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4 Reflection paper on the current regulatory testing
5 requirements for veterinary medicinal products and
6 opportunities for implementation of the 3Rs
7 Draft

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8 Comments should be provided using this EUSurvey [form](#) . For any technical issues, please contact the [EUSurvey Support](#).

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

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29 **1. Executive summary**

30 The "Reflection paper on the current regulatory testing requirements for veterinary medicinal products
31 and opportunities for implementation of the 3Rs" has been revised to incorporate new developments
32 and/or approaches in the field of 3Rs that are acceptable for regulatory decision-making during the
33 assessment of veterinary medicinal products.

2. Introduction

In December 2016, the CHMP and CVMP published the “Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches” (EMA/CHMP/CVMP/JEG-3Rs/450091/2012). This reflection paper has been developed as a follow up to that guideline and provides an overview of the main animal tests required for 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. It should be emphasised that the latter comprises areas or approaches that are currently under investigation, and these will necessitate data review and further in-depth consideration before applicability to the assessment of veterinary medicinal products and/or their impact on 3Rs can be fully appraised. This document should encourage sponsors to develop new 3Rs methodologies and submit them for regulatory review and acceptance.

The information is presented in tabular format and divided into sections based on the areas of responsibility of the relevant EMA working parties. In certain areas, essential initiatives or contributions were provided by the European Directorate for the Quality of Medicines & Healthcare (EDQM), in particular the Biological Standardisation Programme (BSP) Group 15 (V) ('Vaccines, sera for human and veterinary use').

The EMA working parties involved are:

- 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 veterinary medicinal products;
- The CVMP Novel Therapies and Technologies Working Party (NTWP), which develops guidance on matters relating to veterinary novel therapies and technologies;
- The CVMP Immunologicals Working Party (IWP), which develops guidance on quality, safety and efficacy testing of immunological veterinary medicinal products (IVMPs);
- The CVMP Environmental Risk Assessment Working Party (ERAWP), which develops guidance on environmental testing of veterinary medicinal products;
- The CVMP Efficacy Working Party (EWP-V), which develops guidance on efficacy of veterinary medicinal products, including target-animal safety;
- The CHMP Methodology Working Party (MWP), which develops guidance in non-clinical and clinical areas such as biostatistics, modelling and simulation, pharmacokinetics, pharmacogenomics and real-world evidence.

The tables presented cover data requirements to demonstrate quality, safety and efficacy of pharmaceuticals, including biological and immunological veterinary medicinal products. The tabled studies present the standard testing requirements to allow robust decision-making based on a benefit-risk evaluation that will be driven by product type and therapeutic indication and will consequently be developed on a case-by-case basis. Importantly, 3Rs should be considered in the design of all studies conducted in animals, including efficacy studies, taking into account section I.1.7 of Annex II of Regulation (EU) 2019/6, notwithstanding the place of conduct of the experiments. Alternative test methods should be employed whenever possible.

It is important to note that, for the tests enumerated in the tables below, applicants may deviate from guidelines as long as they are able to provide data (new data or published literature) or argumentation to scientifically demonstrate that the 3Rs approach provides an equivalent level of information on quality, safety or efficacy. If an applicant considers waiving of a particular test requiring the use of animals, or using a 3Rs-compliant methodology, advice on the acceptability of the proposed approach can be requested through the [EMA's scientific advice procedure](#). In addition, developers of 3Rs testing approaches, including novel approach methodologies (NAMs)¹, can contact [EMA's Innovation Task Force \(ITF\)](#) for an early dialogue with scientific experts from the European Regulatory Network to discuss regulatory, technical and scientific issues related to method development, data collection required for regulatory acceptance of the method, and finally on future qualification of the 3Rs-compliant testing method for a particular context of use.

The tables below include 3Rs-compliant methods that may serve in the decision-making in the frame of the assessment of veterinary medicinal products in a regulatory context. Furthermore, approaches for reduction or refinement have been included. The current reflection paper provides a snapshot of animal testing requirements and possibilities to include 3Rs-compliant approaches at the time of publication. It is to be expected that, over time, new 3Rs-compliant testing approaches will become accepted, and the tables should be interpreted accordingly.

In reviewing these tables, the reader should remember that the fundamental responsibility of the CVMP is to ensure the quality, safety and efficacy of veterinary medicinal products. In doing so, its role is to safeguard the health of target animals, human users administering the products, and exposed household members, including children, and to protect the environment, human consumers of food derived from treated animals, as well as human society in the event of zoonoses unrelated to food and the environment. 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, consumers or the environment.

¹ The term 'new approach methods/methodologies' (NAMs) refers to 3Rs-compliant methods which may be incorporated in the assessment of the safety and efficacy of new medicines to replace or reduce animal use. Examples include *in vitro* (cell-based) systems and computer modelling.

3. Overview of regulatory animal testing requirements

3.1. CHMP/CVMP Quality Working Party and European Pharmacopoeia (Ph. Eur.)

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 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Pyrogens (rabbits)* * test also applicable to biological medicinal products	Ph. Eur. chapter 2.6.8 Pyrogens <i>(note: chapter 2.6.8 will be suppressed from the Ph. Eur as of 1 January 2026).</i> Ph. Eur. chapter 5.1.13. <i>(note: chapter 5.1.13 pyrogenicity is to be implemented on 1 July 2025)</i>	Amikacin-sulfate, calcium levulinate dihydrate, colistimethate sodium, chloramphenicol sodium succinate, dicloxacillin sodium, flucloxacillin sodium, glucose, glucose monohydrate, kanamycin acid sulphate, kanamycin monosulfate, polymyxin B sulphate, sodium citrate. Besides the active substances in this table, the test was used in case of derived medicinal products and some older products.	In June 2021, the European Pharmacopoeia Commission took the decision to completely replace the rabbit pyrogen test (RPT) 2.6.8 in the Ph. Eur. with Monocyte-activation test (MAT) (2.6.30) or bacterial endotoxins test (BET) (2.6.14/2.6.32) within approximately 5 years. Subsequently, in June 2024, the Ph. Eur. Commission adopted revised text for 57 monographs where the RPT has been deleted with an implementation date of 1 July 2025. Accordingly, the requirement to carry out the RPT in the monographs for Amikacin-sulfate, Calcium levulinate dihydrate, Colistimethate	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			sodium, Chloramphenicol sodium succinate, Dicloxacillin sodium, Flucloxacillin sodium monohydrate, Glucose, Glucose monohydrate, Kanamycin acid sulfate, Kanamycin monosulfate, Polymyxin B sulfate, and Sodium citrate has been deleted. As a result, the new requirements for pyrogenicity in the revised Ph. Eur. general monograph 2034 Substances for pharmaceutical use will apply. The new requirement in the general monograph 2034 refers to the general chapter 5.1.13 (to be implemented on 1 July 2025), which provides guidance for the selection and implementation of a suitable test for pyrogenicity: MAT (as described in 2.6.30) or BET (as described in 2.6.14/2.6.32).	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Bacterial endotoxins (amoebocyte lysate from <i>Limulus polyphemus</i> or <i>Tachypleus tridentatus</i>)* * test also applicable to biological medicinal products	Ph. Eur. Chapter 2.6.14 Bacterial endotoxins	Active substances of endotoxin-free grade and most of medicinal products intended for parenteral administration.	The bacterial endotoxins test (BET) is used to detect or quantify endotoxins from Gram-negative bacteria using limulus amoebocyte lysate (LAL) obtained from blood cells (amoebocytes) of horseshoe crabs (<i>Limulus polyphemus</i> , <i>Tachypleus tridentatus</i>). Ph. Eur. chapter 2.6.32 describes a test for bacterial endotoxins using recombinant factor C (rFC) that may be used as an alternative to LAL-based methods.	
Abnormal toxicity test (ATT) (mice)* * test also applicable to biological medicinal products	Ph. Eur. chapter 2.6.9 Abnormal toxicity was removed from the Ph. Eur. in Supplement 9.6 of July 2018 (implementation date: 1 January 2019).	ATT was originally developed to detect external contaminants causing adverse events in biological products.	At its session in November 2017, the Ph. Eur. Commission endorsed the complete suppression of the ATT from the Ph. Eur. The ATT was removed from all Ph. Eur. monographs referring to it. The corresponding general chapter 2.6.9 Abnormal toxicity was suppressed in Ph. Eur. Supplement 9.6 of July 2018 (implementation date: 1 January 2019).	

