13 October 2023
EMA/CVMP/NTWP/230544/2023
Committee for Veterinary Medicinal Products

Overview of comments received on 'Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy' (EMA/CVMP/NTWP/32862/2022)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	Anna Pistocchi and Massimo Aureli - University of Milan, Italy
2	Gregory Resch - Laboratory of Bacteriophages and Phage Therapy, Lausanne University Hospital, Switzerland
3	SIMV Syndicat de l'Industrie du Médicament et Diagnostic vétérinaires, France
4	Universitat Autònoma de Barcelona, Spain
5	Phage Germany, NGO consortium consisting of various phage experts (microbiologists, virologists, physicians, pharmacists) and solicitor
6	AnimalhealthEurope
7	German Pharmaceutical Industry Association (BPI), Germany
8	Centro de Calidad Avícola y Alimentación Animal de la Comunidad Valenciana, Spain
9	Dr. Hans Petter Kleppen, CSO - ACD Pharmaceuticals AS, Norway
10	M. Canaval, J. Sacre, V. Garcia & G. Scherer (Global Regulatory Affairs team) - PhageLAB Chile SpA.
11	Salmon Scotland
12	Vesale Bioscience, Belgium
13	MEDEA Biopharma GmbH, Germany
14	Federation of Veterinarians of Europe (FVE)

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1. General comments – overview

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1	The main point that we think is missing in the guidelines is a mention of a phage pre-testing system with regard to safety, toxicity and degree of preparation. Such a system would ensure that we would get to testing phages in target animals already with some degree of safety and could also be certified. Thus, it would no longer be necessary to conduct *ad hoc* tests on the type of animal to be treated, which is different each time, nor on the various routes of administration, nor to refer to a validated literature. In addition, a pre-test system including a model animal and human cell lines would avoid potential adverse effects of phage therapy in the "tester" animal. If this occurred, we imagine that the use of phages in clinic or veterinary medicine would surely be blocked. Moreover, these adverse effects then might be totally species-specific and, therefore, absent in other animals or humans. A pre-testing system would make it possible to do all the screening, dose-dependence testing of the various preparations, to develop a certification system for the quality of phage preparations, and, crucially, to reduce the number of "tester" animals used in accordance with the 3Rs policy. Consideration of validation of concomitant use of phages and antibiotics could be highly variable from time to time depending on the antibiotic(s) previously used in animals. In addition, bacteria may also evolve very variably following various antibiotic and/or phage treatments. In addition, phage therapy is always subsequent to antibiotic treatment so far and is indeed more effective if subsequent or concomitant to it. Furthermore, it has been shown in	It is not missing. The text gives flexibility to applicants to avoid running studies in laboratory animals, if safety information can be obtained by other means, such as, bibliographic information, in vitro tests or any kind of previous experience with the product (including pre-testing systems). Bacteriophages must be well characterised before their use in animals and the characterisation implies in vitro analysis/studies including pre-testing systems. Thus, studies in target animal species are expected to be done with selected and well-characterised bacteriophages (in agreement with 3R principles). On the other hand, studies in target animal species are needed to demonstrate the safety and efficacy of the product. Pre-testing systems need to be adequately validated.

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	humans that phage treatment results in resensitization of bacteria toward antibiotics for which they had developed resistance.	
3	This guideline is of great value to the field and we would like to thank the CVMP group for this effort. From a general point of view, the document is well constructed and provides all the necessary aspects to develop phage-based veterinary products. Nevertheless, we would like to suggest that parts IIIa.3A.4.4. and IIIa.4.A.2. bring more detailed information to applicants	The guideline is drafted at high level and it is not possible to give more details. Applicants are invited to ask for a scientific advice to EMA in case they need further clarifications for a specific bacteriophage product.
5	1. It is generally appreciated that there shall be an individual GMP Guideline for the production of phages apart from the general EU Guidelines to GMP on Medicinal Products for Human and Veterinary Use (SANCO/C8/AM/sl/ares(2010)1064597), since phages are natural, biological agents and thus very different from fixed chemical formulations. The general context for the necessity of phage products is the worldwide AMR-disaster into which mankind is steering actually, and which is to a certain extent due to the abusive use of chemical antibiotics in animal farming, above all in the industrial animal farms, where chemical antibiotics have been administered regardless to its resistance-promoting effect. Since the abusive use of chemical antibiotics is estimated to be the cradle of multiresistancies in human bacterial infections, too, it seemed logical to us, that in a One-Health-scenario, the use of phages in vet. medicine, particularly in industrial animal farms, is	1. No comments 2. No comments 3. GMPs are not under the remit of the CVMP. The corresponding group (inspector´s working group, IWG) is currently updating Annex 4 and 5. 4. Not agreed. The scope of the GMPs and GLPs are different and complementary. 5. Phage treatments prescribed as magistral formula are out of the scope of this Guideline and applicable regulation is stated at national level. 6. If bacteriophages are part of the parental preparations they can be changed to be adapted to the epidemiological situation. 7. and 8. Agree. However, this will be established by GMPs rules.

