1 21 April 2016
2 EMA/CHMP/CVMP/JEG-3Rs/164002/2016
3 Committee for Medicinal products for Veterinary Use (CVMP)

4 Reflection paper providing an overview of the current 5 regulatory testing requirements for veterinary medicinal 6 products and opportunities for implementation of the 3Rs

7 Draft

Draft agreed by JEG 3Rs following review by respective WPs (QWP, SWP-V, IWP, ERAWP and EWP-V)	March 2016
Adopted by CVMP for release for consultation	21 April 2016
Start of public consultation	29 April 2016
End of consultation (deadline for comments)	31 October 2016

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Reflection paper on providing an overview of the current regulatory testing requirements for veterinary medicinal products and opportunities for implementation of the 3Rs

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1. Introduction

In October 2014 the CHMP and CVMP agreed a draft guideline for consultation on regulatory acceptance of 3R (replacement, reduction, refinement) testing approaches (EMA/CHMP/CVMP/JEG-3Rs/450091/2012).

The current reflection paper has been developed as a follow up to that draft guideline and provides an overview of the main animal tests required for the regulatory testing of veterinary medicinal products. A parallel document has been developed in relation to human medicinal products (EMA/CHMP/CVMP/JEG-3Rs/742466/2015). It includes information on opportunities for limiting animal testing that can already be implemented, where appropriate, as well as information on opportunities that may become available in the future. In so doing, it is hoped that the document may stimulate future submissions for CVMP advice on the regulatory acceptance of new 3R approaches.

The information is presented in tables and divided into sections based on the main working party responsible for development of relevant guidance. Separate tables are provided for guidance developed by:

- the joint CHMP/CVMP Quality Working Party (QWP), which develops guidance on quality testing for medicinal products for human and veterinary use
- the CVMP Safety Working Party (SWP-V), which develops guidance on safety and residues testing for pharmaceutical products
- the CVMP Immunologicals Working Party (IWP), which develops guidance on quality, safety and efficacy testing of immunological products
- the CVMP Environmental Risk Assessment Working Party (ERAWP), which develops guidance on environmental testing of pharmaceutical products

The tables presented cover tests needed to demonstrate quality and safety of pharmaceutical and immunological veterinary medicinal products. In addition, according to the applicable legislation, there is a need to demonstrate efficacy of a veterinary medicinal product. However, the programme of studies required to demonstrate efficacy will be driven by the product type and therapeutic indication and will consequently be developed on a case by case basis. As there is not a set of standard endpoints to be addressed or a battery of tests to be performed an overview of the efficacy testing requirements is not presented (although, for immunological veterinary medicinal products, information is provided on laboratory based efficacy tests (but not on field studies)). However, it is expected that the 3Rs will be considered in the design of efficacy studies, as in the design of all studies conducted in animals.

It is important to note that for the tests enumerated in the tables below, and for efficacy tests, applicants may deviate from guidelines as long as they are able to provide data or argumentation to scientifically demonstrate that the alternative approach provides an equivalent level of quality, safety or efficacy. If an applicant considers that a particular test is not necessary or if it would like to use an alternative methodology it can use the scientific advice procedure to obtain advice on the acceptability of its proposed approach. In addition, where appropriate, published literature can be used as a substitute for new tests.

The current reflection paper provides a snapshot of the animal testing requirements at the time of publication. It is to be expected that, over time, new testing approaches will become accepted and the tables will become out of date.

While examining these tables it should be borne in mind that the fundamental responsibility of the CVMP is to ensure the quality, safety and efficacy of veterinary medicinal products and so to safeguard the health of the target animals as well as that of the human users administering the products and the human consumers ingesting food commodities derived from treated animals. While the CVMP is committed to encouraging use of 3Rs approaches wherever possible, these cannot be accepted at the expense of safety and efficacy for the target animal or safety for the users and consumers or safety of the environment.

