REPLACEMENT, REDUCTION, REFINEMENT (3Rs)

Animal Welfare Progress In European Pharmacopoeia Monographs

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The European Convention on the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes was opened for signature on 18 March 1986. This marked the beginning of an intensification of the activities of the European Pharmacopoeia Commission to review all animal tests in monographs with a view to applying the precepts of the Convention for replacement, reduction and refinement of the use of animals for test purposes.

After 20 years, the amount of progress is considerable and this paper gives a summary of this in the form of tables shown below. The tables summarise the improvements in 5 fields where animal testing is or in the past has been a common feature:

- biological and biotechnological products;
- blood products;
- antibiotics;
- vaccines for human use;
- vaccines for veterinary use.

There have been improvements in other fields, notably with replacement of the rabbit pyrogens test by the test for bacterial endotoxins wherever possible.

The European Convention takes over what has now become a standard classification of progress in animal testing, 'the 3Rs':

- Replacement: animals are no longer used for the test;
- Reduction: fewer animals are used to achieve the defined aim of the test;
- Refinement: a test is carried out that causes less distress to the animals used.

Although replacement will always be seen as the highest aim and was the Commission's first priority, attention is increasingly paid to reduction and refinement, and often to a combination of the two, especially where, for the time being at least, replacement of the animal test is not feasible.

Opportunities for application of the 3Rs arise in different forms as far as Pharmacopoeia tests are concerned. The following have been largely exploited by the Commission:

- review of all aspects of the test to assess its current relevance;
- developments in analytical methods and scientific understanding;
- validation studies that lead to monograph revision.

Many animal tests were introduced at a time when GMP production of medicinal products was not current. Generalisation of GMP conditions has led to a review of the need for some animal tests.

What remains to be done? It is clear from the tables below that an enormous amount has already been achieved. There are and perhaps for a long time will be opportunities for further progress. What is common to most of the outstanding tests is that applications of the 3Rs will require extensive research and then validation work, with the consequent need for funding. The level of funding needed is such that priorities have to be set. The basis for these priorities should be:

- the amount of distress caused by the test;
- the number of animals used annually;
- the likelihood of success in application of one or more of the 3Rs.

For the European Pharmacopoeia, this clearly delineates a number of priority items, including:

- pyrogen testing of blood products;
- potency testing of inactivated vaccines;
- extension of the coverage of humane end-points.

Implementation. For some products, monographs have introduced the possibility of application of the 3Rs and this has an effect only if manufacturers and regulatory authorities make use of the opportunity. An example is the waiving of the safety for veterinary vaccines, where manufacturers can propose waiving of the test after testing a sufficient number of batches (usually 10). This possibility has not been widely applied for various reasons. Manufacturers have concerns over liability in case of adverse reactions and since this possibility has been introduced only in Europe and not in other regions there has been reluctance to apply it on the part of manufacturers who supply a vaccine in different regions. This attitude does not appear to be in conformity with the European Convention and Directive. Persuasive action on the part of regulatory authorities would promote its use.

Biological Standardisation Programme. One of the aims of this programme, established some 15 years ago jointly by the EDQM and the European Union, is the development and validation of methods that promote the application of the 3Rs. This programme has provided the means of carrying out studies that would not otherwise have been possible within the framework of the European Pharmacopoeia. The results of the studies have been promptly incorporated into the monographs and general chapters of the European Pharmacopoeia thus fostering their use. Items where a contribution from the Biological Standardisation Programme have led to changes to monographs and general chapters are indicated (*) in the tables below.

Validation approaches. Pharmacopoeia monographs are public standards and are intended to provide a quality specification applicable to all products on the market. Application of the 3Rs to animal testing in existing monographs has been seen to require development of an alternative method applicable without modification to all existing products. For finished products, notably

vaccines, this aim has rarely been achieved in a way that leads to direct application of the 3Rs. The existing products were developed at a time when the animal model was the standard method and the products were necessarily developed in such a way as to comply with the regulatory requirement. Demonstration of equivalence of an alternative method is not simply problematic in many instances, it is also of limited relevance. This implies that a complete re-evaluation of the aims of the test needs to be made to define the relevant aspects that have to be validated.

