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The Target Animal Safety Test—Is it Still Relevant?

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Abstract. In Europe, the target animal safety test (TAST) is stipulated by 52 European Pharmacopoeia monographs, by three European Union (EU) *Directives* and a number of EU guidelines as a routine test for veterinary immunologicals, to be carried out on the finished product. TAST data from seven European Official Member States Control Laboratories (OMCLs) and 14 manufacturers were retrospectively analysed. During 1994–1997, 11 185 vaccine batches had been submitted for batch release, and the OMCLs had tested 670 batches in the TAST (665 passed, 4 passed after retesting, 1 failed). In total, 82 of these batches were not released; however, in only one case this was due to failure in the TAST. The data received from the 14 manufacturers covered the years from 1997 to 1999. 11 386 batches were tested in the TAST, of which 215 passed after retesting and 7 failed. Although only 30% of the OMCLs provided data and the data of the manufacturers are not complete they clearly indicate that the TAST does not contribute to the safety of veterinary vaccines and should therefore not be required as a routine batch test. In cases, where it appears to be necessary, detailed guidance on the test design and evaluation should be given.

Introduction

During the last few years, the relevance of the target animal safety test (TAST) was increasingly questioned.[1–10] The introduction of Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) into the manufacture of vaccines has significantly increased their safety and quality. Thus, some of the animal tests carried out for purity and safety purposes appear to be superfluous. The European Centre for the Validation of Alternative Methods (ECVAM; Institute for Health & Consumer Protection, Joint Research Centre, European Commission, Ispra, Italy) took up this issue and commissioned the Advisory Group on Alternatives to Animal Testing in Immunobiologicals (AGAATI) to perform the study, *Evaluation of the relevance of the target animal safety test for the quality control of veterinary immunological medicinal products* (*contract 134 10-97-11F 1 EI ISP NL*). The main objectives of the study were: (a) to identify the monographs, directives and guidelines in the European regulatory framework, which stipulate the TAST; (b) to analyse and critically review the purpose of the TAST; (c) to perform a retrospective analysis of TAST data; and (d) to give recommendations, based on the outcome of the retrospective analysis, for modifying the relevant monographs and guidelines.

Regulatory framework

Europe

The TAST was introduced some decades ago, during the development of the first veterinary vaccines.[3] To date, it is stipulated by 52 European Pharmacopoeia (*Ph. Eur.*) monographs on veterinary immunologicals (Table 1a), by three European Union (EU) *Directives* (Table 2)[11–13] and various EU guidelines (Table 3),[14], to be carried out on the finished product. Seven *Ph. Eur.* monographs do not require the TAST (Table 1b).

At least two animals of the target species are injected with the twice (inactivated vaccines) or ten times (live vaccines) the recommended dose of the vaccine to be tested. None of the animals should show abnormal or systemic reactions during a given observation period. Significant differences in the numbers of animals required (mammals: 2 animals; poultry and fish: at least 10 animals), the administration scheme and the period of observation are evident between the individual *Ph. Eur.* monographs, and also between the number of animals

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Table 1a. *Ph. Eur.* monographs which stipulate the TAST*

