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***A**dvisory **G**roup on **A**lternatives to **A**nimal **T**esting in **I**mmunobiologicals*

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EVALUATION OF THE RELEVANCE OF THE SAFETY TEST FOR THE QUALITY CONTROL OF VETERINARY IMMUNOLOGICAL MEDICINAL PRODUCTS

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LIST OF ABBREVIATIONS

Abbreviations	Meaning
ADR	Adverse Drug Reaction
AHP	Animal Health Products
ATT	Abnormal Toxicity Test
BVD	Bovine Viral Diarrhoea
CFR	Code of Federal Regulations
CPMP	Committee for Proprietary Medical Products
CVMP	Committee for veterinary medicinal products
e.g.	For example
EC	European Community
EDQM	European Division for the Quality of Medicines
EEC	European Economic Community
EMEA	European Agency for the Evaluation of Medicinal Products
EU	European Union
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GRIMV	General requirements for inactivated mammalian vaccines
GRLMV	General requirements for live mammalian vaccines
i.e.	That is
IVMP	Immunological Veterinary Medicinal Product
IWP	Immunologicals Working Party
MS	Member States
NCA	National control authority
OECD	Organisation for Economic Cooperation and Development
OIE	Office International des Epizooties
OMCL	Official Member States Control Laboratory
Ph. Eur.	European Pharmacopoeia
R & D	Research and Development
SOPs	Standard Operating Procedures
SPC	Summary of Product Characteristics
SPF	Specific pathogen free
SR	Specific Requirements

TAST	Target animal safety test
VICH	Veterinary International Committee for Harmonisation of Technical Requirements for the Registration of Pharmaceuticals
WHO	World Health Organisation
WP	Working Party

I. Introduction

Infectious diseases are a constant threat to the health of animals. It is undeniable that vaccines are important for maintaining the health of all farm animals and also for the increasing number of animals kept as companion animals.

Size of Immunological Veterinary Medical Product Markets in the EU Member States in 1992

Country	Ratio IVMP / AHP*
Belgium / Luxembourg	29 %
Denmark	26 %
France	18 %
Germany	22 %
Greece	19 %
Ireland	14 %
Italy	24 %
Netherlands	20 %
Portugal	12 %
Spain	18 %
U.K.	26 %
Average	**21 %**

AHP*: Animal Health Products

The market for Immunological Veterinary Medical Products (IVMPs) is still growing. This is due to

- concerns over drug residues favouring the use of vaccines rather than pharmaceuticals
- expanding markets in countries which are attempting to increase agricultural production
- increasing emphasis on prevention of disease rather than treatment
- increasing number of pets in countries, where spending on pet health care is relatively high
- development of new products

- improvement of existing products and administration techniques using both conventional and biotechnological methods
- better understanding of diseases and mechanism of immune response
- new diseases

There are still several animal infectious diseases for which vaccines are not yet available. This may be because it has not been possible to grow the micro-organism in a practical manner, or to isolate the protective antigen, or because the induction of protective immunity is very complex and difficult to achieve.

Biotechnology has provided tools to tackle these difficulties. This is one of the reasons why diagnostics and vaccines are one of the most active areas for veterinary biotechnology R&D (FEDESA, 1994).

A reliable supply of pure, safe, potent, and effective vaccines is essential for maintenance of animal health and successful operation of animal health programmes. Immunisation of animals with high quality vaccines is the primary means of control for many animal diseases. In other cases, vaccines are used in conjunction with national disease control or eradication programmes.

It is generally accepted that the quality of vaccines must be controlled and the animal-based tests may be necessary for this purpose. The most obvious way to assess the safety of veterinary vaccines is to use target animals for those experiments.

The safety test in the target species was introduced some decades ago during the development of the first veterinary vaccines (Zeegers et al., 1997).

Whenever a safety test is deleted from a monograph or other requirement, the rationale is that the test has been used for decades and has not shown beneficial results (Castle, 1993). This has recently been the case for the abnormal toxicity test (ATT) in all veterinary vaccine monographs of the *Ph.Eur.* and for the mouse safety test for erysipelas vaccines. However, manufacturers and control authorities still seem to feel uneasy about omitting safety tests even if there is no scientific justification of their value. For human vaccines it was even stated that it would be unethical to inject a biological product into a human being unless it has first been tested in animals (Castle, 1993). For albumin and immunoglobulins some people only accepted deletion of the ATT because there was still a rabbit pyrogen test in the monograph (Castle, 1993).

It must be remembered that quality control is a dynamic, not a static process. Scientific knowledge accrues with time, and quality control tests can change accordingly. Unfortunately, tests do not always keep pace with production methods. Sometimes old quality control tests are still applied, even though in principle new

techniques and developments are available for more precise tests, which might sometimes yield even more information.

Without animal experiments the quality of vaccines would not have been as good as it is today, and their successful use on such a large scale would never have been possible. However, the ethics of animal experiments have increasingly been questioned.

II. Monographs and Guidelines which directly stipulate the target animal safety test (TAST)

As with all other medicines, veterinary biologicals are subject to statutory regulations concerning their production, quality and use. Various official commissions and other institutions are responsible for formulating and implementing these regulations. While some regulations are legally binding (e.g. EU Directives or Pharmacopoeia Monographs) others provide “only” guidance (e.g. WHO and OIE Requirements or EMEA and EU Guidelines). Other International regulations may become important in the near future when these issues are discussed at the VICH (see below). Therefore it seems necessary to give an overview on the most important European and international regulations which require the TAST. A list of the most important regulatory documents is given in the Annex.

II.1 European Union Directives and Guidelines

The pharmaceutical legislation of the European Community, which has evolved over a 30-year period, covers both medicinal products for human and veterinary use. Harmonisation of requirements in the area of veterinary medicines began in 1981 with the adoption of Directives 81/851/EEC and 81/852/EEC, laying down common requirements for manufacturing and marketing authorisation, based on the evaluation of the quality, safety and efficacy of the product.

In 1995, a new European system for the authorisation of medicinal products came into force and the European Agency for the Evaluation of Medicinal Products (EMEA) was established. This agency formulates opinions and, apart from the administrative staff and the management board, is composed of two scientific committees, the CPMP (Committee for Pharmaceutical Medicinal Products) in charge of medicinal products for humans and the CVMP (Committee for Veterinary Medicinal Products) in charge of the animal health sector.

The CVMP is responsible for the evaluation of applications for marketing authorisation for products derived from biotechnology, for productivity enhances, new chemical entities and other innovative new products. Among the working parties, the Immunological Veterinary Medicinal Working Party (IWP) has to advise the CVMP for example on general policy issues such as the elaboration and revision of guidelines

on immunological products. The guidelines for the testing of veterinary medicinal products are listed in Volume VII of the *Rules Governing Medicinal Products in the European Union*.

II.1.1 European Union Directives

In accordance with Directive 81/851/EEC, authorisation is required for the manufacture of veterinary medicinal products, including immunologicals. This directive provides for regular inspections and stipulates that manufacture must be supervised by a 'qualified person', who certified that each batch is in conformity with the approved specifications for the product including safety tests.

In the special case of immunological veterinary medicinal products, Directive 90/677/EEC allows those Member States that consider it necessary to ask for the submission of samples of each production batch of the bulk and/or finished product for examination by a control laboratory before that batch is placed on the market. In this context OMCLs are also performing animal experiments such as the TAST. Except in specially justified circumstances, batch release carried out by one national control laboratory must be recognised without repetition by the other Member States. Directive 81/852/EEC describes the requirements for the demonstration of the quality, safety and efficacy of veterinary medicinal products.

II.1.2 European Guidelines

"The Rules Governing Medicinal Products in the European Union" incorporates testing guidelines prepared within the EU. Volume 7 lists the Guidelines for Veterinary Medicinal Products. The guidelines on the quality of IVMPs are included in Volume 7B.

These guidelines are intended to provide a basis for a practical harmonisation of the manner in which the Member States (MS) and the EMEA interpret and apply the detailed requirements for the demonstration of quality, safety and efficacy stipulated by the Community directives.

Table 1: European Guidelines stipulating the TAST

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*

The use of guidelines, which are not legally binding, rather than a formal legal instrument, such as a directive, has been preferred in order to maintain an element of flexibility and not to place undue legislative restraints on scientific progress. It is recognised that in some cases, as a result of scientific developments, an alternative approach may be appropriate. However, where an applicant chooses not to apply a guideline, that decision must be explained and justified in the Expert Reports submitted by the company in support of the application.

The guidelines have been prepared by the CVMP of the EMEA, in consultation with the competent authorities of the MS.

It is the purpose of the guidelines to provide more details on standards that are likely to be acceptable and they are therefore supplementary to the Directives. The two general guidelines “General Requirements for Live Mammalian Vaccines” (GRLVM) and “General Requirements for Inactivated Mammalian Vaccines” (GRIVM) are building a framework for eight specific requirements (SR). The guidelines were adopted between 1992 and 1994. They are produced on a species basis and include information on live and inactivated vaccines. Serum derivates are covered by a separate SR. They are to be as detailed as possible so as to provide as much guidance as possible to manufacturers and to increase harmonisation of requirements within Member States. They contain sections on in-process and final product tests. Each guideline specifically mentions the TAST as a final product test. Thus the TAST is a general requirement in the EU guidelines.

By their very nature, the guidelines must be updated in the light of scientific and technical progress. However, so far no revisions or updates have been made.

Table 2: TAST requirements in EU guidelines for the finished product

Guideline	Text
GRLMV	Using at least the recommended route of administration posing the greatest risk, each batch of vaccine shall be shown to be safe in at least two susceptible animals from the most sensitive target species for which the vaccine is recommended. A ten-fold dose of vaccine shall be administered. For products in liquid form, a double-dose may be acceptable. The animals shall be observed for at least 14 days for adverse effects attributable to the vaccine.
GRIMV	The vaccine shall be shown to be safe in two susceptible animals of one species and one class for which the vaccine is recommended. The animals are given a double dose of vaccine. Rectal temperatures and any adverse local or systemic reactions should be recorded. Where required in specific *Ph.Eur.* monographs: a) the double dose shall be followed by administration of a single dose 14 days later; and/or b) serum samples shall be obtained from the animals two weeks after the last vaccination and test for the absence of antibodies to organisms pathogenic for the species and antibodies to other organisms handled on the premises. Additional tests may be imposed at times of serious disease in the vicinity of the manufacturing premises. It is the responsibility of the manufacturer to notify the competent authorities of the circumstances.
SR Avian Live Vaccines	The batch of vaccines to be tested shall be diluted in the batch of diluent with which it is to be marketed, if appropriate. **Vaccines for use in chickens from 2 weeks of age** For vaccines recommended for use in chickens, but not in birds under two weeks of age, satisfactory results from the test for safety and extraneous agents in chicks will normally be sufficient evidence for the safety of each batch of vaccine provided that a post mortem examination is carried out on any birds which die during the period of the test. **Vaccines for use in chickens under 2 weeks of age** Each batch of vaccine recommended for use in chickens under two weeks of age shall be shown to be safe in at least 10 SPF day-old chicks unless a different number is prescribed in a specific monograph of the *Ph.Eur.* and the vaccine shall be administered by the route stated in the monograph. Where no monograph exists, vaccines to be administered by spray or drinking water shall be given by eye-drop. Otherwise, the vaccine shall be administered by one of the recommended routes. Each bird shall receive at least 10 field doses of vaccine. The inoculated birds shall be observed for at least 21 days for adverse effects attributable to the vaccine. **Vaccines for use in birds other than chickens** Using one of the recommended routes of administration, each batch of vaccine recommended for use in avian species other than chickens shall be shown to be safe in at least 10 birds of the target species and of the most sensitive target class and age. Each bird shall receive at least 10 field doses of vaccine. The inoculated birds shall be observed for at least 21 days for adverse effects attributable to the vaccine. If any of the birds develop significant local or systemic reactions, or if any respiratory or nervous signs or other of disease are seen, other than mild signs that are attributable to the vaccine, then that batch is unsatisfactory and shall be discarded.

SR Avian Inactivated Vaccines	In the safety test, 10 healthy birds, at least 14-28 days of age, or of the youngest age recommended for vaccination if less than 14 days of one of the target species shall be inoculated with two field doses by a recommended route and observed for three weeks for signs of abnormal local or systemic reactions.
SR Bovine Live and Inactivated Vaccines	To comply with the requirements of GRIMV and GRLMV, the vaccine shall be injected to two susceptible calves aged two to five months, or the minimum age for vaccination where this is less than two months of age.
SR Ovine and Caprine Live and Inactivated Vaccines	To comply with the requirements of GRLMV or GRIMV, the vaccine shall be injected to two susceptible lambs or kids, as appropriate, aged two to five months or the minimum age for vaccination where this is less than 2 months of age. If the product is not to be used in animals younger than 5 months, the safety test may be carried out in two susceptible animals aged 6 to 12 months.
SR Live and Inactivated Vaccines for Fish	The test shall employ the target species of fish. If a vaccine is intended for use in several species, the species most sensitive to infectious agents in question should be used. For vaccines intended for individual administration, at least 30 susceptible fish should be vaccinated and for vaccines intended for oral-, spray-m bath- or dip-vaccination, at least 50 fish should be employed. Fish of minimum age and/or size recommended for vaccination should be used. Vaccination should be carried out according to label instructions. A control group of a composition similar to the vaccinated group should undergo mock-vaccination. During the following 21 days, vaccinates and controls should be held at an environmental temperature relevant to the species and observed every day for mortality and adverse effects. Mortality among vaccinates should not exceed that of controls, and no clinical symptoms of ill health should appear in any surviving vaccinated. The cause of mortality should be assessed. If in the case of 30 vaccinated, more than 2 fish die or if more than 3 out of 50 vaccinates die during the observation period, the safety test must be repeated once. If in the second test the specified limits for mortality are again exceeded, the batch is considered unsafe and must be discarded.
SR Live and Inactivated Vaccines for Cats and Dogs	The requirements for GRLMV or GRIMV of the appropriate section shall apply
SR Immunosera and Colostrum Substitutes	See GRIMV 3.2 In general, for this test, one of the species for which the product is to be recommended shall be given a dose of product equivalent to a double dose for that species. Where it is considered that double dose testing is inappropriate, a safety test using at least a single dose should be performed.

II.2 European Pharmacopoeia

The European Pharmacopoeia (*Ph.Eur.*) has the usual functions of the various national pharmacopoeias and, in addition, has the role of harmonising the specifications contained in the pharmacopoeias of its MS.

The purpose of the European Pharmacopoeia is to promote public health by the provision of recognised common standards for use by health care professionals and others concerned with the quality of medicines. Such standards are to be of appropriate quality, as a basis for the safe use of medicines by patients and consumers.

European Pharmacopoeia monographs and other texts are designed to be appropriate to the needs of:

- regulatory authorities;
- those engaged in the control of quality;
- manufacturers of starting materials and medicinal products.

For a long time the *Ph.Eur.* took a 'classical' view of vaccine monographs, concentrating on the finished product available as a commercial article that an independent analyst could obtain and test according to the specifications. Recently it was decided that monographs would, in future, treat all aspects of quality throughout the manufacturing process. A new Production section has been added to each monograph which sets out the essential features and control requirements along the manufacturing process. This section includes animal tests for the demonstration of safety and immunogenicity under the heading "choice of vaccine composition".

The production of new, and the revision of existing monographs has accelerated with the concomitant harmonisation of European registration legislation. New or revised monographs, or general chapters, are constantly being added. Before their adoption, all monographs are published for inquiry and comments in the Forum of the European Pharmacopoeia, *Pharmeuropa*. In addition, the European Pharmacopoeia endorsed the aims of the European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purposes, and encouraged its groups of experts to take into account the 'three Rs rule' and review all existing animal tests.

The *Ph.Eur*. set mandatory standards for all products covered by monographs. The general monograph 62 on *Vaccines For Veterinary Use* includes the requirement for safety testing during the development of a vaccine which is described in detail in the section *5.2.6 Evaluation of safety of veterinary vaccines*. In the section TESTS this monograph also requires the performance of a safety test using animals of the target species (see below). This requirement is repeated but also specified in most monographs for IVMPs (see Table 3).

Monograph 062: Vaccines for veterinary use

Safety. In general, two doses of an inactivated vaccine and/or ten doses of a live vaccine are injected by a recommended route. For combined vaccines, the test may be carried out on the separate components when the presentation of the vaccine permits this; if this is not possible, it may be necessary to reduce the prescribed number of doses of a live vaccine for use in the test if the injection of ten doses would necessitate injection of an unacceptably high amount of an inactivated component. The animals are observed for the longest period stated in the specific monographs. No abnormal local or systemic reaction occurs.

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Indeed, the specific monographs vary considerably in the outline for the test. Furthermore, there are some monographs where no TAST is required (for details see Section IV.1). As the TAST is different in many *Ph.Eur.* monographs it is difficult to discuss this as a general issue. Indeed, product or monograph specific aspects have to be taken into account.

Table 3a: *Ph.Eur.* monographs which are not stipulating the TAST

- Tetanus vaccine for veterinary use (1997; 697)
- Rabies vaccine (live, oral) for foxes (1997; 746)
- Immunosera for veterinary use (1997; 30)
- Clostridium novyi alpha antitoxin for vet. use (1997; 339)
- Clostridium perfringens beta antitoxin for vet. use (1997; 340)
- Clostridium perfringens epsilon antitoxin for vet. use (1997; 341)
- Gonadotropin, equine serum for vet. use (1997; 719)

Table 3 b: *Ph.Eur.* monographs which are stipulating the TAST

***General* Monograph**	**Monographs for *Inactivated Vaccines***
Vaccines for Veterinary Use	Aujeszky‘s disease vaccine for pigs
Monographs for *Live Vaccines*	Avian infectious bronchitis vaccine
Aujeszky‘s disease vaccine for pigs	Avian infectious bursal disease vaccine
Avian infectious bronchitis vaccine	Avian paramyxovirus 3 vaccine
Avian infectious bursal disease vaccine	Canine adenovirus vaccine
Avian infectious encephalomyelitis vaccine	Canine parvovirosis vaccine
Avian infectious laryngotracheitis vaccine	Clostridium botulinum vaccine
Bovine parainfluenza virus vaccine	Clostridium chauvoei vaccine
Bovine respiratory syncytial virus vaccine	Clostridium novyi (type B) vaccine
Brucellosis vaccine (Brucella melitensis Rev. 1 strain)	Clostridium perfringens vaccine
Canine contagious hepatitis vaccine	Clostridium septicum vaccine
Canine distemper vaccine	Egg drop syndrome ’76 vaccine
Canine parvovirosis vaccine	Equine influenza vaccine
Distemper vaccine for mustelids	Feline calicivirosis vaccine
Duck viral hepatitis vaccine	Feline infectious enteritis vaccine
Feline calicivirosis vaccine	Feline leukaemia vaccine
Feline infectious enteritis vaccine	Feline viral rhinotracheitis vaccine
Infectious bovine rhinotracheitis vaccine	Foot-and-mouth disease vaccine
Marek‘s disease vaccine	Furunculosis vaccine for salmonids
Feline viral rhinotracheitis vaccine	Leptospira vaccine
Fowl-pox live vaccine	Neonatal piglet colibacillosis vaccine
Newcastle disease vaccine	Neonatal ruminant colibacillosis vaccine
Swine-fever vaccine, classical	Newcastle disease vaccine
	Porcine actinobacillosis vaccine
	Porcine influenza vaccine
	Porcine parvovirosis vaccine
	Porcine progressive atrophic rhinitis vaccine
	Rabies vaccine
	Swine erysipelas vaccine
Monograph for Immunosera	Vibriosis (cold-water) vaccine for salmonids
Swine erysipelas Immunoserum	Vibriosis vaccine for salmonids

II.3 International Organisations

II.3.1 O.I.E.