107 **3.2. CVMP Safety Working Party**

108 Overview of animal testing requirements for safety studies for establishment of **maximum residue limits (MRLs)** for pharmacologically active substances
 109 (Safety Working Party — CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Non-biologicals, chemical-like biologicals and chemical-unlike biologicals for which it was concluded that an MRL evaluation is required				
Pharmacodynamics	Regulation (EC) No 470/2009 for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin Regulation (EU) 2018/782 establishing the methodological principles for the risk assessment and risk management recommendations referred to in Regulation (EC) No 470/2009 Guideline on the approach to establish a pharmacological ADI (EMA/CVMP/SWP/355689/2006)	The main aim of such studies is to determine a no observed effect level (NOEL) or a benchmark dose (lower confidence limit) (BMDL) for pharmacological effects for use in determining a pharmacological acceptable daily intake (ADI). Pharmacodynamics studies may also provide mechanistic information that can aid the understanding of effects seen in toxicology studies.	Where appropriate human data are available, these can be used for the establishment of a no observed adverse effect level (NOAEL). A pharmacological ADI is not required if residues in foodstuffs are devoid of pharmacological activity, if the substance is not bioavailable by the oral route in humans, for substances for which the only expected pharmacodynamic activity is an antimicrobial activity, if it is clear that it would be higher than the toxicological ADI or 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, i.e. do not	Consider use of <i>in vitro/in silico</i> modelling, if scientifically justified.

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			occur at doses below those required to produce toxicological effects.	
Pharmacokinetics in laboratory animals	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 VICH Guideline 47 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)	The aim is to provide ADME (absorption, distribution, metabolism and excretion) 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 the species used in toxicology studies.	In some cases, human data can be used, if available, for example if data exist that demonstrate absence of oral absorption or metabolism. <i>In vitro/in silico</i> modelling can be used if scientifically justified.	
Single dose toxicity	Regulation (EC) No 470/2009 Regulation (EU) 2018/782	Not required for the establishment of MRLs but, if available, relevant data should be provided.	Not relevant, as single dose studies are not required and, therefore, there is no need for generation of new data.	
Repeat dose (90 day) toxicity	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 VICH Guideline 31 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.	One species could be acceptable on a case-by-case approach and if clearly justified. Inclusion of additional <i>in vivo</i> endpoints in repeat dose toxicity studies in order to reduce animal use is acceptable, if scientifically justified (e.g. by integration of safety pharmacology or genotoxicity endpoints).	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Repeat dose (chronic) toxicity	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 VICH Guideline 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 Guideline 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".	
Reproductive toxicity including developmental toxicity	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 VICH Guideline 22 on Studies to evaluate the safety of residues of veterinary drugs in human food: Reproduction testing (CVMP/VICH/525/00-FINAL) VICH Guideline 32 on Studies to evaluate the safety of residues of veterinary drugs in human food: Developmental toxicity testing (CVMP/VICH/485/02-FINAL)	Reproduction testing: a multigeneration test in at least one species, normally rat (VICH Guideline 22). Developmental toxicity testing: The tiered approach begins with developmental toxicity testing in the rat in accordance with VICH Guideline 32. If no teratogenicity is observed (or in the case of equivocal results), then developmental toxicity testing in a second species (usually rabbit) is required.	Both VICH Guidelines 22 and 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. An extended one generation reproductive toxicity study can be provided as an alternative to the standard multi-generation study (to be included in VICH Guideline 22). In relation to developmental toxicity, no second species is required if teratogenicity is observed in the first species, except when the ADI would be based on the teratogenicity study.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Genotoxicity studies	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 VICH Guideline 23(R) 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 (<i>in vitro</i>) or an <i>in vitro</i> mouse lymphoma tk gene mutation assay. - An <i>in vivo</i> test for chromosomal effects using rodent haematopoietic cells.	In principle, the choice of tests can be modified, if appropriate. Option 1 of the standard battery of tests in VICH Guideline 23 provides for only one <i>in vivo</i> study for genotoxicity testing. Genotoxicity endpoints can be incorporated into other <i>in vivo</i> tests (such as repeat dose toxicity studies).	Unless there are other concerns, waiving of <i>in vivo</i> testing if all <i>in vitro</i> tests are clearly negative might be considered.
Carcinogenicity	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 VICH Guideline 28 on Studies to evaluate the safety of residues of veterinary drugs in human food: Carcinogenicity testing (CVMP/VICH/645/01-Rev.1-FINAL)	A 2-year rat bioassay and an 18-month mouse bioassay (in accordance with OECD Test Guideline 451 and 453) when data from (quantitative) structure-activity relationship ([Q]SAR) or toxicity studies (e.g. preneoplastic or genotoxic effects) suggest potential carcinogenicity.	Carcinogenicity studies are not required if there is no reason to suspect possible carcinogenicity (based on [Q]SAR data or the absence of 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, unless a threshold-based	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			mechanism of action can be identified.	
Immunotoxicity	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 VICH Guideline 33 on Studies to evaluate the safety of residues of veterinary drugs in human food: General approach to testing (EMA/CVMP/VICH/486/02-Rev.2)	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 in other tests).	Not routinely required. It is up to the applicant to justify the nature and extent of additional studies. Testing of developmental immunotoxicity can be integrated into the extended one generation reproductive toxicity test (in accordance with OECD Test Guideline 443).	OECD test guidance (TG) 360 <i>Detailed review paper on in vitro test addressing immunotoxicity with a focus on immunosuppression</i> : this paper defines an <i>in vitro</i> tiered approach to testing and assessment.
Neurotoxicity	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 VICH Guideline 33 on Studies to evaluate the safety of residues of veterinary drugs in human food: General approach to testing (EMA/CVMP/VICH/486/02-Rev.2)	Required for certain groups of substances known to be associated with neurotoxicity and for substances which have shown relevant toxicological effects in other toxicity tests. Possible tests to consider include a neurotoxicity test in rodents (in accordance with 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. Testing on developmental neurotoxicity can be integrated in the extended one generation reproductive toxicity test (OECD Test Guideline 443).	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Testing for effects on the human intestinal flora	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 VICH Guideline 36(R) on Studies to evaluate the safety of residues of veterinary drugs in human food: General approach to establish a microbiological ADI (EMA/CVMP/VICH/467/2003)	VICH Guideline 36(R) recommends possible <i>in vitro</i> and <i>in vivo</i> approaches.	Only required for compounds with antibacterial properties. <i>In vitro</i> approaches, i.e. minimum inhibitory concentration (MIC) tests, faecal slurries, semi-continuous and continuous cultures, and fed-batch cultures of faecal inocula, are identified in the guideline and can be employed to derive a microbiological ADI.	
Chemical-unlike biologicals				
	Regulation (EC) No 470/2009 Regulation (EU) 2018/782 Draft Guideline on determination of the need for an MRL evaluation for biological substances (EMA/CVMP/SWP/591282/2021)		Chemical-unlike biologicals can be included in the 'list of biologicals considered as not requiring an MRL evaluation' following a specific safety assessment on a case-by-case basis. Consequently, most animal studies required for chemical active substances and chemical-like biologicals might not be required for the assessment.	