Ph. Eur. monographs
General monograph
Vaccines for veterinary use (62)
Live vaccines
Anthrax spore vaccine (live) for veterinary use (441)
Aujeszky's disease vaccine for pigs (745)
Avian infectious bronchitis vaccine (442)
Avian infectious bursal disease vaccine (587)
Avian infectious encephalomyelitis vaccine (588)
Avian infectious laryngotracheitis vaccine (1068)
Bovine parainfluenza virus vaccine (1176)
Bovine respiratory syncytial virus vaccine (1177)
Brucellosis vaccine (Brucella melitensis Rev. 1 strain) (793)
Canine contagious hepatitis vaccine (446)
Canine distemper vaccine (448)
Canine parvovirosis vaccine (964)
Distemper vaccine for mustelids (449)
Duck viral hepatitis vaccine (1315)
Feline calicivirosis vaccine (1102)
Feline infectious enteritis vaccine (251)
Feline viral rhinotracheitis vaccine (1206)
Fowl-pox live vaccine (649)
Infectious bovine rhinotracheitis vaccine (696)
Marek's disease vaccine (589)
Newcastle disease vaccine (450)
Swine-fever vaccine, classical (65)
Inactivated vaccines
Aujeszky's disease vaccine for pigs (744)
Avian infectious bronchitis vaccine (959)
Avian infectious bursal disease vaccine (960)
Avian paramyxovirus 3 vaccine (1392)
Canine adenovirus vaccine (1298)
Canine parvovirosis vaccine (795)
Clostridium botulinum vaccine (360)
Clostridium chauvoei vaccine (361)
Clostridium novyi (type B) vaccine (362)
Clostridium perfringens vaccine (363)
Clostridium septicum vaccine (364)
Egg drop syndrome '76 vaccine (1202)
Equine influenza vaccine (249)
Feline calicivirosis vaccine (1101)
Feline infectious enteritis vaccine (794)
Feline leukaemia vaccine (1321)
Feline viral rhinotracheitis vaccine (1207)
Foot-and-mouth disease vaccine (63)
Furunculosis vaccine for salmonids (1521)
Leptospira vaccine (447)
Neonatal piglet colibacillosis vaccine (962)
Neonatal ruminant colibacillosis vaccine (961)
Newcastle disease vaccine (870)
Porcine actinobacillosis vaccine (1360)
Porcine influenza vaccine (963)
Porcine parvovirosis vaccine (965)
Porcine progressive atrophic rhinitis vaccine (1361)
Rabies vaccine (451)
Swine erysipelas vaccine (64)

*Numbers in parentheses relate to the corresponding monograph in *European Pharmacopoeia*, 3rd Edition, Council of Europe, Strasbourg, France.

Table 1b. *Ph. Eur.* monographs which are *not* stipulating the TAST*

Ph. Eur. monographs
General monograph
Immunosera for veterinary use (30)
Live vaccines
Rabies vaccine (live, oral) for foxes (746)
Inactivated vaccines
Tetanus vaccine for veterinary use (697)
Immunosera
Clostridium novyi alpha antitoxin for vet. use (339)
Clostridium perfringens beta antitoxin for vet. use (340)
Clostridium perfringens epsilon antitoxin for vet. use (341)
Hormones
Gonadotropin, equine serum for vet. use (719)

*Numbers in parentheses relate to the corresponding monograph in *European Pharmacopoeia*, 3rd Edition, Council of Europe, Strasbourg, France.

stipulated in the *Ph. Eur.* monographs on fish vaccines (10 fish) and the EU guidelines (30 fish).

Non-European Countries

According to United States Code of Federal Regulations (CFR),[15] veterinary biologicals must meet certain basic criteria, including safety: the product must be safe in the target species and, if it contains live agents, in species exposed to shed organisms. However, safety tests are also carried out in laboratory animals. The detailed requirements for each type of product are listed in CFR Title 9, Part 113. Standard procedures are given for safety tests in mice, guinea-pigs, cats, dogs, calves, pigs and sheep.

In Japan,[16] medicinal products that are exclusively used for animals, including veterinary biologicals, are under the jurisdiction of the Ministry of Agriculture, Forestry and Fisheries, and ensuring their quality, efficacy and safety is included in the Pharmaceutical Affairs Law. It stipulates batch safety testing in the target species for all vaccines, with the exception of inactivated vaccines for large animals. Inactivated vaccines for other species are tested with a single dose injection, and live vaccines for all species are tested with an overdose. Batch safety tests in target animals are also included in the monographs of the Japanese Pharmacopoeia.