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	the only agent which is able to stop this vicious circle. The production and commercialization of phages should therefore be promoted and facilitated best possible. Phageproducts must therefore remain cheap and easy to get without harming men, animal and environment. 2. We checked the draft Guideline therefore for its ability to provide easy, quick and cheap access to phage therapeutics for individual farmers, industrial animal farms, zoos and pet keeping individuals. It is obvious, that the users have a tendency to stick to cheap and easy to get chemical antibiotics. If, due to the GMP-framework, the phage products will in future be difficult to get, if the production process will be time-consuming and if the costs will exceed the purchase price for chemical antibiotics by far, things won't change. It is therefore paramount that phage products are quick and easy to get and cheap enough to motivate the users to replace chemical antibiotics by phage products. 3. We then tested a quick and easy production process against the need for safety of the product for the animal, the keepers, the consumers and the environment, and came to the conclusion that - phages are natural agents which are deriving from nature, which have been omnipresent in the entire environment, including the guts of animals and men since ages and will go on to do so,	

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	- phages have no pathogenic effect neither for mammals in general nor for humans in particular, - phages develop unique strategies to overcome bacterial resistances quite naturally themselves all the time, and that - phages keep changing their genome persistently and naturally themselves all the time as well. Since the major concern of the Agency is to identify the actual risks on a scientific base and minimize eventual risks significantly, a proper scientific risk assessment would be advisable aforehand. Upon scientific advice, the Agency will probably come to the conclusion that phages do no harm neither to the affected animals nor to the keepers nor to the environment, nor to the consumers if it comes to comestible livestock animals, even if there is a contamination of men or/and a re-insertion of phages into the environment. The Agency will probably come to the conclusion that a production under GMP-standards will cause very high costs without adding significantly to the safety aspect, and that the GMP-rules will thus prove to be prohibitive. The Agency will eventually be satisfied that a manufacturing following e.g. the actual Belgian Monograph, resp. a monograph or a chapter included into the Eu. Pharmacopoeia at GLP-levels would grant sufficient safety. We would therefore like to suggest that the Agency shall first have an entire, scientific risk & cost assessment done by microbiologists,	

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	virologists, infectiologists, veterinarians, physicians and health engineers, who should advise on the question, - are there any risks to be addressed, - to which degree these risks can be tolerated (compared to the ongoing use of chemical antibiotics), - which GMP-measures would add significantly to the safety of the API compared to mere pharmacopoeian and GLP-standards, and - to which extent they would increase the costs of the end-product and affect the commercialization of the product and hinder the AMR-defeating effect. The Agency should identify specific, indispensable GMP-rules only after this assessment. 4. In the meantime, and in consideration of the fact that phages are meant to defeat an actual emergency (shortage and inefficiency of chemical antibiotics and a corresponding, inexorable expansion of AMR-infections also in humans), the Agency is invited to provide a GMP-free production of lytic phages for an intermediate period of 3-5 years under mere GLP conditions. The Agency should arrange for an evaluation process after 3-5 years upon the entering into force of the provisional approval, and assess at the end of the period,	