2. Overview of testing requirement

2.1. CHMP/CVMP Quality Working Party

Overview of animal testing requirements for active substances of synthetic, semi-synthetic, fermentation origin as well as medicinal products (Quality Working Party - CHMP/CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Pyrogens (Rabbits)	European Pharmacopoeia (Ph. Eur.) Chapter 2.6.8	Amikacin-sulfate, Bacitracin zinc, Calcium levulinate dehydrate, Colistimethate sodium, Chloramphenicol, Chloramphenicol sodium succinate, Dicloxacillin sodium, Flucloxacillin sodium, Glucose anhydrous, Glucose monohydrate, Kanamycin acid sulphate, Kanamycin monosulfate, Polymyxin B sulphate, Sodium citrate. Besides the ASs in the table the, test is used in case of derived medicinal products and some older products.	According to specific Ph. Eur. monographs, this test should be used if the active substance is intended for administration by spraying into internal body cavities (Bacitracin zinc) or use in the manufacture of parenteral preparations without further appropriate procedure for the removal of pyrogens. So in practice, the pyrogen test is seldom performed on the active substances. In addition, the latest version of Chapter 2.6.8 (published in edition 8.8) includes the following text: 'In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. Wherever possible and after product specific validation, the pyrogen test is replaced by the monocyte-activation test (2.3.60).	To communicate that the test shall be used only in the justified and authorised cases when the test for Bacterial Endotoxins (BET) cannot be performed (see general monograph Substances for pharmaceutical use). Monocyte-activation test (MAT, 2.6.30.) should be taken into consideration as an alternative as well. Therefore the pyrogen test should be substituted by the BET or MAT in the current applications for marketing authorisation of medicinal products, and in the case of older products via variation procedures.

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Bacterial Endotoxins (amoebocyte lysate from *Limulus polyphemus* or *Tachypleus tridentatus)*	Ph. Eur. Chapter 2.6.14.	Active substances of endotoxin-free grade and most of medicinal products intended for parenteral administration.	Often used as an alternative to the pyrogen test. The test for Bacterial endotoxins (BET) is used to detect or quantify endotoxins from Gram-negative bacteria using Limulus Amoebocyte Lysate obtained from blood cells (amoebocytes) of horseshoe crabs (*Limulus polyphemus, Tachypleus tridentatus*). As invertebrates, horse shoe crabs do not fall under the scope of Directive 2010/63/EU.	
Abnormal toxicity (Mice)	Ph. Eur. Chapter 2.6.9. General test	Dihydrostreptomycin Sulphate, Streptomycin sulphate, Griseofulvin, Kanamycin acids sulphate, Kanamycin monosulfate, Nystatin, Rifamycin sodium.	This test is included in the Production section of the Ph. Eur. monographs of some active substances, therefore its performance is not required as a routine, since the Pharmacopoeia establishes that "the method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test".	Communicate that test should not be used routinely since manufacturing methods should be suitably validated to assure compliance "if tested. In addition, due to concerns regarding animal welfare linked to excessive injection volume and the scientific relevance of the test, the concerned Ph. Eur. expert groups should consider the removal of the abnormal toxicity test. Submit relevant data to the Ph. Eur. to support this change.

2.2. CVMP Safety Working Party

Overview of animal testing requirements for safety studies to be submitted in support of applications for maximum residue limits (Safety Working Party - CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Pharmaco-dynamics	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the European Union Guideline on the approach to establish a pharmacological ADI (EMA/CVMP/SWP/355689/2006)	No specific requirements. Main aim is to determine a no adverse effect level (NOAEL) for pharmacological effects, for use in determining a pharmacological ADI. Pharmacodynamic studies may also provide mechanistic information that can aid the understanding of effects seen in toxicology studies.	Where appropriate data from use in humans are available these can be used for the establishment of a NOAEL. A pharmacological ADI is not required if residues in foodstuffs are devoid of pharmacological activity. A pharmacological ADI is not needed if the substance is not bioavailable by the oral route in humans. A pharmacological ADI is not required for substances for which the only expected pharmacodynamic activity is an antimicrobial activity. A pharmacological ADI is not needed if it is clear that it would be higher than the toxicological ADI.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
			A pharmacological ADI is not required if the mode of action is not relevant for humans. A separate pharmacological ADI is not needed if the relevant pharmacological effects are included in toxicology studies.	
Pharmaco-kinetics in laboratory animals	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the European Union VICH GL47 on Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: laboratory animal comparative metabolism studies (EMA/CVMP/VICH/463104/2009)	No specific requirements. Aim is to provide ADME data modelling the fate of the substance in humans following oral ingestion and to demonstrate that residues present in food of animal origin were also present in species used in toxicology studies.	In some cases human data can be used if available, for example if data exists to demonstrate that there is no oral absorption or metabolism. In vitro/in silico modelling can be used where scientifically justified.	Increased use of in vitro/in silico modelling if scientifically justified.
Single dose toxicity	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the	Not required for the establishment of MRLs but, if available, relevant data to be provided.	No need for generation of new data.	Not relevant as single dose studies are not required.