Opportunities for regulatory authorities. Manufacturers and other control laboratories do not always carry into practice changes in monographs regarding animal welfare progress, particularly where the change requires validation studies before implementation. Information on the control procedures actually used is available to regulatory authorities but not necessarily to the European Pharmacopoeia Commission. Regulatory authorities should be encouraged to request from manufacturers information on measures taken to comply with the European Convention and Directive in the light of changes in monographs and general chapters. The EDQM is of course willing to provide assistance in this.

Future action. The EDQM will continue to examine critically all animal tests included or proposed for inclusion in the Pharmacopoeia and will encourage and support studies that lead to progress in animal welfare. In order to benefit fully from the current achievement, a partnership between the EDQM, regulatory authorities and manufacturers is needed to facilitate implementation. For some products, guidelines on the way to introduce improved methods from the point of view of animal welfare have been included in the Pharmacopoeia (for example, *2.7.20. In vivo assay of poliomyelitis vaccine (inactivated)*). Further such guidelines can be developed in the future wherever this is considered useful.

TABLE 1 - BIOLOGICAL AND BIOTECHNOLOGICAL PRODUCTS

Monograph	Replacement, reduction, refinement of animal tests
Aprotinin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test R2: replacement of the batch tests for abnormal toxicity and histamine by upstream validation requirements
Aprotinin concentrated solution	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test R2: replacement of the batch tests for abnormal toxicity and histamine by upstream validation requirements
Buserelin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Calcitonin (salmon)	R1: replacement of the assay in animals by liquid chromatography R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Chymotrypsin	R2: replacement of the batch test for histamine by an upstream validation requirement
Dalteparin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Danaparoid sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Desmopressin	R1: replacement of the assay in animals by liquid chromatography R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Enoxaparin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Erythropoietin concentrated solution	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Felypressin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Glucagon human	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Gonadorelin acetate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Gonadotrophin, chorionic	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Gonadotrophin equine serum for veterinary use	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Goserelin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Heparin calcium	R1: deletion of the abnormal toxicity test based on historical review R1: deletion of the test for depressor substances based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

Heparin sodium	R1: deletion of the abnormal toxicity test based on historical review R1: deletion of the test for depressor substances based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Heparins, low-molecular-mass	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Hyaluronidase	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Insulin, bovine	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Insulin, human	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Insulin, porcine	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Interferon alfa-2 concentrated solution	R1: deletion of the test for depressor substances based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Interferon gamma-1b concentrated solution	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Leuprorelin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Molgramostim concentrated solution	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Nadroparin calcium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Oxytocin	R1: replacement of the assay in animals by liquid chromatography R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Oxytocin concentrated solution	R1: replacement of the assay in animals by liquid chromatography R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Parnaparin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Protamine hydrochloride	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test R2: replacement of the batch test for abnormal toxicity by an upstream validation requirement
Protamine sulphate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test R2: replacement of the batch test for abnormal toxicity by an upstream validation requirement
Protirelin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Sodium hyaluronate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Somatostatin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Somatropin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Somatropin concentrated solution	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Somatropin for injection	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Streptokinase bulk solution	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test R2: replacement of the batch test for abnormal toxicity by an upstream validation requirement
Tetracosactide	R1: replacement of the assay in rat primary cells by liquid chromatography
Tinzaparin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Trypsin	R2: replacement of the batch test for histamine by an upstream validation requirement
Urofollitropin	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

