For abnormal toxicity tests (ATTs) or general safety tests (GSTs), deletion without replacement is being called for, whereas *in vivo* potency tests could be replaced by non-animal test methods. For many *in vivo* potency tests, that had been implemented for vaccine and other biological QC before the principles of *Good Manufacturing Practice (GMP) for ensuring the quality of biological products* had been established almost twenty-five years ago (WHO, 1991), non-animal methods have become available. As C. Stirling explained in further detail, modern analytical techniques are generally more appropriate for QC because they have the potential to enhance product characterisation, and they are generally more consistent, rapid and cost effective. Most importantly, the so-called ‘consistency approach’ has great potential to replace the need for *in vivo t*esting. The consistency approach is founded upon a thorough characterisation of the products during their development and the principle that the quality of subsequent batches is ensured by their consistent production and the strict application of an enforced quality system (De Mattia *et al.,* 2011; Stirling, 2012). Subsequently, I.

Wilk-Zasadna described that, depending on the type of biological, non-animal methods may need to meet different specifications: For well-established inactivated vaccines, such as inactivated rabies vaccines, generic tests may be suitable for all products from different producers. For novel vaccines that are, e.g. based upon reverse vaccinology (Sette and Rappouli, 2010), product-specific QC assays will be required. Nevertheless, for specific classes of new vaccines that have common potency-related attributes, e.g. conjugated vaccines with similar advanced production processes, common non-animal QC tests may be developed using a harmonised approach with further product-specific 'fine-tuning'.

2 Case studies

Two breakout groups were formed to discuss two case studies each **(Anna Szczepanska** (EFPIA, Belgium), *Introduction to the breakout groups).* Case studies 1 and 2 addressed the possible deletion of GSTs from all human biologicals requirements and of GSTs and target animal batch safety tests (TABSTs) from the veterinary vaccines requirements, respectively, and case studies 3 and 4 discussed non-animal methods for the potency testing of specific vaccines, i.e. human diphtheria and tetanus (DT) vaccines and veterinary swine erysipelas vaccines, respectively. As an outcome of each case study, concrete recommendations to internationally foster the 3Rs principle in these areas were spelled out. The recommendations were further discussed and approved during the final plenary session. The following sections of Chapter 2 summarise the individual case study discussions, and the subsequent Chapter 3 *How to speed up the uptake and harmonisation of the 3Rs principle in regulatory testing requirements for biologicals?* presents the outcome of the final plenary discussion that put a number of case study-specific recommendations into a more general, overarching perspective.

Case study 1: Deletion of the abnormal toxicity test / general safety test / test for innocuity from international recommendations and national regulatory requirements

Moderator: **Marlies Halder** (EU Commission JRC, Italy)

Introduction to case study 1

Klaus Cussler (Paul-Ehrlich-Institut (PEI), Germany), *The scientific relevance of the abnormal toxicity test – today and from a historical perspective;* **Martin Bopst** and **Joerg Garbe** (F. Hoffmann-La Roche Ltd, Switzerland), *A case study of false positive results and the ATT in the context of modern QC;* **Robin Levis** (Food and Drug Administration (FDA), USA), *Status of the GST in the USA.*

The ATT (*Ph. Eur.*, 2015), also referred to as GST (US FDA, 2015) or test for innocuity (WHO, 1990), is carried out in mice and/or guinea pigs and aims at detecting non-specific contaminants or toxins. The term ATT will be used consistently throughout this text unless a specific form of the test is addressed. As presented by K. Cussler, the use of safety tests in mice and guinea pigs dates back to the early 1900s. Mice were used to detect potentially toxic phenol levels in

diphtheria antisera. Guinea pigs were used to detect contamination with tetanus spores or toxin. In the 1940s, these two specific safety tests were combined to a general safety test, and consequently incorporated into most national or international pharmacopoeias and requirements. The test design of the ATT varies between countries and product classes with regard to injection volume, injection route and observation period. Depending on the test design required, a product could pass the ATT in one country and fail it in another country.

