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Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy

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Executive summary

The aim of this guideline is to establish the regulatory/technical and scientific requirements applicable to veterinary medicinal products (VMPs) specifically designed for phage therapy and composed of bacteriophages.

Bacteriophages are viruses that infect bacteria and do not have the capacity to infect eukaryotic cells. Their action is linked to their lytic activity, generally restricted to specific bacterial strains. Additionally, the interaction bacteriophage-host bacteria is a dynamic process and host bacteria might develop resistance against bacteriophages with some frequency.

Consequently, VMP based on bacteriophages are expected to require frequent changes in composition for the bacteriophage strain(s) in order to maintain efficacy/circumvent resistance development in relation to the intended indication.

Phage therapies are defined as novel therapies (NTs) by Regulation (EU) 2019/6. Additionally, Regulation (EU) 2021/805 amending Annex II to Regulation (EU) 2019/6 includes general and specific requirements applicable to NTs, and specific provisions for phage therapy.

This guideline addresses how these provisions can be applied in practice for bacteriophage-based products for phage therapy.

1. Introduction (background)

Bacteriophages are present in the whole biosphere: in waters, soils, plants, food, on the skin, mucous membranes and in the digestive tract. They are present in large quantities wherever bacteria can be found.

The vast majority (96%) of known bacteriophages belong to the Caudovirales order (tailed viruses) and are most often non-enveloped viruses with double stranded DNA. Bacteriophages of current interest in phage therapy predominantly belong to three morphotypes: myo-, podo- and siphoviruses (Monribot A et al. 2021), although other morphotypes could be of interest in the future.

Bacteriophages as therapy have been used since the beginning of the past century, both in humans and animals. Although their use in humans was abandoned in Western countries in favour of antibiotic therapies, phage therapy continued to be practiced in Eastern Europe (Chanishvili N, 2016). In some countries (e.g. Georgia), the use of phage therapy in humans has never stopped and it is still applied, primarily against antimicrobial resistant (AMR) pathogenic bacteria. Within veterinary medicine, phage therapy has been used in chickens, cattle and pigs (Loponte R et al. 2021).

Currently, bacteriophages are reappearing in the therapeutic arsenal as a potential alternative to antibiotic therapy (or to complement the latter) as a salvage therapy in therapeutic dead end, due to increasing antibiotic resistance.

Some studies show phage-antibiotic synergies, often characterised by a reduced emergence of antibiotic and/or phage resistance in bacteria (Chaudhry WN et al. 2017).

Pursuant to Regulation (EU) 2019/6 veterinary medicines composed of bacteriophages are considered novel therapies and, as such, the following sections of Annex II of Regulation (EU) 2019/6 apply:

- Requirements for biological veterinary medicinal products other than immunological veterinary medicinal products (section IIIa).

- General requirements for novel therapies veterinary medicinal products (sections V.1.1. to through V.1.4.).
- Veterinary medicinal product specifically designed for phage therapy (sections V.1.5.4.).

It is recognised in Annex II of Regulation (EU) 2019/6 that due to the specific nature of bacteriophage products, adaptation of the general rules may be acceptable, and the regulatory framework is expected to be flexible because:

- Phage therapy VMPs may consist of monophage or multiphage preparations whose composition may require to be regularly updated/reconditioned, due to the narrow bacterial host ranges, the development of resistance against the bacteriophages, and the immune response of the treated animal (against both, the bacteriophages and the bacteria).
- Technical/scientific requirements for novel therapy products should be proportionate to the risks associated with their intended uses, that are dependent on: The target animal species (i.e. pets or livestock animals), the indication (prophylaxis, treatment and/or metaphylaxis), the intended treatment (i.e. individual and/or customised, first line or last resort treatments), the route of administration, dosage form and concomitant use with other medicines e.g. antibiotics.

This current guideline addresses, among other aspects, the regulatory, technical and scientific basis applicable to the quality, safety and efficacy of phage therapy veterinary medicinal products where a variable composition of the final product is expected. The authorisation of phage products with flexible qualitative and quantitative composition is expected to require suitable scientific knowledge, risk based approaches, appropriate quality risk management and pharmaceutical quality systems.

Due to the biological complexity and nascent nature of veterinary medicinal products specifically designed for phage therapy (none have yet been centrally authorised in the EU), the advice given in this document is general and does not enter into details. Developers are encouraged to seek early advice at the national or European level to guide product development.

Where animal studies are needed, they should be selected and conducted in strict adherence to the 3R principles.

2. Scope

The guideline specifically concerns bacteriophage products for prophylactic, metaphylactic and/or therapeutic treatment of one or more specific bacterial infection(s) or infectious disease(s) caused by bacteria, or dysbiotic conditions, where efficacy of treatment is linked to the lytic activity of bacteriophages that confers bactericidal activity with specificity for the bacterial strains concerned.

The lytic bacteriophages included in VMPs may be natural, or optimised, for e.g. enhanced potency or broader bacterial host range by classical microbiological *in vitro* selection methods, or genetic engineering (molecular biology) methods.

Other uses of bacteriophages in veterinary medicine, e.g. use of bacteriophage particles as display platforms for vaccines or use of temperate/integrating bacteriophages to modulate bacterial phenotypes, are outside the scope of this guideline.

Likewise, bacteriophage-derived products (e.g. lysins or other enzymes), or magistral formulae composed of bacteriophage(s) are outside the scope of this guideline.

3. Legal basis

This guideline should be read in conjunction with Regulation (EU) 2019/6, Regulation (EU) 2021/805 amending Annex II to Regulation (EU) 2019/6, and supportive texts listed in References.

4. Initial marketing authorisation application requirements for phage therapy VMPs

In general, the requirements stated in Annex II of Regulation (EU) 2019/6 should be followed. The particularities applicable to an initial marketing authorisation for phage therapy are described in this Section 4.

One of the innovations in Annex II of Regulation (EU) 2019/6 is the application of risk-based principles for NT VMPs. Thus, if scientifically justified and based on the required specific product properties and appropriate identification and assessment of risks to target animals, users, consumers and the environment, requirements in Annex II of Regulation (EU) 2019/6 may be adapted.

Due to the complexity of NT VMPs such as phage therapy products, apart from the adapted requirements listed in this guideline, there may be further instances where requirements may be adapted but this cannot be pre-specified and it must be evaluated on a case-by-case basis, based on specific characteristics of the product.

The principles in the proactive risk-based approach used to determine whether adaptations of Annex II of Regulation (EU) 2019/6 requirements are possible are described in section V.1.1.4. through V.1.1.6 of Annex II of Regulation (EU) 2019/6.

When proposed adaptations of Annex II of Regulation (EU) 2019/6 requirements cause risks to quality, safety, efficacy or traceability of the product, control/mitigation measures should be established to ensure that such risks remain at acceptable levels.

This risk management approach is clarified and detailed in this guideline. To facilitate direct, practical use of the guidance provided when drafting dossiers for marketing authorisation applications, this guideline uses the same structure (headings) of Annex II of Regulation (EU) 2019/6. Only sections for which advice is given in this guideline are included below.

Finally, Section 5 describes the requirements applicable to variations of an initial marketing authorisation (for example, addition of new bacteriophage strains not previously authorised in the marketing authorisation).

4.1. Administrative information (Part 1)

Product information

The qualitative and quantitative composition of the product should include all monophages that may be present in the product. The host bacterial species should also be indicated.
The monophages included in the final product are to be stated on the label.

The indication is expected, in general, to be for prophylactic, metaphylactic and/or therapeutic use, of one or more specific infection(s) or infectious disease(s) caused by specific bacteria or dysbiotic conditions.

While phage therapy may be intended as an alternative to antibiotics, in some cases, concomitant use of phage products and antibiotics may be relevant in the field. If intended, this must be supported with appropriate data and the conditions defined in the dossier and in the product information. If no data

are presented, a corresponding text should be included in the product information to prevent the concomitant use of phage products and antibiotics.
For multiphage products, if individual monophage components intended for concomitant administration are provided in different primary containers, information on such use should be provided. If mixing of the monophage components by the end user is required, information on mixing prior to administration and in-use shelf life after mixing should be provided.

4.2. Quality documentation (Section IIIa.2 Part 2)

The principles in the proactive risk-based approach essentially corresponds to the quality risk management principles laid out especially in ICH Q8, Q9 and Q11 guidelines. These guidelines are not directly applicable to VMPs but they could be used for additional guidance.