Purpose of the TAST

Historically, the use of animals for safety testing purposes was very important, since the availability of non-animal testing methods was limited and adequate regulatory requirements were not yet established. Thus, the safety test in mice was originally introduced at the beginning of the last century, to control the amount of phenol in immunosera. However, the licensing of veterinary vaccines was introduced later; for example, in Germany, only in 1978.

Table 2. European union directives

Directive	Scope
Council Directive 81/851/EEC of 28 September 1981 on the approximation of the laws of the Member States relating to veterinary medicinal products[11]	Marketing authorisation; regular inspections; each batch must be in conformity with the approved specifications for the product including safety tests
Council Directive 81/852/EEC of 28 September 1981 on the approximation of the laws of the Member States relating to analytical, pharmaco-toxicological and clinical standards and protocols in respect to the testing of veterinary medicinal products[12]	Requirements for demonstrating the quality, safety and efficacy of veterinary medicinal products
Council Directive 90/677/EEC of 13 December of 1990 extending the scope of Directive 81/851/EEC on the approximation of the laws of the Member States relating to veterinary medicinal products and laying down additional provisions for immunological veterinary medicinal products[13]	National control laboratories can repeat batch tests; batch release tests carried out by one national control laboratory must be recognised without repetition by the other Member States

Table 3. European guidelines stipulating the TAST[14]

General requirements for the production and control of
Inactivated mammalian bacterial and viral vaccines for veterinary use
Live mammalian bacterial and viral vaccines for veterinary use
Specific requirements for the production and control of
Equine live and inactivated viral and bacterial vaccines
Bovine live and inactivated viral and bacterial vaccines
Pig live and inactivated viral and bacterial vaccines
Ovine and caprine live and inactivated viral and bacterial vaccines
Live and inactivated vaccines for dogs and cats
Avian live and inactivated viral and bacterial vaccines
Live and inactivated vaccines for fish
Immunosera and colostrum substitutes

Nowadays, the safety testing of immunologicals is mandatory. This can still involve the use of animals and, in the case of veterinary immunologicals, it might appear logical to perform the safety test in the target species.

The objectives of the TAST have never been clearly defined. The requirement in the *Ph. Eur.* monographs, *no local or systemic reactions should occur*, implies that the TAST should demonstrate sufficient attenuation of live vaccines or reveal any harmful side-effects of adjuvants, which are used in inactivated vaccines. Thus, the outcome of the TAST seems to be crucial for the safety of a vaccine. However, the test is not designed to provide this information. It is a general safety test and, in fact, more-appropriate *in vivo* and *in vitro* tests which cover specific safety aspects have become available in the meantime and have been incorporated into the monographs and guidelines, such as tests for extraneous agents, and tests for specific or residual toxicity.

Therefore, the necessity for a general safety test can be questioned. Another general safety test, the abnormal toxicity test (ATT), has already been deleted from all of *Ph. Eur.* monographs for the veterinary vaccines and from most of the *Ph. Eur.* monographs for human vaccines, because it could be demonstrated that the ATT was no longer relevant for the safety of these vaccines.[17] Zeegers *et al.*[3] concluded from their survey on various veterinary vaccines from the same vaccine manufacturer, that the TAST did not contribute to the safety of the tested vaccine batches. Roberts and Lucken[2] proposed that a risk-benefit analysis should be conducted on a product-to-product basis, to determine whether the routine testing of batches of vaccines in the target animal was beneficial and could be justified. The wide-ranging discussion about the need and the relevance of this test has recently been summarised by Weisser and Hechler.[5]

TAST design

Comparison of the (non-mandatory) EU guidelines and the mandatory *Ph. Eur.* monographs reveals differences in the TAST design. For example, the EU guidelines stipulate the use of 30 fish whereas the relevant *Ph. Eur.* monographs only 10 fish. More striking are the differences between the *Ph. Eur.* monographs, where even vaccines of comparable product classes are not tested in the same way. For no obvious reasons, the requirements on TAST design and evaluation vary for the following spects: definition of target species; abnormal or significant reaction, duration of the observation period (14 to 21 days); number of test animals (10 animals are used for chicken and fish vaccines, whereas two are used for the other species); requirement for control animals (only fish vaccines); repeated application of a single dose; and use of antibody-free or susceptible animals. In addition to these points, further issues will be addressed in the following paragraphs:

Definition of "target species"

The safety test has to be carried out in the target species. However, the term ''target species'' is often not sufficient, because many products are only used in specific animal categories within the target species. For example, it is not appropriate to test vaccines which are only used for the vaccination of pregnant cows or sows, in calves or fattening pigs. However, this is common practice, and, for example, is stipulated by the *Ph. Eur.* monograph on

Escherichia coli vaccines for pigs (*Neonatal piglet colibacillosis vaccine [inactivated];[962]*).

Test requirement: no abnormal/no significant reaction

The terms *abnormal reaction* and *significant reaction* are not defined. Therefore, controversies arise when companies provide detailed test data and competent authorities have to decide, for example, whether or not a temperature increase of 1° C, 1·5° C, or even 2° C, is significant. The test requirements (pass/fail-criteria) have to be clearly defined and specified for each product/product group (for example, no local or general reaction exceeding the information in the summary of product characteristics [SPC] is acceptable). The fail criteria for the most critical reactions (for example, the degree of local reactions, the increase of body temperature) should be established during the licensing procedure or when a licence is renewed.

Repeated application of the vaccine in a single dose

In some monographs, the repeated injection of a single dose is required in the TAST, although this is already investigated during the licensing procedure and, in cases where sensitisation occurs, it must be mentioned in the SPC. So far, experience with the products concerned shows that there had been no case where a batch caused problems. Since there is no evidence to support the performance of the TAST with repeated single doses, this expensive and time-consuming test design should be modified.

Seronegative/susceptible animals

The monographs deviate with regard to specification of the immune status of the animals to be used. Terms such as *susceptible* or *most sensitive* are frequently used, whereas some monographs require the use of antibody-free animals and others do not specify their status at all. If the immune status or the susceptibility of animals is important for the use of a vaccine, then this problem should be addressed during the development of the product. In routine batch control, the TAST should reflect the status of animals in the environment where the product will be used. Thus, the specification of immune status or susceptibility should not be necessary, unless there is a very special reason for it.

Overdose

Currently, the animals are injected with twice (inactivated vaccines) or ten times (live vaccines) the dose recommended for the product. However, there is no scientific reason for this. In fact, the effect of overdosing has to be intensively investigated during the licensing procedure. For some vaccines, it is well known from the licensing procedure that the injection of an overdose may induce severe local reactions (for example, oil-adjuvanted products, such as Johne's disease vaccine, fish vaccines). This information is included in the SPC. Therefore, the routine application of a potentially harmful overdose of a product, without gaining safety-relevant information, is a matter of animal welfare concern.

If the TAST could be performed with the recommended dose, it would be possible to combine the TAST with the batch potency testing in many cases. This would result in a considerable reduction of the numbers of animals used, at least for some vaccine categories, such as poultry and fish vaccines.

Requirements for the performance of the TAST

In general, safety tests have to be performed according the principles of GLP. This should apply to species which are considered as laboratory animals according to the Annex 1 of Article 21 of *Directive 86/609/EEC*.[18] However, for farm animals and companion animals, the manufacturer may perform the test in agreement with the competent authority under conventional conditions. The manufacturer then has to provide sufficient evidence that the test was performed in accordance with Appendix A (Guidelines for accommodation and care of animals), Article 5 of the *European Convention 123*[19] and Article 5 of *Directive 86/609/EEC*, and GLP-like conditions and results can be guaranteed.