In Europe, the scientific validity and rationale of the ATT has been criticised for many years. After introduction of GMP and, most importantly through the use of adequate and stringent QC measures, the relevance of the ATT has become highly questionable. The outcome of a retrospective analysis of ATT data provided scientific evidence that the test is neither specific, reproducible, reliable, nor suitable for the intended purpose (Duchow *et al.,* 1994). Consequently, it has been deleted as a routine batch release test from more than 80 monographs of the European Pharmacopoeia (*Ph. Eur*.) (Schwanig *et al.,* 1997). Nevertheless, manufacturers producing for the global market may still need to perform the ATT to satisfy WHO recommendations and many national regulatory requirements (such as the ones enforced in China, Japan, and Russia).

M. Bopst and J. Garbe reported an example of an excipient (benzyl alcohol) used in human biologicals that may cause false positive results in the ATT depending on the injected volume and route of administration (Xie *et al.*, 2015). In addition to the general lack of a scientific rationale for the test, this example provides further evidence to question the validity of the ATT as a modern QC test. Today, drug development and control of contaminants occur at several levels. An extended product characterisation takes place during process development and validation where degradation profiles, etc., are investigated. Advanced process understanding, in-process controls, validation of the manufacturing process and release testing complying with international standards are also part of modern vaccine development. Contaminants are controlled *via* a number of validated and specific tests that aim at detecting, e.g., microbiological contaminants or residual contaminants (Garbe *et al.*, 2014). By contrast, the ATT does not fulfil the International Conference on Harmonisation (ICH) validation criteria (specificity, reproducibility, detection limit) for a QC test, and it is not possible to validate it. For example, the ATT lacks explicit acceptance criteria and specific endpoints in relation to its objective of detecting contaminants.

Animal welfare as well as legal considerations to comply with the 3Rs principle should also be applied in the context of the ATT. In accordance with Directive 2010/63/EU, a solid scientific rationale and justification is required to support any testing requirements using live animals (EP and Council of the EU, 2010). In the case of the ATT, this premise is no longer met. More

adequate QC measures (including non-animal tests) are already in place, and omission of ATTs does not compromise the safety of human vaccines or any other human pharmaceutical. Both presentations concluded that all different kinds of ATTs or GSTs in mice or guinea pigs lack scientific relevance and should be completely deleted from national or international pharmacopoeias and requirements both as final product tests as well as during development and production. This step has recently been taken by the United States (US FDA, 2015), and R. Levis provided an overview on the underlying motivations. In essence, the US FDA decision is based upon the rationale that GST requirements are *no longer appropriate to help ensure the safety, purity, and potency of licensed biological products*.

Outcome of the case study 1 discussion

The participants of the case study 1 discussions agreed that all forms of the ATT lack scientific relevance and that omission of the ATT does not compromise the safety of human vaccines, or any other human pharmaceutical, since more adequate QC measures are in place. Workshop participants from various countries and international organisations reported the deletion of ATT requirements (e.g. Brazil), the possibility to waive the ATT after the testing of three batches (India) or upon approval by the given national regulatory authority (WHO recommendations). Even though the ATT for batch release has been deleted 20 years ago, some *Ph. Eur.* monographs still require compliance with the ATT for product development. Members of the *Ph. Eur.* Group of Experts 15 (human vaccines) highlighted that complete deletion of the ATT is a matter of ongoing discussions.

The mentioning of the ATT in internationally recognised pharmacopoeias or WHO requirements may result in the assumption that the ATT is necessary to ensure the safety of a product. Accordingly, the ATT continues to be required and performed. The deletion of the ATT should be addressed at a global level. In this respect, the WHO is a key player, due to its outstanding global position. The global deletion of the ATT should be voiced by regulators and manufacturers in equal measure. All jurisdictions and regulatory bodies that still include the ATT in their monographs, regulations, etc., should be addressed.