TABLE 2 - BLOOD PRODUCTS

Monograph	Replacement, reduction, refinement of animal tests
Albumin solution, human	R1: deletion of abnormal toxicity test based on historical review
Anti-D immunoglobulin, human	R1: deletion of abnormal toxicity test based on historical review
Human anti-D immunoglobulin, for intravenous administration	R1: deletion of abnormal toxicity test based on historical review
Antithrombin III concentrate, human	R1: deletion of abnormal toxicity test based on historical review
Factor VII, human	R1: deletion of abnormal toxicity test based on historical review
Factor VIII, human	R1: deletion of abnormal toxicity test based on historical review
Factor IX, human	R1: deletion of abnormal toxicity test based on historical review
Fibrin sealant kit	R1: deletion of abnormal toxicity test based on historical review
Fibrinogen, human	R1: deletion of abnormal toxicity test based on historical review
Human hepatitis A immunoglobulin	R1: deletion of abnormal toxicity test based on historical review
Human hepatitis B immunoglobulin	R1: deletion of abnormal toxicity test based on historical review
Human hepatitis B immunoglobulin, for intravenous administration	R1: deletion of abnormal toxicity test based on historical review
Immunoglobulin, normal, human	R1: deletion of abnormal toxicity test based on historical review
Immunoglobulin, normal, human, for intravenous administration	R1: deletion of abnormal toxicity test based on historical review
Measles immunoglobulin, human	R1: deletion of abnormal toxicity test based on historical review
Prothrombin complex, human	R1: deletion of abnormal toxicity test based on historical review
Rabies immunoglobulin, human	R1: deletion of abnormal toxicity test based on historical review R1: replacement of mouse potency test
Rubella immunoglobulin, human	R1: deletion of abnormal toxicity test based on historical review
Tetanus immunoglobulin, human	R1: deletion of abnormal toxicity test based on historical review R2: introduction of an in vitro assay to be used after validation
Varicella immunoglobulin, human	R1: deletion of abnormal toxicity test based on historical review
Varicella immunoglobulin, human, for intravenous administration	R1: deletion of abnormal toxicity test based on historical review

TABLE 3 - ANTIBIOTICS

Monograph	Replacement, reduction, refinement of animal tests
Amoxicillin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Amphotericin B	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Ampicillin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Bacitracin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Benzylpenicillin, benzathine	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Benzylpenicillin potassium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Benzylpenicillin, procaine	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

Benzylpenicillin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Bleomycin sulphate	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Cefalotin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Cefamandole nafate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Cefapirin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Cefazolin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Cefoperazone sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Cefotaxime sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Cefoxitin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Ceftazidime	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Ceftriaxone sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Cefuroxime sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Chlortetracycline hydrochloride	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Ciclosporin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Clindamycin phosphate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Cloxacillin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Daunorubicin hydrochloride	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Dihydrostreptomycin sulphate for veterinary use	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Doxorubicin hydrochloride	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Doxycycline hyclate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Epirubicin hydrochloride	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Fosfomycin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Framycetin sulphate	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Gentamicin sulphate	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

Imipenem	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Kanamycin acid sulphate	R2: replacement of the batch test for abnormal toxicity by an upstream validation requirement
Kanamycin monosulphate	R2: replacement of the batch test for abnormal toxicity by an upstream validation requirement
Lincomycin hydrochloride	R1: deletion of the abnormal toxicity test based on historical review R2: replacement of the batch test for abnormal toxicity by an upstream validation requirement
Minocycline hydrochloride dihydrate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Mitomycin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Netilmicin sulphate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Nystatin	R2: replacement of the batch test for abnormal toxicity by an upstream validation requirement
Oxacillin sodium monohydrate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Oxytetracycline hydrochloride	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Piperacillin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Potassium clavulanate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Rifamycin sodium	R1: deletion of the test for depressor substances based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test R2: replacement of the batch test for abnormal toxicity by an upstream validation requirement
Spectinomycin dihydrochloride pentahydrate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Spectinomycin sulphate tetrahydrate for veterinary use	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Streptomycin sulphate	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test R2: replacement of the batch test for abnormal toxicity by an upstream validation requirement
Sulbactam sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Tetracycline hydrochloride	R1: deletion of the abnormal toxicity test based on historical review R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Tiamulin for veterinary use	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Ticarcillin sodium	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Tobramycin	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test
Vancomycin hydrochloride	R1: replacement of the rabbit pyrogens test by the bacterial endotoxins test