Briefly, for phage products, evaluation of the quality risks associated with any proposed adaptations of Annex II of Regulation (EU) 2019/6 requirements should take into consideration the following factors:

- The variable composition of the final product.
- The intended quality, safety and efficacy characteristics of the product, considering e.g., the indications, epidemiological situation in the field (development of bacterial resistance against phages or changes in the epidemiology of bacterial pathogen(s) in the field), route of administration, dosage form, bioavailability, strength, concomitant use with other products and stability, etc (see under quality target product profile QTPP in Definitions).
- The critical quality attributes (CQA) of active substances and final product (see under CQA and specifications in Definitions).
- The characteristics of the manufacturing process(es) (see under critical process parameters in Definitions).
- The defined and controlled quality of the starting materials, including characterisation and specification of phage and bacteria banks and the characterisation of the active substances.
- The stability of the active substance and the finished product.
- The accumulated commercial manufacturing knowledge and post-authorisation pharmacovigilance database for this or similar products.
- Current scientific knowledge.

To ensure a consistent quality of phage products, a comprehensive control strategy is necessary considering the abovementioned aspects.

Manufacturers should document the ability of their quality systems to ensure that throughout the entire product lifecycle, the proposed control/mitigation measures are reviewed, updated and corrected on a continuous basis, to remain fit-for-purpose.

IIIa.2A1. Qualitative and quantitative composition

According to Annex II of Regulation (EU) 2019/6, a flexible composition of phage products is expected to be the usual situation; in this section, this principle is detailed and clarified.

<u>Phage products with fixed qualitative and quantitative composition:</u>

Depending on product characteristics, declaration of a fixed qualitative and quantitative composition may remain relevant for certain phage products.

Briefly, this comprises listing of (i) active substance(s), (ii) excipients, (iii) accompanying reconstitution solvent(s), (iv) container(s) and container closure(s) for finished product and any accompanying solvent(s), and (v) devices required for delivery.

Phage products with flexible qualitative and quantitative composition:

Where a flexible composition of the phage product is sought applicants should provide the following information for the parental phage product (see Definitions):

1. Qualitative composition: Description of all different bacteriophage strains which may be included in the composition of the final product, including phages not used in key safety and efficacy studies during product development, but where existing knowledge is sufficiently predictive to justify their registration as part of the flexible product composition.
2. Range for the quantitative composition:
    a. Minimum and maximum number of monophage components in the final product.
        (i) Justification should be provided for the inclusion of each monophage component.
        (ii) For each monophage as well as the phage product as a whole, minimum and maximum levels of bacteriophage per unit or dose should be defined.

The customization of phage products based on monophage components included in the approved dossier for the parental product means that manufacturers may pick monophage components from those included in the approved dossier for the parental product, to match the geographical distribution and phage resistance patterns of targeted bacterial pathogens in different countries, or even on a case-by-case basis for individual bacterial disease outbreaks. Thus, different compositions of the parental product can be marketed at the same time or at different times in the same/different country(ies) to address different epidemiological needs (see under product updates in Definitions).

Such customisation of phage products does not require variation applications.

IIIa.2A2. Product development

The justification of product composition and manufacturing process robustness may be particularly complex for phage products. Special attention to these issues during development of the parental product may leverage maximal flexibility for any product, and ease product updates.

These issues are therefore detailed and clarified below.

Justification of the composition:

Regardless of whether the final product contains one or more monophage components, justification for the choice of the monophage components (phage strains) should be provided.

When flexibility in quantitative and qualitative composition is proposed, it should not carry with it unacceptable risks for quality, safety, efficacy and traceability of the phage composition of the final product.

See annex I of this guideline for further details.

Robustness of manufacturing processes and associated analytic technologies towards changes in the identity and quantity of monophage components:

The documentation required to support a flexible composition is minimised if the anticipated changes to the product composition do not cause substantial changes to manufacturing processes, and

associated analytical technologies (e.g. assays used for process quality control, batch release and stability studies).

At the same time, it is acknowledged that even in the simplest cases of flexible composition, e.g. exchange of a monophage component by another, the upstream part of the manufacturing process (amplification of phage strains in bacterial hosts) may have to be changed, for example, if a new bacterial host may be needed.

Yet, it is expected that it may be technically possible to ensure that the downstream part of manufacturing processes (purification and formulation) is relatively robust towards upstream changes in phage strains and bacterial hosts.

Also, for multiphage products, it may be possible to design the blending and final formulation steps so that changes in the manufacture of monophage components are less likely to have adverse effects on the overall quality of the final product.

Thus, the scientific understanding of the composition and manufacturing process(es) for the parental product, as well as a proactive, rational design of the process(es) to ensure robustness towards changes which may be required, are expected in order to allow a degree of regulatory flexibility.

Knowledge-based and documented understanding of the relationship between manufacturing process CPP and CQA for phage products is expected to increase the flexibility when updates need to be made to multiphage products, to overcome development of resistance (such as for example changes in the total number of monophage components or exchanges of one monophage component for another).

This may optionally involve enhanced approaches to product development, to gain a deeper understanding of the relationship between critical parameters in the manufacturing process(es) and product quality, thereby allowing flexibility in operating conditions for manufacturing processes without adverse effects on product quality, safety and efficacy (see under traditional and enhanced manufacturing process development in Definitions section).

IIIa.2A3. Characterisation

Based on current experience with bacteriophage products, the following set of requirements is expected to be sufficient for characterisation of monophage preparations in most cases (see definition of monophage preparation in Definitions):

- Genetic characterisation (see Annex II to this guideline).
- Phenotypic characterisation (using appropriate *in vitro* microbiology methods, as scientifically justified).
- Host range (i.e., the ability of a bacteriophage to form plaques on a set of bacterial pathogens).
- Absence of lysogenic activity.
- Potency for relevant bacterial pathogens.

Determination of some of the abovementioned characteristics may be omitted, if scientifically justified.

On the other hand, depending on intended product use (see under quality target product profile in Definitions and Annex I), it may be appropriate to determine other phenotypic phage characteristics, for example:

- Activity on bacterial biofilms.
- Antagonism/synergy with antibiotics, if relevant.

For genetically engineered bacteriophages and chemically modified bacteriophages, the modifications must be described and their effects characterised.

Determination of phage morphology is generally recommended (e.g. by electron microscopy), and considered especially relevant where bioinformatic analysis is not sufficient for phage classification.

The bacterial hosts used to amplify bacteriophages should be free of nucleic acid sequences coding for (i) toxins, (ii) elements conferring antibiotic resistance, (iii) prophages, and (iv) any other genetic elements considered to be predictive for detrimental effects on safety or efficacy of the product. If freedom from these elements is not possible, it should be justified that this has no negative effects on the safety and efficacy of the bacteriophage product. An adequate threshold of the maximal amount of these elements in the host bacteria should be set.

Process- and product-related impurities shall be addressed, as stated in Annex II of Regulation (EU) 2019/6.

IIIa.2B. Production and control of starting materials

Bacteriophages may be isolated from any relevant source (environmental, clinical or other relevant sources). The used bacteriophages must be strictly lytic, and the source must be adequately described to the extent possible. The origins of bacteriophages and matched bacterial hosts should be described, including, as far as possible, isolation procedures and subsequent manipulations the materials may have undergone.

Monophage preparations should be manufactured from characterised and quality-controlled seed lots of phages. Similarly, characterised and quality-controlled seed lots of matched bacterial host are used. For bacteriophages as well as matched bacterial hosts, the seed lot systems should ensure the genetic and phenotypic stability together with the viability of the material, and the maximal allowable number of passages of seed lots must be established.

Antibiotics are not expected to be used during production, and toxic chemicals traditionally used for phage purification should be avoided. If this is not possible, these substances should be quantified and controlled in the final product.

IIIa.2C. Control tests on the finished product

An illustrative guidance example for a quality control test panel on finished phage product is given in the table below. For all quality control tests, *in vitro* methods are expected to be sufficient.