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
	European Union			
Repeat dose (90 day) toxicity	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the European Union VICH Topic GL31 on Studies to evaluate the safety of residues of veterinary drugs in human food: repeat-dose (90 days) toxicity testing (CVMP/VICH/484/02-FINAL)	90 day testing in one rodent and one non-rodent species.		
Repeat dose (chronic) toxicity	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the European Union VICH GL 37 on studies to evaluate the safety of residues of veterinary drugs in human food: repeat-dose (chronic) toxicity testing (CVMP/VICH/468/03-FINAL)	Chronic testing in one species.	VICH GL 37 states that "this guideline does not preclude the possibility of alternative approaches that may offer an equivalent assurance of safety, including scientifically based reasons as to why chronic toxicity testing may not need to be provided".	

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Reproductive toxicity including developmental toxicity	Annex V of Regulation 2377/90 VICH GL 22 on Studies to evaluate the safety of residues of veterinary drugs in human food: reproduction testing (CVMP/VICH/525/00-FINAL) VICH GL 32 on Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: Developmental Toxicity Testing (CVMP/VICH/485/02-FINAL; VICH GL32)	A multigeneration reproductive toxicity study in one species (normally rat) Developmental toxicity testing in the rat. If no teratogenicity is observed (or in the case of equivocal results) then developmental toxicity testing in a second species (usually rabbit) is required. If teratogenicity is observed in the rat then testing in a second species is not required.	Both VICH GL 22 and GL 32 state that they do not preclude the possibility of alternative approaches that may offer an equivalent assurance of safety, including scientifically based reasons as to why data may not need to be provided. Currently no implemented alternatives to the multigeneration study in one species. A tiered approach applies in relation to developmental toxicity, with no second species required if teratogenicity is observed in the first species.	Consider use of the extended one generation reproductive toxicity study as an alternative to the standard multigeneration study (ongoing activity at VICH).
Genotoxicity studies	Annex V of Regulation 2377/90 VICH GL 23 on Safety studies for veterinary drug residues in human food: Genotoxicity testing (EMA/CVMP/VICH/526/2000)	The following standard battery of tests is recommended: - a test for gene mutation in bacteria. - a cytogenetic test for chromosomal damage (in vitro) or an in vitro mouse lymphoma tk gene mutation assay. - an in vivo test for chromosomal effects using rodent haematopoietic cells.	In principle the choice of tests can be modified if appropriate but an in vivo test is expected.	Consider modification of the standard battery to remove the default requirement for an in vivo test (e.g., if all in vitro results are clearly negative) or to allow this test to be incorporated into another in vivo test (such as repeat dose toxicity).

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Carcinogenicity	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the EU VICH GL 28 on Studies to evaluate the safety of residues of veterinary drugs in human food: carcinogenicity testing (CVMP/VICH/645/01-Rev.1-FINAL) VICH Guideline on Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Testing (EMEA/CVMP/VICH/486/02-Rev.2; VICH GL33)	2 year rat bioassay and 18 month mouse bioassay (OECD TGL 451 & 453) when SARS, preneoplastic or genotoxic effects suggest potential carcinogenicity.	Carcinogenicity studies are not required if there is no reason to suspect possible carcinogenicity (based on SARS or observed preneoplastic or genotoxic effects). With appropriate scientific justification, carcinogenicity studies may be carried out in one rodent species, preferably the rat. In practice carcinogenicity studies are rarely required as genotoxic substances are generally not accepted for use in food producing animals.	Reconsider continued need for carcinogenicity in two species.
Immunotoxicity	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the EU VICH Topic GL33 on studies to evaluate the safety of residues of	No specific requirements. Only required in those cases where there is a particular concern relating to potential immunotoxicity (e.g., if a potential hazard is identified from other tests).	Not routinely required. It is up to the applicant to justify the nature and extent of additional studies.	Acceptance of the extended one generation reproductive toxicity test would allow integration of developmental immunotoxicity testing, where appropriate, into reproductive toxicity testing.