The 4th edition of the OIE Manual of Standards for Diagnostic Tests and Vaccines, edited by the Standards Commission, was published in 2000 and has been distributed world-wide. It contains recommendations for (a) “prescribed tests” for diagnosis, and (b) requirements for vaccines for list A and B diseases (OIE Manual, 2000).

The chapter of the OIE Manual concerning vaccines contains information on recommended vaccines, data on seed management, characteristics of the vaccine strains, culture, validation as a vaccine, manufacture, in-process control, sterility tests, safety tests, and potency tests. At present, OIE is distributing information contained in the OIE Manual and annual reports of the OIE Standards Commission which undoubtedly contribute to international harmonisation.

In 1994, the OIE set up an Ad hoc Group on the harmonisation of veterinary medicines, which was the first step towards the creation of the ‘Veterinary International Conference on Harmonisation’ (VICH).

II.3.2 WHO

WHO has a unit “Biologicals” which is responsible for biological standardisation, mainly for human medicinal products (among them vaccines), but also for some veterinary vaccines against zoonotic diseases. This unit receives advice from the WHO Expert Committee on Biological Standardisation. With regard to products for veterinary use or of animal origin, this committee has established requirements for rabies vaccines and tuberculins (human and bovine), for anthrax spore vaccine (live for veterinary use), for immune sera of animal origin, for rinderpest cell culture vaccine (live) and rinderpest vaccine (live), and for *Brucella abortus* strain 19 vaccine and *Brucella melitensis* Strain Rev.1 vaccine (live for veterinary use). WHO is editing and distributing general guidelines for international vaccine standardisation as well.

II.4 United States Code of Federal Regulations

In the United States of America, veterinary biologics or veterinary biological products are defined as all viruses, sera, toxins or analogous products that are intended for use in the treatment (prevention, diagnosis, management, or cure of diseases) of animals and that act primarily through the direct stimulation, supplementation, enhancement, or modulation of the immune system or immune response.

The Virus-Serum-Toxin Act of 1913 (‘VST Act’), as amended, 21 U.S.C. Section 151-159, provides the legal authority for the regulation of immunologicals and biologicals

for animal use in the USA. The VST Act requires that products governed by the Act that enter channels of commerce be ''not worthless, contaminated, dangerous or harmful'. The regulatory programme implementing the requirements of the VST Act is administered by the Center for Veterinary Biologics. Administrative regulations, duly promulgated and with effect of law, are published in Title 9. Code of Federal Regulations (9 CFR) Parts 101-118.

Veterinary biologicals must meet certain basic criteria including safety requirements: the product must be safe in the target species and, if live, in species exposed to shed organisms. However, in the US several other safety tests in laboratory animals are required. The detailed requirements for each type of product including safety tests are described in Title 9 CFR Part 113. Standard procedures are given for mouse, guinea pig, cat, dog, calf, pig and sheep safety tests:

- In general, US made killed bacterial vaccines for mammalian species are tested for safety in mice and/or guinea pigs. Killed bacterial vaccines for poultry are tested for safety by observing the potency test vaccinates.
- In general, killed virus vaccines for mammalian species are tested for safety in mice and guinea pigs, and by observing the potency test vaccinates. Killed virus vaccines for poultry are tested for safety by observing the potency test vaccinates.
- In general, live bacterial vaccines for mammalian species are tested for safety in the target species (2 animals, 2X or 10X doses), and in mice if not inherently lethal. Live bacterial vaccines for poultry are tested in the target animal (typically 10 animals, 10X dose).
- In general, live virus vaccines for mammalian species are tested for safety in the target species (2 animals, 10X doses), and in mice if not inherently lethal. Live virus vaccines for poultry are tested in chickens. The latter test appears to be equivalent to the EU's safety and extraneous agents tests combined.
- In general, serum products are tested for safety in mice, and diagnostic products are tested in guinea pigs.

Regarding the 9 CFR's, the mouse safety test for inactivated products and live bacterial products is 113.33(b); 8 mice, 0.5ml IP or SQ. The mouse safety test for live virus products is 113.33(a), 8 mice, 0.5ml IP and 8 mice, 0.03ml IC. The guinea pig test is 113.38; 2 guinea pigs, 2ml IM or SQ

As in the EU, there are numerous minor exceptions specific to individual products. Table 9 gives some examples.

II.5 Japanese Requirements

Medicinal products that are exclusively used for animals, including veterinary biologicals, are under the jurisdiction of the Ministry of Agriculture, Forestry and

Fisheries, and securing their quality, efficacy and safety is included in the Pharmaceutical Affairs Law.

The minimum requirements of veterinary biological products provide information about the consistency of the manufacturing process and the quality of the product. Any product that does not conform to these product standards cannot be manufactured, imported or marketed.

In Japan the general tests have to be done in each target species of the most sensitive category using a single dose, an overdose (maximum 10-fold dose for killed vaccines, and maximum 100-fold dose for live vaccines) and a repeated single dose given with a two-month interval. A minimum of three mammals, or ten birds or twenty fish has to be used. Necropsy and histopathology examinations, haematology and, if necessary, blood chemical examination are required.

For vaccines to be used for pregnant animals, offspring are observed for adverse effects.

The presence and depletion of adjuvant residues needs to be examined visually (MAFF guideline of safety tests).

For live vaccines, the tests to demonstrate absence of reversion to virulence, vaccine virus dissemination and shedding are required. However, the tests are not categorised as part of the safety study and GLP standard is not explicitly required.

A batch safety test using the target species is required except for killed vaccine for large animals. A single dose is sufficient for most killed vaccines but overdoses are required for live vaccines.

Studies on safety using target animals are included in the Japanese Pharmacopoeia.

In principle, studies with livestock and cultured fish should be conducted on the new animal drug.

1. Animals:
   Vaccine, serum and drug for diagnosis should be tested as follows.
   a) Animal of expected age and most sensitive ages for reactions with drug should be used.
   b) Species, line criteria and situation for antibody should be clarified.
   c) In case of those drugs for pregnant animals, both pregnant and non-pregnant animals should be used.

2. Number of Animals:

Livestock and cultures fish for which the test drug is administered should be employed as test animals. For mammal, three heads or more should be used for each dose group, for poultry, 10 birds or more for each dose group and for cultured fish, 20 tails or more for each dose group.

3. Administration route:
   In principle, administration route should be in accordance with the expected clinical route. In the case of the plural administration routes, the administration route by which the strongly Adverse Reactions (ADRs) to the test drug are observed should be employed.
   However, vaccine containing adjuvant should be tested with all the expected clinical route.

4. Dose levels:
   At least two dose levels or more plus control group should be set up.
   Dose levels of test group should be employed at least adverse effect dose and non-adverse effect dose.
   - Live vaccines need preparation when it is administered ··· 100 dose.
   - Others ··· 10 doses

5. Administration period:
   - In case of vaccine, after expected administration period and frequency of administration are done vaccine should be administered more than once with interval of 2 months. When frequency of administration is limited within twice in lifetime, administration period and frequency is followed as it planned.
   - Serum and drug for diagnosis used directly to animals are administered more than twice with interval of 2 months.

6. Observation:
   - All animals in each group should be observed daily for general signs. If necessary, all or some animals of each group should be performed for haematological examinations and blood chemical analysis.
   - The animals which died during the administration period should be autopsied immediately, and gross observations, weight measurement and histopathological examinations on organs and tissues should be performed. Gross observations should be made on all organs and tissues.
   - The animals which should show apparent morbidity during the administration period should be autopsied immediately, and gross observations, weight measurement and histopathological examinations on organs and tissues should be performed.
     Haematological examinations and blood chemical analysis should be conducted on the blood collected at sacrifice.
   - At the end of the administration period, all or some of surviving animals in each group should be autopsied, and gross observation and weight

measurement on organs and tissues and histopathological examinations should be performed (in case of any anomaly in clinical symptoms are not observed at the highest dose or at the local reaction drug respectively, all the animals should not be sacrificed).
Haematological examinations and blood chemical analysis should be conducted on the blood collected at sacrifice.

- Vaccine expected to use for pregnant animals, its litter should be observed according to the tested group.
- Vaccines containing adjuvant should be observed duration of residue of adjuvant by autopsy and histopathology.

III. Other regulatory requirements which have an impact on the necessity for the TAST

III.1 Good Laboratory Practices and Good Manufacturing Practices

III.1.1 Good Laboratory Practices

GLP principles for the toxicity testing of chemicals were set up by the Organisation for Economic Co-operation and Development (OECD). These GLP principles are directly referred to in several other GLP regulations, including the EC Directive 87/18 IEEC on the application of the Principles of Good Laboratory Practice. The OECD principles of GLP which must be complied with during the conduct of safety tests on medicinal products are also included in Volume 7B of “The Rules Governing Medicinal Products in the European Union”.

The GLP regulations are concerned with the organisation of laboratory practice and research and the conditions under which this work is carried out. If GLP is followed, it must be possible to replicate tests. GLP stipulates the laboratory accommodation, equipment and test animals. Persons who carry out research must have a certain level of expertise and the responsibilities for parts of the work are divided between several people. All this leads to well controlled processes in laboratories and standardisation of tests, assuring the reliability of the data generated in the studies. Many laboratories now work according to these GLP directives, as laboratory work must comply with the GLP regulations and suggestions in order to be accepted by regulatory authorities. Governments regularly make GLP inspections.

The published GLP regulations are mainly intended for toxicological research. There are no special GLPs for vaccine research but safety studies must be carried out in conformity with GLP and by laboratories registered for GLP (Lee, 1993). Test procedures are better defined by means of Standard Operating Procedures (SOP). Fewer repetitions of tests will be necessary and test results will be more reliable. This could indirectly lead to fewer animals being used in the quality control tests (van der Kamp 1994).

Animal used for the manufacture and control of biological products shall be accommodated in separate buildings with self-contained ventilation systems. The buildings' design and construction materials shall permit maintenance in a clean and sanitary condition free from insects and vermin. Facilities for animals shall include isolation units for quarantine of incoming animals. Provision shall also be made for animal inoculation rooms, which shall be separate from the post-mortem rooms. There shall be facilities for disinfecting cages, if possible by steam, and an incinerator for disposing of waste and of dead animals.

The health status of animals from which starting materials are derived and of those used for quality control and safety testing should be monitored and recorded. Staff employed in animal quarters must be provided with special clothing, changing facilities and showers.

III.1.2 Good Manufacturing Practices

In many countries, there are both legal and functional frameworks for the application of GMP in the manufacture of veterinary vaccines. Within the EU, legislation specific to GMP is laid down in Directive 91/412/EEC, which requires that manufacturers "shall ensure that the manufacturing operations are carried out in accordance with good manufacturing practice". Similar requirements are also laid upon the importers of any product manufactured outside of the EU.

Chapter II of 91/412/EEC covers the Principles and Guidelines of Good Manufacturing Practice, describing wide-ranging summary requirements. The EU provides for the interpretation of these principles in the form of detailed guidelines published in the form of Volume IV of "The Rules Governing Medicinal Products In The European Community; Guide To Good Manufacturing Practice For Medicinal Products".

The underlying purpose that drives the development and codification of GMP is the provision of assurance that products in the market place have been manufactured in a consistent and suitable manner. Consistency is required to ensure that batches of product manufactured over many years retain the same attributes of safety and efficacy that were claimed and demonstrated by the manufacture at the time of licensing. Commercially, consistency is also required so that manufacturers can be as certain as possible that production processes work and will result in the correct product being available for marketing at the required time.

Suitable manufacturing methods and premises are also a requirement, as it is not possible to test most vaccines effectively in order to demonstrate that they are of a suitable quality. Even a basic, apparently simple, property, such as sterility, cannot be adequately demonstrated in a final batch of vaccine, as the sample size that can

be used is inadequate to detect anything other than gross contaminations. Consequently, the only reasonable assurance of sterility is that the product has been manufactured in such a way that the likelihood of it not being sterile is very small. This requires the conscientious application of very well-defined procedures, and the provision and maintenance of high quality equipment and premises (Luff and Soulebot, 1997).

One of the major influences on GMP has been the need to control the potential biological hazards of vaccine manufacture and use. In recent years much effort has been made by manufacturers, often at the insistence of regulatory authorities, to build and install high quality premises and equipment to enable them to operate at the required level of assurance.

III.2 Pharmacovigilance

The granting of a marketing authorisation for a veterinary medicinal product means that the competent authorities are satisfied that the manufacturer has demonstrated its quality, safety and efficacy in accordance with Community requirements and that the product presents a favourable benefit-risk ratio, based on the information available at that time. However, authorisation of a product does not mark the end of its supervision. On the contrary, it is the beginning of a very active and dynamic process covered by the term ‘pharmacovigilance’.
The safety and efficacy of a product are evaluated prior to marketing authorisation on the basis of the results of tests and trials carried out under defined conditions. Severe adverse reactions are in principle not acceptable for any vaccine, although they may be unavoidable. Vaccines are only permitted to cause side-effects if the disease is serious enough, if no other alternatives are available and the vaccine is sufficiently effective. For example, it is well known that low frequency reactions (generally below 1%) will most likely be overlooked during the pre-authorisation period and that they may result in major consequences when the product reaches its normal distribution level. Such reactions include development of sensitisation and cross-sensitisation or interaction with other medicinal products or contaminants. The economic impact of an unexpected reaction may be considerable.

All veterinary medicinal products are covered by the requirements on pharmacovigilance, including immunological veterinary medicinal products, which have fallen under the scope of the general veterinary pharmaceutical legislation of the EU since the adoption of Directive 90/677/EEC. The importance given to pharmacovigilance reflects the fact that a product is never entirely known and that one should carefully look at its performance in normal practice. The term includes not only spontaneous reporting, but also drug monitoring, post-marketing studies, post-marketing surveillance and field trials.

The marketing authorisation holder should report all serious ADRs occurring within the EU immediately and, in no case, later than 15 calendar days from receipt, to MS in whose territory the incident occurred. Any suspected increase in the frequency of serious reactions should also be reported.

A cumulative list of all reactions should be provided upon request, or at 6-month intervals for 2 years post-authorisation, yearly for the subsequent 3 years, and at the 5-year renewals in what is defined as a 'periodic safety update'.
The marketing authorisation holder is also expected to screen the world-wide literature and report published suspected ADRs in relation to the active substance(s) contained in his veterinary medicinal products.

This guidance is available and published in a new version of the Notice to Applicants (Volume VB of "The rules governing medicinal products in the European Union").

Suspected ADR reports can be divided into the following:
- Serious Suspected ADR Reports
- Periodic Safety Update Reports
- Human Suspected ADR Reports

Serious ADR: An ADR which is fatal, life threatening, disabling, incapacitating, or which results in permanent or prolonged signs in the animals tested.

In veterinary medicine the existence of a variety of animal species and husbandry conditions require a modified approach top the classification of a 'serious ADR'. For example in intensive animal production with species such as poultry or fish, a certain level of mortality rate is considered as 'normal' or 'expected'. These species are usually treated as a group and only an increased incidence of mortality, or severe signs, or variations of animal production levels exceeding the rates normally expected should be considered as a 'serious ADR'.

However, in species like dogs, cats or horses a single death constitutes a 'serious ADR'. This also applies to cases of individual deaths in cattle, sheep, pigs, goats and rabbits even of they are kept in herds or flocks in intensive animal production because treatment is often performed on the individual animal and therefore a single death or severe symptoms have to be considered on an individual basis.

For all species if they are kept as individual companion animals a single death constitutes a 'serious ADR'.

III.2.1 Periodic Safety Update

A periodic safety update is intended to provide competent authorities with an update of the world-wide safety experience with a veterinary medicinal product at defined

time-period after marketing authorisation. These reports should provide summary information, together with a critical evaluation of the benefit/risk of the product in the light of new or changing post-marketing information, in order to ascertain whether further investigations need to be carried out and whether changes should be made to the summary of product characteristics (SPC).

A safety update must address the relationship of sales volume of a product related to numbers of suspected ADRs reported.

For vaccines it is suggested to express this in numbers of vaccine doses.
The incidence of reactions should be calculated by dividing the total number of animals reacting during the period by the number of doses sold during the period of the report and multiplying by 100.

$$\text{Incidence} = \frac{\text{No of animals reacting during period} \times 100}{\text{No of doses sold during period}}$$

A periodic safety update summary report should be prepared at the following intervals:

- 6-monthly for the first 2 years after authorisation
- annually for the subsequent 3 years
- thereafter 5-yearly at the time of renewal.

These safety updates are carefully examined to reassess the safety profile of a particular product.

III.3 VICH

Although, agencies and regulations differ on a global basis , all strive to ensure that products offered to the end-consumer conform to basic standards. There are many procedural differences between the EU, Japan and the USA. Harmonisation between the systems should be established where possible, on the recognition of equivalence for tests and procedures that are performed to assess a vaccine and that ensure quality, safety and efficacy of the product.

VICH will focus on harmonising registration requirements for veterinary medicinal products in the EU, USA and Japan. Countries not involved in VICH will be kept informed of its progress through the OIE. The objectives of the VICH are:

- Provide a forum for a constructive dialogue between regulatory authorities and the veterinary medicinal products industry on the real and perceived differences in the technical requirements for product registration in the EU, Japan and the USA, with the expectation that such a process may serve as a catalyst for wider international harmonisation;

- VICH should be conducted in a transparent and cost-effective manner and should provide the opportunity for public comment on recommendations at the draft stage.