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	whether specific GMP-rules have shown necessary, and a more expensive version of the product is desirable. 5. In consideration of the fact that the general GMP-Guideline SANCO/C8/AM/sl/ares(2010)1064597 states that "*The principles of GMP and the detailed guidelines are applicable to all operations which* *require the authorisations referred to in Article 40 of Directive 2001/83/EC, in Article 44 of* *Directive 2001/82/EC and Article 13 of Directive 2001/20/EC, as amended. They are also* *relevant for pharmaceutical manufacturing processes, such as that undertaken in hospitals*." the draft should state explicitly that the pharmaceutical manufacturing process of individualized phage therapeutics is not subject to any GMP-framework, be it the general or a specific framework. 6. The mutability of the bacteria, the broad variety and mixture of bacterial infections and an imminent exacerbation of the infection often require that a quick and flexible, effective action must be taken. For this purpose, it seems reasonable that phage products, which have been manufactured in respect of the (remaining) GMP-rules, may be mixed on the farm or by the veterinarian to fight an	

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	individual bacteria-mix on the spot without the mixture resp. the new product being subject to an individual resp. entirely new GMP-assessment. 7. For the above-mentioned reasons, and in consideration of the fact that the effectiveness of a phage is made obvious by the plaque-test and/or by PCR-sequencing, it is reasonable to exempt the isolation- and identification process of the API from GMP rules and let a reasonable GMP-process start with the proliferation and purification of the phages. 8. We think it is advisable to establish different, cascade-like GMP-standards with respect to the different application of the phages, like for - enteral application (feed) - local application (lavage) - inhalation (pneumonia) and instillation (UTI) - i.v. application Particular with respect to the pyrogenic contents (pathogen LPS, endotoxins and exotoxins), the enteral application tolerates a higher level, whereas the level for an i.v. application should be kept as low as possible (5 PFU/ml).	

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	This would allow a better stability of the product (and lower costs) for the enteral application, which in vet. medicine will be the most common application, and medium stability and higher costs for the other applications. 9. Please find more detailed annotations to the draft GMP-Guideline as follows:	
6	AnimalhealthEurope welcomes this guideline regarding phages as a start to preparing the regulatory environment for such products. Indeed, whilst known for decades, no phages have been registered as medicinal products to date, both on human and animal health sides. It is acknowledged that the concept of an industrial product, as classically understood, is considered not suitable for phages as these in general are used on an individual basis. In section V.1.1.3 of paragraph "Novel therapies VMPs" of annex II, it is stated that "*the manufacturing processes for novel therapy medicinal products shall comply with the principles of Good Manufacturing Practice (GMP) adapted where necessary*". It is not in the scope of this guideline to list these specific GMP requirements for this type of product, nevertheless this possibility should appear in the guideline, keeping practical manufacturing/specificities of phages in mind. Moreover, from a general point of view the number of studies required according to the guideline to demonstrate toxicology, immunology and efficacy for every target species is considered too exhaustive and is expected to block any future developments in veterinary medicines (as it has largely the case for in human	In relation to the proposal of adding the text stated in section V.1.1.3 about GMPs in the guideline, it is not agreed as this information is already in the Regulation (EC) 805/2021 and GMPs are not under the remit of the CVMP. Studies mentioned in the Guideline related to toxicology, immunology and efficacy are the ones required in the Regulation (EC) 805/2021. The guideline describes a flexible framework to fulfil the corresponding requirements. Finally, bacteriophages and bacterial hosts characterisation are highly relevant for the safety and the efficacy of the product. The advice given in the Guideline related to freedom of certain genetic components and thresholds should be followed.

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	medicine). In the guideline, it is stated that missing studies should be justified by scientific literature. This would also have to be considered for the characteristics required for the phages and their bacterial hosts, *e.g.* with regard to freedom of certain genetic components and thresholds.	
7	The BPI welcomes the intention to enable the use of bacteriophages by the so far missing regulation in the EU. We also consider the approach to keep the main features of the regulation as general as possible and to realize this under consideration of a risk-based quality management approach as reasonable, especially against the background that phages have to be handled case-specific and users do not have to work through the whole range of regulation in every use of phages, but it is ultimately always a case-by-case procedure with the least possible regulatory approach. However, this should be regulated from the outset in such a way that the relevant procedures in the EU member states are as simple and quick as possible, because otherwise a disease risks ending in the worst case with a fatal outcome before the authorities decide on the use of the phage therapy required in each case. Then the best NT-VMP is of no use. One point of criticism is that the requirements for phage-based and phage-derived products are not brought together in one guideline.	Noted.
9	The global battle against AMR must be fought on several fronts, and the use of bacteriophages may certainly be among the most important measures; both directly by saving animals and herds for whom antibiotics no longer work, and indirectly, by preventing	Fully agree.