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
	veterinary drugs in human food: general approach to testing (EMEA/CVMP/VICH/486/02-Rev.2)			
Neurotoxicity	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the EU VICH Topic GL33 on studies to evaluate the safety of residues of veterinary drugs in human food: general approach to testing (EMEA/CVMP/VICH/486/02-Rev.2)	Required for certain groups of substances known to be associated with neurotoxicity as well as for other substances which have shown relevant toxicological effects in other toxicity tests. Possible tests to consider include a neurotoxicity test in rodents (OECD test guideline 424), developmental neurotoxicity testing (usually in rats) (OECD test guideline 426), delayed neurotoxicity of organophosphorus substances following acute exposure in hens (OECD test guideline 418) or repeated exposure (OECD test guideline 419).	Not routinely required.	Acceptance of the extended one generation reproductive toxicity test would allow integration of developmental neurotoxicity testing, where appropriate, into reproductive toxicity testing.
Testing for effect on the human intestinal flora	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the EU VICH Topic GL36(R) on studies to	The VICH guideline recommends possible in vitro and in vivo approaches.	Only required for compounds with antibacterial properties. In vitro approaches are already identified in the guideline.	In vitro approaches are already identified in the guideline.

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
	evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI (EMA/CVMP/VICH/467/2003)			

78 Overview of animal testing requirements for residue studies to be submitted in support of applications for the establishment of maximum residue limits and
79 marketing authorisation for veterinary medicinal products for use in food producing species (Safety Working Party - CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Pharmacokinetics in the target species	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the EU VICH GL46 on studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: metabolism study to determine the quantity and identify the nature of residues (EMA/CVMP/VICH/463072/2009)	Relates to MRL applications only. Aim is to identify and quantify residues of concern in food derived from treated animals and to monitor changes over time. The standard study is one using radiolabelled drug in the target animal species.	In cases where MRLs have already been established in one species, and if scientifically justifiable, it may be possible to use the same MRL values in other species (extension/extrapolation of MRLs).	For well characterised substances where suitable physicochemical and pharmacokinetic data as well as model assumptions are available, physiologically based pharmacokinetic modelling may be used to predict pharmacokinetic behaviour in the target species.
Residue depletion studies in the target species	Annex V of Regulation 2377/90 Volume 8 of The rules governing medicinal products in the EU VICH GL46 on studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: metabolism study to determine the quantity and identify the nature of residues	For MRL applications the study using radiolabelled drug in the target animal species (as per VICH GL46) provides critical information relating to depletion of residues as well as pharmacokinetics in the target species. Aim of studies conducted to VICH GL48 is to monitor the depletion of the marker residue over time in the	For the purpose of establishing MRLs, reduced data requirements apply for minor species (extension/extrapolation of MRLs). In cases where MRLs have already been established in one species, and if scientifically justifiable, it may be possible to use the same MRL values in other species.	For well characterised substances where suitable physicochemical and pharmacokinetic data as well as model assumptions are available, physiologically based pharmacokinetic modelling may be used to predict residue depletion in the target species.

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
	(EMA/CVMP/VICH/463072/2009) VICH GL48 on studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: marker residue depletion studies to establish product withdrawal periods (EMA/CVMP/VICH/463199/2009)	target animal species. This type of study is used both for the establishment of maximum residue limits and for the establishment of withdrawal periods required for marketing authorisation.		

80 Overview of animal testing requirements for safety studies to be submitted in support of applications for marketing authorisation for veterinary medicinal
81 products for use in food producing species (Safety Working Party - CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Single dose toxicity	Directive 2009/9/EC	No specific test requirements have been defined. Single dose toxicity data is intended to characterise signs of overdose in target animal species and for user safety assessment. May provide information useful for setting doses to be used in repeated dose studies.	Data can be bibliographic. Data from repeated dose studies may provide an alternative.	
Repeat dose toxicity, reproductive toxicity testing including developmental toxicity, genotoxicity testing, carcinogenicity testing, immunotoxicity testing, neurotoxicity testing and testing for effects on the human intestinal flora	Directive 2009/9/EC	The requirements are the same as specified for safety studies to be submitted in support of applications for the establishment of maximum residue limits.	The published Summary Report/European Public MRL Assessment Report may be submitted in place of data.	
Other tests required for the user risk assessment, possibly including skin and eye irritation, sensitisation and inhalation toxicity	Directive 2009/9/EC CVMP guideline on user safety for pharmaceutical veterinary medicinal products	The legislation requires an evaluation of user safety but does not specify the tests to be undertaken. The guidance provides information on how to undertake a user risk assessment but does not specify	The guidance indicates that toxicity data presented in other areas of the dossier as well as data from published literature and information from human use should be used wherever possible. Where original studies	Improved understanding of mechanisms underlying skin sensitisation has allowed advances in development of alternative methods - see Adverse Outcome Pathway