In order to achieve harmonisation on the selected topics, the VICH Steering Committee will appoint working groups to draft recommendations. Each working group will normally comprise six experts – one representing each VICH full member. Additional experts from observer countries – or even other countries – may be appointed by the Steering Committee if deemed appropriate. Recently a new working group was established to harmonise the safety test requirements for veterinary medical products including IVMPs (VICH-Target Animal Safety WP) which met in November 2000 for the first time. In the context of the VICH, US and the Japanese regulations (Chapter II.4 and II.5) are also important.

III.4 Experimental Evaluation of Safety in the Development of a Vaccine

Manufacturers of veterinary vaccines have an advantage over those who produce human vaccines: a veterinary vaccine can be tested in animals of the target species at any stage of development.

In the last decade, the *Ph.Eur.* and the EU guidelines have introduced requirements which call for a long list of studies for an experimental evaluation of safety to be carried out in the target species during R & D of an animal vaccine. The goal is to evaluate the potential risks from use of the vaccine. The dose used should be that quantity of the product recommended for use and containing the maximum titre of potency.

Table 4: The safety testing of veterinary vaccines during the licensing procedure (after requirements of EU guidelines and European Pharmacopoeia)

Laboratory studies - safety of administration of one dose - safety of one administration of an overdose - safety of the repeated administration of one dose - examination of reproductive performance - examination of immunological functions - special requirements for live vaccines: spread of the vaccine strain dissemination in the vaccinated animal reversion to virulence of attenuated vaccines biological properties of the vaccine strains recombination or genomic reassortment of strains - study of residues - interactions
Field studies

The different types of safety tests are shown in Table 4. It should be noted that, for live vaccines, specific additional safety tests are required. The specific tests for live vaccines are intended to determine the risk of excretion of the vaccine virus into the environment by the faeces, urine, milk, eggs, etc. This can be a potential source of infection of other species of animals which could be highly susceptible to the live vaccines virus, e.g. Aujeszky's disease virus in dogs.

It has also be to ascertained that the excreted attenuated vaccine virus will not become virulent again by passaging through animals in the vaccinated herd or flock. These tests are, of course, performed with the vaccine strain, but not necessarily with the product itself. These tests relate only to the properties of the strain, the number of organisms, and the passage level and not to the final product itself.

In the case of adjuvanted vaccines, it is necessary to study the effect of the vaccine at the injection site, e.g. residues, tissue lesions, etc., and their duration.

The batch used in the safety studies should contain maximum potencies of all components but it is not usually possible to achieve this. Batches as close to the requirements as possible should be used and any deficiencies should be justified. If necessary other supporting data may be used to address deficiencies.

The safety studies carried out in the laboratory may have to be complemented by field studies. These studies are of importance because the laboratory studies are always carried out in a limited number of animals. The safety of the product will, in any case, be monitored in the post-marketing surveillance system, also called pharmacovigilance.

III.4.1 Safety of the Administration of One Dose

Susceptible animals receive either a normal dose of vaccine or a placebo by the recommended routes of administration. They need to be observed for signs of systemic and local reactions for at least 14 days post vaccination. The rectal temperature of each animal is measured the day before vaccination and on the four following days. If possible, performance measurements (e.g. weight gain, feed intake, milk production) should be recorded. Any abnormal clinical signs observed by a qualified veterinarian should be reported. The most frequent ADRs observed are loss of appetite, change in behaviour, trembling, vomiting, and diarrhoea. The injection site of each animal is palpated for several days or until any site reaction disappears. If a reaction is observed, especially in food animals, histopathological examination of the reaction site is performed at the time of slaughter or at relevant time intervals. If needed, blood samples are withdrawn for blood cell counts and blood chemistry analyses.

Testing is done with the dose of vaccine recommended for use. It shall contain the maximum titre or have the maximum potency as specified for the product. In the case of inactivated vaccines, it is usually not possible to meet the requirement precisely; in that case, a representative batch of the vaccine shall be used. In order to obtain representative data, the tests are done with a batch of vaccine produced in exactly the same manner as the normal commercial product.

For animal welfare and economic reasons, this study is very often combined with others, such as safety of a repeated administration of one dose, examination of immunological functions, or spread of the vaccine strain.

III.4.2 Safety of One Administration of an Overdose

The goal is to investigate the potential risks associated with an accidental overdosing of animals. The study design and requirements are similar to those of the previous study. The overdose is two doses for an inactivated product and 10 doses for a modified live vaccine.

III.4.3 Safety of the Repeated Administration of One Dose

This study is required mainly to detect adverse allergic reactions which could occur after the administration of booster injections in the lifetime of the animal. It is conducted under the same conditions and requirements as the safety if administration of one dose. The monitoring should pay attention to allergic signs such as elevation of body temperature, depression, trembling or change in white blood cell count. This test is particularly important for an adjuvanted inactivated vaccine (Vannier, 1992).

III.4.4 Examination of Immunological Function

Where the vaccine is expected to have the potential to adversely affect the immune system of the vaccinated animal, the appropriate tests on the immunological functions are carried out.
In the absence of clearly defined guidelines from any regulatory agencies, various possibilities exist to prove lack of interference with the immune system. For example, comparison of the lymphoblastic proliferative response to mitogens or antigens in control and vaccinated animals at various time intervals (weekly for 6 weeks, for example) is possible. In the Ph.Eur. Monograph on Avian Infectious Bursal Disease Vaccine (live) two challenge tests are included for immunosuppression (Bruckner et al., 2000).

III.4.5 Demonstration of Safety in Pregnant Animals

The effect on reproductive performance needs to be assessed if the product is intended for use in pregnant animals. The studies are conducted in the target animals using the recommended route of administration. The design of study depends on the product and its intended use, and on the species.
In laboratory studies, animals will receive a single or overdose of a vaccine, according to the intended use before or during pregnancy. The teratogenic and abortifacient effect are carefully investigated. Abortion rate, litter size and survival of offspring at 2 weeks of age are common parameters reported. All abnormalities should be described in detail. Animals can be vaccinated at a specific time during pregnancy for vaccines aimed at increasing colostrum antibody level (Rotavirus, Coronavirus, *E. coli* vaccine given during the last trimester in cattle) or at various times during pregnancy if it is claimed that vaccinating animals during pregnancy is safe. It is often difficult under laboratory conditions to obtain numbers with sufficient statistical significance. The second step is to complement those results by data generated during the field trial. In all cases, the vaccinates should be compared with controls.

III.4.6 Specific Safety Aspects for Modified Live Vaccines

Modified live vaccines are preparations of one or more virus, bacterium, or parasite of attenuated virulence or of natural low virulence for the target species. Attenuation can be accomplished using conventional technology (heat, serial passages, culture under abnormal conditions), or by constructing a strain by genetic manipulation. Modified live vaccines stimulate the best immune response, but because they may present hazards as a result of residual virulence or genetic recombination, the genetic stability of the vaccine strains has to be monitored carefully. During the development of a new vaccine, additional studies may have to be carried out in the target species.

Reversion to Virulence

The first set is referred to as being the reversion to virulence of attenuated vaccines, or the back passage studies. The goal of these studies is to determine if the attenuated vaccine organism will revert to a more pathogenic type upon serial passaging through susceptible animals. The vaccine material used in such studies must come from the passage level, which is least attenuated between the master seed and the final product.
The dose administered is the quantity of product recommended for use and containing the maximum titre of potency. The animals used in the study must come from the target species and from the most susceptible category of animals for which the vaccine is recommended. The first administration is done in at least two susceptible animals and by the recommended route of administration most likely to lead to reversion to virulence. Samples are then collected daily for a period of time, usually for as long as the organism is shed by the animals. All animals should be monitored closely to detect any clinical signs which might be indicative of reversion to virulence. If five serial passages are carried out in the target species, an equal number of animals receive a placebo. Clinical signs are observed by a qualified veterinarian before vaccination, and daily for 14 days post vaccination. The vaccinates are compared with the placebos. Local and systemic reactions are reported. Systemic reactions that may be observed and recorded include fever, loss of appetite, vomiting, diarrhoea, trembling, reluctance to move, and change in behaviour. Body temperatures are recorded for a minimum of 4 days. When applicable, performance parameters (body weight, milk production, litter size, feed efficacy) should be measured.

Recombination of Genomic Reassortment of Strains

The possibility of recombination between a live vaccine strain and a virulent strain, or between vaccine strains, has been demonstrated previously (e.g. Henderson et al., 1990). Therefore, the probability of recombination or genomic reassortment has to be assessed. For example, co-administration of two vaccine strains with complementary gene deletions can be done in order to assess potential recombination.

The regulations on the safety characteristics of recombinant vectors have recently been reviewed by Gay and Roth (1994).

III.4.7 Field Trials

Although the assessment of safety and efficacy of a new product under laboratory conditions is very useful, the numbers used are small (resulting in a lack of statistical power), and the animals are usually from one breed and seronegative. This does not reflect the real life situation. Therefore, field trials form a major part of any dossier for licensing of any vaccine. They are required to prove both safety and efficacy of a new product.

Field studies are designed to demonstrate efficacy under working conditions and to detect unexpected reactions, including mortality that may not have been observed during the development of the product. Under field conditions there are many uncontrollable variables that make it difficult to obtain good efficacy data, but demonstration of safety is more reliable. The tests should be done on the host animal, at a variety of geographical locations, using large numbers of susceptible animals. The test animals should represent all the ages and husbandry practices for which the product is indicated; unvaccinated controls must be included.

The vaccine used needs to be prepared according to the final method of manufacturing, and tested for stability and potency in the same manner as the commercial product will be tested.
The vaccine titre for a safety study needs to include at least one commercial batch with the maximum authorised titre. Trial authorisation needs to be granted by the local veterinary authority before the beginning of the trial.
The safety trials are usually conducted using 3 different serials of product in 500 to 1,000 animals for each serial. An equivalent number of animals should receive a placebo. It is good practice to conduct these studies blind. At least 3 geographic locations are used, and several veterinary clinicians working in veterinary practices are enrolled in the study. The owner of the animals needs to be informed of the experimental nature of the vaccine and to sign a consent form.
The parameters recorded in such trials may include body temperature (monitored before and 3 to 4 days post-vaccination in a representative number of animals), local and general reactions and measurements of performance (weight gain, appetite, milk production). Any significant observations must be recorded. This study should be conducted according the principles of Good Clinical Practice (GCP).

III.5 Animal Welfare Regulations

In general, there has been real progress with animal welfare in the Pharmacopoeia for implementing the three Rs (Artiges, 1999). However, the hard core of the problem is certainly vaccines (Castle, 1993).

There is a legal and ethical obligation for the countries, which have signed the Convention of the Council of Europe and in particular, for the MS of the European Union. Both, the *European Convention for the Protection of Animals Used for Experimental and other scientific purposes, ETS 123* (Council of Europe, 1986) and *Directive 86/609/EEC* (European Union, 1986) claim that

- "an (animal) experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonable and practicably available"; (replacement)
- "in a choice between experiments, those which use the minimum number of animals … cause the least pain, suffering, distress, and lasting harm and which are most likely to provide satisfactory results shall be selected"; (reduction and refinement) and
- "all experiments shall be designed to avoid distress and unnecessary pain and suffering to experimental animals".

These principles, which are based on the 3R concept of Russel and Burch (1959), also apply to safety testing of IVMPs. However, the opportunities for alternatives in the safety evaluation are generally more limited than in other areas such as potency testing. Therefore for safety testing the need for careful reassessment of animal tests was proposed with the aim to abolish those tests which do not contribute to the safety of a product (Cussler, 1999).

IV. Comparison of the requirements for the TAST

The TAST has a general framework in the monograph *Vaccines for Veterinary Use* (see Box page 10). A general outline for the test with regard to the different animal species is also given in the EU guidelines (Table 2). At least two animals of the target species have to be used. The dose to be used shall be twice (inactivated vaccines) or ten times (live vaccines) the quantity of the product recommended for the use. Thus, on the first view the TAST seems to be a general test which has to be applied to all IVMPs. However, detailed comparison of the requirements reveals that they are inconsistent or even contradictary.

IV.1 Inconsistency within *Ph.Eur.* Monographs

At the moment it is unclear whether the TAST is a general requirement of the *Ph.Eur.* for all IVMPs or not. It is required for most vaccines and swine erysipelas antiserum. However, the TAST is not required by the *Ph.Eur.* for the following products:

- Tetanus vaccine for veterinary use (1997; 697)
- Rabies vaccine (live, oral) for foxes (1997; 746)
- Immunosera for veterinary use (1997; 30)

- Clostridium novyi alpha antitoxin for vet. use (1997; 339)
- Clostridium perfringens beta antitoxin for vet. use (1997; 340)
- Clostridium perfringens epsilon antitoxin for vet. use (1997; 341)
- Gonadotropin, equine serum for vet. use (1997; 719)

There are no scientific reasons for these discrepancies. A clear rationale should be given for which biologicals the test is mandatory and where the TAST is not required.

Furthermore there are different requirements for the performance of the TAST between the Ph. Eur. monographs. Without obvious reasons, the requirements for test performance and evaluation vary for the following criteria:

- abnormal or significant reaction
- duration of the observation period
- number of test animals
- requirement for control animals
- repeated application
- category of animals
- antibody-free/susceptible animals

etc.

The background for these modifications is often unknown and the scientific relevance is questionable.

Recently special concern was expressed that the various specifications for the status of animals to be used in the TAST are inconsistent, unscientific, illogical, unjustified and impractical (Roberts, 1999).

Even monographs for comparable vaccines for the same species which are published simultaneously set different requirements for the TAST (see Tables 5 and 6). This is paradox because these dog and sheep vaccines are usually used as combined vaccines. According to the rules of the *Ph.Eur.* for combined vaccines, this means that for each component of the mentioned combined vaccines different TASTs have to be performed.

Moreover, these variations in the specific monographs hinder an overall discussion of the TAST as a general requirement and make it necessary to discuss each individual TAST in the relevant Ph.Eur. monographs.

Table 5: Safety test requirements of Clostridial vaccines (*Ph.Eur.* 3rd ed. Suppl. 2001, 646-653)

***Clostridium chauvoei* vaccine (2000:0361)**		***Clostridium novyi* (type B) vaccine (2001:0362)**		***Clostridium perfringens* vaccine (2001:0363)** and ***Clostridium septicum* vaccine (2001:0364)**	
Safety: Use two healthy susceptible animals of one of the species for which the vaccine is intended. Inject into each animal at a single site by the recommended route twice the maximum dose stated on the label. Observe the animals for 7 days. No abnormal local or systemic reaction is produced.		**Safety:** Inject into each of two sheep by a recommended route twice the maximum dose of the vaccine stated on the label. Observe the animals for not less than 14 days. No abnormal local or systemic reaction is produced.		**Safety:** Use two animals of one of the species for which the vaccine is intended. Inject into each animal, by a recommended route, twice the maximum dose stated on the label. Observe the animals for 14 days. No abnormal local or systemic reaction is produced.	
Species:	species for which the vaccine is intended	Species:	Sheep	Species:	species for which the vaccine is intended
Health status:	healthy susceptible animals	Health status:	not mentioned	Health status:	not mentioned
Dosage:	twice the maximum dose at a single site	Dosage:	twice the maximum dose	Dosage:	twice the maximum
Observation time:	7 days	Observation time:	not less than 14 days	Observation time:	14 days

Table 6: Safety test requirements of canine live vaccines (*Ph.Eur.* 3rd ed. Suppl. 2001, 546-548)

***Canine contagious hepatitis* vaccine (live), freeze dried**		***Canine distemper* vaccine (live), freeze dried**		***Canine parvovirosis* vaccine (live)**	
Safety: Inject by the route stated on the label twice the dose of the reconstituted vaccine into each of two susceptible puppies 8 to 16 weeks old and free from specific neutralising antibodies. Observe the animals for 21 days. The animals remain in good health and do not show signs of keratitis.		**Safety:** Use two puppies of the minimum age recommended for vaccination and which do not have antibodies against canine distemper virus. Administer ten doses of the vaccine to each dog by a recommended route. Observe for 14 days. The dogs remain in good health and no abnormal local or systemic reaction occurs.		**Safety:** Use two puppies of the minimum age for vaccination stated on the label and which preferably do not have haemagglutination-inhibiting antibodies against canine parvovirus. Inject 10 doses of the vaccine into each dog by one of the routes stated on the label. Observe for 14 days. The dogs remain in good health and no abnormal local or systemic reaction occurs.	
Category of animals:	puppies 8 to 16 weeks old	Category of animals:	Minimum age recommended for vaccination	Category of animals:	minimum age for vaccination
Health status:	susceptible puppies, free from specific neutralising antibodies	Health status:	Puppies, which do not have antibodies against canine distemper virus	Health status:	puppies which preferably do not have haemagglutination-inhibiting antibodies against canine parvovirus
Dosage:	twice the dose of the reconstituted vaccine	Dosage:	ten doses of the vaccine	Dosage:	10 doses of the vaccine
Observation time:	21 days	Observation time:	14 days	Observation time:	14 days
Evaluation criteria:	The animals remain in good health and do not show signs of keratitis.	Evaluation criteria:	The dogs remain in good health and no abnormal local or systemic reaction occurs.	Evaluation criteria:	The dogs remain in good health and no abnormal local or systemic reaction occurs.

IV.2 Inconsistency within EU Guidelines

The requirements for the TAST as specified in *The Rules Governing Medicinal Products in the European Union* are listed in Table 2. It is obvious that there are some discrepancies between the several guidelines, for example:

1) Animal numbers
   - Mammalian vaccines — at least 2 animals
   - Avian vaccines — at least 10 animals
   - Fish vaccines — at least 30 animals or at least 50 animals for oral-, spray-, bath- or dip-vaccination

2) Observation period
   - GRLMV — at least 14 days
   - GRIMV — not specified
   - SR Avian vaccines — at least 21 days
   - SR Fish vaccines — 21 days

3) Rectal temperature
   - GRIMV — should be recorded
   - GRLMV — not specified

4) The SR for bovine vaccines requires using calves younger than 5 months for the test irrespective of the category for the use of the vaccine (for example cows for *E. coli* vaccines).

5) The SR for fish vaccines is the only one which requires a control group and specifies when a test has to be repeated.

Again there seem to be no scientific reasons for these differences.

VI.3 Differences between *Ph.Eur.* Monographs and EU Guidelines

Differing requirements between *Ph.Eur.* monographs and EU Guidelines give raise to special concerns (see Table 7).