The following immediate steps were agreed upon: For initiatives at the European level, K. Cussler (PEI, Germany), *via* the German Pharmacopoeia, agreed to submit *requests for revision* to the *Ph. Eur.* asking for deletion of the ATT from all monographs. Subsequent to the international EPAA workshop, this request is now scheduled to be discussed at the next ‘Group 15’ meeting, in Spring 2016, and will also be considered by other relevant EDQM working groups. With regard to the WHO, Dianliang Lei and Ute Anna Rosskopf (both WHO) and Karl-Heinz Buchheit (EDQM) agreed to present the recommendations from the EPAA workshop at the upcoming WHO Expert Committee on Biological Standardisation (ECBS) meeting, proposing to include *deletion of the test*

for innocuity on the agenda for the 2016 ECBS meeting. Generally, industry representatives, in striving for deletion of the ATT, were invited to approach relevant regulators *via* their interest groups. Companies were further encouraged to include proposals for the deletion of these tests in their applications for batch release even though this would necessitate submission of full in-process testing data to justify the deletion. Finally, the EPAA (*via* its member organisations) and, specifically, the EU Commission were invited to explore means to engage legislators from key countries that still maintain ATT requirements in collaborative discussions, e.g. by organising regional meetings or workshops to which targeted groups would be invited.

Case Study 2: Deletion of the target animal batch safety test at the national and Veterinary International Conference on Harmonisation levels

Moderator: **Catrina Stirling** (Zoetis, United Kingdom)

Introduction to case study 2

Harrie Glansbeek (Merck Sharp & Dohme (MSD) Animal Health, The Netherlands), *Waiving the target animal batch safety test at MSD Animal Health.*

The TABST is a routine QC test to demonstrate that a veterinary vaccine batch is safe in the target animal. In most cases, the TABST is the central batch safety test, but it may be combined with potency tests. In Europe, since 2004, performance of the TABST for each batch of veterinary vaccine was allowed to be waived upon provision of consistency data for at least 10 batches. Subsequently, in 2012, the TABST was deleted from the *Ph. Eur.* monographs for all veterinary vaccines with two specific, scientifically justified exceptions and renaming of the test to 'residual toxicity test' to avoid misunderstandings (EDQM, 2012 (rev. 2013) and 2015). As a result, since 2013, for veterinary vaccines produced and sold in the EU, the TABST is generally no longer conducted. However, the TABST is still required in non-EU countries including Argentina, Brazil, Canada, India, Japan, South Korea, China, Taiwan and the USA. Of these, some countries may allow specific waivers, while others do not. The Veterinary International Conference on Harmonisation (VICH) has made progress towards harmonising criteria for waiving the TABST. It has published the VICH guideline GL50 for inactivated vaccines that, however, still requires data on at least 10 consecutive batches and product-specific variations for removal (VICH, 2013). This guideline has been implemented in the various VICH regions, for instance, in the USA with the USDA veterinary services memorandum No. 800.116 (USDA, 2013). Finally, in the USA (just as in, e.g., Japan or Brazil), a routine batch general safety test using mice or guinea pigs is required in addition to the TABST (Kulpa-Eddy *et al.,* 2011).

Outcome of the case study 2 discussions

The participants of the case study 2 discussions agreed that scientific evidence that the TABST contributes to the safety of veterinary vaccines was lacking. Similarly, there was agreement that

the ’10 batches’ waiving approach as it was initially implemented in Europe (EDQM, 2015) and subsequently laid down in the VICH GL50 (VICH, 2013) does not have a scientific basis. Different participants highlighted that, if veterinary vaccines do elicit safety problems, this is recognised at the pharmacovigilence level. However, in different jurisdictions different pharmacovigilance systems are in place, and this post-marketing control may differ and potentially be less stringent than the corresponding EU provisions. Similarly, national quality systems for veterinary vaccines may differ between different jurisdictions, and different countries may further take different approaches for the QC of new versus existing products. In this respect, also the ‘seed lot system’, that requires successive batches of a product to be prepared using the same master seed (EU Commission, 1992), is not mandatory in all countries. However, the most fundamental barrier to deletion appears to be local legal requirements.