111 Overview of animal testing requirements for establishment of **MRLs** and **withdrawal periods** (Safety Working Party — CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Pharmacokinetics in the target species	Regulation (EU) 2018/782 Regulation (EU) 2017/880 on the use of a maximum residue limit established for a pharmacologically active substance in a particular foodstuff for another foodstuff derived from the same species and a maximum residue limit established for a pharmacologically active substance in one or more species for other species, in accordance with Regulation (EC) No 470/2009 VICH Guideline 46 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. The aim is to identify and quantify residues of concern in food derived from treated animals and to monitor changes over time. The standard study uses 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 for other species (extension/extrapolation of MRLs).	For well-characterised substances where suitable and sufficiently robust physicochemical and pharmacokinetics data (obtained from guideline (and GLP)-compliant animal studies) as well as relevant and properly validated/qualified model assumptions are available, physiologically based pharmacokinetic modelling may be used to predict pharmacokinetic behaviour in the target species.

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	Guideline on safety and residue data requirements for the establishment of maximum residue limits in minor species (EMA/CVMP/345236/2020)			
Residue depletion studies in the target species	Regulation (EU) 2018/782 Regulation (EU) 2017/880 VICH Guideline 46 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) VICH Guideline 48(R) 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	For MRL applications, the study using radiolabelled drug in the target animal species (as per VICH Guideline 46) provides critical information relating to the depletion of residues as well as pharmacokinetics in the target species. The aim of studies conducted in accordance with VICH Guideline 48 is to monitor the depletion of the marker residue over time in the 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.	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 the purpose of establishing withdrawal periods, reduced data requirements apply for veterinary medicinal products for limited markets.	For well-characterised substances where suitable and sufficiently robust physicochemical and pharmacokinetic data (obtained from guideline (and GLP)-compliant animal studies) as well as relevant and properly validated/qualified 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 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	(EMA/CVMP/VICH/463199/2009) Guideline on safety and residue data requirements for the establishment of maximum residue limits in minor species (EMA/CVMP/345236/2020) Guideline on safety and residue data requirements for applications for non-immunological veterinary medicinal products intended for limited markets submitted under Article 23 of Regulation (EU) 2019/6 (EMA/CVMP/345237/2020) Guideline on safety and residue data requirements for applications for non-immunological veterinary medicinal products intended for limited markets but not eligible for authorisation under Article 23 of Regulation (EU) 2019/6 (EMA/CVMP/SWP/32027/2022)			

113 Overview of animal testing requirements for safety studies for veterinary medicinal products (Safety Working Party — CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Single dose toxicity	Annex II to Regulation (EU) 2019/6	No specific test requirements have been defined. Single-dose toxicity studies may be used to predict the possible effects of acute overdose in the target species, the possible effects of accidental exposure of humans, and the doses which may usefully be employed in the repeat dose studies.	Data can be bibliographic. Data from repeat dose studies may provide an alternative. Acute oral toxicity studies may be waived also considering the criteria as listed in the OECD Guidance Document No. 237. This needs to be considered on a case-by-case basis.	Data of LD ₅₀ tests are of limited value and normally not useful for quantitative risk assessment. The overall relevance of single-dose testing in safety testing is questionable. Therefore, it is recommended to derive short-term toxicity values from other study types instead of performing specific single-dose toxicity studies.
Repeat dose toxicity	Annex II to Regulation (EU) 2019/6	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 laboratory animal study may be replaced by a study conducted in the target species. Repeat dose toxicity testing may not be needed for topical use veterinary medicinal products for which absorption is negligible.	
Reproductive toxicity including developmental toxicity	Annex II to Regulation (EU) 2019/6	Standard reproductive (only for food-producing species) and developmental toxicity testing is required (i.e. based on established guidance, including VICH Guidelines 22 and 32; see	For target animal safety, the developmental toxicity study in laboratory animals may be replaced with a study in the target species.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		the MRL section above for details) for user safety where significant exposure of users is expected. Reproduction toxicity studies including developmental toxicity are required for veterinary medicinal products intended for use in breeding animals.	Reproductive toxicity studies are not expected for the evaluation of effects on the user. Standard developmental toxicity testing can be waived where significant user exposure is not expected.	
Genotoxicity studies	Annex II to Regulation (EU) 2019/6 VICH Guideline 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 (<i>in vitro</i>) or an <i>in vitro</i> mouse lymphoma tk gene mutation assay. - An <i>in vivo</i> test for chromosomal effects using rodent haematopoietic cells.	In principle, the choice of tests can be modified, if appropriate, but an <i>in vivo</i> test is expected. Option 1 of the standard battery of tests in VICH Guideline 23 provides for only one <i>in vivo</i> study for genotoxicity testing. Genotoxicity endpoints can be incorporated into other <i>in vivo</i> tests (such as repeat dose toxicity).	Unless there are other concerns, waiving of <i>in vivo</i> testing if all <i>in vitro</i> tests are clearly negative might be considered.
Carcinogenicity	Annex II to Regulation (EU) 2019/6	A 2-year rat bioassay and an 18-month mouse bioassay (in accordance with OECD Test Guidelines 451 and 453) when data from (quantitative)	In practice, carcinogenicity studies are rarely required since genotoxic substances are rarely accepted for use in veterinary medicinal products. They are	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		structure-activity relationship ([Q]SAR) or toxicity studies (e.g. preneoplastic or genotoxic effects) suggest potential carcinogenicity.	not required if there is no reason to suspect possible carcinogenicity (based on [Q]SAR data or the absence of 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 veterinary medicinal products for which absorption is negligible. Cell transformation assays (CTAs) and/or 'Integrated Approaches to Testing and Assessment' (IATAs) on non-genotoxic carcinogens can be used in a weight-of-evidence approach to predict carcinogenic potential. Additionally, carcinogenicity testing can be	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			combined with chronic toxicity testing as described in OECD Test Guideline 453.	
Other tests required for user risk assessment, possibly including skin and eye irritation, sensitisation and inhalation toxicity	Annex II to Regulation (EU) 2019/6 Guideline on user safety for pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-Rev.1) Guideline on user safety of topically administered veterinary medicinal products (EMA/CVMP/SWP/721059/2014)	The legislation requires an evaluation of user safety but does not specify the tests to be undertaken. The guidelines provide information on how to assess the risk for the user but does not specify particular test methods.		The guidelines indicate 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 additional original studies are required, these should be performed in accordance with accepted methodology and follow a stepwise approach. - <i>For skin irritation/corrosion testing:</i> OECD Test Guideline 203 and <i>in vitro</i> methods as listed in OECD Test Guidelines 430, 431, 435 and 439 - <i>For eye irritation/corrosion testing:</i> OECD Test Guideline 263 and <i>in vitro</i> methods as listed in OECD