Table 7: Some examples for differing requirements in *Ph. Eur.* and EU guidelines

Monograph(s) on	Ph. Eur.	EU-guidelines
Immunosera	TAST not required	TAST required
Tetanus vaccine	TAST not required	TAST required
Fish vaccines	10 animals	30 or 50 animals

The most prominent example is the TAST for Immunosera. Whereas only the *Ph.Eur.* is legally binding, some National Control Authorities (NCAs) refer to the EU guidelines and insist that these requirements are fulfilled. However, to waive the TAST the applicant has to approach all individual NCAs for permission for this variation. The experience with this procedure so far is very disappointing for the applicants because it seems to be highly unlikely to receive a harmonised decision. In 1997 AGAATI pointed out this problem to the EMEA (see Annex) which passed the letter on to the *Ph.Eur.* The issue is on the agenda of Group 15V at the *Ph.Eur.* and the IWP at EMEA, but so far no official response has been received.

IV.4 Differences between *Ph.Eur.* Monographs and other international Requirements

Nowadays many vaccine manufacturers produce for a global market. Therefore the quality control tests often have to meet different requirements in various parts of the world which are so far not harmonised and thus may require repetition of similar animal tests e.g. for safety. Table 8 lists general batch safety tests required in Europe, United States and Japan.

Table 8: General Batch Safety Tests for Veterinary Vaccines

Europe:	Target animal safety test (*Ph.Eur.* monograph 62: Vaccines for veterinary use and most monographs for specific vaccines)	
United States	Mouse safety tests Guinea pig safety test Cat safety test Dog safety test Calf safety test Swine safety test Sheep safety test	9CFR § 113.33 9CFR § 113.38 9CFR § 113.39 9CFR § 113.40 9CFR § 113.41 9CFR § 113.44 9CFR § 113.45
Japan:	Safety test (no detailed information available)	

As the sales in the EU and North America together account for more than half of the total world market, harmonisation of test requirements between those regions is most important to avoid repetition of animal tests. A comparison of safety test requirements of Ph.Eur. and 9CFR is given in Table 9.

Table 9: Comparison of Routine Batch Safety Test Requirements in *Ph. Eur.* (3rd edition) and 9CFR 1998

Biological *Title in 9CFR* / **Title in *Ph.Eur.***	***Ph. Eur.***		**9 C F R**	
	TAST	**Others**	**TAST**	**others**
Tetanus toxoid Tetanus vaccine	-*	*Toxicity* Guinea pigs 5.0 ml s.c.	-	*Mouse safety* 8 mice, 0.5 ml i.p. or s.c.
Clostridium botulinum type C bacterin-toxoid Clostridium botulinum vaccine	2 animals double dose	*Residual toxicity* 5 mice, 5.0 ml s.c.	-	*Mouse safety* 8 mice, 0.5 ml i.p. or s.c.
Leptospira canicola and icterohaemorrhagiae bacterin Leptospira vaccine	2 dogs double dose	-	-	*Guinea pig safety* 2 guinea pigs 2.5 ml i.m. or s.c.
Erysipelothrix rhusiopathiae bacterin Swine erysipelas vaccine (inactivated)	2 pigs double dose	-	-	*Mouse safety* 8 mice, 0.5 ml i.p. or s.c. **and** *Guinea pig safety* 2 guinea pigs, 2.0 ml i.m. or s.c.
Pseudorabies vaccine, killed virus Aujeszky's disease vaccine (inactivated) for pigs	2 pigs 2 doses	-	5 vaccinated and 5 control pigs From the potency test are used	*Mouse safety* 8 mice, 0.5 ml i.p. or s.c. **and** *Guinea pig safety* 2 guinea pigs, 2.0 ml i.m. or s.c.

Continuation

Biological	***Ph. Eur.*** TAST	***Ph. Eur.*** Others	**9 C F R** TAST	**9 C F R** others
Bovine rhinotracheitis vaccine Freeze-dried infectious bovine rhinotracheitis vaccine	2 calves 10 doses	-	2 calves 10 doses	*Mouse safety* 8 mice, 0.5 ml i.p. **and** 8 mice, 0.03 ml i.c.
Feline Panleukopenia vaccine Freeze-dried feline enteritis (feline panleucopenia) live vaccine	2 cats double dose	-	2 cats 10 doses	*Mouse safety* 8 mice, 0.5 ml i.p. or s.c.
Canine distemper vaccine Freeze-dried canine distemper live vaccine	2 dogs double dose	-	2 dogs 10 doses	*Mouse safety* 8 mice, 0.5 ml i.p. or s.c.
Rabies vaccine, live virus Rabies vaccine (live, oral) for foxes	-*	-	3 target animals	*Mouse safety* 8 mice, 0.5 ml i.p. or s.c. **and** LD_{50} determination in mice
Tetanus antitoxin	-*	-	-	*Mouse safety* 8 mice, 0.5 ml i.p. or s.c.
Erysipelothrix rhusiopathiae antibody Swine erysipelas immunoserum	2 pigs double dose	-	-	*Mouse safety* 8 mice, 0.5 ml i.p. or s.c.

* The EU Guidelines (GRLMV and GRIMV) require a safety test in two animals of the target species

The most obvious difference is the fact that 9CFR still requests general safety tests in mice and guinea pigs for many product groups. In Europe this kind of test (Abnormal Toxicity Test [ATT]) has recently been abolished for all IVMPs (Schwanig et al. 1997). On the other hand 9CFR requires a safety test in the target species only for several kinds of vaccines (mainly live vaccines) whereas the Ph.Eur. requires the TAST for nearly all products.

The Ph.Eur. only requires that “no abnormal local or systemic reactions occur”. The 9CFR gives guidance when the test has to be repeated (see Table 10).

Table 10: Evaluation criteria for target animal safety tests

Ph.Eur.	No abnormal local or systemic reaction occurs
9CFR § 113.39 § 113.40 § 113.41 § 113.44 § 113.45	If unfavourable reactions attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavourable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated: *Provided*, that, if not repeated, the serial shall be unsatisfactory.

The requirements of the OIE for safety tests somehow commute between both positions. The discrepancy between the safety test requirements is most pronounced for live rabies vaccine where the Ph.Eur. does not require a safety test at all, whereas the 9CFR and OIE Manual require the use of three target animals (e.g. foxes) a mouse safety test and an additional LD 50 determination in mice (see Table 9).

V. Objectives and Critiques of the TAST

So far the objectives of the TAST are not clearly defined. It is a legal requirement that immunobiologicals have to be tested for safety. It may be argued that this implies the use of animals and it seems logical to use the target species for veterinary products. That may be the reason why the test is named *Safety* in the Ph.Eur. and in the EU Guidelines.

In the historical context, batch safety testing may have been very important when laboratory testing was limited and adequate regulatory requirements were not yet available. For example, the mouse safety test was originally introduced at the beginning of the last century to check the phenol content of immunosera. Furthermore the requirement for veterinary vaccines to be licensed before the product can be brought into the market is relatively new, e.g. in Germany this became mandatory in 1978.

Nowadays, all IVMPs have to undergo a very stringent licensing procedure where all relevant safety aspects are tackled (see III.4). For those issues which might still cause safety concerns for special products, specific safety tests including animal tests are required before batch release. For example, residual toxicity of tetanus or botulinum toxoids has to be excluded in a laboratory animal test.

However, depending on the kind of product, safety can also be demonstrated completely *in vitro* without any animal use. This is already the case for several immunobiologicals for human use such as immunoglobulins or polysaccharide vaccines. In principle, this is also the case for several veterinary immunobiologicals (see IV.1) where no TAST is required in the Ph.Eur. monograph. The live rabies vaccine is another example.

The rationale behind the TAST seems to be to prove the degree of attenuation of live vaccines or to investigate harmful side-effects of adjuvants which are used in inactivated vaccines. No local or systemic reactions should occur. Thus, the results seem to have a high impact on the safety of the vaccines.

The test cannot provide information on issues for which it is not intended. For example, the testing for extraneous agents, for complete inactivation or sufficient attenuation are in principle other tests which need a different design to fulfil their purpose properly. Nevertheless, these arguments are often given in favour of the TAST (see VI.1 and Annex XII-1-2). Of course the TAST may (by coincidence) detect such problems, however, in most situations this will not be the case (e.g. it cannot be expected that the TAST detects contamination of bovine vaccines with bovine diarrhoea (BVD) virus. This would need a more appropriate, preferably *in vitro* test).

Therefore the necessity for „general safety tests“ has nowadays to be questioned. The safety test in laboratory animals (ATT) has already been abolished for all veterinary and most human vaccines because it was evident that this test does not contribute to the safety of these medicines anymore (Schwanig et al., 1997). It has been argued that the same situation applies for the TAST (Weisser and Hechler, 1997). Indeed the need for this test is increasingly criticised (Roberts and Lucken, 1996, van der Kamp 1994, Zeegers et al., 1997).

From a survey of the TAST on different vaccines within one company, it was concluded that the test did not contribute to an appropriate assessment of the quality of the vaccine batches examined (Zeegers et al., 1997). It was proposed by others that a risk-benefit analysis should be conducted on a product-by-product basis to determine whether the continued application of the TAST to each batch of a product is beneficial and justified (Roberts and Lucken, 1996). The wide-ranging discussion about the need and the relevance of this test has recently been summarised by Weisser and Hechler (1997).

Overall, from the animal welfare point of view there is already ample evidence to abolish the TAST. However, so far all drafts for new or revised vaccine monographs of the Ph.Eur. still stipulate the TAST as a routine test and requests to delete the TAST were rejected. Similar experience has been reported for the ATT. People are very reluctant to remove such an animal test, which seems to be some kind of „comfort factor„ (Griffith, 2000).

In view of the pro and contra arguments it seems necessary to provide more data by new and additional surveys on this topic in order to initiate general re-assessment of the test which could then lead to a deletion of the TAST at least as routine batch control test.

Even if the test is kept only to cover special problems of certain vaccines, guidance has to be provided how to perform and evaluate the TAST properly. In particular, the various specifications and differences for the TAST in various Ph.Eur. monographs have to be criticised:

- ***Definition of target species***

  The safety test shall be carried out in the target species. However, the term target species is often not sufficient because many products are only used in special animal categories of the target species. For example, it is nonsense to test vaccines intended for use in pregnant cows or sows in calves or fattening pigs. However, this is frequently done in practice and e.g. included in the Ph. Eur. monograph on E. coli vaccines for pigs (Neonatal piglet colibacillosis vaccine [inactivated] [1997; 962]).

- ***Necessity to test an overdose***

  The dose to be used shall be twice (inactivated vaccines) or ten times (live vaccines) the quantity of the product recommended for the use. However, there is no logical reason why the safety test should be performed with an overdose. This issue has to be intensively investigated during the licensing procedure.

  For some vaccines it is well known from the licensing procedure that the use of an overdose may cause severe local reactions (e.g. oil-adjuvanted products like Johne's disease vaccine, fish vaccines, etc). This information is included in the summary of product characteristics (SPC). Thus, the routine application of a potentially harmful overdose of a product without gaining safety-relevant information is of animal welfare concern.

  Furthermore, if vaccines are not properly used in practice, this is not the responsibility of the manufacturer or the competent authority. If that were the

case, then it would also be necessary to test pig vaccines that are licensed for s.c. use by the i.m. route because this off-label use is often done in practice.

Again there are several exceptions from the rule. If a vaccine caused problems in the overdose test, often the requirement was modified (e.g. Anthrax spore vaccine [live] for vet. use [1997; 441], Brucellosis vaccine [live] [Brucella melitensis rev. 1 strain] freeze-dried, for vet. use [1997; 793]).

For live viral pet vaccines the situation is even more confusing. The general rule to test a ten-fold overdose does not always apply for specific monographs, e.g. canine hepatitis vaccine. Several other monographs where „only“ a double dose test was required (canine distemper vaccine or feline panleucopenia vaccine in the Ph.Eur. 3rd ed. 1997) were revised and contain now (in the Ph.Eur. Supplement 2000) again the requirement for a ten-fold dose.

The use of the recommended dose in the safety test offers the possibility of combining safety and potency tests for many products and would result in a considerable reduction of animal use at least for some vaccine categories, e.g. poultry and fish vaccines.

- ***Repeated application of the vaccine in a single dose***

The repeated application as required in several monographs should be deleted. Again this issue is already investigated during licensing. So far, the experience with the products concerned has shown that there has been no case where a batch has caused problems. There is no additional safety information available from this specific requirement. Even if an animal shows a pathological reaction due to sensitisation, this only reflects a biological phenomenon inherent to all immunological products which are therefore always mentioned in the leaflet. Obviously, there is no rationale to require this expensive and time-consuming modification in future.

- ***Test requirement: no abnormal/no significant reaction***

It is not defined what a significant or abnormal reaction is. This leads to a very controversial discussion in some cases where companies provide detailed test data (e.g. is a temperature increase of 1°C, 1.5°C or even 2°C significant or not?).

The test requirements (pass/fail-criteria) have to be clearly defined and specified for each product/product group (e.g. no local or general reaction exceeding the information in the SPC is acceptable). The fail criteria in regard to the most critical points (extent of local reactions and increase in body temperature) should be fixed during the licensing procedure or at the renewal of the license.

- ***Requirements for the performance of the test.***

  In general, safety tests have to be performed according to the rules of GLP. This should apply for species considered as experimental animals according to the Annex 1 of Article 21 of Directive 86/609/EEC. However, for large animals and companion animals, the company may perform the test with the agreement of the competent authority under conventional conditions, if sufficient information is provided by the company that the test is performed under circumstances that are in agreement with APPENDIX A (Guidelines for accommodation and care of animals), Article 5 of the European Convention No 123 and Article 5 of Directive 86/609, and guarantee *GLP-like* conditions and results.

  The necessary clinical parameters (clinical signs, body weight, body temperature, local reactions) should be recorded in data sheets which are adapted to the vaccine and the animal species under test. Those data sheets should be part of the batch records to provide the competent authority with all information necessary to avoid repetition of the test.

- ***Test samples***

  At the moment, it is unclear whether one sample or two (several) samples should be tested. In practice, some companies use bulk material for inactivated products to reduce animal use or pool many lyophilised containers of live vaccines.

  The samples used for the safety testing shall be taken from a batch produced according to the manufacturing process described in the application for marketing authorisation, usually using final containers. To increase the sensitivity of the test a minimum of three samples of final containers should be used in at least two animals. However, with the agreement of the competent authority for products where the bulk material is identical with the final product, and contamination during the filling procedure can most likely be excluded, the safety test may be performed using final bulk material.

- ***Re-use of animals***

  At the moment it is unclear whether animals can be re-used or not. Obviously, when seronegative or susceptible animals are required (see below), this cannot be the case for the same type of product. However, it should never be a problem to re-use animals for unrelated products.

- ***Seronegative/susceptible animals***

There is also no consistency for the specification of the antibody status within the monographs. Undefined terms such as „susceptible“ or „most sensitive“ are frequently used. Some monographs require the use of antibody-free animals and others do not specify the status at all. If the immune status or the susceptibility of animals is of relevance for the use of a vaccine, then this problem has to be targeted during the development of the product. In routine batch control, the TAST should reflect the status of animals in the field where the product will be used. Thus, there should be no need to specify the antibody status or susceptibility for the TAST unless there is a very special reason to do so.

Therefore, if the TAST is kept in the Ph.Eur. a guideline should be drafted to give advice how to perform the test in future and to define conditions when the test is not necessary any more. It should provide general guidance how to perform and evaluate the TAST to achieve the required information.

Such a guideline should provide the general scientific principles only for the performance and control of the TAST in the context of batch control and could be added to Ph.Eur. 5. 2. 6 (General Tests on Vaccines) where safety tests and requirements that have to be performed during the licensing of a product are already laid down.

VI. Retrospective analysis of TAST data

VI.1 Experience with the TAST at OMCLs

A detailed inquiry of Official Member States Control Laboratories (OMCLs) was performed in order to gain more information about the TAST. For this reason all OMCLs which mentioned in the EDQM OMCL Directory that they perform the TAST were asked to participate and a detailed questionaire was sent to them. The OMCLs were requested to provide data about batches of IVMPs received during the period 1994-1997.

Overall, seven of 23 OMCLs replied and submitted the compiled detailed questionaire. As they only representabout 30%, the reliability of the data may be questioned. However, the seven laboratories include participants from large, medium-sized and small countries as well as representatives from central, south and northern Europe. Therefore the results of the questionnaire can be seen as representative for Europe. Furthermore it should be kept in mind that this is the only information available so far. Nevertheless, the data should be evaluated with care.

The list of the participating OMCLs and the summary of the answers to the questionaire are attached (see Annex).

VI.1.1 Information about the total number of batches of IVMPs sent to OMCLs for batch release

Tables 11 and 12 give an overview on the total number of batches being submitted to the seven OMCLs. For the interpretation of these data it has to be kept in mind that manufacturers still have to ask for national batch release in every country separately. Therefore it is unavoidable that batches may be counted more than once in these tables. On the contrary only 7 of 23 OMCLs contribute to these figures.

During 1994-1997, more than 11,000 batches were submitted to the seven OMCLs for official batch release. The yearly average for that period is about 2,800 batches. However, a remarkable increase is evident over that period (see Table 11).

With regard to the animal species, most batches were released for use in pigs and chickens. Table 11 gives detailed information about the distribution between the different species.

Table 11: Number of batches of immunologicals sent to 7 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	**2.364**	**2.529**	**3.091**	**3.186**	**11.185**

The distribution of the batches between the various OMCLs is given in Table 12.

Table 12: Number of batches of immunologicals sent to individual OMCLs during 1994-1997

Vaccines for	A	B	C	D	E	F	G
Horses	0	5	133	312	47	33	0
Cattle	0	8	12	1039	107	366	55
Swine	192	7	180	1330	233	692	48

Sheep and goats	0	0	22	44	0	102	12
Dogs	35	4	314	866	141	753	29
Cats	10	2	115	532	41	121	7
Foxes and mink	0	0	88	226	0	0	0
Rabbits	0	0	0	158	13	174	0
Chicken	174	6	111	884	277	866	0
Pigeons	14	0	2	71	11	30	0
Turkeys	0	5	0	20	8	32	0
Other birds	0	1	0	26	0	0	0
Fish	0	0	31	8	0	0	0
Total	425	38	1008	5516	878	3169	151

In addition to the testing by the manufacturers, the OMCLs themselves may repeat laboratory testing of batches. This includes also animal experiments such as the TAST. During the period of 1994-1997 the seven OMCLs re-tested a total of 670 batches (see Table 13) and almost of the batches passed the first test. Only for four batches did the test have to be repeated and three of the batches passed. Only one batch of an inactivated sheep vaccine did not pass the test.