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
	(EMA/CVMP/543/03-Rev.1)	particular tests to be undertaken.	are required these should be performed in accordance with accepted methodology and follow a stepwise approach.	(AOP) developed by the OECD. EURL ECVAM indicates that 3 tests - DPRA (OECD TG 442C), KeratinoSens™ (OECD TG 442D) and h-CLAT (OECD test guideline under development) provide useful information for assessment of skin sensitisation potential when used in combination with other information. Proposed data integration approaches (i.e. ITS, IATA) for both skin sensitisation hazard and potency prediction will be documented as case studies in the OECD guidance document on the reporting of IATA (to become available in 2016).

82 Overview of animal testing requirements for safety studies to be submitted in support of applications for marketing authorisations for veterinary medicinal
83 products for use in companion animals (Safety Working Party - CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Single dose toxicity	Directive 2009/9/EC	No specific test requirements have been defined. Single dose toxicity data is intended to characterise signs of overdose in target animal species and for user safety assessment. May provide information useful for setting doses to be used in repeated dose studies.	Data can be bibliographic. Data from repeated dose studies may provide an alternative.	
Repeat dose toxicity	Directive 2009/9/EC	A study in one species is normally sufficient. The frequency, route of administration and duration of the study should be determined based on the proposed conditions of clinical use.	The study may be replaced by a study conducted in the target species. Repeated dose toxicity testing may not be needed for topical use products for which absorption is negligible.	
Reproductive toxicity including developmental toxicity	Directive 2009/9/EC	For target animal safety: developmental toxicity testing in one species if product is intended for use in female animals that may be used for breeding. For user safety: where exposure of users is expected, standard developmental toxicity testing is required (i.e. based on VICH GL22 - see MRL section above).	For target animal safety: the laboratory animal study may be replaced with a study in the target species. Reproductive toxicity testing may not be needed for topical use products for which absorption is negligible.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Genotoxicity studies	Directive 2009/9/EC VICH GL23 on Safety studies for veterinary drug residues in human food: Genotoxicity testing (EMA/CVMP/VICH/526/2000)	The following standard battery of tests is recommended: - a test for gene mutation in bacteria. - a cytogenetic test for chromosomal damage (in vitro) or an in vitro mouse lymphoma tk gene mutation assay. - an in vivo test for chromosomal effects using rodent haematopoietic cells.	In principle the choice of tests can be modified if appropriate but an in vivo test is expected.	Possible modification of the standard battery to remove the default requirement for an in vivo test (eg, if all in vitro results are clearly negative) or to allow this test to be incorporated into another in vivo test (such as repeat dose toxicity).
Carcinogenicity	Directive 2009/9/EC	2 year rat bioassay and 18-month mouse bioassay (OECD TGL 451 & 453) when SARS, preneoplastic or genotoxic effects suggest potential carcinogenicity.	Carcinogenicity studies are not required if there is no reason to suspect possible carcinogenicity (based on SARs or observed preneoplastic or genotoxic effects). With appropriate scientific justification, carcinogenicity studies may be carried out in one rodent species, preferably the rat. Carcinogenicity testing may not be needed for topical use products for which absorption is negligible.	Reconsider continued need for carcinogenicity in two species.

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
			In practice carcinogenicity studies are rarely required genotoxic substances are rarely accepted for use in veterinary medicinal products.	
Other tests required for the user risk assessment, possibly including skin and eye irritation, sensitisation and inhalation toxicity	Directive 2009/9/EC CVMP guideline on user safety for pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-Rev.1)	The legislation requires an evaluation of user safety but does not specify the tests to be undertaken. The guidance provides information on how to undertake a user risk assessment but does not specify particular tests to be undertaken.	The guidance indicates that toxicity data presented in other areas of the dossier as well as data from published literature and information from human use should be used wherever possible. Where original studies are required these should be performed in accordance with accepted methodology and follow a stepwise approach.	Improved understanding of mechanisms underlying skin sensitisation has allowed advances in development of alternative methods - see Adverse Outcome Pathway (AOP) developed by the OECD. EURL ECVAM indicates that 3 tests - DPRA (OECD TG 442C), KeratinoSens™ (OECD TG 442D) and h-CLAT (OECD test guideline under development) provide useful information for assessment of skin sensitisation potential

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
				when used in combination with other information. Proposed data integration approaches (i.e. ITS, IATA) for both skin sensitisation hazard and potency prediction will be documented as case studies in the OECD guidance document on the reporting of IATA (to become available in 2016).