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

TABLE 4 - VACCINES FOR HUMAN USE

Monograph	Replacement, reduction, refinement of animal tests
BCG vaccine, freeze-dried	R1: replacement of guinea pig test for virulent mycobacteria R1: deletion of abnormal toxicity test based on historical review R2: reduction of number of guinea-pigs used for hypersensitivity testing
Cholera vaccine	R1: deletion of abnormal toxicity test based on historical review
Cholera vaccine, freeze-dried	R1: deletion of abnormal toxicity test based on historical review
Diphtheria vaccine, assay of (2.7.6)	R2: introduction of a one-dilution assay R3: introduction of serological end-point as an alternative to challenge after validation*
Diphtheria and tetanus vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria and tetanus vaccine (adsorbed, reduced antigen(s) content)	R1: replacement of guinea-pig test for residual toxin in bulk toxoid R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria, tetanus and hepatitis B (rDNA) vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria, tetanus and pertussis vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria, tetanus and pertussis (acellular, component) vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria, tetanus, pertussis and poliomyelitis (inactivated) vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria, tetanus, pertussis, poliomyelitis (inactivated) and haemophilus type b conjugate vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria, tetanus, pertussis (acellular, component) and haemophilus type b conjugate vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

Diphtheria, tetanus, pertussis (acellular, component) and hepatitis B (rDNA) vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria, tetanus, pertussis (acellular, component) and poliomyelitis (inactivated) vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria, tetanus, pertussis (acellular, component), poliomyelitis (inactivated) and haemophilus type b conjugate vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria, tetanus, pertussis (acellular, component), poliomyelitis (inactivated), hepatitis B (rDNA) and haemophilus type b vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria vaccine (adsorbed)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Diphtheria vaccine (adsorbed, reduced antigen(s) content)	R1: replacement of guinea-pig test for residual diphtheria toxin in bulk toxoid R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Haemophilus type b conjugate vaccine	R1: deletion of abnormal toxicity test based on historical review R1: deletion of assay in mice
Hepatitis A vaccine (inactivated), assay of (2.7.14)	R2: introduction of an in vitro assay to be used after validation.
Hepatitis A (inactivated) and hepatitis B (rDNA) vaccine (adsorbed)	R1: deletion of abnormal toxicity test based on historical review
Hepatitis A vaccine (inactivated, adsorbed)	R1: deletion of abnormal toxicity test based on historical review
Hepatitis A vaccine (inactivated, virosome)	R1: deletion of abnormal toxicity test based on historical review
Hepatitis B vaccine (rDNA), assay of (2.7.15)	R2: introduction of an in vitro assay to be used after validation.
Hepatitis B vaccine (rDNA)	R1: deletion of abnormal toxicity test based on historical review
Influenza vaccine (split virion, inactivated)	R1: deletion of abnormal toxicity test based on historical review
Influenza vaccine (surface antigen, inactivated)	R1: deletion of abnormal toxicity test based on historical review
Influenza vaccine (surface antigen, inactivated, virosome)	R1: deletion of abnormal toxicity test based on historical review
Influenza vaccine (whole virion, inactivated)	R1: deletion of abnormal toxicity test based on historical review
Measles, mumps and rubella vaccine (live)	R1: deletion of abnormal toxicity test based on historical review