Table 13: Number of batches which were tested by seven OMCLs in the Target Animal Safety Test

Vaccines for:	1994 - 1997	Passed	Passed after retest	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**

A closer look at Table 13 reveals that the pattern of re-testing is not random, but obviously related to the availability of the animal species for testing. Poultry and rabbit vaccines where the target species itself is a laboratory animal are more or less tested routinely, whereas vaccines for pets and large 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, e.g. the company withdraws the application for batch release or the OMCL detects quality deficiencies.

A total of 89 batches was not released during that time period (see Table 5). Only in four cases (~ 5%) was this based on safety concerns and in only one of the four cases the batch was not released due to a failure in the TAST.

Table 14: Number of batches which were not released by national authorities or withdrawn by companies because of inadequate quality

Reason	1994	1995	1996	1997	***Total***
Safety -TAST	0	0	0	1	**1**
-other safety tests	0	3	0	0	**3**
Potency	16	6	1	4	**27**
Others	3	16	21	11	**51**
Total	**19**	**25**	**22**	**16**	**82**

The data received from the OMCLs indicate that the TAST performed at OMCLs has very limited relevance for the safety of IVMPs due to the following reasons:

- only a small percentage of all batches is re-tested
- only a small spectrum of target species is used with a high preference for poultry and rabbits
- because only one out of more than 11,000 batches was not released due to a failure in the TAST (< 0.01%)

Nevertheless, it should be considered that manufacturers will only submit batches for release which passed the TAST and this makes a failure in the TAST at the OMCLs not very likely.

Therefore a second questionnaire was initiated to receive additional data from vaccine manufacturers.

VI.2 Experience with the TAST in Industry

The members of the FEDESA Biologicals Working Group were asked to fill in a form (see Annex) to provide data on the TAST for the time period 1997-1999. If retests or failure in the TAST occurred more details should be given.

Unfortunately not all companies responded and sent in the form. A list of the participating companies is given in the Annex. Not all companies provided data for all years, whereas others gave information for more years than requested.

There were several limitations in the received data;

- Two companies could not provide data for all animal species
- Large international companies with several production sites sent in data from different places which could not be summarised in one table. Therefore different production sites of the same company may be listed as separate „companies“ in the tables.
- The feed-back for repeats and failures in the TAST is not complete because not all companies answered. Therefore only some examples or reasons can be given.

All data from individual companies are listed in the Annex. Table 15 gives an overview of the total number of batches listed for the different animal species in the period 1997-1999.

Table 15: Number of batches tested for different animal species in the TAST by 14 companies

	Total No of batches 1997-1999
Horses	226
Cattle	602
Swine	2.327
Sheep and goats	411
Dogs	1.570
Cats	501
Rabbits	141
Chicken	5.148
Pigeons	64
Turkeys	141
Other birds	170
Fish	85
Total	**11.386**
Yearly average	**3.795**

Far the most vaccine batches are used for chicken followed by pig vaccines and dog vaccines. This extensive use of IVMPs in chicken explains the high number of animals needed for the TAST because five times more animals have to be used for avian vaccines in comparison with mammalian vaccines.

Table 16 gives an overview about repeated tests and test failures.

Table 16: Failures and Repeats of the TAST in Industry (1997 – 1999)

Company-No	No of batches	Passed after retest	Failures
1	43	2 (4,6%)	0
2	95	0	0
3	564	0	0
4	1 036	9 (0,86%)	2 (0,19%)
5	1 386	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	2 108	59 (2,79%)	0
12	2 466	51 (2,06%)	1 (0,04%)
13	1 783	53 (2,97%)	1 (0,05%)
14	358	0	0
All	**11 386**	**215 (1,8%)**	**7 (0,06%)**

Overall more than 98% of all batches pass the TAST without problems. The ratio for those companies who report repeats varies between 0.3% and 5.6%. Most interestingly more than 1/3 of all manufacturers never had to repeat a test and nearly 2/3 never had a failure during this time period.

A look at Table 17 shows a similar pattern for the different animal species. In seven of twelve species, no batch failed in the test and in three species not even a repeat test was necessary. Most interestingly in those species where a high number of animals is requested to perform the TAST (chicken, fish, rabbits and other birds) no failure occurred.

Overall only seven batches failed in the TAST of the companies during 1997-1999 which gives a ratio of 0.06%. Thus more than 99.9% of all batches passed the TAST in the quality control of the manufacturers.

Table 17: Failures and Repeats of the TAST in Industry by animal species

Target animal	No of batches	Passed after retest	Failures
Horses	226	13 (5,75%)	1 (0,44%)
Cattle	602	17 (2,82%)	1 (0,16%)
Swine	2 327	30 (1,28%)	1 (0,04%)
Sheep / Goats	411	7 (1,70%)	1 (0,24%)
Dogs	1 570	45 (2,86%)	3 (0,19%)
Cats	501	9 (1,79%)	0
Rabbits	141	0	0
Chicken	5 148	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
All	**11 386**	**215 (1,8%)**	**7 (0,06%)**

VI.3 Animal Usage for the TAST

Unfortunately it is not possible to provide exact data about the animal usage for the TAST. However, the batch release data from industry allow us to calculate minimum figures for the different species (see Table 18). Keeping in mind

- that the minimal animal number required in the monographs is used
- that not all companies contributed to the questionaire
- and that several companies could not provide data for all species or all years

the real number will be considerable higher.

The batch release figures from the OMCLs should not be used because the same vaccine batch may be send to several authorities for national batch release

Table 18 : Estimated use of animals needed in the TAST for batches tested by 14 companies

	Total No of batches 1997-1999	Estimated use of animals*
Horses	226	452
Cattle	602	1.204
Swine	2.327	4.654
Sheep and goats	411	822
Dogs	1.570	3.140
Cats	501	1.002
Rabbits	141	705
Chicken	5.148	51.480
Pigeons	64	320
Turkeys	141	705
Other birds	170	850
Fish	85	850
Total	**11.386**	**66.184**
Yearly average	**3.795**	**22.061**

* : based on
- 2 animals per test for horses, cattle, swine, sheep and goats, dogs and cats;
- 5 animals per test for rabbits, pigeons, turkeys, ducks, geese, canaries;
- 10 animals per test for chicken;
- 10 fish per test.

The Ph. Eur. monograph 62 requiring the use of at least two animals applies for rabbit and poultry vaccines (other than chicken). However, in practice higher number of animals are used, e. g. for avian vaccines often ten birds are used as required for chicken vaccines (see also Table 19). For fish vaccines different requirements exist in the Ph. Eur. and in the EU guidelines (see III.). The lower numbers of the Ph. Eur. are used for this calculation. However, in practice it is more likely that manufacturers use the higher figures mentioned in the SR for fish vaccines.
Table 19 lists the animal figures of one of the worlds biggest companies for a four-year-period. This company alone needs annually more than 4,800 animals to perform the TAST.

Table 19: Average number of animals used in the TAST
(based on the figures provided by company no. 12 for 1996-1999)

Vaccines for	Total number of Batches	Number of animals used	Average Number/batch
Horses	**65**	**136**	**2,1**
Cattle	**182**	**381**	**2,1**
Swine	**326**	**724**	**2,2**
Sheep	**39**	**88**	**2,3**
Dogs	**642**	**1366**	**2,1**
Cats	**157**	**314**	**2**
Rabbits	**63**	**315**	**5**
Chicken	**1392**	**14205**	**10,2**
Turkeys	**75**	**905**	**12,1**
Ducks	**127**	**1090**	**8,6**
Total	**3068**	**19524**	**6,4**

VI.4 Pharmacovigilance data

In most countries pharmacovigilance data for IVMPs are collected together with other medicines and it is therefore difficult to extract specific data for this group. Furthermore each agency has its own system to summarise and categorise these data. This fact makes it very problematic to compare data from different countries. Only a few countries which have separate agencies for pharmaceuticals and immunobiologicals (e.g. Germany, Switzerland or the United States) differentiate between both groups and provide separate data for „vaccinovigilance“.

Furthermore it is not easy to gain access to pharmacovigilance data. Despite the fact that Guidelines for Pharmacovigilance of Veterinary Medicinal Products have been adopted in 1996 no official data are available for the EU. Only reports from UK are regularly published in an international journal. Much additional data could be gained from the Proceedings of the 2nd International Symposium on Veterinary Pharmacovigilance (Ibrahim, 1998).

Statistics about the market for veterinary medicines reveal that IVMPs share about 13-18% of the total world market value (Wesley, 1999). In Europe, the percentage is higher reaching 20% in 1993 (FEDESA, 1994) and is constantly increasing. Table 20 lists the percentage of pharmacovigilance reports for the three major product groups (antiparasitics, antiinfectives and IVMPs).

Table 20: Percentage of pharmacovigilance reports for product groups (from Ibrahim, 1998)

	Netherlands[1]	**Germany[2]**	**Australia[3]**	**UK[4]**
Antiparasitics	37	29	30	34
Antibiotics / Antifinfectives	28	9	31	13
IVMPs	14	35	17	28
Others	21	27	22	25

[1] = Kamphuis, 1998 [2] = Ibrahim, 1998, see also Annex XII-3-2 for IVMPs
[3] = Savage et al., 1998 [4] = Anon., 1997

It is obvious that the situation is very different between the countries, e.g. whereas the percentage of reports concerning IVMPs is relatively low in the Netherlands, the reports for Germany are relatively high. However, as the percentage of pharmacovigilance reports is not much higher than the market share of IVMPs there seems to be no extraordinary safety risk associated with the use of vaccines. But there are obviously species specific differences which give a relatively high ration of reports for pet animals, horses and cattle.

In this respect it has to be mentioned that IVMPs are mainly used for prophylaxis in healthy animals. Therefore, an ADR will be mostly associated with the vaccination/treatment, which is not always the case when ill animals experience untoward reactions which may be covered by the symptoms of the basic disease. Interestingly the percentage of ADRs for antiparasitics which are also often used in healthy animals for prophylaxis is relatively higher. However, for this group no batch safety test is required.

With regard to the different animal species it is obvious that most ADR reports are received for cats and dogs. On the contrary ADR reports for poultry and pigs are extremely low if compared with the market share of IVMPs for these species.

For this study the pharmacovigilance data from UK which are published annually in „The Veterinary Record“ and the data from Germany (kindly provided by Dr. E. Werner, Paul-Ehrlich-Institut) were evaluated in more detail (see below).

VI.4.1 Pharmacovigilance data from UK

In the UK the Suspected Adverse Reaction Surveillance Scheme records all ADRs to veterinary medicinal products in animals and humans. These are reported to the Veterinary Medicines Directorate (VMD). The system has been in use for more than a decade and the number of reports is constantly increasing - from 329 reports in 1989 (Gray et al., 1990) to 871 suspected reactions in 1999 (Anon., 2000).

Product sales information is used to develop a risk assessment picture by calculating the rate of ADRs. The quality and detail of these reports is variable. Generally the proportions of reactions reported for all veterinary medicines is low. But the number of reports involving vaccines remains relatively high (VMD Annual Report 2000). However, if the market share for IVMPs and the ratio of ADRs is compared for the animal species it is obvious that two different categories exist: Pet animals and horses, where more than 80% of ADRs are reported, account for less than 40% of the market share, whereas the farm animals (pigs, cattle, sheep and poultry) have a market share of more than 50%, but little more than 15% of all ADRs occur in those species.

Table 21: Size of IVMP Markets and ratio of ADRs for different animal species in U.K.

Species	Market share (%)[1]	ADR (%)[2]
Cattle	12,3	9,1
Swine	5,0	1,3
Poultry	20,9	0
Sheep	21,0	4,9
Horses	4,5	20,3
Pets	34,7	61,5
Others	1,4	2,9

[1] from FEDESA (1994) [2] from Vet. Rec., December 3, 1994, p. 544

The reports for the time period 1993-1997 (see Annex XII-3-1) were evaluated. The IVMPs are listed in three therapeutic groups: Antisera, inactivated vaccines and live vaccines (see Table 22). Unfortunately it is not noted how mixed vaccines with live and inactivated components which account for most products (e.g. in dogs) are listed.

The majority of ADRs relate to vaccination in pet animals. In consequence, a working group was set up in 1999 to review the reports in cats and dogs.

Table 22: Pharmacovigilance Reports in UK (1993 – 1997)

	No of reports	%
Total	3038	100,00
Antisera	6	0,19
Live vaccines	496	16,32
Inactivated vaccines	405	13,33
All immunologicals	907	29,85

VI.4.2 Pharmacovigilance data from Germany

In Germany, pharmacovigilance data for pharmaceuticals and IVMPs are collected and evaluated separately. The Paul-Ehrlich-Institut is the responsible authority for IVMPs. The data for 1995-1997 from Germany could be evaluated. The detailed data are attached in the Annex XI-3-2.

Table 23: Summary of Pharmacovigilance Reports for Vaccines in Germany 1995-1997

Species	No of Reports	Related to Safety	%
Dog	203	155	76,3
Cat	110	84	76,3
Horse	46	41	89,1
Bovine	261	173	66,2
Sheep / Goat	55	53	96,3
Pig	94	72	76,6
Rabbit	83	22	26,5
Poultry	27	19	70,3
Total	**899**	**619**	**68,8**

The pharmacovigilance data from Germany provide additional data. They show that approximately 2/3 of all reports concern safety aspects whereas the remaining 1/3 concern lack of efficacy (see Table 23).

There are great species-specific differences in the type of reactions. Whereas the overall ratio of local reactions is about 16% there seem to be a few very sensitive species for such a type of reaction namely rabbits, poultry, sheep and horses. For poultry and sheep this may be related to the adjuvants in vaccines. Especially in poultry many oil-adjuvanted products are used which are well known to induce such types of reaction. In the other species systemic reactions dominate.

Table 24: Summary of Pharmacovigilance Reports for Vaccines in Germany 1995-1997. Type of Safety Reactions

	No of Reactions		
Species	**local**	**systemic**	**Local + systemic**
Dog	25	120	10
Cat	10	73	1
Horse	15	15	11
Bovine	6	167	0
Sheep / Goat	8	5	40
Pig	6	66	0
Rabbit	16	6	0
Poultry	14	5	0
All	**100**	**457**	**62**
%	**16,2**	**73,8**	**10,0**

The most obvious difference between the German statistics for the evaluation period and UK (but also all other reports from Europe) is the dominance of reports in cattle. A high number of ADRs is related to two groups of vaccines, ringworm vaccines which are more popular in Germany than in the rest of Europe and BVD/BRSV vaccines either alone but mostly as combined product (see also IV.4.3 below).

VI.4.3 Case reports

In Germany the pharmacovigilance system noted three products/product groups which caused serious adverse reactions during the period 1992-1996 (Werner 1998).

- In sheep vaccinated with a bacterial vaccine many vaccinees developed wool loss at the injection site, arthritis and lameness, abortion and birth of weak lambs and hyperthermia, paresis and wasting. Overall more than 1,000 sheep died or had to be slaughtered.

- Ringworm vaccines used in cattle induced hypersensitivity and anaphylaxis reactions. Furthermore polyarthritis and lameness was reported.

- Hypersensitivity and anaphylaxis was also reported after use of BVD/BRSV vaccines in cattle but also symptoms of respiratory disease reactions predominated.

All vaccine batches associated with these reactions passed the TAST (Werner, personal communication).

Two recent cases also show that a passed TAST does not guarantee a safe vaccine. As Falcone et al. (2000) report, hundreds of calves died after vaccination because the IBR vaccine used had been contaminated with BVD. The IBR vaccine had been tested according the Ph. Eur. monograph and had been released. Since the two calves used in the TAST had been BVD positive, the contamination with BVD could not be detected.

In France, the marketing authorisation for a dog vaccine has been withdrawn, since several puppies died of maladie de Carre after vaccination (Anon., 2000).

VII. Discussion

There is no doubt that safety has first priority in the quality control of medicines. Today a thorough basic research has to be done on a veterinary medicinal product for registration with special emphasis on safety testing. In contrast to pharmaceuticals, after licensing IVMPs are subject to governmental batch control which requires amongst many others safety testing in the TAST.

Nowadays IVMPs are produced according to a seed lot system and GMP/GLP regulations. The introduction of GMP and GLP should indirectly reduce the number of animals used in the quality control of veterinary vaccines, as adhering to GMP and GLP increases consistency in production, leading to a more reliable product, and furthermore, tests are standardised and the results become more reliable. Thus the situation has markedly changed compared to the times when the TAST was introduced. Therefore, the usefulness of the TAST for routine release of batches of IVMPs has recently been questioned.

Manufacturers have stated that they hardly ever have rejected vaccine batches on the grounds of failing the TAST. The safety tests sometimes have to be repeated, but this usually seems to be because of causes other than the vaccine itself. However, so far only general reports lacking precise data or only data from a single company were available. For clostridial vaccines EDQM asked manufacturers to supply data on results of the test. Furthermore, pharmacovigilance data for each product should be send in as an indicator of field safety (EDQM, 1997).

To provide this kind of data our study aimed

- To collect and compare requirements and guidelines which stipulate the TAST
- To collect and evaluate data about the TAST from OMCLs
- To collect and evaluate data about the TAST from manufacturers
- To collect and evaluate pharmacovigilance data of IVMPs

Requirements and guidelines which stipulate the TAST

The TAST is required in the general Ph.Eur. monograph „Vaccines for veterinary use“ and in the EU Guidelines. Therefore this animal test has to be performed routinely on every single batch. Furthermore the TAST is outlined in most individual Ph.Eur. monographs with several monograph specific modifications. Even though the animal numbers for one test is comparably low, the high number of batches results in considerable high figures. Especially the relatively high numbers of large animals and companion animals give raise to concern.

A comparison between the EU Guidelines and the Ph.Eur. monographs also show discrepancies. As this also includes the general requirement for the test (e.g. immunosera) or affects animal numbers (e.g. fish vaccines) there is an urgent need for harmonisation.

With respect to the VICH negotiations it is important to highlight that the requirements for safety testing e.g. in the United States vary considerably from the European legislation.

Data about the TAST from manufacturers

Most companies represented in the Biologicals Working Party of FEDESA participated in the study and sent in data about the TAST. In more than 98% of all tests the batch passed. Only 7 batches (< 0,05%) failed in the test. Indeed there are companies where not even a repeat of the test was necessary during the three year period. No vaccine batch intended for use in poultry, fish, cats or rabbits failed.