2.3. CVMP Immunologicals Working Party

Overview of animal testing requirements for immunological veterinary medicinal products - tests required during licensing (Immunologicals Working Party - CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Product development	Dir. 2001/82/EU, Annex 1, Title 2, Part 2	Dose finding studies in target animals.	Refinement: selection of dosages based on already existing comparable products.	
Starting materials: antigen seed	Dir. 2001/82/EU, Annex 1, Title 2, Part 2	Freedom of extraneous agents requires in some cases animals.		Replacement: Relevant Ph. Eur. provisions currently under revision.
Finished product	Dir. 2001/82/EU, Annex 1, Title 2, Part 2	Development of routine testing for batches.	See table on finished product testing.	
Stability	Dir. 2001/82/EU, Annex 1, Title 2, Part 2	Real time stability studies In-use-stability study.	Products are tested in regular intervals according to VICH –GLs and Ph. Eur. provisions. For inactivated vaccines the batch potency test is used. Saving of animals depends on the development of replacement methods for these tests.	
Safety	Dir. 2001/82/EU, Annex 1, Title 2, Part 3	Laboratory trials (performed on target species, with some exceptions made by Ph. Eur.).		
Safety	Dir. 2001/82/EU, Annex 1, Title 2,	Environmental risk assessment.	Phase 1 of this assessment is based on all data provided in Dir. 2009/9/EU, Annex 1,	Not very relevant as, to date, additional animal trials for phase 2 trials have

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
	Part 3		Title 2, Part 3. For IVMPs containing or consisting GMOs the documents required by Dir. 2001/18/EU, Art.2 and Part C are assessed in addition. Phase 2 is only required, when Phase 1 assessment allows no final conclusion in the safety of the IVMP. Up to now, additional animal trials for a phase 2 assessments have never been required.	never been required.
Efficacy	Dir. 2001/82/EU, Annex 1, Title 2, Part 4	Laboratory trials (performed on target species, with some exceptions made by Ph. Eur.		
Quality, Safety	Dir. 2001/18/EU, Art.2 and Part C Council Dir. 90/220/EU (GMOs)	Laboratory trials.	IWP was not involved in the setting of these requirements. Therefore no proposal could be made for 3R activities. The products containing or consisting of GMOs represent less than 10% of the IVMPs licensed within the EU.	
Extraneous agents in seed lots of avian virus vaccines	Ph. Eur. Chapter 2.6.64	Test for extraneous agents using chicks.		A request for revision was made to revise the whole chapter, including the deletion of the test in chickens. The revision is currently under discussion in Group 15V.

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Safety of IVMPs	Ph. Eur. Chapter 2.5.6	Laboratory trials (performed on target species, with some exceptions made by Ph. Eur.)		
Safety of IVMPs	Ph. Eur. Chapter 2.5.6	Environmental risk assessment	This assessment is based on all data provided; no additional tests in animals are required.	
Antibody production in animals	Ph. Eur. monograph 0030 (Immunosera for veterinary use)	Health status of animals	Currently, there is no alternative the production of polyclonal sera in animals.	
Stability	Ph. Eur. monograph 0062 (Vaccines for veterinary use)	Real time stability studies In-use-stability study	Products are tested in regular intervals according to VICH guidelines and Ph. Eur. provisions. For inactivated vaccines the batch potency test is used. Saving of animals depends on the development of replacement methods for these tests.	Revised text published in Ph. Eur. 9th Edition. Details on how to use stability studies, what is expected for stability as regards intermediates and the definition of appropriate formulation and release parameters have been added.
Safety	Ph. Eur. monograph 0062 (Vaccines for veterinary use)	see Ph. Eur. Chapter 5.2.6		
Efficacy	Ph. Eur. monograph 0062 (Vaccines for veterinary use)	see Ph. Eur. Chapter 5.2.7		