To achieve global deletion of the TABST, the corresponding requirements have to be deleted from the respective legislations that have been enforced in different jurisdictions. This requires lobbying at the legislator level to encourage and support countries to change the relevant legislation. To obtain an overview on the type of country-specific lobbying required, IFAH-Europe agreed to collaborate with veterinary vaccine companies in preparing a consolidated list of countries that do or do not accept deletion of the TABST, further indicating the reasons for non-acceptance, if available. Additionally, it was decided to invite the *Ph. Eur.* (*via* its Group of Experts 15V that deals with veterinary vaccines) to prepare a detailed white paper or peer reviewed publication explaining the rationale for removal of the TABST from the *Ph. Eur*. monographs. Such a document could be shared with other international pharmacopoeias. Finally, the OIE was identified as pivotal international organisation to foster lobbying at the national level for removal of the TABST, even more so since the OIE stands in direct contact with the chief veterinary officers of its member countries. Judy Mac Arthur Clark (Home Office, United Kingdom) agreed to establish contacts at the OIE level. It was suggested that the OIE, together with the EPAA and Health for Animals (formerly IFAH-global), convene a joint workshop to promote the global deletion of the TABST. Such a joint workshop could further be conceived to generally address 3Rs-relevant issues related to veterinary vaccines including the harmonisation of veterinary vaccine potency testing.

Case Study 3: Potential harmonisation pathways for in vivo testing requirements for diphtheria and tetanus vaccines and perspectives for 3Rs implementation

Moderator: **Dean Smith** (Health Canada, Canada)

Introduction to case study 3

Sylvie Uhlrich (Sanofi Pasteur, France), *Towards replacement of in vivo potency assays for diphtheria and tetanus vaccines;* **Paul Stickings** (National Institute for Biological Standards and Control (NIBSC), United Kingdom), *Antigen quantification as an in vitro indicator of potency in diphtheria and tetanus vaccines*.

To date, the potency testing of DT vaccines involves assessing the immune response induced in mice and guinea pigs. Depending on the required test method, the severity of the animal tests ranges from mild (serological assay with *in vitro* titration of immune sera) to severe (challenge assay or serological assay with *in vivo* titration of immune sera). In some laboratories, refinement of testing procedures has resulted in a reduction of animal numbers and/or the severity of the procedures. However, despite the fact that *in vivo* potency assays may suffer from poor precision and poor reproducibility, to date, no non-animal method for DT vaccine potency testing has gained regulatory acceptance in the EU (McFarland *et al.,* 2011). The global regulatory environment is complex and different countries or regions may require different versions of an *in vivo* potency test. This places an additional burden on manufacturers who produce products that are registered in a large number of countries or regions. For some products, it significantly increases the total number of animals required to release a vaccine batch. In addition, because of the inherent variability of many of the required *in vivo* potency tests, re-testing may delay release and affect the supply chain creating the potential for vaccine shortages in the market.

One of the barriers preventing the replacement of the *in vivo* potency tests with *in vitro* assays is that this requires a demonstration of correlating outcomes. Due to the high variability of the *in vivo* DT vaccine potency tests, this is an unrealistic expectation. The *in vitro* assays are generally more powerful in detecting quality differences between different vaccine batches than the corresponding *in vivo* assays. Therefore, the evaluation of the *in vitro* assays (as replacements for *in vivo* tests) should focus on their ability to detect changes in the quality attribute(s) that are relevant for the biological function of the vaccine, rather than on a demonstration of concordance between pass and fail results (that are based on criteria that are related to the traditional *in vivo* assay). DT vaccines are formulated based on the measurement of antigen content using *in vitro* immunoassays, typically the flocculation test. Other *in vitro* immunoassays (ELISA-based methods) have also been developed for the quantification and/or characterisation of D and T antigens. Such *in vitro* immunoassays have been shown to be suitable for monitoring the batch-to-batch consistency of DT vaccines in terms of antigen content and degree of adsorption (Coombes *et al.,* 2009, 2012). The assays presented in case study 3 involve the use of monoclonal antibodies that recognise functional epitopes on the D or T toxoid. An analysis of the data demonstrated that the *in vitro* assays were either equal to or superior to the *in vivo* methods at detecting vaccine stability (i.e. product (non-)degradation under heat stress), which forms an essential part of DT vaccine potency testing. Following up on the presentations from S. Uhlrich and P. Stickings, the case study 3 discussions addressed the potential for assays of the mentioned type to function as *in vitro* potency tests. Furthermore, the principles of comparing the responses obtained in a proposed *in vitro* method with the results obtained with an existing *in vivo* method were discussed.