The crucial clinical parameters (clinical signs, body weight, body temperature, local reactions) should be recorded in data sheets, which take into account the characteristics of the tested vaccine and of the animal species used. These data sheets should be part of the batch records and should be submitted to the competent authority, thus avoiding unnecessary repetition of the TAST.

Number of test samples

At the moment, how many samples should be tested is not clear. In practice, some manufacturers test, in the case of inactivated vaccines, the bulk material or, in the case of live vaccines, pool a number of vials of the finished product, to reduce the numbers of animals used.

The test samples should be taken from a batch produced according to the manufacturing process

Table 4. Number of batches of immunologicals sent to seven OMCLs during 1994–1997

Vaccines for	1994	1995	1996	1997	Total
Horses	119	127	141	143	530
Cattle	353	384	399	436	1572
Swine	546	609	733	794	2682
Sheep and goats	61	43	35	41	180
Dogs	390	422	668	662	2142
Cats	172	194	239	223	828
Foxes and mink	61	67	116	70	314
Rabbits	70	81	73	121	345
Chicken	518	551	624	625	2318
Pigeons	36	24	35	33	128
Turkeys	14	15	15	21	65
Other birds	10	5	7	5	27
Fish	14	7	6	12	39
Total	2364	2529	3091	3186	11 185

described in the application for marketing authorisation, which is usually a vial of the finished product. To increase the sensitivity of the test, a minimum of three samples of the finished product should be tested in at least two animals. However, with the agreement of the competent authority, the TAST might be performed with final bulk material for products, where the bulk material is identical with the final product and contamination during the filling procedure can virtually be excluded.

Re-use of animals

At the moment, it is not clear whether or not animals can be re-used. When seronegative or susceptible animals are required (see above), re-use of animals is not possible; however, it should be allowed for products, which are not related.

Retrospective analysis of TAST data

Data collected from OMCLs

A detailed questionnaire was sent to the 23 OMCLs, which according to the OMCL Directory of the European Directorate of the Quality of Medicines (EDQM; Council of Europe, Strasbourg, France), are performing the TAST. The OMCLs were asked to provide data on batches of veterinary vaccines submitted during 1994–1997. Seven of 23 OMCLs replied and returned the completed questionnaire. As they only represent about 30% of the total, the reliability of the data might be questioned. However, the seven laboratories were from large, medium-sized and small countries, in central, southern and northern Europe. Therefore, the results of the questionnaire can be considered as representative for Europe, although should be evaluated with care. Furthermore, it should be borne in mind that these are the only available data so far.

During 1994–1997, more than 11 000 batches were submitted to the seven OMCLs for official batch release, i.e. 2800 batches per year (Table 4). Most of the batches were released for use in pigs and chickens. Table 4 gives detailed information on the distribution between the different species. For the interpretation of these data, it has to be borne in mind that manufacturers still have to ask for national batch release in each country. Therefore, some batches may have been counted more than once in these tables.

In addition to the testing by the manufacturers, the OMCLs are entitled to repeat the tests performed for batch release. Thus, a total of 670 batches (Table 5) were re-tested by the seven OMCLs during 1994–1997. 665 batches passed the first TAST, three the second test and one failed. A closer look at Table 5 reveals that the pattern of re-testing was not random, but was clearly related to the availability of the animal species. Poultry and rabbit vaccines, where the target species itself is a laboratory animal, are more-or-less routinely tested, whereas vaccines for pets and larger animals (cattle and horses) are very rarely tested. The OMCLs were also asked to specify the reasons why batches did not receive a batch release certificate; for example, whether the company withdrew the application for batch release or the OMCL detected quality deficiencies. A total of 89 batches were not released during the indicated time period. Only in four cases

Table 5. Number of batches which were tested in the TAST by the seven OMCLs

Vaccines for:	1994–1997	Passed	Passed after test repetition	Failed
Horses	5	5		
Cattle	11	11		
Swine	132	131	1	
Sheep and goats	120	116	3	1
Dogs	4	4		
Cats	2	2		
Foxes and mink	—	—		
Rabbits	148	148		
Chicken	229	229		
Pigeons	13	13		
Turkeys	5	5		
Ducks and geese	1	1		
Fish	—	—		
Total	670	665	4	1

(~5%) was this based on concerns about safety, and in only one of the four cases, was the batch not released due to failure in the TAST.