Data about the TAST from OMCLs

Seven OMCLs participated in the study. They received more than 11,000 batches of IVMPs during the evaluation period and repeated the TAST for 670 batches (~ 6% of all batches). More than 99% of the batches passed. Only one batch failed in the test which gives a ratio of 0,15%.

It has to be noted that >50% of all tests were performed in chickens and rabbits, laboratory animals which are easily available for such kind of testing. On the contrary, only 6 tests were performed in pet animals (0,9%) and only 16 tests were performed in horses or cattle (2,4%). Thus the retesting of the OMCLs is not random and is not representative for the whole spectrum of products.

It has to be highlighted that the OMCLs concentrated their retesting on those animal species where (at least in the reported period) no batch failed in the quality control of manufacturers. However, in farm animals and dogs where a few failures occurred retesting by OMCLs was very rarely performed. Thus retesting of safety in the TAST at the OMCLs is of questionable value.

Pharmacovigilance data of IVMPs

Much emphasis has been placed to establish a pharmacovigilance system for veterinary medicines in the EU. In 1995 only four MS had developed a structured system: Germany, UK, France and Ireland. In the meantime, many more MS have provided their first statistics and the system is rapidly developing in most MS. But it was only possible to receive data from UK and Germany for the time period investigated in this study.

The rate of underreporting of ADRs is estimated to be very high. There is a need to stimulate reporting of ADRs in all species but especially in poultry, pigs and aquaria fish (Gray, 1991).

However, the data available so far already provides valuable information about individual products or product groups which need further attention. Apart from the spontaneous reports, the periodic safety updates which have to be submitted by the companies at regular intervals will add a lot more product specific safety information. Furthermore, the rapid alert system which is now established in Europe should guarantee that adequate actions are taken immediately if serious ADRs occur in the field. For example, the contamination of a live bovine vaccine which was not detected in the TAST became obvious because a rapid alert was initiated (Falcone et al. 2000).

Within the pharmacovigilance system more attention should be given to specific problems with vaccines at European level. For example, the action of the UK in

establishing a working group to investigate vaccination ADRs in pets seems to be an appropriate approach. Similar activities could be initiated to investigate the background for the relatively high proportion of hypersensitivity/anaphylactic reactions in cattle or the injection site reactions in horses.

Another possibility to investigate safety problems of a specific product would be the performance of a post-marketing surveillance study. However, this approach which is quite common for human medicines has so far not been used for IVMPs.

Conclusion

Overall the data of this study support the position that the routine performance of the TAST as it is required at the moment does not contribute to the safety of IVMPs. Vaccine batches are hardly ever rejected as a result of safety tests. Therefore, it should be possible to abandon the TAST completely as a routine batch control test.

It must be considered whether the TAST may still be required under certain circumstances, e.g. for new products, for a certain period after licensing or for vaccines which caused serious pharmacovigilance problems. If this is the case, clear guidance how to perform and evaluate the test is necessary. This includes the following criteria:

number of animals
dose to be used
acceptable/non-acceptable local and systemic reactions
conditions for repeating the test.

At the moment, the specifications for the TAST in the different monographs vary considerably in many aspects. Therefore, the creation of a guideline which gives a common framework and a standardised approach for this kind of testing would be very helpful. Particular aspects of individual vaccines could then be specified in the specific monographs. As there are considerable differences between mammalian, avian and fish vaccines, these groups should be treated separately.

VIII. Summary

The strict and extensive regulations for IVMPs and the efforts of the vaccine manufacturers have resulted in great improvements in the production and quality control of biological products. Today an extensive safety evaluation has to be performed during R&D of a new product which has to be documented in the licensing application. GMP ensures that each batch of IVMPs is produced at a high standard and that batch-to-batch consistency is preserved. Furthermore national and international networks for pharmacovigilance are now established which ensure that

fast and efficient measures are taken in any case of serious adverse reactions in the field.

The existing kind of safety testing for routine batch control is expensive and requires large numbers of animals. Overall, there is no evidence that the test in the current form leads to a real improvement of product safety. Therefore a deletion of the TAST as a standard requirement for batch control of IVMPs is strongly recommended. For those products/product groups where the TAST is considered to be still necessary in the future, a new concept is urgently needed. This would replace the uncritical routine use of the safety test by a product specific state-of-the-art concept which reflects the actual standards of vaccine production and control, includes a risk-benefit-analysis and respects animal welfare legislation.

IX. Recommendations

The following recommendations result from the report:

- A request for revision of Ph.Eur. Monograph 62 „Vaccines for Veterinary Use“ should be initiated to delete the requirement that the TAST is a routine batch control test and to provide guidance how to perform and evaluate the TAST.
- The CVMP should be asked to revise the EU Guidelines for IVMPs and to delete the requirement that the TAST is a routine batch control test.
- Requests for revision should be initiated for all individual Ph.Eur. monographs of IVMPs where sufficient information is available to support the deletion of the TAST (e.g. for clostridial vaccines, erysipelas vaccines, immunosera).
- The Ph.Eur. Group 15V and the CVMP-IWP should be asked to harmonise the TAST requirements in the Ph.Eur. and in the EU Guidelines and to provide general guidance how to perform and evaluate the TAST for those products or circumstances where the TAST is still considered to be necessary in future.
- The CVMP-Pharmacovigilance WP should be asked to initiate an annual pharmacovigilance report for all IVMPs in the EU and to discuss the results with the IWP to ensure that existing and arising safety concern for specific products/product groups can be adequately addressed.
- The VICH WP on Safety Testing should receive this report and should be asked to include harmonisation of batch safety testing of IVMPs into their work programme.
- The results of this project should be presented to the OMCLs at the next annual meeting.
- The results of this report should be presented at an international conference about regulatory requirements for IVMPs, e.g. the next IABS conference.

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A N N E X E S

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XII-4 Proposals for deletion of the TAST for several IVMPs

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XII-4-3 Proposal for deletion of the TAST for Immunosera from the EU Guideline

XII-1 Data from OMCLs

XII-1-1 Questionnaire

The following questionnaire was send to all OMCLs listed in the EDQM directory which mentioned that they perform the TAST for quality control of IVMPs.

XII-1-2 Summary of answers

Seven OMCLs answered and sent the questionnaire back. Only one laboratory used the possibility to send the data to EDQM. The individual answers are available on request.

AGAATI

Advisory **G**roup on **A**lternatives to
Animal **T**esting in **I**mmunobiologicals

Relevance of the Target Animal Safety Test

Results of the OMCL questionaire

7 OMCLs answered: Belgium
Finland
Germany
Italy
Portugal
Switzerland
The Netherlands

A. OMCL - questionnaire:

In which way do you regularly control and check the incoming batch release documents and test samples of the immunobiologicals which the companies want to release in your country.

1. Do you check the batch release documents and release the batches on an administrative basis.
   *: *one country: both*

Yes	No
6 x*	2 x*

2. Do you check the batch release documents and perform laboratory tests ?
   : once - only for certain Immunobiologicals

Yes	No
6 x*	1 x

Do the laboratory tests include the Target Animal Safety Test (TAST) ?

Yes	No
3 x	3 x

When you sometimes perform animal tests, what are the Criteria for testing:

	Yes	No
- ad random:	3 x	
- suspicion:	3 x	
- others:	2 x	

3. Do you control the quality of batches on a Quality Assessment inspection basis?
   the governm. authority is not responsible

Yes	No
	7 x

4. Has there been a change in your quality control system recently ?

Yes	No
1 x*	6 x

If yes, in which way? ** the governm. authority's assistance is given to local authority*

5. Are there any differences in the treatment of batches depending on whether they come from the USA, Switzerland or from a European Country?

Yes	No
2 x*	4 x

If yes,
please specify: *: *see EU rules and directives, & more stringent protocol for batches from USA*

6. Do you treat batches differently depending on whether they come from a centralised, a mutual recognised or from a national procedure?

Yes	No
1 x*	5 x

If yes,
please specify: *: *centralised ≡ only tests which have not been performed by another MS before*

7. This number of batches of immunologicals were sent to the agencies (7) in the year

Vaccines for	1994	1995	1996	1997	Total	Estimated use of animals*
Horses	119	127	141	143	530	1.060
Cattle	353	384	399	436	1587	3.174
Swine	546	609	733	794	2682	5.364
Sheep and goats	61	43	35	41	180	360
Dogs	390	422	668	662	2142	4.284
Cats	172	194	239	223	828	1.656
Foxes and mink	61	67	116	70	314	628
Rabbits	70	81	73	121	345	1.725
Chicken	518	551	624	625	2318	23.180
Pigeons	36	24	35	33	128	640
Turkeys	14	15	15	21	65	325
Other birds	10	5	7	5	27	135
Fish	14	7	6	12	39	390
Total SUM	2.364	2.529	3.091	3.186	11.185	42.921
Batches without TAST	10	5	3	5	23	0

* : based on
2 animals per test for horses, cattle, swine, sheep and goats, dogs, cats, foxes and mink;
5 animals per test for rabbits, pigeons, turkeys, ducks, geese, canaries;
10 animals per test for chicken;
10 fish per test.

These are the figures of how many batches of immunologicals were sent to the different agencies A – G between 1994-1997.

Vaccines for	A	B	C	D	E	F	G*
Horses	0	5	133	312	47	33	0
Cattle	0	8	12	1039	107	366	55
Swine	192	7	180	1330	233	692	48
Sheep and goats	0	0	22	44	0	102	12
Dogs	35	4	314	866	141	753	29
Cats	10	2	115	532	41	121	7
Foxes and mink	0	0	88	226	0	0	0
Rabbits	0	0	0	158	13	174	0
Chicken	174	6	111	884	277	866	0
Pigeons	14	0	2	71	11	30	0
Turkeys	0	5	0	20	8	32	0
Other birds	0	1	0	26	0	0	0
Fish	0	0	31	8	0	0	0
Batches without TAST	0	0	0	0	0	0	23
Sum	425	38	1008	5516	878	3169	174

* only batches from companies of the country without international shipping

8. These are the number of batches which were tested in the OMCL-Labs using the Target Animal Safety Test.

Vaccines for:	1994 - 1997	Passed	Passed after retest'	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	-	-		

* inactivated bacterial vaccines

9. These batches were not released /drawn back by the company.

Reason	1994	1995	1996	1997	***Total***
Safety -TAST*	0	0	0	1	**1**
-other safety tests	0	3	0	0	**3**
Potency	16	6	1	4	**27**
Others	3	16	21	11	**51**
Total	**19**	**25**	**22**	**16**	**82**

* : Target Animal Safety Test

10. Do you carry out other in vivo tests for batch release than the TAST which reveal / cover safety aspects?

Yes	No
3 x	4 x

If yes,
please specify: *Potency : 3 x*
Extraneous Agents Test: 1 x

B. Your comments / suggestions

1. Necessity for the test

Do you think that the TAST .... (You may choose more than one option)

[1x] Is a necessary safety test for all immunobiologicals
currently to be carried out by the company

[1x] Is nowadays an unnecessary animal test which should be completely removed.

[5x] Is only necessary in the following cases

[ ] For certain species (e.g. poultry)
Please specify species:

Your reasons (please use a separate sheet of paper if necessary)

[5x] For certain types of immunologicals
Please specify type of immunological -→ *e.g. with high residual virulence, live vaccines in target species (SPF), oily vaccines*

Your reasons:
Residual pathogenicity, biological characteristics of strains etc.,

[2x] For special products
Please specify products: *Vaccines containing antigens : footroot, Aujeszky*
Your reasons :

[1x] When no other test based on animals is performed for batch control

[3x] Only for a limited period or limited number of batches of a new product (e.g. the first 10 batches after licensing)

If yes, please specify:
[1x] Time period
} *depends on product*
[2x] Number of batches *(canary pox only small number of batches)*

[1x] Or other criteria

[3x] When pharmacovigilance reports show a potential safety risk for a product

[1x] Others:
A safety test has to be applied during licensing of a product but not for single batches

2. Modification of the test

If you think that the TAST is still required which modifications would you suggest:

[ ] No modification at all

[1x] Reduction of the number of animals to two animal per test for animal welfare Reasons (e.g. chicken vaccines)

[1x] Increase of the number of animals for statistical reasons;
Suggested number of animals: *for a limited time period*

[3x] Clearly defined pass / fail criteria for systemic and local reactions

[ ] Test performance should be harmonised as far as possible for all products

[4x] Test should be individually specified for each monograph

[4x] Test should be reduced to a single dose application to be able to combine Safety and Potency Testing (e.g. for chicken vaccines)

[ ] Other modifications; please specify:

3. Other comments

A safety test has to be applied during licensing of a product but not for single batches.

For chicken vaccines: problems – potency tests of inactivated chick vaccines:
ND – 1/50 dose / animal
Others - 1 dose / animal

TAST is, in my opinion, mainly useful for contaminating viruses in live vaccines (or batches).
The recent matter with BHV1 vaccine contaminated with BVD virus (pestivirus) shows that a TAST is only useful if the animals used for testing are negative for antibodies against possible contaminating viruses. For some species, this will be difficult (e.g. horses). TAST could be replaced by other tests such as PCR if available considering that contaminating viruses are detected (even in very low quantity).

XII-1-3 List of participants

List of participating OMCLs in alphabetical order which is not identical with the listing in the tables

Belgium	Faculty of Vet Medicine Kristien van Reelfs Salisburglaan 133 B-9820 Meselbeke
Italy	Instituto Superiore de Sanita Maria Tollis Viale Regina Elena 299 I-00161 Roma
Finland	National Veterinary and Food Research Institute Martti Nevalainen PO Box 368 FI-00231 Helsinki
Germany	Paul-Ehrlich-Institut Veterinary Department Dr. M. Moos Paul-Ehrlich-Straße 51-59 D-63225 Langen
The Netherlands	Institute for Animal Science and Health Department International Registration and Requirements Dr. Hok Oei ID-DLO Edelhertweg 15 NL-8200 AB Lelystad
Portugal	Laboratório National de Investigação Veterinária Antonio Valadares Estrade de Benfica No. 701 P-1500 Lisboa
Switzerland	Institute for Virology and Immunoprophylaxis Dr. Lukas Bruckner Sensemstraße 293 CH-3147 Mittelhäusern

XII-2 Data from industry

XII-2-1 Questionnaire

The members of the Biologicals Working Party of FEDESA were asked to provide data on the TAST performed during 1997 – 1999 for internal batch release.

Target Animal Safety Test: Number of Batches of Immunologicals tested

Vaccines for:	Manufacturers' quality control				Batches rejected by authorities because of the TAST[1]
	year: 199...	passed	passed after retest[1]	failed[1]	
Horses					
Cattle					
Swine					
Sheep and goats					
Dogs					
Cats					
Foxes and minks					
Rabbits					
Chicken					
Pigeons					
Turkeys					
Ducks and geese					
Fish					

[1] Please, specify product groups [e.g. erysipelas vaccine (inactivated) or combined erysipelas/parvovirosis vaccine]

XII-2-2 Data from individual companies

Target Animal Safety Test
Number of batches of immunologicals tested 1997 – 1999
by Company No 1

Vaccines	**1997**	**1997**	**1997**	**1997**	**1998**	**1998**	**1998**	**1998**	**1999**	**1999**	**1999**	**1999**	**1997-99**	**1997-99**	**1997-99**	**1997-99**
for	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed
Fishes	20	19	1		8	8			15	14	1		43	41	2	
Total	**20**	**19**	**1**	**0**	**8**	**8**	**0**	**0**	**15**	**14**	**1**	**0**	**43**	**41**	**2**	**0**

Target Animal Safety Test
Number of batches of immunologicals tested 1997 – 1999
by Company No 2

Vaccines	**1997**	**1997**	**1997**	**1997**	**1998**	**1998**	**1998**	**1998**	**1999**	**1999**	**1999**	**1999**	**1997-99**	**1997-99**	**1997-99**	**1997-99**
for	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed
Horses	2	2			3	3			3	3			8	8		
Cattle	12	12			24	24			39	39			75	75		
Dogs	2	2			2	2			2	2			6	6		
Cats	2	2			2	2			2	2			6	6		
Total	**18**	**18**	**0**	**0**	**31**	**31**	**0**	**0**	**46**	**46**	**0**	**0**	**95**	**95**	**0**	**0**

Target Animal Safety Test
Number of batches of immunologicals tested 1995 – 2000
by Company No 3

Vaccines	**1995-2000**	**1995-2000**	**1995-2000**	**1995-2000**
for	total	passed	passed after retest	failed
Swine	564	564		
Total	**564**	**564**	**0**	**0**

Target Animal Safety Test
Number of batches of immunologicals tested 1997 – 1999
by Company No 4

Vaccines for	**1997** total	**1997** passed	**1997** passed after retest	**1997** failed	**1998** total	**1998** passed	**1998** passed after retest	**1998** failed	**1999** total	**1999** passed	**1999** passed after retest	**1999** failed	**1997-99** total	**1997-99** passed	**1997-99** passed after retest	**1997-99** failed
Cattle	1	1			3	3			2	2			6	6		
Swine	42	41		1	32	32			50	50			124	123		1
Sheep/Goats	56	56			57	57			45	39	5	1	158	152	5	1
Rabbits	7	7			8	8			4	4			19	19		
Chicken	244	241	3		178	177	1		243	243			665	661	4	
Turkeys					4	4			6	6			10	10		
Ducks/Geese	15	15			23	23			16	16			54	54		
Total	**365**	**361**	**3**	**1**	**305**	**304**	**1**	**0**	**366**	**360**	**5**	**1**	**1036**	**1025**	**9**	**2**

Target Animal Safety Test
Number of batches of immunologicals tested 1997 – 1999
by Company No 5

Vaccines for	**1997** total	**1997** passed	**1997** passed after retest	**1997** failed	**1998** total	**1998** passed	**1998** passed after retest	**1998** failed	**1999** total	**1999** passed	**1999** passed after retest	**1999** failed	**1997-99** total	**1997-99** passed	**1997-99** passed after retest	**1997-99** failed
Cattle	6	6			3	3			6	6			15	15		
Swine	45	45			49	49			52	52			146	146		
Sheep/Goats	17	17			21	21			19	19			57	57		
Rabbits	18	18			15	15			26	26			59	59		
Chicken	353	353			330	330			384	384			1067	1067		
Fishes	11	11			15	15			16	16			42	42		
Total	**450**	**450**	**0**	**0**	**433**	**433**	**0**	**0**	**503**	**503**	**0**	**0**	**1386**	**1386**	**0**	**0**

Target Animal Safety Test
Number of batches of immunologicals tested 1999
by Company No 6

Vaccines	**1999**	**1999**	**1999**	**1999**
for	total	passed	passed after retest	failed
Swine	43	43		
Sheep/Goats	12	12		
Rabbits	9	9		
Chicken	24	24		
Total	**88**	**88**	**0**	**0**