Table: Illustrative guidance example for a minimal test panel on finished phage product

Quality control test on the finished product	Comments
Identity of individual bacteriophage active substance(s)	Can be established using for example nucleic acid amplification technologies. For multiphage products, identity of each monophage component should be documented. Products should not contain bacteriophages other than those intentionally added as active substances. If contaminating phages cannot be avoided in products (e.g. prophages derived

Quality control test on the finished product	Comments
	from bacterial host cells), maximum acceptable levels should be set.
Potency of individual bacteriophage active substance(s)	Can be documented as viable phage per unit or dose, using for example titration on appropriate bacterial host cells (plaque-forming units per mL), or other potency assays, as scientifically justified. If possible, evidence that the selected potency assays correlate with clinical efficacy should be provided. For multiphage products, potency should be determined for each monophage component. This is expected to be technically possible in the majority of cases. For monophage components where specific bacterial host cells are not available, it may be possible to adapt alternative approaches, if scientifically justified.
Pyrogen content	Content of gram-negative endotoxins and/or gram-positive pyrogens, depending on bacterial host(s) used for phage propagation. *In vivo* pyrogen tests should be avoided.
Total protein concentration	No comments.
Residual/free nucleic acid content (DNA and RNA, if relevant)	Residual free nucleic acid content (unpackaged DNA/RNA from viral origin and bacterial DNA/RNA residues).
Host cell DNA	DNA derived from bacterial host cells, excluding phage DNA. May be omitted if manufacturing process(es) have been validated as providing sufficient clearance.
Other impurities	Chemicals used during manufacture, etc.
General tests	pH, osmolality etc, as relevant.
Water content	If lyophilised.
Sterility	No comments.

It is recognised that in cases where the robustness of manufacturing process(es) towards anticipated post-marketing product updates has been explored already during the development of the parental product by means of enhanced process development approaches (see Definitions), this may justify the use of wider and more flexible specification ranges than traditionally used (see EMA/CHMP/CVMP/QWP/354895/2017).

IIIa.2D. Batch-to-batch consistency

It is generally recommended that batch-to-batch consistency is documented based on three commercial-scale batches.

For multiphage products where product compositions are expected to be flexible, it is acceptable to document manufacturing consistency using batches with the maximal number of monophage components.

IIIa.2E. Stability tests

It is generally recommended that stability of the product is documented based on three commercial-scale batches, using batches with the maximum number of monophage components.

If scientifically justified, stability data may be partly provided post-authorisation. The shortest shelf life for the currently authorised strains is applied in the meantime.

4.3. Safety documentation (Safety and residue tests; Section IIIa.3 Part 3)

Requirements for a marketing authorisation application are established in Regulation (EU) 2019/6 and are specified in Annex II of Regulation (EU) 2019/6, Section IIIa for biological VMPs other than immunological VMPs. The documentation accompanying the application for a marketing authorisation shall be presented in accordance with Annex II of Regulation (EU) 2019/6.

In line with the requirements for NT VMPs detailed in Annex II of Regulation (EU) 2019/6, the requirements for safety may be adapted if scientifically justified and based on the required specific product properties. Specific safety concerns may be related to natural, engineered or synthetic type bacteriophages. These risks should be pro-actively identified applying a risk profiling methodology and taking into account the quality risk management approaches detailed in the section on quality documentation. If safety risks cannot be excluded, it may be possible to reduce such risks to acceptable levels by instating control/mitigation measures. As a general principle, the CVMP and VICH guidelines concerning safety are applicable.

To obtain a marketing authorisation for bacteriophage VMPs in food-producing species, the MRL status shall be considered in accordance with Regulation (EC) No 470/2009 in advance, for all pharmacologically active substances for the concerned food-producing animal species and relevant tissues or products (e.g., milk, eggs, honey). These include the active substance(s)[1] and excipient(s)[2]. Additionally, a withdrawal period should be established (even when the withdrawal period is zero). The European Medicines Agency (EMA) should be consulted for the need for an MRL evaluation.

Finally, it should be mentioned that the requirements presented in this guideline only address the active substance bacteriophages. Should a product contain excipients or active substances other than bacteriophages, their safety has to be shown according to requirements presented in Annex II of Regulation (EU) 2019/6.

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[1] The establishment of maximal residue limits (MRL), as set out in Commission regulation (EU) 470/2009 for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, is not addressed in this guideline.
Two positive outcomes of the MRL assessment will be anticipated:
1) The inclusion of the substance(s) in the list of (chemical-unlike) biological substances considered as not requiring an MRL evaluation following Annex I of Regulation (EU) No. 2018/782, with regard to residues of veterinary medicinal products in foodstuffs of animal origin.
2) The inclusion of the substance(s) in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

[2] Excipients can either be included in Regulation (EU) No 37/2010 or in the list of "Substances considered as not falling within the scope of Regulation (EC) No 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin" (EMA/CVMP/519714/2009)

IIIa.3A. Safety tests

Safety tests should address target animal safety, user safety and environmental risk.

For details on target animal safety studies, please see the section on tolerance in the target animal species in section 4.4.1 of this guideline.

Requirements from Annex II of Regulation (EU) 2019/6, chapter IIIa are applicable to a representative monophage or multiphage preparation for which the full safety package should be provided, in principle.

Studies (including toxicology and special studies) could be carried out with representative monophage or multiphage preparations representing worst case scenarios in terms of safety concerns (for example, containing the maximum amount of protein and/or titre in any phage combination). The final product could be used.

Extrapolation between comparable strains of bacteriophages, or between target animal species, or different routes of administration may be possible based on representative/validated *in vitro* or *in vivo* parameters or in well justified cases, based on scientific justification for respective safety studies.

IIIa.3A1. Precise identification of the product and of its active substance(s)

Requirements detailed for *biological veterinary medicinal products other than immunological veterinary medicinal products* in Annex II of Regulation (EU) 2019/6 for this point might not be adequate for bacteriophage VMPs.

The identification of the active substance(s) should be tailored for biological (viruses) entities and based on the requirements for identification used in the quality part.

The formulation of the product should be in line with section 2.A.1. (Qualitative and quantitative composition) and it is recommended to be included in this section. Cross reference should be avoided.

IIIa.3A2. Pharmacology

It is necessary to provide pharmacological data for the bacteriophage VMP to characterize the mechanism of action and pharmacodynamic findings relevant for the safety evaluation. However, absence of studies in laboratory animals could be justified by reference to existing data and data from target animal species studies.

Since bacteriophages are actively replicated only when they encounter their target bacteria, the pharmacological data needed for the safety could be drawn from studies in the target animal species submitted in part 4 of the dossier, provided that these have been adequately designed to address this evaluation. Whilst conventional absorption, distribution, metabolism and excretion (ADME) studies may not be appropriate/possible, the applicant should provide information concerning the absorption from the site of administration and dissemination to other anatomical locations, together with information concerning expected degradation pathways, and should take into account situations when the product will be used in target animals without active bacterial infection and when the product will be used for treatment in target animals with presence of the host bacteria of the bacteriophage strain(s). The data should be derived from appropriate sources (e.g., dedicated PK/PD studies, *in vitro* models, pilot efficacy studies, or a combination of these), as scientifically justified by product characteristics.

Due to the novel and complex nature of phage products, it is not possible to provide guidance beyond the general principles outlined above.

IIIa.3A3. Toxicology

Bacteriophage products within the scope of this guideline are biologicals which do not propagate in eukaryotic cells and are not expected to exert direct pharmacologic effects in target animals (as opposed to for example cytokines, hormones, autoantibodies, etc), i.e., there is no mechanism-based concern for toxicity in target animals or humans.

Furthermore, target animals as well as humans are naturally exposed to high amounts of bacteriophages produced locally in the gastrointestinal tract, as well as from external sources (water, food, environment) throughout their lifespan.

However, due to the potential presence of microbiological contamination in bacteriophage products, endotoxins or exotoxins are considered a safety concern. Therefore, the control of these aspects is an essential element of the manufacturing process.

Thus, considering the reasons above and in agreement with 3R principles, and in order to provide appropriate flexibility for bacteriophage products as foreseen in Annex II of Regulation (EU) 2019/6, a satisfactorily controlled manufacturing process, target animal safety studies (preclinical or clinical) and/or literature data according to the current state of science are expected to be sufficient to address single-dose toxicity, repeated dose toxicity, and effects on reproduction and developmental toxicity. In cases where reference to the aforementioned studies or literature data is not directly relevant for the specific phages or if a specific safety concern is identified, supplementary studies should be submitted.