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

Measles vaccine (live)	R1: deletion of neurovirulence test for seed lots R1: deletion of abnormal toxicity test based on historical review
Meningococcal group C conjugate vaccine	R1: deletion of abnormal toxicity test based on historical review
Meningococcal polysaccharide vaccine	R1: deletion of abnormal toxicity test based on historical review
Mumps vaccine (live)	R1: deletion of neurovirulence test for seed lots R1: deletion of abnormal toxicity test based on historical review
Pertussis vaccine	R1: deletion of abnormal toxicity test based on historical review R2: reduction of the number of animals in the test for specific toxicity
Pertussis vaccine (acellular, component, adsorbed)	R1: deletion of abnormal toxicity test based on historical review
Pertussis vaccine (acellular, co-purified, adsorbed)	R1: deletion of abnormal toxicity test based on historical review
Pertussis vaccine, adsorbed	R1: deletion of abnormal toxicity test based on historical review
Pneumococcal polysaccharide vaccine	R1: deletion of abnormal toxicity test based on historical review
Poliomyelitis vaccine (inactivated), *in vivo* assay of (2.7.20)	R2: introduction of a guideline on waiving of the assay in routine testing
Poliomyelitis vaccine (inactivated)	R1: deletion of abnormal toxicity test based on historical review
Poliomyelitis vaccine (oral)	R1: deletion of abnormal toxicity test based on historical review R2: introduction of genome analysis for screening prior to neurovirulence testing in animals
Rabies vaccine for human use prepared in cell cultures	R1: deletion of abnormal toxicity test based on historical review R2: introduction of a one-dilution potency test R3: introduction of an annex on humane end point
Rubella vaccine (live)	R1: deletion of neurovirulence test for seed lots R1: deletion of abnormal toxicity test based on historical review
Tetanus vaccine (adsorbed)	R1: deletion of abnormal toxicity test based on historical review R2: replacement of specific toxicity test in guinea-pigs by an upstream validation requirement
Tetanus vaccine, assay of (2.7.8)	R1: deletion of abnormal toxicity test based on historical review R3: introduction of humane end-point for challenge assay R3: introduction of serological end-point as an alternative to challenge after validation*
Tick-borne encephalitis vaccine (inactivated)	R1: deletion of abnormal toxicity test based on historical review
Typhoid polysaccharide vaccine	R1: deletion of abnormal toxicity test based on historical review
Typhoid vaccine	R1: deletion of abnormal toxicity test based on historical review
Typhoid vaccine, freeze-dried	R1: deletion of abnormal toxicity test based on historical review
Typhoid vaccine (Ty21a strain, live, oral)	R1: deletion of abnormal toxicity test based on historical review
Vaccines for human use	R3: addition of a requirement for use of humane end-points wherever possible
Varicella vaccine	R1: deletion of neurovirulence test for seed lots R1: deletion of abnormal toxicity test based on historical review
Yellow fever vaccine (live)	R1: deletion of abnormal toxicity test based on historical review