Outcome of the case study 3 discussions

The participants of the case study 3 discussions agreed that *in vitro* assays may be suitable for the potency testing of either D or T if they are capable of detecting changes in the product characteristics and/or quality attributes that are relevant for immune protection. It follows that suitable *in vitro* assays should enable assessment of the stability of the vaccine (WHO, 2006). As such, they should be stability indicating, or they should form part of a quality strategy that is stability indicating. While harmonised assays that would be applicable to products from all manufacturers would have their merits, product-specific assays are equally acceptable. In the end, manufacturers should be allowed to choose the appropriate potency test for their product since they are in the best position to evaluate its suitability.

Potential *in vitro* assays for DT vaccine potency testing have been developed, and further investigation of their suitability for different products should be encouraged. It was highlighted that any comparison between an *in vitro* and *in vivo* method should be considered in the context of what is scientifically meaningful. Demonstration of agreement between two methods in terms of the ability to identify 'failing' batches can be challenging in some cases – for reasons that are not related to the suitability of the *in vitro* method. This may be particularly relevant where the potency of a given product is close to the minimum requirement for the animal potency test so that a single *in vivo* potency test will not reliably discriminate between in-specification and out-of-specification batches. Further, when the potency of a product significantly exceeds the minimum requirement for the *in vivo* assay, focusing only on that minimum requirement for *in vitro-in vivo* comparisons may be inappropriate because of the difficulty in (deliberately) producing (experimental) vaccine batches that fail to meet this requirement. The need to perform truly quantitative *in vivo* assays for comparison purposes was also acknowledged since single dilution models and other limit tests have little or no discriminative power and are therefore not useful for assessing the potential suitability and relevance of a proposed *in vitro* potency test.

Even though some jurisdictions are willing to accept appropriately validated *in vitro* vaccine potency tests, reluctance in other jurisdictions poses a major disincentive for global manufacturers to develop product-specific *in vitro* potency tests. Hence, the lack of global regulatory acceptance is the main barrier to their international implementation. International collaborative studies for validation (or pre-validation) of proposed *in vitro* potency tests, involving regulatory authorities, control authorities and industry, would be of great value and would facilitate the further development of non-animal methods. Additionally, collective action between key regulatory groups from major jurisdictions focusing on convergence on the scientific principles of the use of appropriately validated *in vitro* methods will be an effective means to promote the international regulatory acceptance of non-animal methods for vaccine potency testing. Such action could be led by, e.g., the *Ph. Eur.* 'Group 15' or the EMA at the European level, the US FDA or the Pan-

American Health Organisation (PAHO) at the American level, or, at the global level, by the WHO ECBS or the Network of WHO Collaborating Centres. The driving force for any engagement in fostering a change in the willingness of international regulators to accept *in vitro* vaccine potency assays (or other new methodologies) should be an acceptance of the scientific limitations of the *in vivo* assays and an understanding that the best science will offer superior QC and product availability. This best science approach to the development of *in vitro* methods also offers the potential to reconcile divergent testing approaches that have evolved globally between different regions.