It is not expected that the bacteriophages within the scope of this guideline interact directly with DNA or other chromosomal material. Furthermore, it is recognised that for biologicals, the range and type of genotoxicity studies routinely conducted for pharmaceuticals are not applicable (see ICH S6 Rev1 which applies to human medicines but gives useful guidance for VMPs). Thus, the standard battery of genotoxicity tests can be omitted.

An influence on eukaryotic cells is considered unlikely, as bacteriophages are not expected to interact with eukaryotic cells. Therefore, carcinogenicity studies can most likely be omitted.

IIIa.3A4. Other requirements

IIIa.3A4.1. Special studies (immunogenicity, immunotoxicity, neurotoxicity, endocrine dysfunction)

A specific safety or tolerance concern that needs to be considered is potential immunogenicity (see ICH S6 Rev.1 for more information) and immunotoxicity of bacteriophage products. It is envisaged that data from target animal studies, combined with the proposed posology and existing knowledge on immunogenicity and immunotoxicity of phages, could be used to assess this risk. Please refer to section IIIa.4A1 Pharmacology.

For bacteriophage VMPs for which skin and eye exposure may occur the general requirements in Annex II of Regulation (EU) 2019/6 apply.

IIIa.3A4.2. Development of resistance and related risk in humans

Bacteriophages are a normal component of mammalian environment including food and gut microbiota. Therefore, it is unlikely that there is a risk for humans and hence, specific studies might be omitted if appropriately justified.

However, over time, bacteria most likely develop resistance to bacteriophages. The applicant should reflect upon the risk of developing/spreading resistance in the environment and the related risks to humans associated with the use of the product.

IIIa.3A5. User safety

Currently, no specific guidance on user safety is available for biological products other than immunologicals. Nevertheless, the general principles on user safety assessment lined out in GL EMA/CVMP/543/03-Rev.1 (hazard identification and characterisation, exposure assessment and risk assessment) should apply to phage products in order to derive appropriate warnings or other risk management measures when required.

The information obtained from the assessment of hazard identification and exposure will be considered for the risk characterisation. In cases where no information on dose response relationship is available, a qualitative risk characterisation might be sufficient.

IIIa.3A6. Environmental risk assessment

IIIa.3A6.1. Environmental risk assessment of veterinary medicinal products not containing or consisting of genetically modified organisms

Bacteriophages used as VMPs enter the environment after application either by direct excretion into the environment or by application of manure from treated animals to agricultural land. Only limited research has been conducted on anthropogenically released bacteriophages that are non-native to their receiving environments. Therefore, there are uncertainties about the fate and effects of such bacteriophages in the environment. So far, studies indicate that changes in the microbial community composition with effects on the natural ecosystem function are to be expected (Meaden S et al. 2013; Kowalska JD et al. 2020) and have already been reported in laboratory experiments (Braga LP et al. 2020). The applicant should reflect upon the environmental impact to soil bacteria and soil function associated with the use of the product. The performance of studies in accordance with or based on OECD test guidelines might be required, such as OECD GL 216. Genetically modified bacteriophages need to be additionally assessed like genetically modified organisms according to IIIa.3A6.2 of the Commission Delegated Regulation (EU) 2021/805.

4.4. Efficacy documentation (Pre-clinical studies and clinical trial(s); Section IIIa.4 Part 4)

The general requirements for a marketing authorisation application are laid down in Regulation (EU) 2019/6, and are specified in Annex II of Regulation (EU) 2019/6, Section IIIa for biological VMPs other than immunological VMPs, and the documentation accompanying the application shall be presented in accordance with the general principles of this Annex.

In addition, as a general principle, the CVMP and VICH guidelines concerning efficacy are also applicable to bacteriophage VMPs.

When any proposed adaptations of Annex II of Regulation (EU) 2019/6 requirements could present risks to the expected efficacy of the products and the safety in the target animal, control/mitigation measures should be proposed to ensure that such risks remain at acceptable levels.

A full efficacy package should be provided, as specified below, for a representative monophage or multiphage preparation. Extrapolation of efficacy for alternative combinations to the representative one

for which efficacy is demonstrated may be based on validated *in vitro* or *in vivo* data or, in well justified cases, based on a scientific justification.

The efficacy and safety of the VMP designed for phage therapy should normally be demonstrated by studies in the target animal species under laboratory conditions (pre-clinical studies) and supported by field conditions.

4.4.1. IIIa.4A. Pre-clinical studies

Pre-clinical studies aim to document the safety and efficacy of bacteriophage products in the target animal species. In principle, studies in target animal species are required for pharmacokinetics, target animal safety (TAS) studies, dose determination (DD) studies, and dose confirmation (DC) studies. Their omission or replacement by studies conducted in non-target animal species or by *in vitro* data may be possible when sufficiently scientifically justified.

Studies in non-target animal species and validated *in vitro* models may be used for e.g. the demonstration of the mode and mechanisms of action.
These pre-clinical studies support the use of the product under the recommended conditions (recommended route(s) of administration, dose, dosing interval, resistance), considering the epidemiology of the targeted bacterial pathogen(s).

If data from *in vitro* models are used to support efficacy, it should be demonstrated in pre-clinical study(ies) in the target animal species or in clinical trials that a sufficient correlation exists between the *in vitro* model readout and the claimed effect in the target animal species. However, it should be noted that as a general requirement, *in vivo* proof of principle in the target animal species would be necessary, in particular for the representative monophage or multiphage preparation.

In vitro data may be considered of more relevance to support the efficacy of alternative combinations to the representative monophage or multiphage preparation, based on scientifically valid extrapolation and initial demonstration of effectiveness of the primary monophage or multiphage preparation.

IIIa.4A1. Pharmacology

Mode and mechanism of action

The mode and mechanism of action of the bacteriophage strain on the target bacteria should be described. Bacteriophages should be well characterised: It has to be demonstrated that they are lytic and do not contain genetic determinants that confer lysogeny to the phage, or virulence or antibiotic resistance to bacteria. Please see details in product characterisation from the quality documentation part.

Range of host bacteria and in vitro susceptibility test

The host range of each bacteriophage strain included in a product should be defined by the activity against the target pathogen(s), in addition to representative non-targeted bacteria as appropriate. Phage host range should support the claims that are made.

In vitro susceptibility tests could be used to test bacteriophage activity against a range of host bacteria (e.g., bacterial growth inhibition in 96-microwell plates and formation of plaques), considering the concentration of bacteria and the multiplicity of infection (MOI).

The isolates of the target bacteria to be tested should be justified by the applicant as being clinically representative of the strains found in the field. Isolates from samples collected during clinical trials or

strains of bacterial pathogens used in *in vitro* and *in vivo* models should be characterised and these details should be included in the marketing authorisation application.

Posology

It is suggested to demonstrate, that the recommended dose and dosage and the administration route of the representative monophage or multiphage preparation results in a productive bacteriophage infection at the site of bacterial infection in the target animal species e.g. by means of PK/PD models. A representative *in vivo* model of infection might also be useful. If sufficiently justified, providing such pre-clinical data can be very valuable to limit or avoid a number of unsuccessful clinical trials.

The immune response to the effect of bacteriophage treatment in target bacteria

The immune response in the target animal species to the bacteriophage effect in the host bacteria should be addressed. Relevant data from the literature, if available, may be considered sufficient to evaluate any potential adverse effects on immunological functions.

In some circumstances (for example, if repeated treatment is recommended), it may be necessary to assess immune response following treatment against bacteriophages, to document that the responses do not negatively impact the therapeutic effect.

Bacteriophages can kill bacterial cells within minutes. However, studies suggest that bacterial lysis caused by bacteriophages is not expected to be associated with higher endotoxin releases or inflammatory responses as compared to treatment with antibiotic VMPs (Dufour N et al., 2019). Thus, it is not expected that this issue needs to be addressed by the applicant, as it is considered only a theoretical concern.

Comparability data to support a flexible composition of monophage or multiphage preparations

For alternative bacteriophage combinations to the parental one, demonstration of efficacy may be possible based on representative/validated *in vitro* or *in vivo* data or parameters or based on a scientific justification.

Data or robust scientific justification showing comparable biodistribution, immune clearance and MOI support should be provided to demonstrate comparability between representative and alternative preparations.