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

TABLE 5 - VETERINARY VACCINES FOR VETERINARY USE

Monograph	Replacement, reduction, refinement of animal tests
Anthrax spore vaccine (live) for veterinary use (441)	R2: waiver for safety test after testing of initial batches
Aujeszky's disease vaccine (inactivated) for pigs (744)	R2: waiver for safety test after testing of initial batches
Aujeszky's disease vaccine (live) for pigs for parenteral administration (745)	R2: waiver for safety test after testing of initial batches
Avian infectious bronchitis vaccine (inactivated) (959)	R2: waiver for safety test after testing of initial batches
Avian infectious bronchitis vaccine (live) (442)	R1: replacement of test for extraneous agents in chicks by cell culture test R2: waiver for safety test after testing of initial batches
Avian infectious bursal disease (Gumboro disease) vaccine (live) (587)	R1: replacement of test for extraneous agents in chicks by cell culture test R2: waiver for safety test after testing of initial batches
Avian infectious bursal disease vaccine (inactivated) (960)	R2: waiver for safety test after testing of initial batches
Avian infectious encephalomyelitis vaccine (live) (588)	R1: replacement of test for extraneous agents in chicks by cell culture test R2: waiver for safety test after testing of initial batches
Avian infectious laryngotracheitis vaccine (live), for chickens (1068)	R1: replacement of test for extraneous agents in chicks by cell culture test R2: waiver for safety test after testing of initial batches
Avian paromyxovirus 3 vaccine (inactivated) (1392)	R2: waiver for safety test after testing of initial batches
Bovine parainfluenza virus vaccine (live) (1176)	R2: waiver for safety test after testing of initial batches
Bovine respiratory syncytial virus vaccine (live) (1177)	R2: waiver for safety test after testing of initial batches
Bovine viral diarrhoea vaccine (inactivated) (1952)	R2: waiver for safety test after testing of initial batches
Brucellosis vaccine (live) (Brucella melitensis Rev. 1 strain), for veterinary use (793)	R2: waiver for safety test after testing of initial batches
Calf coronavirus diarrhoea vaccine (inactivated) (1953)	R2: waiver for safety test after testing of initial batches
Calf rotavirus diarrhoea vaccine (inactivated) (1954)	R2: waiver for safety test after testing of initial batches
Canine adenovirus vaccine (inactivated) (1298)	R2: waiver for safety test after testing of initial batches
Canine distemper vaccine (live) (448)	R2: waiver for safety test after testing of initial batches
Canine leptospirosis vaccine (447)	R1: introduction of an *in vitro* batch potency test for use with non-adjuvanted vaccines after validation R2: waiver for safety test after testing of initial batches R3: introduction of a serological model for the batch potency test to be used after validation
Canine parainfluenza virus vaccine (live) (1955)	R2: waiver for safety test after testing of initial batches
Canine parvovirosis vaccine (live) (964)	R2: waiver for safety test after testing of initial batches
Canine parvovirosis vaccine (inactivated) (795)	R2: waiver for safety test after testing of initial batches
Clostridium botulinum vaccine for veterinary use (360)	R2: waiver for safety test after testing of initial batches

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

Clostridium chauvoei vaccine for veterinary use (361)	R2: waiver for safety test after testing of initial batches R2: modification of the potency test to avoid unnecessary repetitions due to invalidity
Clostridium novyi alpha antitoxin for veterinary use (339)	R2: waiver for safety test after testing of initial batches
Clostridium novyi (type B) vaccine for veterinary use (362)	R2: waiver for safety test after testing of initial batches R2: waiver for the test for residual toxicity test on the final product by the manufacturer R3: introduction of a serological evaluation for the batch potency test
Clostridium perfringens beta antitoxin for veterinary use (340)	R2: waiver for safety test after testing of initial batches
Clostridium perfringens epsilon antitoxin for veterinary use (341)	R2: waiver for safety test after testing of initial batches
Clostridium perfringens vaccine for veterinary use (363)	R2: waiver for safety test after testing of initial batches R2: waiver for the test for residual toxicity test by the on the final product by the manufacturer R3: introduction of a serological evaluation of the batch potency test
Clostridium septicum vaccine for veterinary use (364)	R2: waiver for safety test after testing of initial batches R2: waiver for the test for residual toxicity test by the on the final product by the manufacturer R3: introduction of a serological evaluation of the batch potency test
Colibacillosis inactivated vaccine, neonatal ruminant (961)	R2: waiver for safety test after testing of initial batches
Colibacillosis inactivated vaccine, neonatal piglet (962)	R2: waiver for safety test after testing of initial batches
Distemper vaccine (live) for mustelids (449)	R2: waiver for safety test after testing of initial batches
Duck viral hepatitis vaccine (live) (1315)	R1: replacement of test for extraneous agents in chicks by cell culture test R2: waiver for safety test after testing of initial batches
Egg drop syndrome ’76 vaccine (inactivated) (1202)	R2: waiver for safety test after testing of initial batches
Equine herpesvirus vaccine (inactivated) (1613)	R2: waiver for safety test after testing of initial batches
Equine influenza vaccine (inactivated) (249)	R2: waiver for safety test after testing of initial batches
Feline calicivirosis vaccine (inactivated) (1101)	R2: waiver for safety test after testing of initial batches
Feline calicivirosis vaccine (live) (1102)	R2: waiver for safety test after testing of initial batches
Feline infectious enteritis (feline panleucopenia) vaccine (inactivated) (794)	R2: waiver for safety test after testing of initial batches
Feline infectious enteritis (feline panleucopenia) vaccine (live) (251)	R2: waiver for safety test after testing of initial batches
Feline leukaemia vaccine (inactivated) (1321)	R2: waiver for safety test after testing of initial batches
Feline viral rhinotracheitis vaccine (inactivated) (1207)	R2: waiver for safety test after testing of initial batches
Feline viral rhinotracheitis vaccine (live), freeze-dried (1206)	R2: waiver for safety test after testing of initial batches
Foot-and-mouth disease (ruminants) vaccine (inactivated) (63)	R2: waiver for safety test after testing of initial batches
Fowl-pox vaccine (live) (649)	R1: replacement of test for extraneous agents in chicks by cell culture test R2: waiver for safety test after testing of initial batches