87 Overview of animal testing requirements for immunological veterinary medicinal products - tests required for routine finished product (batch) testing
88 (Immunologicals Working Party - CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Identification	Dir. 2001/82/EU, Annex 1, Title 2, Part 2	Tests according Ph. Eur. requires test in animals for most inactivated vaccines.	Replacement: Relevant Ph. Eur. provisions currently under revision.	
Batch titre or potency	Dir. 2001/82/EU, Annex 1, Title 2, Part 2	Tests according Ph. Eur. requires test in animals for most inactivated vaccines.		Major field of development of 3R: Some new methods already developed or currently under development.
Safety test	Dir. 2001/82/EU, Annex 1, Title 2, Part 2	Target animal safety test.	Test no longer required, already deleted in Ph. Eur. with some exceptions. (Guideline on data requirements for removing the target animal batch safety test for immunological veterinary medicinal products in the EU no longer applies).	Not relevant as test not required in Europe. However, the EU is leading the development of International guidelines (VICH GL 50 and VICH GL 55) on harmonization of criteria to waive target animal batch safety testing for inactivated vaccines and for live vaccines.
Purity	Dir. 2001/82/EU, Annex 1, Title 2, Part 2	Test for extraneous agents according Ph. Eur. for a number of product groups.		Replacement strategies under discussion.
Extraneous agents in batches of finished products of avian virus vaccines	Ph. Eur. Chapter 2.6.25	Test for EA using chicks.		A request for revision was made to revise the whole chapter, including the deletion of the test in chickens. The revision is currently under discussion in Group 15V.

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Specified extraneous agents test	Ph. Eur. Specific monographs for inactivated vaccines	Serological response to a list of specific agents Monographs: 0249: Equine influenza 0959: Avian Infectious Bronchitis 0960: Avian Infectious Bursal Disease 1392: Avian Paramyxovirus 3 1202: Egg Drop Syndrome 0870: Newcastle Disease 0963: Porcine Influenza 2324: Feline chlamydiosis		The introduction of a reference to the Ph. Eur. general chapter *5.2.13. Healthy chicken flocks for the production of inactivated vaccines for veterinary use*, which sets quality requirements upstream in the production of inactivated vaccines that will provide guarantees with regard to extraneous agents contamination, makes the test for Specified extraneous agents performed on each batch of final product obsolete. As a consequence, the test for Specified extraneous agents has been deleted in the monographs concerned.
Identification	Ph. Eur. Specific monographs (all inactivated vaccines)	Tests according Ph. Eur. require test in animals for most inactivated vaccines Revised text: "3-1 Identification: The antigen is identified by suitable methods such as nucleic amplification techniques (2.6.21). For inactivated vaccines, the test may be combined with the batch potency test."		Replacement: Relevant Ph. Eur. provisions laid down in the specific monographs for inactivated vaccines for veterinary use, text published in Ph. Eur. 9th Edition "In the interest of animal welfare, the antibody induction test has been replaced by suitable methods for all inactivated vaccines."

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Batch titre or potency	Ph. Eur. monograph 0062 (Vaccines for veterinary use)	Tests according Ph. Eur. requires test in animals for most inactivated vaccines Revised text: "2-4-2: ...For inactivated vaccines, development of in-vitro methods is recommended,...".		Revised text currently in Ph. Eur. 9th Edition: Major need for development in this field: Some new methods already developed: Rabies inactivated, Erysipelas inactivated, Newcastle disease inactivated, Leptospirosis for cattle, dogs, inactivated
Safety test	Ph. Eur. monograph 0062 (Vaccines for veterinary use)	Target animal safety test already deleted.		When specific batch associated risk identified, an overdose in target species test called "residual toxicity" was kept in some specific monographs 1360: Porcine Actinobacillosis 1361: Porcine Progressive Atrophic Rhinitis
Residual live virus/ bacteria/detoxification	Ph. Eur. Specific monographs	Tests in animals not required, with the following exceptions (in vitro methods not available): 2325: rabbit haemorrhagic disease (residual live virus test in rabbits) 0744: Aujeszky (residual live virus test in rabbits if not possible in cell cultures) 0360: Cl. botulinum (residual toxicity test in mice) 0362: Cl. novyi (Type B) (residual toxicity		For clostridial vaccines, validation work involving the EDQM to replace the test in mice by a test in cells BSP130: Validation of cell line assays for in-process testing of Clostridium septicum vaccine antigens BSP 136: Validation of the BINACLE assay for in vitro detection of toxicity in tetanus toxoids