IIIa.4A2. Development of phage resistance and related risk in animals

Where possible, information on the coevolution of bacteriophages and host bacteria, the risk of appearance and dissemination of resistant bacteria, the resistance mechanisms and the molecular genetic basis of resistance should be provided. This information may come from literature, peer-reviewed journals or proprietary studies.

Measures to limit the development of resistance in bacteria of clinical relevance for the intended use of the veterinary medicinal product shall be proposed by the applicant.

IIIa.4A3. Dose determination and confirmation studies

The minimum effective dose, the proposed dosing interval, the duration of treatment and, where relevant, any proposed repeated treatment should be provided for the representative monophage or multiphage preparation. This should be documented for each target bacterium in each target animal species and for the recommended route of administration. These studies could be performed using experimental models of infection in the target animal.

A justification based on literature data may be considered acceptable provided that the posology is supported in a preclinical or clinical study in the target animal species or by a clinical trial.

The choice of the representative monophage or multiphage preparation should be justified considering the indication of the product.

These studies may also serve to evaluate any potential impact on immunological functions depending on the range of follow-up parameters included within the studies, as discussed under section IIIa.4A1.

IIIa.4A4. Tolerance in the target animal species

The implementation of a Target Animal Safety (TAS) study is considered necessary to gain information to appropriately characterise the safety profile of the product before introducing it in the field.

The TAS study should be designed on the basis of the route of administration and dosage, including repeated administration and treatment duration, intended for use of the product in its final formulation.

The representative monophage or multiphage preparation should be composed to represent the worst-case scenario in terms of safety. The 1X dose is acceptable and overdose studies are not expected to be necessary.

Normally, post-mortem examinations could be omitted. In case unexpected or severe adverse events occur, these are to be clarified by other means, e.g. specific clinical or laboratory examinations.

Generally, healthy animals shall be used in TAS studies; however, bacteriophage products within the scope of this guideline are biologicals which do not propagate in eukaryotic cells, and there is no mechanism-based concern for toxicity in target animals.

Additionally, bacteriophages can increase in number in the presence of their bacterial host, and therefore safety data derived from use of bacteriophages in diseased animals is generally expected to be more informative than data from healthy animals.

However, when specific risks are identified, target animal safety studies in healthy animals could be required (for example, when the targeted bacteria are also commensal bacteria).

4.4.2. IIIa.4B Clinical trials

IIIa.4B1. General principles

Clinical trials should examine, under field conditions, the target animal safety and efficacy of the veterinary medicinal product.

Clinical trials should be conducted in accordance with the principles of good clinical practice (GCP) (VICH GL9).

Clinical trials should be performed with the final formulation including a representative preparation and the study endpoints should support each proposed indication and targeted bacteria in each target animal species claimed. The diagnostic methods of the disease and clinical condition of the animals should be appropriately and fully described. Whenever possible, established methods for diagnosis should be applied. Strictly defined clinical and microbiological inclusion and exclusion criteria as appropriate for the claimed indication/s and the intended target population should be incorporated. When the aim is to confirm efficacy against one or several specified bacteria, isolation of the target pathogen(s) from the animals or a representative sample is required through microbiological sampling performed at the time of inclusion.

For collected bacterial isolates, susceptibility to the test product should be tested *in vitro*.

Endpoints (i.e. clinical cure rate and/or microbiological cure rate) and timing of efficacy assessment should be established and adequately justified taking into consideration the characteristics of the infection/disease and the nature of the intended claims. Principally, there are three different kinds of claims: Treatment, metaphylaxis and prophylaxis of specific infectious diseases or infections caused by one or several specific bacterial species.

Appropriate statistical methods should be used (see CVMP guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals) (EMA/CVMP/EWP/81976/2010-Rev.1*).

However, other designs for the clinical trials could be also accepted, if appropriately justified.

Special considerations for metaphylaxis claims

The administration of a phage therapy with the aim of treating clinically diseased animals and controlling the spread of the disease to clinically healthy animals, that are likely to be in the incubation phase due to close contact with diseased animals or exposed to the same external factor, may be justified from an epidemiological point of view. In these cases, the presence of the disease in the group should always be confirmed before starting the phage therapy.

A metaphylaxis claim is considered to be acceptable in conjunction with a treatment claim. The need for metaphylaxis should be discussed and the threshold for the initiation of metaphylactic treatment (e.g. the proportion of clinically diseased animals at a certain time point within a group and the severity of clinical signs) should be justified on epidemiological and clinical grounds. In the justification reference may be made to published literature.

Special considerations for prophylaxis claims

Prophylaxis claims refer to the administration of a veterinary medicinal product to healthy animals before clinical signs appear. The need for prophylaxis must be fully justified for each target species and indication.

4.5. Concomitant use of bacteriophages with conventional antibiotics

The potential use of bacteriophages with antibiotics should be addressed. Any specific claims that are proposed for inclusion in the product information for associated use of a bacteriophage with antibiotics are required to be supported by data. Taking into account the need of the development of alternatives to antibiotics together with the need to preserve the efficacy of the antibiotics available, the use of bacteriophages together with antibiotics could be considered if a significant therapeutic benefit is demonstrated and the risks of development of antibiotic/phage resistance are addressed appropriately. This concurrent use should be adequately justified for each claim and supported by bibliographic data and data from clinical studies.

5. Post marketing authorisation changes

It is recognised in Annex II of Regulation (EU) 2019/6 that phage products will likely need to be updated on a regular basis due to development of resistance or changes in the epidemiology of bacterial pathogen(s) in the field.

Thus, it may be necessary to use trained versions of monophage components for the parental product, or new monophage components (see under trained phages and parental and updated products in the Definitions section).

Such updates will occur to the already authorised product.

The assessment and authorisation of updated phage products can be streamlined based on some of the studies conducted with the parental product, combined with other relevant data which may have been generated during the commercial lifespan of the parental product (e.g. improved understanding of commercial-scale manufacturing processes, pharmacovigilance data, and supplementary scientific knowledge, to enhance the impact on the quality, safety and efficacy).

The development and consequent authorisation of parental products may proceed through different scientific and regulatory paths:

(i) traditional or enhanced product development approaches or a combination of these (see section on product development, and Definitions section),

(ii) fixed or flexible compositions may be specified (see section of qualitative and quantitative composition),

(iii) post-approval change management protocols may or may not have been filed (see EMA/CHMP/CVMP/QWP/586330/2010).

It is up to applicant´s responsibility to pursue the development paths considered optimal for their specific parental products.

It should however be stated that the scientific standards for data supporting product updates are the same: applicants should demonstrate that the proposed product update will have the intended effect (restored efficacy) without negatively impacting on the quality, safety, efficacy and traceability of the product.

Due to the abovementioned different development and regulatory paths open to applicants, and the complexity of phage products, it is not possible to specify exact data requirements for product updates. Therefore, it is recommended to consult with the Agency regarding specific requirements on a case-by-case basis.

Early consideration and description of anticipated product characteristics may guide not only an optimal development path of the parental phage product, but also provide maximal flexibility for any post-authorisation product updates which may be required to address development of resistance or changes in the epidemiology of bacterial pathogen(s) in the field.

Below, the regulatory pathway to be followed on such post-authorisation updates are addressed, giving examples to illustrate the applicable requirements.

Product update by variation requiring assessment (VRA):

Updates may impact the quality and safety of the product and any such changes need to be addressed. Furthermore, the restored activity against the bacterial pathogen(s) which developed resistance against the parental product should be documented; all these issues requiring scientific assessment by the Agency (Regulation (EU) 2019/6, article 60, and Regulation (EU) 2021/17).

Therefore, for such phage product updates, it is anticipated that (i) applications for changes to the terms of the marketing authorisation will be submitted to the Agency (a VRA), and (ii) these variation applications will require approval before implementation of the proposed product updates.

Post-approval change management protocols

Unnecessary updates to phage products should be avoided.

Therefore, it is recommended that, where possible, plans and protocols for anticipated product updates are formulated, and formalised in post-approval change management protocols (see EMA/CHMP/CVMP/QWP/586330/2010).

Post-approval change management protocols may be included as part of the application for the parental product or be submitted after authorisation of the parental product. In the former case, the plans are based on data generated during development of the parental product; in the latter case, the plans may also benefit from scientific knowledge and understanding gained from pharmacovigilance activities and post-authorisation studies for the parental product.