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points

Furunculosis vaccine (inactivated, oil-adjuvanted, injectable) for salmonids (1521)	R2: waiver for safety test after testing of initial batches
Immunosera for veterinary use (30)	R2: waiver for safety test after testing of initial batches
Infectious bovine rhinotracheitis vaccine (live) (696)	R2: waiver for safety test after testing of initial batches
Mannheimia vaccine (inactivated) for cattle (1944)	R2: waiver for safety test after testing of initial batches
Mannheimia vaccine (inactivated) for sheep (1946)	R2: waiver for safety test after testing of initial batches
Marek's disease vaccine (live) (589)	R1: replacement of test for extraneous agents in chicks by cell culture test R2: waiver for safety test after testing of initial batches
Myxomatosis vaccine (live) for rabbits (1943)	R2: waiver for safety test after testing of initial batches
Neonatal piglet colibacillosis vaccine (inactivated) (962)	R2: waiver for safety test after testing of initial batches
Neonatal ruminant colibacillosis vaccine (inactivated) (961)	R2: waiver for safety test after testing of initial batches
Newcastle disease vaccine (inactivated) (870)	R1: introduction of an in vitro potency test to be used after validation* R2: waiver for safety test after testing of initial batches
Newcastle disease vaccine (live) (450)	R1: replacement of test for extraneous agents in chicks by cell culture test R2: waiver for safety test after testing of initial batches
Pasteurella vaccine (inactivated) for sheep (2072)	R2: waiver for safety test after testing of initial batches
Porcine actinobacillosis vaccine (inactivated) (1360)	R2: waiver for safety test after testing of initial batches
Porcine influenza vaccine (inactivated) (963)	R2: waiver for safety test after testing of initial batches
Porcine parvovirosis vaccine (inactivated) (965)	R2: waiver for safety test after testing of initial batches
Porcine progressive atrophic rhinitis vaccine (inactivated) (1361)	R2: waiver for safety test after testing of initial batches
Rabies vaccine (inactivated) for veterinary use (451)	R2: waiver for safety test after testing of initial batches R2: introduction of a one-dilution potency test R3: introduction of an annex on humane end point
Swine erysipelas vaccine (inactivated) (64)	R2: waiver for safety test after testing of initial batches R3: introduction of a serological evaluation for the batch potency test*
Swine-fever vaccine (live), classical (65)	R2: waiver for safety test after testing of initial batches
Tetanus antitoxin for veterinary use (343)	R2: waiver for safety test after testing of initial batches
Tetanus vaccine for veterinary use (697)	R2: waiver for safety test after testing of initial batches R3: introduction of a serological evaluation for the potency test
Vibriosis (cold-water) vaccine (inactivated) for salmonids (1580)	R2: waiver for safety test after testing of initial batches
Vibriosis vaccine (inactivated) for salmonids (1581)	R2: waiver for safety test after testing of initial batches
Vaccines for veterinary use (62)	R2: general waiver for safety test after testing of initial batches R3: addition of a requirement for use of humane end-points wherever possible

R1 = replacement of a test by an *in vitro* test or deletion of test
R2 = reduction the number of animals required
R3 = refinement of test to cause less distress, for example by use of humane end-points