Data collected from vaccine manufacturers

Fourteen manufacturers of the Biologicals Working Group of the European Federation of Animal Health (FEDESA) provided data on the TAST for 1997–1999. Not all of the manufacturers provided data for all the requested period, whereas others gave information for longer periods.

The following limitations of the data collected should be considered: two manufacturers could not provide data for all the animal species; and international manufacturers with several production sites sent in data from various sites, which could not be summarised in one table. Therefore, the various production sites of the same manufacturers are listed as individual manufacturers in the tables; the information on test repetitions or failures in the TAST is not complete since not all manufacturers provided it. Therefore, only few examples or reasons can be given.

Table 6 gives an overview on the total number of batches tested in 1997–1999, and lists them according to different animal species. Almost 50% of the batches were chicken vaccines, followed by pig vaccines and dog vaccines. This explains the high number of chickens used, since the monographs on chicken vaccines stipulate 10 animals for the TAST, in contrast to only two animals for mammalian vaccines. The incidence of test repetition, pass and failure of re-tested samples is shown in Table 7. More than 98% of the batches passed the TAST without problems. The percentage of test repetitions reported by manufacturers was between 0·3% and 5·6%. Most interestingly, more than one-third of the manufacturers never had to repeat a test and nearly two-third of them never had a failure during this time period. Table 8 reveals a similar pattern for the different animal species. For seven species, no batch failed the TAST, and for three species not even a repetition was necessary. In those species, where testing in a high number of animals is requested (chicken, fish, rabbits and other birds), no failure occurred. In total, only seven (0·06%) batches failed the TAST during 1997–1999, which means that

Table 6. Number of batches tested for different animal species in the TAST by 14 manufacturers (1997–1999)

Species	Total number of batches
Horses	226
Cattle	602
Swine	2327
Sheep and goats	411
Dogs	1570
Cats	501
Rabbits	141
Chicken	5148
Pigeons	64
Turkeys	141
Other birds	170
Fish	85
Total	11 386
Average *per annum*	3795

Table 7. TAST repetitions and failures at vaccine manufacturers (1997–1999)

Manufacturer no	Number of batches	Repetition: pass	Repetition: failure
1	43	2 (4·6%)	0
2	95	0	0
3	564	0	0
4	1036	9 (0·86%)	2 (0·19%)
5	1386	0	0
6	88	0	0
7	355	10 (2·81%)	2 (0·56%)
8	497	28 (5·63%)	0
9	324	1 (0·30%)	1 (0·03%)
10	283	2 (0·70%)	0
11	2108	59 (2·79%)	0
12	2466	51 (2·06%)	1 (0·04%)
13	1783	53 (2·97%)	1 (0·05%)
14	358	0	0
Total	11 386	215 (1·8%)	7 (0·06%)

Table 8. TAST repetitions and failures at vaccine manufacturers, listed by species (1997–1999)

Species	Number of batches	Repetition: pass	Repetition: failures
Horses	226	13 (5·75%)	1 (0·44%)
Cattle	602	17 (2·82%)	1 (0·16%)
Swine	2327	30 (1·28%)	1 (0·04%)
Sheep/goats	411	7 (1·70%)	1 (0·24%)
Dogs	1570	45 (2·86%)	3 (0·19%)
Cats	501	9 (1·79%)	0
Rabbits	141	0	0
Chicken	5148	85 (0·16%)	0
Pigeons	64	0	0
Turkeys	141	7 (4·96%)	0
Other birds	170	0	0
Fish	85	2 (2·35%)	0
Total	11 386	215 (1·8%)	7 (0·06%)

99·9% of the batches passed the TAST in the quality control conducted by the manufacturers.