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
		test in mice) 0363: Cl. perfringens (residual toxicity test in mice 0364: Cl. septicum (residual toxicity test in mice) 0697: Tetanus (residual toxicity test in guinea pigs) 0451: Rabies (residual live virus in mice for adjuvanted vaccines only)		

2.4. CVMP Environmental Risk Assessment Working Party

Overview of animal testing requirements for environmental risk assessment of veterinary medicinal products (Environmental Risk Assessment Working Party - CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Fish acute study - freshwater	VICH GL 38 on environmental impact assessment for veterinary medicinal products phase II (CVMP/VICH/790/03 -FINAL)	Required as part of phase II tier A ERA risk assessment (otherwise not needed). Acute toxicity testing in one fish species, in line with OECD guideline 203.	The limit test as described in OECD 203 should be used to demonstrate that LC50 > 100 mg/l, allowing a reduction from (at least) 42 fish to 14 fish. The use of the threshold approach as described in OECD guidance document 126 should be considered. This allows a tiered testing strategy which has the potential to significantly reduce the number of fish used. It is based on the fact that the LC50/EC50 value of the most sensitive of the three test species (fish, algae and invertebrates) is commonly used for hazard and risk assessment and fish is often not the most sensitive test species.	The European Union Reference Laboratory for Alternatives to Animal Testing has recommended the Zebrafish embryo acute toxicity test (OECD 236) for acute aquatic toxicity testing. However, the data allows comparison of acute fish toxicity data and acute fish embryo toxicity data with only a limited number of pharmaceuticals (8), and consequently more evidence is considered necessary before the method can be accepted as an alternative to acute toxicity testing in fish. Developments will be monitored.

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
				There is an ongoing OECD project to revise OECD 203, which would incorporate humane endpoints. This will also be monitored.
Fish acute study - saltwater	VICH GL 38 on environmental impact assessment for veterinary medicinal products phase II (CVMP/VICH/790/03 -FINAL)	Required as part of phase II tier A ERA risk assessment (otherwise not needed). Acute toxicity testing in one fish species - no international guidance in place but the guideline "Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians" (E&29-96 (2002)) available from the American Society for Testing of Materials (ASTM) and the Office of Prevention, Pesticides and Toxic Substances (OPPTS) guideline "Fish acute toxicity test, freshwater and marine (850.1075).	This is rarely performed as the freshwater test tends to be the preferred option.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
Studies on birds	VICH GL 38 on environmental impact assessment for veterinary medicinal products phase II (CVMP/VICH/790/03 -FINAL)	Required as part of phase II tier B ERA risk assessment (otherwise not needed). Testing in one bird species, in line with OECD guideline 205.	Studies on toxicity to birds are rarely required – it is only in those cases where there is both high toxicity and potential exposure through the food chain that they might be considered appropriate (secondary poisoning – ERA Phase II Tier B). If relevant toxicity data in mammals are available, studies in birds are not necessary.	
Fish early life stage	VICH GL 38 on environmental impact assessment for veterinary medicinal products phase II (CVMP/VICH/790/03 -FINAL)	Required as part of phase II tier B ERA risk assessment (otherwise not needed) Testing in one fish species in line with OECD guideline 210		
Fish chronic toxicity/reproduction	VICH GL 38 on environmental impact assessment for veterinary medicinal products phase II (CVMP/VICH/790/03 -FINAL)	Required as part of phase II tier B ERA risk assessment (otherwise not needed) Testing in one fish species - no specific guidance is available although there are several OECD test guidelines available for testing of endocrine disruptor related effects:		

Topic	Regulatory provision	Animal testing requirements	Implemented 3R opportunities	Newly identified opportunities for 3R implementation
		OECD 229 (fish short-term reproduction assay), OECD 230 (21-day fish assay: a short term screening for oestrogenic and androgenic activity, and aromatase inhibition), OECD 234 (fish sexual development test), OECD 240 (Medaka extended one-generation reproduction test). Applicants are recommended to seek regulatory advice.		
Bioconcentration in fish	VICH GL 38 on environmental impact assessment for veterinary medicinal products phase II (CVMP/VICH/790/03 -FINAL)	Required as part of phase II tier B ERA risk assessment, or for a PBT assessment (otherwise not needed). Testing in one fish species in line with OECD 305.	The 2012 version of OECD 305 allows for a reduction in the number of fish used under certain conditions, using the minimised aqueous exposure fish test.	

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