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
				Test Guidelines 437, 438, 460, 491 and 492 - <i>For skin sensitisation testing: In vitro</i> methods as listed in OECD Test Guidelines 442C, 442D, 442E and 497
Limited market (LM) VMPs	Article 23 of Regulation (EU) 2019/6 Guideline on safety and residue data requirements for applications for non-immunological veterinary medicinal products intended for LM submitted under Article 23 of Regulation (EU) 2019/6 (EMA/CVMP/345237/2020) Guideline on safety and residue data requirements for applications for non-immunological veterinary medicinal products intended for LM but not eligible for authorisation under Article 23 of		No general recommendation for omission of animal studies is given, but standard requirements may be waived or varied on a case-by case basis, if scientifically justified. In addition, it is possible to use 'New Approach Methodologies' (NAMs) for required endpoints or a combination of approaches, if scientifically justified and valid.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	Regulation (EU) 2019/6 (EMA/CVMP/SWP/32027/2022)			

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115 **3.3. CVMP Novel Therapies and Technologies Working Party and European Pharmacopoeia**

116 Overview of animal testing requirements for safety studies for veterinary medicinal products — tests required for authorisation (CVMP Novel Therapies and
117 Technologies Working Party)

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Quality requirements	Annex II to Regulation (EU) 2019/6	Data requirements for novel therapy VMPs classified as a biological or an immunological product shall in general be in accordance with those for biological or immunological medicinal products, including the need for a relevant potency test. Additional requirements might be applicable, e.g. for cells and vector gene constructs. Also, for novel therapy VMPs constructed by chemical	Opportunities for implementation of 3Rs-compliant approaches as identified for other VMPs normally apply also to novel therapy VMPs depending on their classification as pharmaceutical, chemical like or unlike biological or immunological.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		synthesis, additional requirements might be applicable, e.g., relevant potency tests.		
Safety requirements	Annex II to Regulation (EU) 2019/6	Generally, data requirements outlined in Annex II to Regulation 2019/6/(EU) apply. Depending on the nature of the product and its intended use, further data could be relevant as determined by a risk analysis in each case.	Opportunities for implementation of 3Rs-compliant approaches as identified for other VMPs normally apply also to novel therapy VMPs depending on their classification as pharmaceutical, chemical like or unlike biological or immunological.	
Efficacy requirements	Annex II to Regulation (EU) 2019/6	Efficacy data requirements differ primarily depending on the intended indications for use in the target species. Depending on the novel therapy VMP categorisation and the intended use in the target species, efficacy requirements set out in Sections II or III of Annex II to Regulation (EU) 2019/6 may be		

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		applicable for a novel therapy veterinary medicinal product.		
Specific data requirements for particular types of novel therapy products				
Gene therapy veterinary medicinal products	Annex II to Regulation (EU) 2019/6	For quality/safety testing: In addition to standard data requirements, off-target insertions (leading, for example, to tumours/cancer, metabolic dysfunctions) and insertional mutagenesis and genotoxicity (insertion of genetic elements and the expression of DNA-modifying proteins as mediators of genotoxic side effects) in target species need to be considered. Germline transmission studies shall be provided, unless otherwise justified.		
Regenerative medicine, tissue engineering and cell therapy veterinary medicinal products	Annex II to Regulation (EU) 2019/6	For quality/safety testing: In addition to standard data requirements, relevant information shall be provided on the characterisation of the cell population or cell mixture in	According to ADVENT Q&A document (EMA/CVMP/ADVENT/791465/2016) the risk of tumorigenicity should be primarily addressed through control of	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		terms of identity, purity (for example, extraneous agents and cellular contaminants), viability, potency, karyology, tumourigenicity and suitability for the intended medicinal use.	manufacturing and quality aspects. In vivo testing for tumorigenicity testing may only be needed if an increased risk of tumour formation is anticipated, e.g., due to extensive manipulation of the MSCs, changes of the culture conditions and/or in case of expected biodistribution.	
VMPs specifically designed for phage therapy	Annex II to Regulation (EU) 2019/6 Draft Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy (EMA/CVMP/NTWP/32862/2022 2) Ph. Eur. general chapter 5.31 Phage therapy medicinal products has been adopted in March 2024 and published on the EDQM website pending its publication in Supplement 11.6 (implementation date: 1 January 2025).	For safety testing: Standard data requirements for biological VMPs other than immunological VMPs apply to a representative mono-phage or multi-phage preparation representing worst case scenarios in terms of safety concerns.	For quality testing: (<i>In vitro</i>) pyrogenicity testing is a requirement (<i>note: the revised monographs omitting the rabbit pyrogen test are to be implemented on 1 July 2025</i>). For safety testing: A satisfactorily controlled manufacturing process, target animal safety studies (pre-clinical or clinical) and/or scientific literature data are expected to be sufficient to address single-dose toxicity, repeat dose toxicity, potential immunogenicity and immunotoxicity and effects on	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			reproduction and developmental toxicity. Supplementary studies should be submitted where reference to existing studies or literature data is not directly relevant for the specific phages or if a specific safety concern is identified. The standard battery of genotoxicity tests and carcinogenicity studies can most likely be omitted. Extrapolation between comparable strains of bacteriophages, between target animal species or different routes of administration may be possible based on representative/validated <i>in vitro</i> or <i>in vivo</i> parameters or in well-justified cases. Absence of studies in laboratory animals could be justified by reference to existing data and	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			data from studies in target animal species.	
VMPs issued from nanotechnologies	Annex II to Regulation (EU) 2019/6	For safety testing: The impact of nanoparticles for drug delivery shall be investigated in the corresponding organ(s) when physiological barriers are crossed, i.e. the blood-brain barrier. The impact of agglomerates shall be investigated in relevant target organs, in particular regarding the risk of embolism in the smaller blood vessels. Safety issues might be perceived at cellular level (cytotoxicity, especially by induction of oxidative stress). Toxicological assays shall be able to assess this cytotoxicity and related aspects, e.g. the generation of toxic free radicals and biopersistence.	For quality testing: Size distribution of particles shall be determined and a suitable <i>in vitro</i> test for their function and possible delivery capacity (if used as drug delivery system) shall be used.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		Immunosafety issues, inflammatory capacity and haemocompatibility shall be considered.		
RNA antisense therapy and RNA interference therapy products	Annex II to Regulation (EU) 2019/6	In addition to standard data requirements, for certain antisense therapy products, a potency bioassay may be needed for their release testing. For RNA antisense therapy products, the possible harmful effects due to on- or off-target binding shall be addressed as well as possible non-antisense harmful effects due to, for example, accumulation, pro-inflammatory responses and aptamer binding. For RNAi therapy products, the possible harmful effects of off-target interference (due to the positive RNAi strand) shall be addressed, as well as the possibility of crossing the blood-		

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		brain barrier and causing central nervous system disorders. For RNA antisense therapy and RNAi therapy products intended for gene therapy, the requirements for gene therapy VMP shall be considered.		