Unfortunately, it was not possible to calculate the exact number of animals needed for the TAST by the manufacturers. However, the batch release data from manufacturers permitted the calculation of the minimum numbers for the individual species (Table 9). Taking into consideration that the minimum numbers of animals stipulated in the monographs were used, that not all of the manufacturers contributed to the data collection, and that several manufacturers could not provide data for all the species or for the whole period, the total number of animals used would have been significantly higher.

Pharmacovigilance data of immunologicals

It was only possible to get pharmacovigilance data from the U.K. and Germany for the time period investigated in this study.

In Germany, the pharmacovigilance system noted three products/product groups which caused serious adverse reactions during the period 1992–1996.[20] A bacterial vaccine for sheep caused wool loss at the injection site, arthritis and lameness, abortion and birth of weak lambs, hyperthermia, paresis and wasting. More than 1000 sheep died or had to be slaughtered. Ringworm vaccines used in cattle induced hypersensitivity and anaphylaxis reactions,

Table 9. Estimated number of animals required during 1997–1999 by 14 manufacturers for the testing of 11 386 vaccines batches in the TAST

Species	Total number of batches	Estimated numbers of animals*
Horses	226	452
Cattle	602	1204
Swine	2327	4654
Sheep and goats	411	822
Dogs	1570	3140
Cats	501	1002
Rabbits	141	705
Chickens	5148	51 480
Pigeons	64	320
Turkeys	141	705
Other birds	170	850
Fish	85	850
Total	11 386	66 184
Average *per annum*	3795	22 061

*Based on minimum *Ph. Eur.* requirements.
—2 animals per test for immunologicals for horses, cattle, pigs, sheep and goats, dogs and cats.
—5 animals per test for immunologicals for rabbits, pigeons, turkeys, ducks, geese, canaries.
—10 animals per test for immunologicals for chickens.
—10 animals per test for immunologicals for fish.

polyarthritis and lameness were also reported. Vaccination of cattle with BVD/BRSV vaccines led to hypersensitivity, and symptoms of respiratory disease reactions. However, all of the vaccine batches associated with these reactions had passed the TAST (Werner, personal communication).

Two recent cases also show that passing the TAST does not guarantee a safe vaccine. As Falcone *et al.*[21] reported, hundreds of cattle died after vaccination in the Netherlands, because the infectious bovine rhinotracheitis (IBR) vaccine used had been contaminated with bovine viral diarrhea (BVD) virus. The IBR vaccine had been tested according the *Ph. Eur.* monograph and had been released. Since the serological status of the two calves used in the TAST had not been BVD negative, the contamination with BVD could not be detected. In France, the marketing authorisation for a dog vaccine was withdrawn, when several puppies died of distemper after vaccination.[22]

Conclusions

General

- There is no doubt that safety must be the first priority in the quality control of immunologicals. Nowadays, however, immunologicals are produced according to a seed lot system and in line with GMP/GLP regulations. The introduction of GMP and GLP should indirectly reduce the number of animals used in the quality control of veterinary vaccines, since adhering to GMP and GLP principles increases consistency in production, and furthermore, the tests used are standardised and the results have become more reliable. Thus, the situation has significantly changed since the TAST was introduced, so the relevance of the TAST as a routine batch test has recently been questioned.

European and international guidelines, directives and monographs

- The TAST is required in the general *Ph. Eur.* monograph *Vaccines for veterinary use*, and in EU directives and guidelines, and has to be performed routinely on every single batch. Furthermore, the TAST is stipulated by most individual *Ph. Eur.* monographs, but which differ in test design.
- EU Guidelines and *Ph. Eur.* monographs are not harmonised and differ with regard to the necessity of the TAST (for example, immunosera) and the number of animals requested (for example, fish vaccines).