The generally expected content in such post-approval change management protocols is outlined in EMA/CHMP/CVMP/QWP/586330/2010. The following may also be considered (illustrative guidance examples and questions):

- Pre-defined monitorable and quantifiable criteria which may trigger product updates (How will development of bacterial resistance be detected? What level of resistance is acceptable?).
- What is the expected nature of future product updates? (exchange of individual monophage components with similar substitute components with higher activity without affecting total number of monophage components in the product, introduction of new monophage components, thus increasing the number of monophage components in the product, etc.).
- How are potential substitute monophage components expected to be generated? (training or engineering of phage strains which have lost activity, identification of new phage strains overcoming resistance but being otherwise comparable to strains which have lost activity, etc).
- Which data are expected to be required to document that apart from overcoming the developed resistance, the updated product is comparable to the parental product?

  E.g. product quality data only, quality data combined with *in vitro* surrogate data for efficacy, quality data combined with *in vivo* data for clinical safety and efficacy in target animal species, etc (see under comparability and *in vitro* surrogate endpoints in Definitions section, and considerations in evaluating the comparability of phage strains in the text immediately below).

Post-approval change management protocols should be realistic (feasible), i.e. should be based on relevant scientific knowledge and understanding of manufacturing processes and product characteristics, coupled with appropriate quality risk management and pharmaceutical quality systems.

Thus, post-approval change management protocols such as these may not be possible for particularly complex phage products and are in any case optional.

Yet, where possible, post-approval change management protocols are expected to provide a level of predictability and transparency in terms of the requirements and studies expected to be needed to implement product updates, facilitating faster and more flexible implementation of said updates.

<u>Considerations for evaluation of the comparability of monophage components and bacteriophage products:</u>

Such product updates may comprise the use of trained or new bacteriophage components (see under trained bacteriophages and updated phage products in Definitions), and in this case, data requirements depend on the comparability between the monophage components involved in the update (see under comparability and updated phage products in Definitions).

Comparability between monophage components should be assessed following the principles established in ICH Q5E (comparability of biotechnological/biological products subject to changes in their manufacturing process); the guideline on similar biological medicinal products may also be consulted

(CHMP/437/04 Rev 1, 23 October 2014). In the following, the application of ICH Q5E and CHMP/437/04 guidelines to bacteriophage products are illustrated.

For parental and substitute monophage components to be considered comparable, the following conditions must be met:

(i) when assayed on variants of bacterial pathogens which are susceptible and resistant to the parental monophage component, the potency of substitute monophage components to the resistant bacteria should be comparable to the potency of parental monophage components against the susceptible bacteria. Higher potency of substitute monophage components is expected to be acceptable.

(ii) the parental/substitute monophage components should be biochemically and biologically comparable, meaning that their critical quality attributes are highly similar, and

(iii) the existing knowledge is sufficiently predictive to ensure that any differences between the monophage components have no adverse impact upon existing analytical assays, and quality, safety or efficacy of the bacteriophage product as a whole.

Determination of comparability should start with a pro-active assessment of the potential risks that the planned post-authorisation update might have for product quality, safety, efficacy or traceability.

In most cases, this will be followed by appropriate analytical studies (so called comparability exercise) comprising as a minimum quality data, and potentially also safety and efficacy data.
Parental/substitute monophage components should initially be compared based on the pre-defined and established characteristics of the parental monophage component (see section on characterisation).

Additional data e.g. data showing comparable stability, biodistribution and immune clearance may be required.

Parental/updated bacteriophage products should initially be compared based on the pre-defined and established specifications for the parental product.

If the results from the risk assessment and initial comparability exercise as outlined above indicate relevant differences between monophage components and/or products, additional studies on quality, safety and efficacy may be required to document comparability.

If it is concluded that monophage components and/or products are comparable, and if the existing knowledge is sufficiently predictive to ensure that the planned product update has no adverse impact upon the quality, safety or efficacy of the bacteriophage product as a whole, documentation of safety and efficacy of the updated product by *in vivo* studies in target animal species may not be required.

On the other hand, if differences in critical quality attributes are so significant that monophage components and/or products cannot be concluded to be comparable, safety and efficacy studies in target animal species may be required for the updated product.

Notably, it is not possible to pre-define absolute thresholds for differences in critical quality, safety and efficacy attributes above which updated products could no longer be considered comparable to parental products; this will require evaluation on a case-by-case basis.

If comparability of quality, safety and efficacy cannot be concluded based on *in vitro* studies, documentation of safety and efficacy of the updated product by *in vivo* studies in target animal species will be usually required (please see further details in ICH Q5E).

Illustrative guidance examples of quality, safety and efficacy data requirements for phage product updates:

For guidance purposes only, illustrative examples of likely data requirements for different categories of product updates are provided in Annex III to this guideline.

Definitions

- **Active substance (AS)**: Any substance or mixture of substances intended to be used in the manufacture of a VMP and that, when used in the production of a VMP, becomes an active substance of the VMP (Ph. Eur. 10000, general notices).

  In the case of bacteriophages, preparations of individual bacteriophages (preparations of individual bacteriophage strains, termed monophage AS or monophage components or monophage preparations, all these terms being synonymous) comprise the basic AS for phage products, and for manufacturing reasons, monophage AS may be mixed to produce multiphage AS, prior to final formulation and filling to produce phage VMPs.

- **Bacteriophage**: Viruses which infect bacteria and do not have the capacity to infect eukaryotic cells.

- **Biobank (of bacteriophages)**: Physical collection of characterised phage strains (qualified repository of bacteriophages), sometimes referred to as phage library (illustrative examples in Gibson SB et al. 2019 and Lin RC et al. 2021).

- **Characteristics of the product**: See quality target product profile.

- **Chemically modified bacteriophages**: Bacteriophage preparations where the infectious particles have been chemically modified e.g. to improve pharmacokinetic/-dynamic properties.

- **Cocktail of bacteriophages**: See multiphage preparation.

- **Comparability (between bacteriophage products or monophage components)**: In this context, similarity between a bacteriophage product having undergone post-authorisation updates to overcome bacterial resistance or changes in the epidemiology of bacterial pathogens in the field (updated product) and the pre-update product.

  A conclusion that updated and pre-updated products are comparable means that they have highly similar quality attributes, and that no adverse impact on the safety or efficacy of the product is expected (ICH Q5E).

  The definitions above apply regardless of whether the comparability term is applied to bacteriophage products, monophage components, or other constituents of bacteriophage products.

  Comparability should be assessed considering the principles established in ICH Q5E (comparability of biotechnological/biological products subject to changes in their manufacturing process); the guideline on similar biological medicinal products may also be consulted (CHMP/437/04 Rev 1, 23 October 2014). See further details in the chapter on post-authorisation product updates in this guideline.

- **Control Strategy**: A planned set of controls, derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control (ICH Q10).

- **Critical Process Parameter (CPP)**: A manufacturing process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces a product of the desired quality (ICH Q8). Critical manufacturing process parameters are controlled by process controls with appropriate acceptance criteria.

In the case of bacteriophages, knowledge-based and documented understanding of the relationship between manufacturing process CPP and critical quality attributes for CQA for phage products is expected to increase the flexibility when updates need to be made to multiphage products, to overcome development of resistance (such as for example changes in the total number of monophage components or exchanges of one monophage component for another).