Case Study 4: Swine erysipelas vaccine: In vitro ELISA to replace the in vivo immunisation-challenge test

Moderator: **David John** (IFAH-Europe, Belgium)

Introduction to case study 4

Pieter-Jan Serreyn (Huvepharma, Belgium), *Swine erysipelas vaccine – in vitro ELISA to replace the in vivo immunisation-challenge test.*

Swine erysipelas vaccines were amongst the first vaccines used for the immunisation of animals, and thus, they were also amongst the first to require batch potency testing. Already in the 1940s, a scarification test using pigs was published thereby presumably constituting the first reliable model for vaccine efficacy testing. For a very long time, a mouse challenge method was the globally accepted golden standard for swine erysipelas vaccine potency testing. In Europe, this method was replaced by a mouse serology test in 2004 after extensive validation work (Rosskopf-Streicher *et al.,* 2000; *Ph. Eur.,* 2004). In the USA, a non-animal assay was published in 2009. This ELISA allows detection of the protective 659kD antigen of *E. rhusiopathiae* (Supplemental Assay Method (SAM) 613; USDA, 2009a). With its publication, the SAM 613 replaced three *in vivo* potency tests (SAM 601, 605, 606; USDA, 2009b).

Outcome of the case study 4 discussions

For the 'SAM 613 ELISA' that the USDA has accepted for swine erysipelas vaccine potency testing, the next step in facilitating global implementation is striving for its regulatory acceptance and use in Europe. In fact, even though the mouse serology test is described in the corresponding monograph, the *Ph. Eur.* provisions for swine erysipelas vaccines do not prevent application of the ELISA. Instead, they explicitly permit use of *suitable validated alternatives*. Therefore, the major hurdle constitutes the need for product-specific validation of the ELISA. While this hurdle is minor when it comes to new vaccines, for existing products that may have been on the market for 15-20 years the expenditure required to validate the new potency test requires profound economic justification. Accordingly, to promote the validation and acceptance of product-specific ELISAs for swine erysipelas vaccine potency testing, industry would benefit from in-advance certainty on the

specific requirements for test validation. In individual cases, companies have gained regulatory acceptance for their product-specific potency test by submitting a number of batch release applications that made use of both the *in vivo* and the *in vitro* assay. Even though only the *in vivo* data were used for the respective marketing procedure, this scheme provided evidence of the applicability of the ELISA for further batches.

While recognising that product-specific validation is imperative to ensure that the respective ELISA provides the necessary level of assurance, it was recommended to initiate a collaborative study to identify critical quality attributes of swine erysipelas vaccines that might be used for a general evaluation of the non-animal method thereby limiting the need for its product-specific validation. Additionally, collaborative studies for veterinary vaccines could serve to explore whether part of the required evidence may be provided by post-implementation monitoring instead of submission of full validation data. It was recommended that industry and regulators work closely together in designing such studies. This would ensure that the outcome will provide industry the desired certainty and authorities the required confidence in the new method. In addressing the EPAA, it was suggested that, e.g., its members of the biologicals project team engage in scientific 'pre-work' laying the foundation for collaborative studies to evaluate, e.g., the applicability of the potency test under investigation for different vaccine strains (or even the suitability of swine erysipelas vaccines, as such, as the focus for a precedence-setting collaborative study).

3 Workshop conclusions: How to speed up the uptake and harmonisation of the 3Rs principle in regulatory testing requirements for biologicals?

Moderator: **Robin Levis** (FDA, USA)

Katrin Schutte (EU Commission, Belgium), *Closing remarks and key take-away messages.*

The following actions flowing from the case study recommendations were agreed upon during the plenary sessions following the specific case study discussions.

Actions following from case study recommendations

SAFETY TESTS

- Encourage deletion of GSTs and TABST from all national / jurisdictional legal requirements and international guidance (e.g. *Ph. Eur*. monographs/ WHO recommendations, OIE guidelines)
  - re. GSTs for human vaccines and other biologicals: Submit proposals to relevant EDQM expert groups and WHO ECBS group
  - re. GST and TABST for veterinary vaccines and other biologicals: Initiate discussion at OIE level
- EPAA (*via* member organisations) / EU Commission: Explore means to contact key countries at legislator level, potentially *via* local meetings or targeted group workshops

POTENCY TESTS

- Goal: Achieve convergence on the scientific principles of the use of appropriately validated *in vitro* assays in place of *in vivo* methods