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119 **3.4. CVMP Immunologicals Working Party and European Pharmacopoeia**

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

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Product development	Annex II to Regulation (EU) 2019/6	Dose-finding studies in target animals.	Refinement: Selection of dosages based on already existing comparable products.	
Starting materials: Antigen seed	Annex II to Regulation (EU) 2019/6	Freedom of extraneous agents (EA) requires animals in some cases.		See reference to EA below
Finished product	Annex II to Regulation (EU) 2019/6	Development of routine testing for batches.	See table below on finished product testing.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Stability	Annex II to Regulation (EU) 2019/6	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 as one parameter among others. Reduction of animal use 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 (pre-clinical)	Annex II to Regulation (EU) 2019/6 VICH Guideline 41 on Target animal safety: Examination of live veterinary vaccines in target animals for absence of reversion to virulence (EMA/CVMP/VICH/1052/2004)	Pre-clinical trials (performed in target species, with some exceptions made outlined in the Ph. Eur.).	Requirements to provide laboratory data defined. Reference to animal welfare, alternative methods and rescue treatment included in the guideline.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	VICH Guideline 44 on Target animal safety for veterinary live and inactivated vaccines (EMA/CVMP/VICH/359665/2005)			
Safety (batch testing)	Annex II to Regulation (EU) 2019/6, Ph. Eur. monograph 0062 Vaccines for veterinary use VICH Guideline 50 on the harmonisation of criteria to waive target animal batch safety testing for inactivated vaccines for veterinary use — Revision 1 (EMA/CVMP/VICH/582610/2009) VICH Guideline 55 on the harmonisation of criteria to waive target animal batch safety testing for live vaccines for veterinary use (EMA/CVMP/VICH/313610/2013)	Target animal batch safety test and laboratory animal batch safety test already deleted from Ph. Eur.	Test no longer required, already deleted in Ph. Eur. with some exceptions (the Guideline on data requirements for removing the target animal batch safety test for immunological veterinary medicinal products in the EU [EMA/CVMP/IWP/107173/2015] no longer applies). Two VICH guidelines on harmonisation of criteria to waive target animal batch safety testing for inactivated vaccines (VICH Guideline 50(R)) and for live vaccines (VICH Guideline 55) came into effect in May 2018. VICH Guideline 59 was implemented in November 2021.	When specific batch associated risk is identified, an overdose in target species test called residual toxicity is included in some specific Ph. Eur. monographs as follows: 1360 Porcine actinobacillosis, 1361 Porcine progressive atrophic rhinitis. The residual toxicity test (former TABST) in both monographs might be deleted in the future if a reliable endotoxin test will become available.

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	VICH Guideline 59 on the harmonisation of criteria to waive laboratory animal batch safety testing for vaccines for veterinary use (EMA/CVMP/VICH/677723/2016)			
Safety	Ph. Eur. Chapter 5.2.6 Evaluation of veterinary vaccines and immunosera	Laboratory trials (performed in target species, with some exceptions outlined in the Ph. Eur.).	Canine parvovirus vaccine (live) (0964) to reduce to a minimum the number of dogs used in the safety testing.	
Safety (environmental risk assessment)	Ph. Eur. Chapter 5.2.6 Evaluation of veterinary vaccines and immunosera	Environmental risk assessment.	This assessment is based on all data provided, no additional tests in animals are required.	
Safety (environmental risk assessment)	Annex II to Regulation (EU) 2019/6 Note for Guidance: Environmental risk assessment for immunological veterinary medicinal products (EMA/CVMP/074/95) Guidance on environmental risk assessment for veterinary	Environmental risk assessment.	The assessment consists of two phases. The first phase of the assessment shall always be performed. The details of the assessment shall be provided in accordance with guidance published by the Agency. In the case of live vaccine strains which may be zoonotic,	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	medicinal products consisting of or containing genetically modified organisms (GMOs)		the risk to humans shall be assessed. For immunological veterinary medicinal products containing or consisting of GMOs, the documents required by section IIIb.3E. of Annex II of Regulation (EU) 2019/6 are assessed in addition.	
Efficacy	Annex II to Regulation (EU) 2019/6 Guideline on clinical trials with immunological veterinary medicinal products (EMA/CVMP/IWP/260956/2021) Ph. Eur. monograph 5.2.7 Evaluation of efficacy of veterinary vaccines and immunosera	Pre-clinical and clinical studies (performed in target species, with some exceptions outlined in the Ph. Eur.)	Requirements to provide laboratory and field data defined. Reference to animal welfare, alternative methods and rescue treatment is included in the guideline.	
Extraneous agents	Ph. Eur. monograph 0062 Vaccines for veterinary use Ph. Eur. monograph 0030 Immunosera for veterinary use	In some cases, the test for extraneous agents requires the use of animals. The decision to maintain or introduce an animal test in a testing strategy must	All requirements have been collated and harmonised and are compiled in Ph. Eur. chapter 5.2.5. It includes a list of extraneous agents to be considered for risk assessment	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	Ph. Eur. chapter 5.2.5 Management of extraneous agents in immunological veterinary medicinal products Ph. Eur. chapter 5.2.4 Cell cultures for the production of vaccines for veterinary use Around 40 vaccine-specific Ph. Eur. monographs Ph. Eur. chapter 2.6.37 Principles for the detection of extraneous viruses in immunological veterinary medicinal products using culture methods	be justified by the risk assessment.	(Annex I) and refers to methods involving molecular techniques (e.g. Nucleic acid amplification techniques (2.6.21)) or using culture methods in accordance with the new general Ph. Eur. chapter 2.6.37 Principles for the detection of extraneous viruses in immunological veterinary medicinal products using culture methods.	
Therapeutic antibody production in animals	Ph. Eur. monograph 0030 Immunosera for veterinary use	Health status of animals.		Alternatives to animal-derived reagents, e.g. antibodies, should be used whenever possible, though it is acknowledged that not many off-the-shelf recombinant reagents are available at present. Development of recombinant therapeutic antibodies should be prioritised.

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
				EURL ECVAM recommends that animals should no longer be used for the development and production of antibodies including for therapeutic use. Identified 3Rs opportunities include e.g. phage display methodologies. (European commission, 2020) (3)

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

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Identification	Annex II to Regulation (EU) 2019/6 Specific monographs in the Ph. Eur. (all inactivated vaccines)	Testing according to the Ph. Eur. requires use of 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	Replacement: Relevant Ph. Eur. provisions currently under revision to incorporate <i>in vitro</i> methods. Relevant Ph. Eur. provisions laid down in the specific monographs for inactivated vaccines for veterinary use. Text published in Ph. Eur. 9 th edition: "In the interest of	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		may be combined with the batch potency test."	animal welfare, the antibody induction test has been replaced by suitable alternative methods for all inactivated vaccines."	
Batch titre or potency	Annex II to Regulation (EU) 2019/6, Ph. Eur. monograph 0062 Vaccines for veterinary use Specific Ph. Eur. monographs for inactivated vaccines	The Ph. Eur. requires testing in animals for most inactivated vaccines. Revised text: "2-4-2: [...] For inactivated vaccines, development of in-vitro methods is recommended [...]. Accordingly, an <i>in vitro</i> method for batch potency testing should be the first option of choice, the second option should be serology, and when not avoidable, other <i>in vivo</i> test formats should be used as the very last option. All refinement options should be used.	Ph. Eur. monograph 0062 Vaccines for veterinary use: 3Rs approaches specifically encouraged under '3. Batch tests'. Reference to general Ph. Eur. chapter 5.2.14 Substitution of <i>in vivo</i> method(s) by <i>in vitro</i> method(s) for the quality control of vaccines: Possibility to upstream testing mentioned, reference to antigen content and consistency added. Some new methods for inactivated vaccines already developed, e.g. rabies, erysipelas, Newcastle disease, Leptospirosis for cattle and dogs.	Major field of development of 3Rs approaches: Some new methods already developed or currently under development (e.g. antigen quantification by immunochemical methods instead of challenge procedures or serology; use of cell culture assays instead of Toxin Neutralisation Test (TNT))