Target Animal Safety Test
Number of batches of immunologicals tested 1998 – 1999
by Company No 7

Vaccines	**1998**	**1998**	**1998**	**1998**	**1999**	**1999**	**1999**	**1999**	**1998-99**	**1998-99**	**1998-99**	**1998-99**
for	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed
Horses	12	12			13	13			25	25		
Cattle	30	30			23	23			53	53		
Dogs	97	92	4	1	96	91	4	1	193	183	8	2
Cats	59	59			25	23	2		84	82	2	
Total	**198**	**193**	**4**	**1**	**157**	**150**	**6**	**1**	**355**	**343**	**10**	**2**

Target Animal Safety Test
Number of batches of immunologicals tested 1998 – 1999
by Company No 8

Vaccines	**1998**	**1998**	**1998**	**1998**	**1999**	**1999**	**1999**	**1999**	**1998-99**	**1998-99**	**1998-99**	**1998-99**
for	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed
Horses	28	25	3		46	40	6		74	65	9	
Cattle					3	2	1		3	2	1	
Swine	62	60	2		56	53	3		118	113	5	
Dogs	48	44	4		17	16	1		65	60	5	
Cats	2	2			2	2			4	4		
Chicken	65	62	3		113	108	5		178	170	8	
Pigeons	29	29			26	26			55	55		
Total	**234**	**222**	**12**	**0**	**263**	**247**	**16**	**0**	**497**	**469**	**28**	**0**

Target Animal Safety Test
Number of batches of immunologicals tested 1997 – 1999
by Company No 9

Vaccines	**1997**	**1997**	**1997**	**1997**	**1998**	**1998**	**1998**	**1998**	**1999**	**1999**	**1999**	**1999**	**1997-99**	**1997-99**	**1997-99**	**1997-99**
for	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed
Horses	19	19			20	20			29	29			68	68		
Cattle	5	5			14	14			14	12	1	1	33	31	1	1
Swine	10	10			16	16			10	10			36	36		
Dogs	45	45			41	41			28	28			114	114		
Cats	25	25			23	23			25	25			73	73		
Total	**104**	**104**	**0**	**0**	**114**	**114**	**0**	**0**	**106**	**104**	**1**	**1**	**324**	**322**	**1**	**1**

Target Animal Safety Test
Number of batches of immunologicals tested 1997 – 1999
by Company No 10

Vaccines	**1997-1999**	**1997-1999**	**1997-1999**	**1997-1999**
for	total	passed	passed after retest	failed
Cattle	24	24		
Swine	19	19		
Sheep/Goats	153	151	2	
Chicken	77	77		
Turkeys	10	10		
Total	**283**	**281**	**2**	**0**

Target Animal Safety Test
Number of batches of immunologicals tested 1997 – 1999
by Company No 11

Vaccines for	**1997** total	**1997** passed	**1997** passed after retest	**1997** failed	**1998** total	**1998** passed	**1998** passed after retest	**1998** failed	**1999** total	**1999** passed	**1999** passed after retest	**1999** failed	**1997-99** total	**1997-99** passed	**1997-99** passed after retest	**1997-99** failed
Chicken	667	652	15		621	602	19		736	715	21		2024	1969	55	
Pigeons	3	3			2	2			4	4			9	9		
Turkeys	22	21	1		18	18			20	17	3		60	56	4	
Ducks/Geese	2	2			5	5			8	8			15	15		
Total	**694**	**678**	**16**	**0**	**646**	**627**	**19**	**0**	**768**	**744**	**24**	**0**	**2108**	**2049**	**59**	**0**

Target Animal Safety Test
Number of batches of immunologicals tested 1997 – 1999
by Company No 12

Vaccines	**1997**	**1997**	**1997**	**1997**	**1998**	**1998**	**1998**	**1998**	**1999**	**1999**	**1999**	**1999**	**1997-99**	**1997-99**	**1997-99**	**1997-99**
for	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed
Horses	13	13			15	14	1		23	19	3	1	51	46	4	1
Cattle	48	46	2		50	44	6		33	33			131	123	8	
Swine	78	78			116	110	6		76	75	1		270	263	7	
Sheep/Goats	11	11			8	8			12	12			31	31		
Dogs	173	170	3		184	182	2		178	175	3		535	527	8	
Cats	32	31	1		45	43	2		42	42			119	116	3	
Rabbits	18	18			18	18			18	18			54	54		
Chicken	398	388	10		383	378	5		332	329	3		1113	1095	18	
Turkeys	16	14	2		23	22	1		22	22			61	58	3	
Ducks/Geese	32	32			43	43			26	26			101	101		
Total	**819**	**801**	**18**	**0**	**885**	**862**	**23**	**0**	**762**	**751**	**10**	**1**	**2466**	**2414**	**51**	**1**

Target Animal Safety Test
Number of batches of immunologicals tested 1997 – 1999
by Company No 13

Vaccines for	**1997** total	**1997** passed	**1997** passed after retest	**1997** failed	**1998** total	**1998** passed	**1998** passed after retest	**1998** failed	**1999** total	**1999** passed	**1999** passed after retest	**1999** failed	**1997-99** total	**1997-99** passed	**1997-99** passed after retest	**1997-99** failed
Cattle	87	86	1		76	74	2		99	95	4		262	255	7	
Swine	334	322	12		400	394	6		273	273			1007	989	18	
Dogs	119	118	1		120	111	8	1	169	154	15		408	383	24	1
Cats	36	35	1		40	37	3		30	30			106	102	4	
Total	**576**	**561**	**15**	**0**	**636**	**616**	**19**	**1**	**571**	**552**	**19**	**0**	**1783**	**1729**	**53**	**1**

Target Animal Safety Test
Number of batches of immunologicals tested 1998 – 1999
by Company No 14

Vaccines	**1998**	**1998**	**1998**	**1998**	**1999**	**1999**	**1999**	**1999**	**1998-99**	**1998-99**	**1998-99**	**1998-99**
for	total	passed	passed after retest	failed	total	passed	passed after retest	failed	total	passed	passed after retest	failed
Dogs	126	126			123	123			249	249		
Cats	50	50			59	59			109	109		
Total	**176**	**176**	**0**	**0**	**182**	**182**	**0**	**0**	**358**	**358**	**0**	**0**

XII-2-3 Data from industry by animal species

Batches of horse vaccines tested by companies in the TAST

Company-No	Years	Animal	Passed	after retest	failed
2	97-99	8	8		
7	98-99	25	25		
8	98-99	74	65	9	
9	97-99	68	68		
12	97-99	51	46	4	1
Total		**226**	**212**	**13**	**1**

Batches of cattle vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
2	97-99	75	75		
4	97-99	6	6		
5	97-99	15	15		
7	98-99	53	53		
8	98-99	3	2	1	
9	97-99	33	31	1	1
10	97-99	24	24		
12	97-99	131	123	8	
13	97-99	262	255	7	
Total		**602**	**584**	**17**	**1**

Batches of swine vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
3	total 95-00	564	564		
4	97-99	124	123		1
5	97-99	146	146		
6	99	43	43		
8	98-99	118	113	5	
9	97-99	36	36		
10	97-99	19	19		
12	97-99	270	263	7	
13	97-99	1007	989	18	
Total		**2327**	**2296**	**30**	**1**

Batches of sheep/goat vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
4	97-99	158	152	5	1
5	97-99	57	57		
6	99	12	12		
10	97-99	153	151	2	
12	97-99	31	31		
Total		**411**	**403**	**7**	**1**

Batches of dog vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
2	97-99	6	6		
7	98-99	193	183	8	2
8	98-99	65	60	5	
9	97-99	114	114		
12	97-99	535	527	8	
13	97-99	408	383	24	1
14	98-99	249	249		
Total		**1570**	**1522**	**45**	**3**

Batches of cat vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
2	97-99	6	6		
7	98-99	84	82	2	
8	98-99	4	4		
9	97-99	73	73		
12	97-99	119	116	3	
13	97-99	106	102	4	
14	98-99	109	109		
Total		**501**	**492**	**9**	**0**

Batches of rabbit vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
4	97-99	19	19		
5	97-99	59	59		
6	99	9	9		
12	97-99	54	54		
Total		**141**	**141**	**0**	**0**

Batches of chicken vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
4	97-99	665	661	4	
5	97-99	1067	1067		
6	99	24	24		
8	98-99	178	170	8	
10	97-99	77	77		
11	97-99	2024	1969	55	
12	97-99	1113	1095	18	
Total		**5148**	**5063**	**85**	**0**

Batches of pigeon vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
8	98-99	55	55		
11	97-99	9	9		
Total		**64**	**64**	**0**	**0**

Batches of turkey vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
4	97-99	10	10		
10	97-99	10	10		
11	97-99	60	56	4	
12	97-99	61	58	3	
Total		**141**	**134**	**7**	**0**

Batches of duck/geese vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
4	97-99	54	54		
11	97-99	15	15		
12	97-99	101	101		
Total		**170**	**170**	**0**	**0**

Batches of fish vaccines tested by companies in the TAST

Company-No	Years	Animal	passed	after retest	failed
1	97-99	43	41	2	
5	97-99	42	42		
Total		**85**	**83**	**2**	**0**

XII-2-4 List of participants

List of participating companies
(in alphabetical order which is not identical with the company-No in the Tables)

Alpharma	Alpharma AS POB 158 Sköyen 0212 Oslo Norway
Bayer	Bayer AG Zentrum Landwirtschaft Monheim D-51368 Leverkusen Germany
Boehringer	Boehringer Ingelheim Vetmedica GmbH 55216 Ingelheim am Rhein Germany
Ceva	CEVA-PHYLAXIA Szallas u. 5 H-1107 Budapest Hungary
Fatro	FATRO SpA Via Emilia, 285 I-40064 Ozzano Emilia (BO) Italy
Fort Dodge	Fort Dodge Animal Health Huizerstraatweg 117 NL-1411 GM Naarden The Netherlands
Hoechst	Hoechst Roussel Vet Vertriebs GmbH Postfach 33 11 65023 Wiesbaden Germany
Intervet	Intervet International B.V. Wim de Körverstraat 35 P.O. Box 31 5830 AA Boxmeer The Netherlands
Merial	Mérial Avenue Tony Garnier, 27 F-69348 Lyon Cedex 07 France
Pfizer	Pfizer Animal Health Mercuriusstraat, 20 B-1930 Zaventem Belgium
Virbac	Virbac Laboratoires F-06511 Carros Cedex France

XII-3 Pharmacovigilance data

XII-3-1 Pharmacovigilance data from UK

published in: The Veterinary Record. July 23, 1994: 77-81
The Veterinary Record. December 3, 1994: 544
The Veterinary Record. January 13, 1996: 826
The Veterinary Record. June 8, 1996: 557-558
The Veterinary Record. July 5, 1997: 4
The Veterinary Record. July 18, 1998: 60-61

Pharmacovigilance reports in UK 1993 – 1997

(Possible reactions received under the Suspected Adverse Reaction Surveillance Scheme)

Year	Group of Medicines	Dog	Cat	Horse	Bovine	Sheep	Pigs	Rabbit	Chicken	Other	Total
1997	Total	337	378	67	39	16	4	24	-	6	871
	Antisera	-	-	1	-	-	-	-	-	-	1
	Live vaccines	63	113	2	1	-	-	11	-	-	190
	Inactivated vaccines	7	28	24	-	6	2	13	-	-	81
1996	Total	219	234	58	43	21	7	18	-	8	608
	Antisera	-	-	-	2	-	-	-	-	-	2
	Live vaccines	36	49	-	1	-	-	2	-	-	88
	Inactivated vaccines	8	17	28	3	3	5	16	-	-	80
1995	Total	211	218	69	54	13	9	8	-*	11	593
	Antisera	-	-	1	-	-	-	-	-	-	1
	Live vaccines	34	26	-	1	-	-	6	-	1	68
	Inactivated vaccines	7	25	34	-	4	3	2	-	-	75
1994	Total	219	148	71	54	8	2	7	-*	2	512
	Antisera	-	-	-	-	1	-	-	-	-	1
	Live vaccines	65	27	-	1	1	1	4	-	-	99
	Inactivated vaccines	13	24	32	2	4	-	2	-	-	77
1993	Total	170	122	96	43	23	6	-*	-*	14	474
	Antisera	-	-	1	-	-	-	-	-	-	1
	Live vaccines	32	14	-	1	-	-	-	-	4	51
	Inactivated vaccines	3	31	53	1	3	-	-	-	1	92

* Data not separately listed (included under Others)

Pharmacovigilance Reports in UK (1993 – 1997) for individual animal species

- for dogs

	No of reports	%
Total	1156	100,00
Antisera	-	0,00
Live vaccines	230	19,89
Inactivated vaccines	38	3,28
All immunologicals	268	23,18

- for cats

	No of reports	%
Total	1100	100,00
Antisera	-	0,00
Live vaccines	229	20,81
Inactivated vaccines	125	11,36
All immunologicals	354	32,18

- for horses

	No of reports	%
Total	361	100,00
Antisera	3	0,83
Live vaccines	2	0,55
Inactivated vaccines	171	47,36
All immunologicals	176	48,75

- for cattle

	No of reports	%
Total	233	100,00
Antisera	2	0,85
Live vaccines	5	2,14
Inactivated vaccines	6	2,57
All immunologicals	13	5,57

- for sheep

	No of reports	%
Total	81	100,00
Antisera	1	1,23
Live vaccines	1	1,23
Inactivated vaccines	20	24,69
All immunologicals	22	27,16

- for pigs

	No of reports	%
Total	28	100,00
Antisera	-	0,00
Live vaccines	1	3,57
Inactivated vaccines	10	35,71
All immunologicals	11	39,28

- for rabbits

	No of reports	%
Total	57	100,00
Antisera	-	0,00
Live vaccines	23	40,35
Inactivated vaccines	33	57,89
All immunologicals	56	98,24

XII-3-2 Pharmacovigilance data from Germany

Kindly provided by Dr. Esther Werner, Paul-Ehrlich-Institut

Pharmacovigilance Reports for Vaccines in Germany in 1997

Year	Vaccine group	Dog	Cat	Horse	Cattle	Sheep/goat	Swine	Rabbit	Poultry	Total
1997	total	66 (83)	23 (26)	20 (23)	62 (74)	3 (4)	18 (16)	15 (40)	15 (21)	222 (301)
	*									
	inactivated	15	11	18	25	3	13	9	1	95
	live	4	5	2	34	0	5	6	14	70
	inact. + live	47	7	0	3	0	0	0	0	57
										222
	bacterial	1	0	0	11	3	12	3	0	30
	viral	8	21	13	32	0	6	12	1	93
	bact. + viral	57	0	6	3	0	0	0	14	80
	fungal	0	0	0	15	0	0	0	0	15
	serum	0	2	1	1	0	0	0	0	4
										222
	local reaction	16	5	7	0	2	1	11	14	56
	systemic reaction	49	18	7	62	1	17	4	1	159
	local + systemic	1	0	6	0	0	0	0	0	7
										222

*Number of reports related to safety(total number of reports)

Pharmacovigilance Reports for Vaccines in Germany in 1996

Year	Vaccine group	Dog	Cat	Horse	Cattle	Sheep/goat	Swine	Rabbit	Poultry	Total
1996	total	50 (68)	37 (47)	6 (6)	66 (120)	39 (39)	44 (59)	7 (32)	1 (1)	250 (375)
	*									
	inactivated	14	14	5	25	5	20	4	0	87
	live	1	16	1	35	34	24	3	1	115
	inact. + live	35	7	0	6	0	0	0	0	48
										250
	bacterial	0	0	0	7	39	14	0	1	61
	viral	6	30	4	31	0	28	7	0	106
	bact. + viral	43	4	2	6	0	1	0	0	56
	fungal	0	0	0	22	0	0	0	0	22
	serum	1	3	0	0	0	1	0	0	5
										250
	local reaction	6	5	0	2	1	3	5	0	22
	systemic reaction	38	31	5	64	4	41	2	1	186
	local + systemic	6	1	1	0	34	0	0	0	42
										250

*Number of reports related to safety (total number of reports)

Pharmacovigilance Reports for Vaccines in Germany in 1995

Year	Vaccine group	Dog	Cat	Horse	Cattle	Sheep/goat	Swine	Rabbit	Poultry	Total
1995	total	39 (52)	24 (37)	15 (17)	45 /67)	11 (12)	10 (19)	0 (11)	3 (5)	147 (223)
	*									
	inactivated	6	11	15	17	0	10	0	1	60
	live	1	9	0	27	11	0	0	2	50
	inact. + live	32	4	0	1	0	0	0	0	37
										147
	bacterial	0	0	1	4	11	3	0	1	20
	viral	3	16	8	27	0	5	0	2	61
	bact. + viral	36	1	6	4	0	1	0	0	48
	fungal	0	0	0	9	0	0	0	0	9
	serum	0	7	0	1	0	1	0	0	9
										147
	local reaction	3	0	8	4	5	2	0	0	22
	systemic reaction	33	24	3	41	0	8	0	3	112
	local + systemic	3	0	4	0	6	0	0	0	13
										147

*Number of reports related to safety (total number of reports)

XII-4 Proposals for deletion of the TAST for several IVMPs

XII-4-1 Proposal for deletion of the TAST for Clostridial vaccines

An evaluation of batch safety test data for clostridial vaccines was sent to the European Pharmacopoeia Commission when the revised text for the following monographs was published in PHARMEUROPA:

Clostridium perfringens vaccines
Clostridium septicum vaccines
Clostridium novyi Type B vaccines

Only the data for *C. perfringens* are attached. The other documents are available on request.

Advisory **G**roup on **A**lternatives to
Animal **T**esting in **I**mmunobiologicals

Paul-Ehrlich-Institut
Bundesamt für Sera und Impfstoffe

Evaluation of the batch safety test for *Cl. perfringens* - vaccines

(*Ph. Eur.* Monograph No. 363)

Conclusion:

For this study the available data have shown that all batches of *Cl. perfringens* vaccines were in compliance with the target animal safety test. Furthermore, there are no pharmacovigilance reports related to the safety of these products.

In conclusion, it is obvious that the production and control of *Cl. perfringens* vaccines have reached such a high quality level that the target animal safety test appears no longer relevant for routine batch control purposes. Therefore it is recommended that this animal test should be deleted in the revised version of the *Ph. Eur.* monograph 363.

This study has been carried out by AGAATI and the PEI. The research group at the PEI is supported by a grant of the German Ministry of Education, Science, Research and Technology (BMBF grant No. 0311 423). We thank the members of the In Vitro Group for providing individual safety data.