- **Critical quality attribute (CQA)**: A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. In practice, there is often a significant overlap between the terms CQA and product specifications (see ICH Q8 pharmaceutical development, and VICH GL40 acceptance criteria for new biotechnological/biological veterinary medicinal products).
- **Engineered bacteriophages**: Bacteriophages which have been modified by molecular biology techniques, e.g., to enhance bactericidal activity, enhance host range, improve pharmacokinetics properties, etc. Examples of engineered phages are given in Palacios Araya D et al. 2021 and Dedrick RM et al. 2019.
- **Enhanced approach to manufacturing process development**: In an enhanced approach, risk management and more extensive scientific knowledge are used to select process parameters and unit operations that impact critical quality attributes (CQAs) for evaluation in further studies to establish any design space(s) and control strategies applicable over the lifecycle of the drug substance. This can create the basis for more flexible regulatory approaches e.g. in cases of post-authorisation changes to manufacturing processes. The degree of regulatory flexibility is generally dependent on the level of relevant scientific knowledge provided in the application for marketing authorisation. This enhanced approach is thus sometimes referred to as "designing quality into product" or "quality by design". Traditional and enhanced approaches are not mutually exclusive. A company can use either traditional or enhanced approaches, or combine both (paraphrased from ICH Q11). Illustrative scientific examples of enhanced approaches to development of manufacturing processes for biologicals are given in Li X et al. 2019, Nie J et al. 2019, and A-VAX. For regulatory information regarding implementation of quality by design principles to manufacturing process development, see EMA/430501/2013, EMA/603905/2013, EMA/59240/2014 and EMA/CHMP/CVMP/QWP/354895/2017.
- **Excipient (auxiliary substance)**: Any constituent of a medicinal product that is not an active substance. Adjuvants, stabilisers, antimicrobial preservatives, diluents, antioxidants, for example, are excipients (Ph. Eur. 10000, general notices).
- **Finished product (Drug product)**: The dosage form in the final immediate packaging intended for marketing (ICH Q7). In the case of bacteriophages, bacteriophage cocktail appropriately formulated with required excipients, in the final immediate packaging intended for marketing.
- **Library (of bacteriophages)**: See biobank.
- **Lysogenic bacteriophages**: See temperate bacteriophages.
- **Lytic bacteriophages (virulent bacteriophages)**: Bacteriophages which are only able to sustain replicative cycles ending in bacterial lysis. Only such strictly lytic bacteriophages are used for phage therapy.
- **Metaphylaxis**: Administration of a medicinal product to a group of animals after a diagnosis of clinical disease in part of the group has been established, with the aim of treating the clinically sick animals and controlling the spread of the disease to animals in close contact and at risk and which may already be sub-clinically infected (Regulation (EU) 2019/6).

- **Monophage preparation**: Pharmaceutical preparation of a single, characterised bacteriophage strain, starting from clonal, characterised and quality-controlled seed lots of phage and matched and similarly quality-controlled bacterial host. Bacteriophages used for veterinary medicinal products are isolated from e.g. environmental or clinical sources, and purified by appropriate means to ensure clonality (homogeneity). Characterisation comprises documentation for factors such as e.g. (i) required activity and potency against target bacterial pathogen(s), (ii) strictly lytic lifestyle, (iii) absence of transducing ability, (iv) absence of toxin genes, etc. See section on characterisation, illustrative literature examples in e.g. Pirnay JP et al. 2018, Lehman SM et al. 2019, and Gibson SB et al. 2019.
- **Multiphage preparation (multiphage composition, bacteriophage cocktail)**: Qualitatively and quantitatively characterised mix of the number of monophage components which is required to obtain the required product characteristics (see quality target product profile). The term multiphage product is used for any product containing more than one monophage component. The terms multiphage preparation, multiphage composition and bacteriophage cocktails may apply to active principle ingredient as well as to final phage product and are used mainly in situations where the monophage components are pre-mixed.
- **Multiphage product**: The term may be applied to multiphage preparations (see definition above) as well as products where the monophage components are filled separately for mixing prior to use.
- **Multiplicity of infection**: Ratios of phages to bacteria.
- **Post-approval change management protocol (PACMP)**: A post-approval change management protocol describes specific changes that a company would like to implement during the lifecycle of the product and how these would be prepared and verified. It is a step-wise approach in the assessment of changes, which allows an early evaluation of the strategy for the change and a later separate evaluation of the data produced based on the agreed strategy. Such a stepwise approach is expected to lead to faster and more predictable implementation of changes post-approval, since the company will have obtained agreement from the Agency about the proposed strategy and tests to verify the effect of the change on product quality (EMA/CHMP/CVMP/QWP/586330/2010).
- **Parental phage product (prototype phage product)**: Originally authorised veterinary phage product, also termed P0. Manufacturers may pick from the monophage components included in the approved dossier for the parental product to match the geographical distribution and phage resistance patterns of targeted bacterial pathogens in different countries, or even on a case-by-case basis for individual bacterial disease outbreaks. Thus, different compositions of the parental product (P0-a, P0-b, P0-c, etc) can be marketed at the same time or at different times in different countries to address different epidemiological needs (see also product updates).
- **Phage products (phage medicines, bacteriophage products)**: Final veterinary medicinal product containing bacteriophage(s).
- **Phage therapy**: Use of bacteriophage products for prophylactic, metaphylactic and/or therapeutic use of one or several specific infection(s) or infectious disease(s). Efficacy of treatment is linked to the lytic activity of bacteriophages that confers bactericidal activity on those bacteriophages with specificity for the bacterial strain concerned (Annex II of Regulation (EU) 2019/6). Other uses of bacteriophages in veterinary medicine, e.g. use of bacteriophage particles as display platforms for vaccines or use of temperate/integrating bacteriophages to modulate bacterial phenotypes, are outside the scope of this guideline.
- **Post-authorisation update (to phage products)**: See under parental phage product and updated phage products.

- **Prophylaxis**: Administration of a medicinal product to an animal or group of animals before clinical signs of a disease, in order to prevent the occurrence of disease or infection (Regulation (EU) 2019/6).
- **Quality by design**: A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. See also under enhanced approach to manufacturing process development.
- **Quality risk management**: A systematic process for the assessment, control, communication, and review of risks to the quality of the VMP across the product lifecycle (ICH Q9).
- **Quality Target Product Profile (QTPP)**: A prospective summary of the quality characteristics of a VMP that ideally will be achieved to ensure the desired quality, safety and efficacy characteristics of a VMP, considering e.g., the indications, epidemiological situation in the field (epidemiology of bacterial pathogens targeted by the phage medicine), route of administration, dosage form, bioavailability, strength, development of resistance, concomitant use with other medicines and stability (paraphrased from ICH Q8). QTPPs are typically formulated very early in the product development.
- **Representative phage preparations (representative phage cocktails)**: Multiphage compositions which were used for key safety or efficacy studies during development of the parental product, and therefore support (are representative for) the full list of monophage components which are authorised in the dossier for the parental product.
- **Residual risk**: Specified and acceptable level of risk to target animals, consumers and the environment from authorised medicines (paraphrased from ICH Q9). Essentially all authorised medicines carry such (acceptable) residual risks (see for example conclusions for benefit/risk balance for authorised pharmaceuticals in public assessment reports).
- **Risk**: In the context of phage medicines, any potential unfavourable effects that may be attributed to the use of the novel therapy product which are of concern to the target population and/or the user, the consumer, and/or the environment (Annex II of Regulation (EU) 2019/6). See also glossary to ICH Q9.
- **Robustness**: As applied to manufacturing processes, the ability to tolerate variability of materials and changes of the process and equipment without negative impact on quality (ICH Q8).
- **Specification**: List of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance, medicinal product or materials at other stages of its manufacture should conform to be considered acceptable for its intended use. "Conformance to specification" means that the drug substance and medicinal product, when tested according to the listed analytical procedures, will meet the acceptance criteria. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval (VICH GL40 acceptance criteria for new biotechnological/biological veterinary medicinal products).
- **Surrogate endpoint**: An endpoint where experimental data and mechanistic rationales support that a sufficient quantitative correlation exists between the endpoint and the claimed safety or efficacy in the target species, such that the surrogate endpoint can be assumed to be predictive for safety or efficacy in the target species with reasonable confidence (paraphrased from EMA/CVMP/IWP/105506/2007 Rev. 2). For example, in situations where bacterial resistance against a monophage component is addressed by training or engineering said monophage

component to regain bactericidal activity, the bactericidal activity of the trained monophage *in vitro* might be considered a surrogate endpoint for efficacy in target animals (assuming that the training/engineering does not substantially change for example stability and pharmacodynamics/-kinetics and if otherwise adequately justified).