- Include key regulators and manufacturers from the beginning in the corresponding discussions
- Collaborative studies could result from such discussions
- New assays as means to unify different regulatory approaches in different jurisdictions
- Harmonised assays desirable, where possible, but product-specific assays may also be acceptable
- Leadership for international collaboration through EDQM Groups 15 / 15V and EMA (JEG 3Rs); FDA, USDA and PAHO; WHO ECBS, Network of WHO Collaborating Centres, and OIE; and manufacturers

Generally, there was consensus that the time is right to strive for the global deletion of GSTs, ATTs, and TABSTs and the international regulatory acceptance of appropriately validated non-animal approaches for vaccine batch potency testing. In many cases, the *in vivo* tests had been established before vaccine licencing procedures had been implemented. Furthermore, many *in vivo* methods only allow 'pass-fail' assessments, whereas, *in vitro* assays that use advanced analytical methods potentially enable more reproducible qualitative and quantitative product assessments. In selecting the best test method for a given purpose, the best science should prevail and application of appropriately validated non-animal methods should ensure the supply of vaccines of acceptable quality. Building the regulatory authorities' confidence in the new methods is key to promoting their global acceptance. In principle, regulators and industry are willing to accept appropriately validated non-animal methods. In striving for international acceptance of a specific assay or an overarching testing strategy (including the deletion of no longer relevant tests), it is imperative to ensure that all relevant regulators are involved. The WHO and the OIE are key players in pursuing this task since national authorities are required to make communications to these organisations. From the European perspective, all initiatives should further take into account ongoing 3Rs-relevant work at the EDQM and the EMA *via* its *Joint Committee for Medicinal Products for Veterinary Use/Committee for Medicinal Products for Human Use Ad-hoc Expert Group on the Application of the 3Rs in Regulatory Testing of Medicinal Products* (JEG 3Rs).

Also after uptake of a new method, e.g. in the respective pharmacopoeia monographs, further initiatives may be necessary to provide the product specific evidence to individual authorities that the new method is indeed able to detect inconsistent batches. As discussed by the participants, approaches to product-specific validation may vary for human and veterinary vaccines, and product-specific validation is generally the norm for human vaccines. Industry plays an important role in furthering the use of the new methods, for instance, by collaborating with those responsible for the pharmacopoeias or with national control laboratories, such as, in Europe, the Official Medicines Control Laboratories (OMCLs).

For vaccines that have been on the market for more than a decade, production and market data should be available that would define the history of manufacturing, safety and (potentially) the

effectiveness of the product. Using the consistency approach to gain acceptance of a product-specific potency test, it may be shown on a case-by-case basis that the new assay is relevant for the given product. To ensure adequate control, the new methodologies have to capture the key quality parameters that are essential to maintain the safety and efficacy of the product established at the time of licensure. There was consensus that the consistency approach is key to promoting the regulatory acceptance of new assays. Importantly, it was also mentioned that, provided adequate information was available, in principle a transition to *in vitro* methods could be achieved without additional clinical trials or a requirement for a so-called one-to-one comparison of the *in vivo* and *in vitro* method. Discussions that are ongoing at different expert groups, such as the EDQM Group 15, to provide a conceptual framework in support of the replacement of *in vivo* methods without a requirement for clinical trials or one-to-one method comparisons when scientifically justified, were described.

Finally, also financial incentives were addressed as supplementary tools to promote the validation and use of new, non-animal test methods on the manufacturer's side. For instance, in Germany and in the United Kingdom fees for the variation of the marketing authorisation may be reduced or waived in case 3Rs methods are introduced for the QC of the product. While it was confirmed that such measures would contribute to alleviating the economic hurdle to validate new product-specific methods, the need for global harmonisation of testing requirements was recognised as a core element in promoting the 3Rs principle in the area of vaccine QC. Striving for global harmonisation was seen as a combined evolutionary process during which the regulators in charge of approval of animal testing, the regulators in charge of assessing the vaccines and the manufacturers engage in continuous collaborative discussions. The EPAA was confirmed as unique platform in facilitating such discussions, as also the workshop itself highlighted.

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