Introduction:

The target animal safety test is required as a final product batch test for nearly all vaccines in the *Ph. Eur.*. The performance of these tests needs a large number of farm animals, i.e. cattle, sheep and pigs (Weißer and Hechler, 1997). In general, the relevance of the test has been questioned by industry and control authorities (Roberts and Lucken, 1996; Weißer and Hechler, 1997; Zeegers et al., 1997). However, no detailed data have been available so far.

At the *Symposium on Clostridial Vaccines and Sera* held at Strasbourg, 4 - 6 February 1997, the usefulness of the target animal safety test for routine batch release was again questioned. Manufacturers were asked to provide data on the results of the safety test for at least the last two years (EDQM PA/PH/BIO [97]2).

This report includes the available data of the target animal safety test on batch release of *Cl. perfringens* vaccines. This test is required by the *Ph. Eur.* monograph No. 363. The present data originate from industry and were provided by the International Veterinary Industry Test Replacement Organisation (In Vitro Group). The batch release documents were made available to the Paul-Ehrlich-Institut. In addition the pharmacovigilance reports of *Cl. perfringens* vaccines for Germany and UK have been evaluated.

Part 1 : Safety test data provided by industry (In Vitro Group)

Four of the world's largest vaccine manufacturers provided data on the target animal safety test for the *Cl. perfringens* vaccines (*Ph. Eur.* Monograph No. 363). Most of the vaccines are combined clostridial products. For all the clostridial vaccine batches cited, 1390 sheep (including 12 sheep for *Cl. perfringens* mono-vaccines), 84 cattle and 6 pigs (mono-vaccines) were listed. Only 3 preparations (of 51) are monovalent vaccines for *Cl. perfringens* (< 5.88 %). Data on a total of 703 batches were provided for clostridial vaccine combinations with the *Cl. perfringens* component, including 30 batches with cattle as target species and 3 batches with pigs as target species. They had been tested at different times during the period of 1991 - 1997. The data of the individual companies are listed in Annex 1.

All of the vaccine batches had passed the safety test. No retesting of any vaccine batch had been reported. 0.57 % of the batches were monovalent vaccines.

Part 2: Safety test data of the PEI

In Germany, only 5 *Cl. perfringens* vaccines are licensed, which are combined and single component clostridial products for sheep and pigs. Forty batches were submitted during 1994 - 1997. According to the batch release documents, all 40 batches were in compliance with the requirements of the target animal safety test specified in the monograph No. 363 (see annex 2).
One additional test has been performed for this kind of vaccine at the PEI during that period.

Part 3: Pharmacovigilance data of the VMD

The following table shows the pharmacovigilance data for the UK as reported in the *Veterinary Record*:

Year	Total number of reports	Total number for inactivated vaccines	Total number for inactivated vaccines in ruminants	Safety problems with *Cl. perfringens* vaccines
1997	871	81	6 (all sheep)	not mentioned
1996	608	80	6 (3 bovid, 3 sheep)	not mentioned
1995	593	75	4 (all sheep)	not mentioned
1994	512	77	6 (2 bovid, 4 sheep)	not mentioned
1993	474	92	4 (1 bovid, 3 sheep)	not mentioned
1992	556	71 *	14 (1 bovid, 13 sheep)	not mentioned
1991	332	16	12 (7 bovid, 5 sheep)	**
1990	317	30	10 (all sheep)	not mentioned

* *mink:* one report with suspected lack of efficacy in a botulinum type C component of a multi - component vaccine
pig: two reports with suspected lack of efficacy in clostridial vaccination

** three of the reports in sheep involved post - vaccinal malaise after injecting clostridial - clostridial/pasteurella vaccines, but no link to the *Cl. perfringens* component is mentioned.

Source:	The Veterinary Record :	July 18, 1998	Page 60 - 61
		July 5, 1997	Page 4
		June 8, 1996	Page 557 - 558
		January 13, 1996	Page 26
		December 3, 1994	Page 544
		July 23, 1994	Page 77 - 80
		January 2, 1993	Page 4 - 6
		July 27, 1991	Page 63 - 65

Part 4: Pharmacovigilance data of the PEI

During 1994 - 1997 eight reports of untoward reactions were related to the clostridial products with a *Cl. perfringens* component. This corresponds to 0,75 % of the total number of reports for immunological veterinary products during that period. None of the reports could be related to a specific safety problem with the *Cl. perfringens* component.

Summary:

All 703 released batches of *Cl. perfringens* vaccines passed the target animal safety test. No retesting of any batch is documented. This result may be explained as follows:

1. The production and control of vaccines have reached a high quality level which guarantees a continuous flow of safe *Cl. perfringens* vaccine batches.
2. The target animal safety test is not suitable for a detection of batches with safety problems..

The first explanation is more likely since no pharmacovigilance data were directly related with safety problems attributed to the *Cl. perfringens* vaccine component.

The target animal safety test for the *Cl. perfringens* vaccines has been useful in the past when consistent production of vaccines was difficult and adequate quality control methods were not available. Nowadays, new vaccines and vaccine batches have to comply with a strict licensing procedure and the older products are revised to guarantee a continuous and consistent production at a high level of safety and quality. Thus, for the *Cl. perfringens* vaccine production the target animal safety test only seems to be a relict of the past.

Considering the large number of farm animals (> 60 cattle, 1382 sheep, 36 pigs) used for this test, the minimal contribution they make to quality control and safety of these products does not justify the continuation of this animal test.

The evaluation of the data indicates that the target animal safety test carried out for batch control purposes could be omitted for the *Cl. perfringens* vaccines. Unless other data become available, showing the relevance of this animal test, the deletion of the test is strongly recommended for the revised *Ph. Eur.* Monograph 363.

Nonetheless, as most of the clostridial products are combined vaccines, only the parallel deletion of the target animal safety test from the other clostridial vaccine monographs would considerably reduce the number of farm animals used. Similar reports will be prepared on the other clostridial vaccine monographs. The data evaluated so far indicate a comparable outcome.

Literature:

[1] Roberts, B., R.N. Lucken (1996): Reducing the use of the target animal batch safety test for veterinary vaccines. Dev. Biol. Stand. 86, 97 - 102.

[2] Weißer, K., U. Hechler (1997): Tierschutzaspekte bei der Prüfung von Immunpräparaten - eine kritische Bewertung der Tierversuche in den Arzneibuchmonographien. BMBF - Projekt Nr. 0310 611 A

[3] Zeegers, J.J.W., W.F. de Vries, R. Remie (1996): Reducing the use of animals by abolishment of the safety test as a routine batch control test on veterinary vaccines. ATLA 24 (Supplement), 138

Annex 1:

Company A
No. of *Cl. perfringens* vaccines: 20
Time period : beginning of 1994 until early 1997

Year	No. of batches	Target species	No. of animals	Test results
1994	155	sheep	310	passed
-	10	cattle	20	passed
1997	3	pigs	6	passed

Company B
No. of *Cl. perfringens* vaccines: 3
Time period: 1995 and 1996

Year	No. of batches	Target species	No. of animals	Test results
1995	45	sheep	90	passed
1996	42	sheep	84	passed

Company C
No. of *Cl. perfringens* vaccines: 6
Time period : 1992 until 1996

Year	No. of batches	Target species	No. of animals	Test results
1992	43	sheep	86	passed
1993	58	sheep	116	passed
1994	36	sheep	72	passed
1995	69	sheep	138	passed
1996	40	sheep	80	passed

Company D
No. of *Cl. perfringens* vaccines: 13
Time period : January 1991 until April 1997

Year	No. of batches	Target species	No. of animals	Test results
1991 -	182	sheep	364	NAD
1997	20	cattle	40	NAD

NAD : nothing abnormal detected

Annex 2:

PEI
No. of *Cl. perfringens* vaccines licensed in Germany: 5
Time period : January 1994 until December 1997

Year	No. of batches	Target species	No. of animals	Test results
1994 - 1997	24	sheep	46	Passed
1994 - 1997	16	pigs	28	Passed

XII-4-2 Proposal for deletion of the TAST for Avian vaccines

An evaluation of batch safety test data for avian vaccines was prepared to be presented at the ECVAM / AGAATI-Workshop “Three Rs Approaches in the Production and Quality control of Avian Vaccines” (see also Bruckner et al. 2000, ECVAM Workshop 41)

Data concerning the following vaccines were presented:

1) Avian infectious laryngotracheitis vaccine (live) for chickens
   Ph. Eur . Monograph No. 1068

2) Avian infectious bronchitis vaccine (live), freeze - dried
   Ph. Eur . Monograph No. 442

3) Newcastle Disease Vaccine (live), freeze - dried
   Ph. Eur . Monograph No. 450

4) Avian infectious bursal disease (Gumboro disease) vaccine (live), freeze - dried
   Monograph No. 587

5) Avian infectious encephalomyelitis vaccine (live)
   Ph. Eur . Monograph No. 588

6) Marek’s disease Vaccine (live)
   Monograph No. 589

7) Evaluation of the batch safety test for poultry in the
   Ph. Eur. Monograph No. 62:
   Vaccines for Veterinary Use

8) Fowl-Pox live Vaccine, freeze - dried
   Ph. Eur . Monograph No. 649

9) Newcastle Disease Vaccine (inactivated)
   Ph. Eur . Monograph No. 870

10) Avian infectious bronchitis vaccine (inactivated)
    Ph. Eur. Monograph No. 959

11) Avian infectious bursal disease vaccine (inactivated)
    Monograph No. 960

Only the data for Avian infectious bronchitis are attached. The other documents are available on request.

Advisory **G**roup on **A**lternatives to
Animal **T**esting in **I**mmunobiologicals

Paul-Ehrlich-Institut
Bundesamt für Sera und Impfstoffe

DRAFT REPORT

Evaluation of the batch safety test for *Avian infectious bronchitis vaccine (live), freeze - dried Ph. Eur . Monograph No. 442*

Conclusion:

The target animal safety test is required in the *Ph. Eur.* Monograph No. 442 using at least 10 chickens. For this study the available data have shown that all batches were in compliance. Furthermore there are no pharmacovigilance reports related to this monograph.

In conclusion, it is obvious that the production and control of these products of vaccines have reached such a high quality level that the target animal safety test appears no longer relevant for routine batch control purposes and may therefore be eliminated as a routine test in the monograph no. 442: *Avian infectious bronchitis vaccine (live), freeze – dried.*

This study has been carried out by AGAATI and the PEI. The research group at the PEI is supported by a grant of the German Ministry of Education, Science, Research and Technology (BMBF grant No. 0311 423). AGAATI is supported by the European Commission, contract No. 13410 – 97 – 11 F 1 EI ISP NI.

Introduction:

The target animal safety test (Tast) needs a large number of animals, i.e. cattle, sheep and pigs, as well as dogs, cats and horses and the poultry (Weißer and Hechler, 1997). Whereas only 2 or 3 animals are required to test mammalian vaccines, at least 10 birds are stipulated for poultry vaccines. In general, the relevance of the test has been questioned by industry and control authorities (Roberts and Lucken, 1996; Weißer and Hechler, 1997; Zeegers et al., 1997). However, no detailed data have been available so far.

This report includes the available data of the target animal safety test on batch release of the different vaccines. This test is required by the *Ph. Eur.* Monograph No. 442: *Avian infectious bronchitis vaccine (live), freeze – dried.* The present data originate from industry and were provided for batch release. In addition pharmacovigilance reports have been evaluated.

Part 1: Wording of the monograph

1.1 Definition:
Freeze dried avian bronchitis vaccine (live) is a preparation of one or more attenuated strains of infectious bronchitis virus.

1.2 Safety:
Use not fewer than ten chickens from a flock free from specified pathogens (5.2.2) and of the minimum age stated on the label for vaccination. Administer by eye-drop ten doses reconstituted so as to obtain a concentration suitable for the test. Observe the chickens for 21 days. For vaccines intended for vaccination of chickens two weeks old or more, use the chickens inoculated in the test for extraneous agents using chicks. If during the period of observation more than two chickens die from causes not attributable to the vaccine, repeat the test. The vaccine complies with the test if no chicken shows serious clinical signs, in particular respiratory signs, and no chicken dies from causes attributable to the vaccine.

Part 2 : Safety test data of the PEI

In Germany, 13 different products are licensed. A hundred batch release certificates were submitted during 1994 - 1997. According to the batch release documents, all batches were in compliance with the requirements of the target animal safety test.

There were seven products not due to governmental batch release (“Freistellung”, exemptions according to German law) during the years 1994 – 1997.

Three additional target animal safety tests have been performed for this kind of vaccines at the PEI during that period.

Table 1: Table of products and released batches

Code of Products	*Number of Animals*	*Species*	*Dead Animals*	*Repetitions*	*Number of Batches*	*Number of Fillings*	*Number of Doses*	*Tast (chicks) / PEI*
2027	120	Chicken	*1*	-	12	205.194	774.290.000	35 chicks in 3 tests
2028	50 (+)	Chicken	-	-	5	88.385	210.419.000	
2029	150*	Chicken	-	-	6	103.832	391.399.000	
2031	100*	Chicken	-	-	4	70.230	127.920.500	
2026	25*	Chicken	-	-	1	16.896	16.896.000	
2076	80 (+)	Chicken	-	-	8	109.199	185.006.000	
2074	150	Chicken	-	-	15	189.040	629.923.000	
2075	40 (+)	Chicken	-	-	4	49.971	98.053.500	
2058	450*	Chicken	3	-	18	219.395	634.485.000	
2057	70	Chicken	3	-	13***	135.135	544.425.000	
2056**	0	Chicken	-	-	0	-	-	
2054	155*	Chicken	1	-	6	36.060	36.060.000	
2055	405*	Chicken	-	-	8	103.973	103.973.000	
SUM	**1795**	**Chicken**	**8**	**-**	**100**	**1.327.310**	**3.752.850.000**	**35 (3)**

*: Increased numbers of animals are due to the requirements of regulations from outside of Europe.
**: No batch release between 1994 and 1997 for this product.
***: Some batches are related and did not need a safety test for each batch.
(+): The test for extraneous agents in chickens was combined with the safety test.

Table 2: Table of products not due to governmental batch release ("Freistellungen", exemptions)

Code of Products	*Number of Animals*	*Species*	*Number of Batches*	*Number of Fillings*	*Number of Doses*
2076	60	Chicken	6	*> 4.044	*> 51.456.000
2074	100	Chicken	10	*> 8.244	*> 251.233.000
2075	60	Chicken	6	*> 3.833	*> 66.179.000
2058	375*	Chicken	15	58.600	232.140.000
2057	200*	Chicken	8	20.700	94.250.000
2054	100*	Chicken	4	18.410	18.410.000
2055	150*	Chicken	6	51.620	51.620.000
SUM	**1045**	**Chicken**	**55**	***> 165.451**	***> 765.288.000**

*>: Numbers of fillings and doses are not yet complete.
*: Increased numbers of animals are due to the requirements of regulations from outside of Europe.

Part 3: Pharmacovigilance data

A) Germany

In Germany 1062 pharmacovigilance reports were received during 1994 – 1997. No report of untoward reactions concerned these products. This corresponds to 0 % of the total number of reports for the immunological veterinary products during that period.

B) other EU countries

Pharmacovigilance data of the VMD

The following table shows the pharmacovigilance data for the UK as reported in the *Veterinary Record*:

Year	Total number of reports	Total number of reports in birds	Total number of reports for chicken vaccines	Safety problems with *chicken IB* vaccines
1997	871	0	0	Not mentioned
1996	608	1	1 (?)	Not mentioned
1995	593	16	9 (?)	Not mentioned
1994	512	8	8 (?)	Not mentioned
1993	474	14	5 (?)	Not mentioned
1992	556	66	6 (Marek, potency)	Not mentioned
1991	332	15	9 (Marek, potency)	Not mentioned
1990	317	3	0	Not mentioned

(?): data not given

Source:	The Veterinary Record :	July 18, 1998	Page 60 – 61
		July 5, 1997	Page 4
		June 8, 1996	Page 557 – 558
		January 13, 1996	Page 26
		December 3, 1994	Page 544
		July 23, 1994	Page 77 – 81
		January 2, 1993	Page 4 – 6
		July 27, 1991	Page 63 – 65

<u>Summary:</u>

All 155 batches of live IB vaccines passed the target animal safety test. No retesting of any batch is documented. No pharmacovigilance data were related to safety problems attributed to this kind of vaccines

Obviously, the production and control of these live, freeze-dried avian bronchitis vaccines have reached a high quality level which guarantees safe batches.

The target animal safety test for these products has been useful in the past when consistent production was difficult and adequate quality control methods were not available. Nowadays, these avian products have to comply with a strict licensing procedure. Older products are reviewed to guarantee a continuously consistent production at a high level for safety and quality. Thus, for their production the target animal safety test only seems to be a relict of the past.

Considering the large number of animals (2875 chickens, and only 8 dead animals without been related to the vaccination) used for this test, the minimal contribution they make to quality control and safety of these products does not justify to continue using this animal test.

The evaluation of the data indicates that the target animal safety test carried out for batch control purposes could be omitted for these products.

The monograph requires other animal tests which would reveal crude impurities.

1. the test for extraneous agents: with a 100 fold dose (i.m.) and a 10 fold dose (eye drop). For some products used in chickens with a minimum age of 14 days for vaccination this test already replaces the safety test (see also Table 1).
2. the potency test (on a representative batch from the same seed lot, subject to agreement by the competent authority) requires the use of not fewer than 20 chickens, vaccinated with one dose.

Unless other data become available, showing the relevance of this animal test, the deletion of the test as a routine batch control is strongly recommended for the monograph no. 442 (*Avian infectious Bronchitis Vaccine (live) freeze-dried).*

Literature:

[1] Roberts, B., R.N. Lucken (1996): Reducing the use of the target animal batch safety test for veterinary vaccines. Dev. Biol. Stand. 86, 97 - 102.

[2] Weißer, K., U. Hechler (1997): Animal Welfare Aspects in the Quality Control of Immunobiologicals. A critical Evaluation of the Animal Tests in Pharmacopoeial Monographs. FRAME; Nottingham

[3] Zeegers, J.J.W., W.F. de Vries, R. Remie (1996): Reducing the use of animals by abolishment of the safety test as a routine batch control test on veterinary vaccines. ATLA 24 (Supplement), 138

XII-4-3 Proposal for deletion of the TAST for immunosera from the EU Guideline

The attached letter was send to EMEA to initiate a revision of the SR for Immunosera and Colostrum Substitutes.