- With respect to international harmonisation, it is important to highlight that the requirements for safety testing, for example, in the United States, considerably differ from those in Europe.

OMCL data

- Seven OMCLs participated in the study. They received more than 11 000 batches of immunologicals during the evaluation period, and repeated the TAST for 670 batches (about 6% of all batches). More than 99% of the batches passed, and only one batch (0·15%) failed the TAST.
- More than 50% of the tests were performed in chickens and rabbits, which are readily available laboratory animals, whereas 6 (0·9%) tests were performed in pet animals and only 16 (2·4%) tests were performed in horses or cattle. This shows that retesting by the OMCLs is not randomly performed and does not represent the whole spectrum of products.
- OMCLs focused their retesting on animal species where (at least in the evaluation period) no batch failed in the quality control by the manufacturers. However, retesting of vaccines for farm animals and dogs, where a few failures occurred when retested by OMCLs, was very rarely performed. Therefore, the retesting of batches with the TAST by the OMCLs is of questionable value.

Data from manufacturers.

- 14 manufacturers of the Biologicals Working Party of the European Federation of Animal Health (FEDESA) participated in the survey and provided data on the TAST. More than 98% of the batches passed in the first test and only seven batches (<0·05%) failed the TAST. Some manufacturers never had to repeat the TAST. No vaccine batch intended for use in poultry, fish, cats or rabbits failed.

Pharmacovigilance data

- Pharmacovigilance data from Germany and U.K. show that all batches which showed adverse reactions had passed the TAST, which means that no *abnormal local or systemic reactions* had been detected.

Relevance of the TAST

- The outcome of this study shows that the TAST as a routine batch test is no longer relevant for the safety of immunobiologicals. Vaccine batches are hardly ever rejected because of failure in the TAST. Therefore, the TAST should be omitted as a routine batch control test.
- In special cases, where the TAST might still be required (for example, for new products or for a certain period after licensing, or for vaccines which caused serious pharmacovigilance problems), clear guidance should be given on the test design (animal number, dosage) and on the evaluation criteria (acceptable/non-acceptable local and systemic reactions, test repetitions).
- At the moment, the design of the TAST and the evaluation criteria given in the *Ph. Eur.* monographs differ considerably. Therefore, a guideline which gives a general framework and a standardised approach for the TAST would be very helpful. Particular aspects of the individual vaccines could then be specified in the relevant monographs. As there are considerable differences between mammalian, avian and fish vaccines, these groups should be treated separately.

Recommendations

1. The general *Ph. Eur.* monograph, *Vaccines for Veterinary Use*, should be revised and the TAST should be deleted as a routine batch control test. In cases where the TAST is considered to be still needed, guidance should be given on the test design and evaluation criteria.
2. The Committee for Veterinary Medicinal Products (CVMP) of the European Medicines Evaluation Agency (London, U.K.) should revise the EU guidelines for immunobiologicals and delete the TAST as a routine batch control test.
3. The TAST should immediately deleted from those *Ph. Eur.* monographs for which sufficient evidence is available to justify its deletion (for example, for clostridial vaccines, erysipelas vaccines, immunosera).
4. The Group of Experts 15V of the European Pharmacopoeia Commission and the Immunological Veterinary Medicinal Working Party (IWP) of the CVMP should work on the harmonisation of the TAST requirements in the *Ph. Eur.* and in the EU guidelines and to provide general guidance on the test design and evaluation criteria for those products or cases where the TAST is still considered to be still necessary.
5. The Pharmacovigilance Working Party of the CVMP should provide an annual pharmacovigilance report for all immunobiologicals in the EU and should discuss the results with the

IWP to ensure that existing and arising safety concerns for specific products and/or product groups can be adequately addressed.

6. The Safety Testing Working Party of the Veterinary International Cooperation on Harmonisation should include harmonisation of batch safety testing of immunobiologicals in its work programme.

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