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			Ph. Eur. monograph 1613 Equine herpesvirus vaccine (inactivated) stresses that an <i>in vitro</i> alternative method should be preferred for the routine batch potency test. Reduction of the number of controls in the batch potency testing for the following Ph. Eur monographs: 1942 <i>Mycoplasma gallisepticum</i> vaccine (inactivated), 1947 <i>Salmonella enteritidis</i> vaccine (inactivated) for chickens, 2361 <i>Salmonella typhimurium</i> vaccine (inactivated) for chickens. Addition of humane endpoints to the following monographs: 2325 Rabbit haemorrhagic disease vaccine (inactivated), 0588 Avian infectious encephalomyelitis vaccine (live), 1945 Fowl cholera vaccine (inactivated), 2151 Winter ulcer vaccine (inactivated, oil	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			adjuvanted, injectable) for salmonids. Addition of alternative endpoints to monograph 0360 Clostridium botulinum vaccine for veterinary use in line with monograph 2113 Botulinum toxin type A injection as follows: 'Potency test' (section 3-4); Batch potency test (section 2-3-1): cell-based assays specifically mentioned as a possible alternative.	
Specified extraneous agents test	Ph. Eur. monograph 0442 Infectious bronchitis vaccine (live)		Revision published in Supplement 10.5. The test for immunogenicity has been revised to remove the description of virus recovery from tracheal swabs (section 2-3-3-2) with the aim to encourage manufacturers to develop and use suitably validated alternative methods such as PCR rather than the method using embryonated hens' eggs.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Residual live virus/bacteria/detoxification	Ph. Eur. specific monographs	Tests in animals not required, with the following exceptions (<i>in vitro</i> methods not available): - Ph. Eur. Monograph 2325 Rabbit haemorrhagic disease vaccine (inactivated)[residual live virus test in rabbits]. - Ph. Eur. Monograph 0360 <i>Clostridium botulinum</i> vaccine for veterinary use [residual toxicity test in mice]. - Ph. Eur. Monograph 0697 Tetanus vaccine for veterinary use [residual toxicity test in guinea pigs]. - Ph. Eur. Monograph 0744Aujeszký [residual live virus test in rabbits when not possible in cell cultures]. - Ph. Eur. Monograph 0451 Rabies [residual live virus	Three animal tests removed from monographs on tetanus vaccines (specific toxicity and residual toxicity on final lot, irreversibility of tetanus toxoid on the bulk purified toxoid) Monographs 0364 <i>Clostridium septicum</i> vaccine for veterinary use, 0363 <i>Clostridium perfringens</i> vaccine for veterinary use and 0362 <i>Clostridium novyi</i> (type B) vaccine for veterinary use, revised in 2022 as follows: (i) replace the use of mice as indicator of toxicity for in-process quality control tests (minimum lethal dose (MLD) and total combining power (TCP) assays); (ii) favour the use of <i>in vitro</i> methods for the routine batch potency test; (iii) switch from an animal test for vaccine identification to an <i>in vitro</i> test; and (iv) delete the test for residual toxicity on the	The BINACLE (Binding and Cleavage) assay is a promising <i>in vitro</i> alternative method for in process testing for absence of tetanus toxin. The method has been found suitable for the detection of residual tetanus toxin in certain toxoids in the EDQM Biological Standardisation Programme project BSP136. Product-specific validation of the test will be required.

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		in mice when the vaccine is adjuvanted].	final product (conditions under which the residual toxicity test may be omitted on final bulk and final batch are stated). Deletion of section 3-3 'Residual live virus' testing for various inactivated porcine, equine and poultry vaccines.	

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127 **3.5. CVMP Environmental Risk Assessment Working Party**

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

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Fish acute study — freshwater	VICH Guideline 38 on Environmental impact assessment for veterinary medicinal products — Phase II (CVMP/VICH/790/03-FINAL)	Required as part of a Phase II Tier A environmental risk assessment (ERA; otherwise not needed). Acute toxicity testing in one fish species in accordance with OECD Test Guideline 203.	The limit test as described in OECD Test Guideline 203 should be used to demonstrate that $LC_{50} > 100$ mg/l, allowing a reduction from (at least) 42 fish to 14 fish. The use of the threshold approach as described in OECD	The European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM) has recommended the zebrafish embryo acute toxicity test (OECD Test Guideline 236) to determine acute aquatic toxicity testing. The applicability of this test to the evaluation of

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			Guidance Document No. 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. Since 2019, OECD Test Guideline 203 contains Annex 4 on sublethal signs.	pharmaceuticals warrants further consideration.
Fish acute study — saltwater	VICH Guideline 38 on Environmental impact assessment for veterinary medicinal products — Phase II (CVMP/VICH/790/03-FINAL)	Required as part of a Phase II tier A ERA (otherwise not needed). Acute toxicity testing in one fish species in accordance with OECD Test Guideline 203.	No additional test with saltwater species needed if a test on fresh water species is available. For saltwater species, the same opportunities for implementation of 3Rs-compliant approaches apply as for fresh water species.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Studies on birds	VICH Guideline 38 on Environmental impact assessment for veterinary medicinal products — Phase II (CVMP/VICH/790/03-FINAL)	Required as part of a Phase II Tier B ERA (otherwise not needed). Testing in one bird species in accordance with OECD Test Guideline 205.	Studies on toxicity to birds are rarely required, i.e. such studies may be considered appropriate only in those cases where there is both high toxicity and potential exposure through the food chain (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 Guideline 38 on Environmental impact assessment for veterinary medicinal products — Phase II (CVMP/VICH/790/03-FINAL)	Required as part of a Phase II Tier B ERA (otherwise not needed) Testing in one fish species in accordance with OECD Test Guideline 210.		
Fish chronic toxicity/reproduction	VICH Guideline 38 on Environmental impact assessment for veterinary medicinal products — Phase II (CVMP/VICH/790/03-FINAL)	Required as part of a Phase II Tier B ERA (otherwise not needed) No specific guidance is available for testing in one fish species, although there are several OECD Test Guidelines available for testing of endocrine-related effects: OECD Test Guideline	During the update of OECD Test Guideline 240 in 2023, a chapter on humane killing was included.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		229 (fish short-term reproduction assay), OECD Test Guideline 230 (21-day fish assay: a short-term screening for oestrogenic and androgenic activity, and aromatase inhibition), OECD Test Guideline 234 (fish sexual development test), OECD Test Guideline 240 (Medaka extended one-generation reproduction test). Applicants are recommended to seek regulatory advice.		
Bioconcentration in fish	VICH Guideline 38 on Environmental impact assessment for veterinary medicinal products — Phase II (CVMP/VICH/790/03-FINAL)	Required as part of a Phase II Tier B ERA or for a PBT assessment (otherwise not needed). Testing in one fish species in accordance with OECD Test Guideline 305. VICH Guideline 38, Tier B: if log $K_{ow} > 4$ and evidence for bioaccumulation from other studies, a study in accordance	OECD Test Guideline 305 allows for a reduction in the number of fish used under certain conditions, for instance by using the minimised aqueous exposure fish test, by only testing one concentration if a preliminary test shows that the bioconcentration factor (BCF) is not concentration dependent.	OECD Test Guideline 321 on the <i>Hyalella azteca</i> bioconcentration test (HYBIT) has recently been finalised. REACH ² R.11 has identified this as a potential alternative to OECD Test Guideline 305. If a substance has a valid and plausible <i>H. azteca</i> BCF $> 2,000$ or $> 5,000$, the substance is defined as 'B' or 'vB', respectively. An <i>H. azteca</i> BCF (3%, w/w) $< 1,200$ and $< 3,000$ indicates 'not B'

² Regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals, note that substances used in medicinal products for human or veterinary use are exempted from the Registration Title of the REACH Regulation (4)

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		with OECD Test Guideline 305 to be performed.		and 'not vB'. Only for a <i>H. azteca</i> BCF between 1,200 and 2,000 and 3,000 and 5,000, further testing will be required. In those cases, OECD Test Guideline 305 should be applied.