- **Synthetic bacteriophages**: Bacteriophages manufactured in completely bacterial cell-free systems using e.g. coupled *in vitro* transcription/translation (an example of this is given in Rustad M et al 2018). In the context of this guideline, the term does not apply to bacteriophages manufactured by synthesis of the genome followed by assembly of particles in bacterial cells.
- **Temperate bacteriophages (bacteriophages exhibiting a lysogenic cycle)**: Bacteriophages which are dually able to sustain dormancy (typically by integration into the bacterial chromosome; lysogeny) as well as lytic replication in host bacteria, depending on e.g. environmental conditions.
- **Traditional approach to manufacturing process development**: In a traditional approach, limits (acceptance criteria) and operating ranges (tolerances) for manufacturing process parameters and analytical tests carried out during manufacture and on final product are established statistically based on (i) validation batches used to demonstrate consistency of commercial-scale production (typically 3 batches), and (ii) batches tested clinically. These limits and tolerances may subsequently be refined on a statistical basis in the light of commercial manufacturing data. Traditional and enhanced approaches are not mutually exclusive. A company can use either traditional or enhanced approaches, or combine both (paraphrased from ICH Q11).
- **Trained bacteriophages**: Traditional technique where phages are co-evolved with bacterial hosts under defined laboratory conditions, in order to reduce risk of development of resistance. An example of this is given in Burrowes BH et al 2019.
- **Updated Phage products**: It is recognised in Annex II of Regulation (EU) 2019/6 that phage products will likely need to be updated on a regular basis (for example by changing the total number of monophage components or exchanging individual monophage components with similar substitute monophage components with higher activity), due to development of phage resistance patterns of targeted bacterial pathogens that can no longer be overcome by use of the monophage components included in the dossier for the parental product or changes in the epidemiology of bacterial pathogen(s) in the field. As a matter of course, such updates will occur to the initially authorised product (parental product, P0), and new monophage components are required (for example trained versions of monophage components included in the dossier for the parental product, or completely new monophage components; The data requirements are detailed in section 5 of this guideline.

References

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Annexes

Annex I

III.A.2A2. Product development

Illustrative examples of questions which may be considered for the justification of phage strains depending on product characteristics (given here for guidance only):

- Which bacterial pathogen(s) are targeted?
- Have the employed phage strains been trained and/or engineered for improved activity against these pathogens?
- Does the clinical indication require activity against bacterial biofilms?
- Why are the proposed phage strains expected to be particularly well suited to target these pathogens, taking into account the epidemiology of the targeted bacterial pathogens, route of administration, dosage form, bioavailability, strength, and stability of the product?
- What are the mechanisms of biological activity of the phages, and by which mechanisms does resistance develop?
- How fast is resistance against the product expected to develop?
- Antagonism/synergy with antibiotics. What is the risk that development of resistance against the phage product may simultaneously cause resistance against antibiotics?
- Are interactions between the phage product and antibiotics anticipated (antagonism, additive effects, synergy)?

Annex II

IIIa.2A3. Characterisation

Genetic characterisation of phages is expected to be based on quality whole-genome sequence, with particular focus on:

- Annotation of genome and taxonomic classification of the phage strain.
    - For illustrative guidance examples of analysis of phage genome sequences, please see Adriaenssens E et al. 2017, Philipson CW et al. 2018 and EFSA 2021.
- Known genetic elements encoding antibiotic resistance.
- Known genetic elements encoding toxins.
- Genetic elements known to indicate ability to transduce (mobilise) genetic elements for antibiotic resistance which may be present in target pathogens.
- Genetic elements known to indicate lysogenic activity.
- Genetic elements predictive for host range and potency (e.g. genes encoding receptor-binding proteins and virulence factors).

- Any other genetic elements considered to be predictive for detrimental effects on safety or efficacy of the individual phage strain (incl. interactions with other phage strains employed in the product).

Annex III

Table: Illustrative examples of data requirements for post-authorisation updates made to phage products in order to overcome bacterial resistance or address changes in the epidemiology of bacterial pathogen(s) in the field.

1

Description of post-authorisation phage product update	Category of phage product update	Level of changes to manufacturing process(es)	Likely quality data requirements for approval of updated phage product	Likely safety data requirements for approval of updated phage product	Likely efficacy data requirements for approval of updated phage product
Addition of a monophage component which is <u>comparable</u> to a component which is authorised with the marketing authorisation application	# Simplest	# Not substantial	§ Minimal	If monophage components are comparable, safety studies are not expected to be required (post-authorisation changes expected to be approvable based on quality data alone). Similarly, user and environmental risk assessment is not expected to be required. It is advised to consult the Agency for the need of a MRL status.	If monophage components are comparable, target animal safety studies are not expected to be required (post-authorisation changes expected to be approvable based on quality data alone).
Addition of one or more new monophage components to a product which <u>are not comparable</u> to a component which is authorised with the marketing authorisation application	$ Complex	$ May be substantial	Unless it can be scientifically justified that the proposed product update does not carry with it unacceptable risks to quality, safety, efficacy and traceability of product, re-validation of manufacturing processes and associated analytical technologies, as well as documentation of comparability between parental and updated product, may be required.	If the impurity profile of the product is not substantially worsened, safety studies might not be required. In case of high level of complexity or lack of alternative evidence, safety data may be required. New user risk assessment and environmental risk assessment might be needed. It is advised to consult the Agency for the need of an MRL.	If the new monophage components are not comparable to monophage components already present in the product, data from laboratory efficacy studies in target animal species or representative species may be required. In worst case scenarios (high level of complexity or lack of alternative evidence), data from new clinical trials in target animal species may be required. To avoid the requirement for a full efficacy package, alternative tools should be established. For example, it is expected that for *in vivo* studies, surrogate efficacy endpoints established and validated for the parental product might be used.

2

Table text:

 # Illustrative examples of situations where product updates might be considered simple and associated changes to manufacturing process(es) might be considered as non-substantial:

- Except for the exchange of inactive monophage components for substitute monophage components which overcome the bacterial resistance, the product composition is not altered.
- The substitute monophage components are comparable to the monophage components which are replaced (see details in main text, subsection on considerations for evaluation of the comparability of monophage components and bacteriophage products).
- The downstream manufacturing process (purification and formulation) for substitute monophage components is not substantially changed compared to the process employed for the monophage components which are being replaced, and the purity, impurity and contamination profiles of the monophage components are comparable (see under comparability in Definitions section).
- New analytical procedures required by the nature of the update are described (e.g. discontinuation of assays for monophage components which are being replaced, and introduction of corresponding new assays for substitute monophage components), and apart from such changes, the existing analytical procedures are minimally affected by the product update.
- The substitute monophage components do not exhibit interactions with other monophage components employed in the product which may adversely impact product quality, safety, efficacy and stability.
- The specifications for the parental product remain appropriate to ensure the quality also of the updated product. It is recognised that product updates may require modification, elimination or addition of specification tests (e.g. addition of tests for new impurity, or exchange of tests for removed monophage components with tests for substitute monophage components); such changes to specifications may be considered non-substantial, if justified by the nature of the product update. Tightening of acceptance criteria and specifications to improve quality are generally expected to be acceptable.

$ Illustrative example of situations where product updates might be considered complex and associated changes to manufacturing process(es) might be considered as substantial:

- The new monophage components are not comparable to the monophage components employed in the parental product (see details in main text, subsection on considerations for evaluation of the comparability of monophage components and bacteriophage products).
- The new monophage components are manufactured on new manufacturing lines not encompassed by the existing process validation data.
- The new monophage components cause worsening of the product impurity profile.
- The new monophage components introduce new potential risks to product quality, safety or efficacy which were not present for the parental product (e.g. interactions with other monophage components which may be employed in product, resistance to new monophage component is associated with antibiotic resistance, etc.).
- The new monophage components require changes to product composition (e.g. change of buffer salts or excipients or other changes to product composition).

§ Illustrative guidance examples of minimal data requirements:

- Description of the monophage components which have been removed from the multiphage product.
- Quality data for the substitute monophage components, with reference to their quality standards (e.g. acceptance criteria for process controls, compendial monographs, product specifications; see CPP and CQA in Definitions).
- Quality data for any new monophage components, with reference to their quality standards (e.g. acceptance criteria for process controls, compendial monographs, product specifications; see CPP and CQA in Definitions).
- Specifications for the updated product (see section 4.2. IIIa.2E. Control tests on finished product).
- The quality documentation which is required to support next product update is revised; for example product characteristics, and post-approval change management protocol.
- Scientific justification is provided that the combined changes do not negatively impact quality, safety, efficacy and traceability of the product (the risk-based approaches recommended to be followed are outlined in section 4.2, quality documentation).
- Changes to quality characteristics of manufacturing processes and product associated with and/or triggered by the product update are described (see under CPP, CQA and QTPP in Definitions).
- Batch-to-batch consistency for manufacture and stability of the updated product is documented based on three commercial-scale batches (expected that stability data can be submitted post-authorisation).
- Stability studies for updated product can be provided post-authorisation, if justified.