130 **3.6. CVMP Efficacy Working Party**

131 Overview of animal testing requirements for efficacy and target animal safety of veterinary medicinal products (Efficacy Working Party — CVMP)

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Target animal safety (TAS)	Regulation (EU) 2019/6 VICH Guideline 43 on Target animal safety for veterinary pharmaceutical products (EMA/CVMP/VICH/393388/2006) Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick	The purpose of TAS studies is to characterise signs of intolerance and to establish an adequate margin of safety using the recommended route(s) of administration. Healthy animals, representative of the species and class in which the product will be used, should generally be used in TAS studies. Treatment groups	Additional safety studies may be appropriate depending on the conditions of use and the characteristics of the test product (e.g. administration site, reproductive, and mammary gland safety studies). Such studies may be combined with the margin of safety evaluation and, in food-producing animals, residue studies.	

	and flea infestation in dogs and cats (EMA/CVMP/EWP/005/2000) Guideline on the conduct of efficacy studies for intramammary products for use in cattle (EMA/CVMP/344/1999)	should include a negative control, the highest recommended dose level (1X), and two multiples of this use dose (in most cases three times (3X) and five times (5X)) for a period of time in excess of the recommended maximum duration of use. In general, it is recommended that each group be treated for at least 3 times the proposed duration up to a max of 90 days. Where product use is expected to exceed 3 consecutive months, longer duration studies may be recommended up to 6 months or longer if appropriate. If multiple routes of administration are proposed, the route that is most likely to cause adverse effects should be selected as the basis for safety study. Randomised and blinded study design should be used. Target animal safety studies typically include relatively small numbers of experimental units (generally 8 per treatment) and	Generic/hybrid ectoparasiticide products for external topical use which are locally acting: Tolerance studies are not considered necessary in the case that the composition (i.e. quality and quantity of the active substance(s) and excipient(s)) and the physico-chemical properties of the candidate and the reference product are identical and the candidate product is to be administered at the same dose and route of administration as the reference product. Generic/hybrid intramammary products: Tolerance studies are not required if the candidate product is identical to the reference product. Tolerance studies may also be waived if the candidate and reference products have the same pharmaceutical form, contain qualitatively and quantitatively the same active substance(s)(salts), the excipients of the candidate	
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		assess large numbers of variables. For products containing systemically absorbed ingredients that are intended for use in breeding animals, specific reproductive safety studies in the target animal species should be provided. Other laboratory safety study designs: Local adverse reactions to topically applied products should be evaluated. Local tolerance should be evaluated.	product are qualitatively and quantitatively very similar compared to the reference product, and the physicochemical properties (e.g. crystalline form, particle size distribution, viscosity, relative density, dissolution profile) of the candidate product are similar to those of the reference product.	
Pharmacokinetics	Guideline on the conduct of pharmacokinetic studies in target animal species (EMA/CVMP/EWP/133/1999)	Depending on the active substance and its use(s), all or some of the following items should be studied: absorption, distribution, metabolism and excretion. When efficacy and target animal safety of a combination product are intended to be supported by data from single-substance products and an absence of interaction is claimed, generally	For established active substances where a range of therapeutic doses is recommended and dose proportionality is documented in the target species, studies with a single dose level, corresponding to the highest intended therapeutic dose, are generally sufficient. Where there is no dose proportionality or a very steep dose/effect curve, studies using three different	The use of <i>in vitro/in silico</i> test systems to replace or reduce animal studies should be considered on a case-by-case basis. The validity and reliability of the used test methods should be demonstrated.

		the results of comparative PK study(ies) should be interpreted together with clinical data ultimately showing the absence of clinically significant interactions, in respect of both safety and efficacy. For an active substance that has not previously been used in a veterinary medicinal product in the target species, pharmacokinetic studies using at least three different dose levels should be performed	dose levels, encompassing the dose range, may be necessary. For established active substances, pharmacokinetic studies with a single dose level may be sufficient where a single dose level is recommended. The accuracy of dosing should be considered when designing such studies, particularly in cases where there is a solid formulation, e.g. tablet. Collection of pharmacokinetics (PK) data may be integrated in early pre-clinical efficacy studies, in order to establish the PK/pharmacodynamics (PD) relationship.	
Bioequivalence	Guideline on the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000)	To allow bridging of safety and efficacy data associated with a reference veterinary medicinal product, bioequivalence studies are often part of applications for generic veterinary medicinal products. Other types of applications may also require demonstration of bioequivalence or other comparative pharmacokinetic data.	It is recommended to ask for scientific advice if it is estimated that a traditional crossover design would not be feasible without the inclusion of a very high number of animals. When <i>in vitro</i> equivalence data are presented for additional strengths, a bioequivalence	Other waivers from bioequivalence studies for certain applications regarding veterinary medicinal products might be considered on a case-by-case basis.

		If two formulations are compared, a randomised, two-period, two-sequence single dose crossover design is recommended. Parameters to be analysed and acceptance criteria for C_{max} and C_{trough} should also generally be within 80% to 125%. Target species: The test animals should be of the target species. Where a veterinary medicinal product is intended for more than one species, bioequivalence studies should normally be performed in each target animal species.	study investigating only one strength may acceptable. For substances with highly variable disposition where it is difficult to show bioequivalence due to high intra-individual variability, different alternative designs have been suggested in the literature (e.g. replicate study design). <i>In vivo</i> bioequivalence studies may not be necessary provided set criteria, as laid out under the relevant section of the Guideline on the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000 Rev.4), are met (waivers from bioequivalence studies). Parameters to be analysed and acceptance criteria: C_{max} and C_{trough}. As these parameters may exhibit a greater intra-individual variability, a maximal widening of the limits to 70% to 143% could, in rare cases, be acceptable if it has been	
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			prospectively defined in the protocol together with a justification from an efficacy and safety perspective. Valid data would be, for example, data on PK/PD relationships for efficacy and safety which demonstrate that the proposed wider range does not affect efficacy and safety in a clinically significant way. Target species: Extrapolation of results from a major species in which bioequivalence has been established to minor species could be acceptable if justified based on scientific information.	
Dose determination and confirmation	Regulation (EU) 2019/6 Guideline for the conduct of efficacy studies for non-steroidal anti-inflammatory drugs (NSAIDs) (EMA/CVMP/EWP/1061/2001) Guideline for the demonstration of efficacy of ectoparasiticides (7AE17a)	Appropriate data shall be provided to justify the proposed dose, dosing interval, duration of treatment and any re-treatment interval. Dose-determination studies should be conducted in the target species using a range of doses selected on the basis of preliminary studies, parameters that are relevant for the	<i>In vivo</i> PK/PD studies in the target species, if conclusive and conducted over a sufficient exposure range, may serve as an aid in the establishment of a dosing strategy and may potentially reduce the need for comprehensive dose-finding data, and if adequately designed, be used in replacement of the latter.	

	Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats (EMA/CVMP/EWP/005/2000)	anticipated effect and a dose range that is considered appropriate for further use. At least two dose confirmation studies are recommended to demonstrate the efficacy of a new product against each ectoparasite species and stage of development as indicated in the labelling.	For generic/hybrid antiparasitic products with local activity only, the efficacy should be confirmed under laboratory conditions in at least one controlled dose confirmation study (GCP) for each parasite species in the target animal.	
Efficacy (for locally acting generic/hybrid products)	Regulation (EU) 2019/6 Guideline on the conduct of efficacy studies for intramammary products for use in cattle (EMA/CVMP/344/1999) Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats (EMA/CVMP/EWP/005/2000)	The results of appropriate pre-clinical studies or clinical trials shall be required when the veterinary medicinal product does not meet all the characteristics of a generic veterinary medicinal product because [...] bioavailability studies cannot be used to demonstrate bioequivalence with the reference veterinary medicinal product.	Generic/hybrid intramammary products: Efficacy studies are not required if the candidate product is identical to the reference product. Efficacy studies may also be waived if the candidate and reference products have the same pharmaceutical form and contain qualitatively and quantitatively the same active substance(s), the excipients of the candidate product are qualitatively and quantitatively very similar compared to the reference product, and the physicochemical properties (e.g. crystalline form, particle size distribution, viscosity, relative	

			density, dissolution profile) of the candidate product are similar to those of the reference product. Generic/hybrid ectoparasiticial products for external topical use which are locally acting: Efficacy [...] studies are not considered necessary in the case that the composition (i.e. quality and quantity of the active substance[s] and excipient[s]) and the physico-chemical properties of the candidate and the reference product are identical and the candidate product is to be administered at the same dose and route of administration as the reference product.	
Limited markets	Guideline on efficacy and target animal safety data requirements for applications for non-immunological veterinary medicinal products intended for limited markets submitted under Article 23 of Regulation (EU) 2019/6 (EMA/CVMP/52665/2020)	Pharmacology: Basic PK data (to characterise the absorption, distribution and elimination) of the active substance should be provided as a complement to the pharmacodynamic studies to support the establishment of the proposed dosage regimen [...].	Pharmacology: Nevertheless, if data [...] is provided to characterise the efficacy and tolerance of the test product in terms of the proposed indication, posology and route(s) of administration, product-specific pharmacokinetic and	

	Guideline on efficacy and target animal safety data requirements for applications for non-immunological veterinary medicinal products intended for limited markets but not eligible for authorisation under Article 23 of Regulation (EU) 2019/6 (EMA/CVMP/EWP/231668/2022)	Dose justification: In principle, specific dose justification and/or confirmation studies in an appropriate and relevant disease model or in naturally diseased animals should be provided to support the dose regimen of the VMP. Target animal safety: Appropriate data to characterise the safety of the target species to the test product following administration by the proposed route(s) should be provided. Typically, target animal safety (local and systemic) should be confirmed in healthy animals of the target species in a negative-controlled TAS study implemented under well-controlled laboratory conditions in line with the principles of VICH Guideline 43 [...]. Efficacy: confirmatory clinical trials to be provided. Pharmacology: The mode of action and the pharmacological effects on which the	pharmacodynamic data can be omitted. Dose justification: Appropriate data should be provided to justify the proposed dose, dosing interval, duration of treatment and any re-treatment interval. [...] Specific dose justification and/or confirmation studies may be omitted where suitable information/data is provided to support the choice of dose. Target animal safety: The absence of a VICH-compliant TAS study may be accepted, if justified, where a comprehensive evaluation of target animal safety is possible by other means, foremostly based on data provided from exploratory and/or clinical trials following administration of the product at the recommended treatment dose and duration of therapy to an adequate number of animals representing the target (sub)species. Safety data on use in the target species may also be supplemented with	
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		recommended application is based shall be adequately described [...]. Dose justification: In principle, dose determination and confirmation studies in an appropriate and relevant disease model and/or in naturally diseased target animals should be provided to support the dosage regimen of the VMP. Target animal safety (TAS): In general, TAS should be confirmed in healthy animals of the target species in a negative-controlled TAS study. Efficacy: Clinical trials should be conducted using the final formulation and carried out in accordance with established principles of good clinical practice.	reference to use in another relevant species in which the safety profile is expected to be similar, data from toxicity studies in laboratory animals, literature reports and pharmacovigilance data. Efficacy: where it is reasonable to conclude that the product is safe for the target population when administered at the recommended treatment dose and by the proposed route(s) of administration, and the product is expected to be effective for the proposed indication in the target diseased animals [...], the provision of comprehensive clinical documentation including confirmatory clinical trial data will not be required. Pharmacology: If appropriate data (dose confirmation study/clinical trial) is available to characterise the efficacy and tolerance of the test product in terms of the proposed indication, posology and route(s) of administration in the	
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			target species, the need to submit PK/PD studies in the target species could be waived. Instead, the respective product-specific pharmacokinetic and pharmacodynamic properties could be established by other means, e.g. by extrapolation from another species for which the product is authorised, appropriate data from literature, or pilot studies. Dose justification: For limited market applications, the number of dose determination and/or confirmation studies may be reduced or omitted depending on whether suitable information/data is provided to support the choice of dose and the adequacy of that information/data. If the efficacy of the product at the recommended dosage regimen has been demonstrated in an adequate and controlled dose confirmation study in the target species, a dose determination study can be omitted.	
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			If a clinical trial has been provided and the selected dose is justified, dose confirmation studies are not required, if the clinical trial includes a control group. Target animal safety: For products where systemic exposure is known to be negligible, and there are no known safety concerns, a specific target animal safety study is not considered necessary, and tolerance can be demonstrated based on the clinical trial(s) or from published literature data. If the test product is approved for another species and is known to have a wide margin of safety in that species, clinical trial data demonstrating satisfactory tolerance in the target species following administration of the test product at the recommended treatment dose for the recommended duration of therapy may be considered	
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			adequate, and a specific target animal safety study may not be required. Where safety in breeding animals of another species is demonstrated, additional safety data in breeding animals of the target species might not be necessary. Efficacy: In specific cases, where the efficacy of the test product has been confirmed in dose determination and/or dose confirmation studies and where adequate and robust data are available relating to target animal safety, clinical trials may not be necessary in that species.	
Refinements	Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats (EMA/CVMP/EWP/005/2000)	During the time period(s) of infestation with ectoparasites, dogs and cats should be kept in individual accommodation, i.e. from the day of infestation until the day of ectoparasite counting (e.g. up to 96 hours at the beginning of the trial from day - 2 to day 2, and up to 48 hours	However, for the other time periods, housing of animals in groups should be considered [...] with sufficient space according to animal species. In order to reduce stress, enrichment of the environment should be considered. The provisions of section I.1.7 of Annex II of	

		after subsequent challenge infestations).	Regulation (EU) 2019/6 should be adhered to.	
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3.7. References

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3. Barroso J., Halder M., Whelan M. EURL ECVAM Recommendation on Non-Animal-Derived Antibodies. Publications Office of the European Union. doi:10.2760/80554, JRC120199.
4. European Chemicals Agency. Guidance on REACH: [Guidance on REACH - ECHA \(europa.eu\).](https://echa.europa.eu/guidance-on-reach)