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	disease and reducing the need for antibiotic treatments. Equally, the vast range of possible applications in areas such as human health, animal health and food safety simultaneously lay the foundation for an important new European industry. We are very positive to the regulatory process on phage therapy initiated by EMA, CVMP and NTWP. Regulation is necessary to create a functional and reliable regulatory framework which will encourage investment into research and development of new phage-based therapies. A regulatory pathway towards licencing a medicine based on bacteriophages needs to be markedly different from current pharma regulation, mainly because of the characteristics of bacteriophages themselves, • Bacteriophages are already ubiquitous and integral parts of all life on earth. • Their strong specificity and efficacy towards quite limited strains of target bacteria, limits the potential revenue for each product. • Bacteriophages are substantially different from classical antibiotics in that they are living and evolving with their hosts, they have narrow host ranges, and differ in fundamentals such as size. A typical effective dose of phage in an aqueous substrate is 1 million pfu/mL (plaque forming units per millilitre), and a typical effective dose of the antibiotic Gentamycin is 9 mg/kg. If administered together, there would be 8.8 billion Gentamycin molecules for every	

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	bacteriophage in the system. This has profound impact on likelihood of interaction between the target bacterium and the therapeutic phage or antibiotic. It is important that both developers and regulators keep minds open and take such fundamental differences into account when adopting existing legislation and guidelines to phage VMPs. • Target bacterial populations are rapidly changing. Post-marketing changes to phage products will need to be handled in a very time-effective manner for the products to be commercially viable. In our experience from the aquaculture sector, large scale use of bacteriophage against a target bacterium can drive short-term changes in target bacterial diversity during treatment through selection for resistance. Such change can be counteracted by using different bacteriophages in a cocktail or a rotational treatment scheme, or by combinatorial use of phage and antibiotics. The more important change in target bacteria populations – one we believe will affect phage-based medicinal products - is the natural (seasonal) diversity fluctuations of target bacteria which happens on a much larger scale. This is similar to diversity fluctuations typically seen for many human pathogens monitored by sequence characterization. (E.g.: which influenza virus variant will we get this year? And which sequence type of *Klebsiella* will cause the next nosocomial outbreak?). Unfortunately, it is not possible to know in advance of a MA application all target bacterial variants which are going to cause future	

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	outbreaks. Therefore, it is essential that phage products can be updated in a time-effective manner. The guideline currently on consultation covers requirements on quality, safety and efficacy for the initial authorization application as well as post MA changes. It is recognized in the Guideline that "due to the specific nature of bacteriophage products, adaptation of the general rule may be acceptable, and the regulatory framework is expected to be flexible." Individually, each requirement makes good sense, however, we have a fear that the sum of requirements, if strictly adopted, will be too much to bear for products which by nature will have very limited market value. A far-reaching, expensive, and overly time-consuming licencing process for bacteriophage based VMPs will curtail commercial viability and stand in the way of the obvious benefits that bacteriophages represent. We were happy to note during the presentation of the Guideline at the Focus group meeting on May 11, that EMA and NTWP recognized that there is no former practical experience with regulating phage VMPs in Europe, and that the road to a large extent must be paved as we walk it. Close collaboration between developers and regulators was encouraged already from a very early product development stage. For phage-based VMPs to become a reality it will be essential that EMA facilitates close and open-minded collaboration with developers to ensure that phage-based VMPs are both medicinally and commercially viable.	

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10	We greatly appreciate the opportunity to participate in this process. We interpret the existence and content of this guide as a genuine demonstration of interest in promoting the development of products based on phage therapy, and in solving the existing obstacles in current regulations.	Noted.
11	We wish to reiterate the significant opportunity that exists with phage therapy, in the context of veterinary health as well as the wider and important One Health agenda. We support the introduction of clear guidelines to oversee the development and licencing on VMPs for phage therapy, but we also urge that controls should avoid being over precautionary, and should, where possible, be enabling to the development of VMPs. They should not be too onerous so as to disincentivise the development of this important opportunity for animal health management (and with knock-on benefits for human health management, through antibiotic stewardship). Although not explicitly covered within the guidelines (as far as we can identify), any assessment of a potential VMP should involve a risk benefit analysis. We wish to note that any such risk benefit analysis for phage-based VMPs must include detailed consideration of the likely benefits in terms of antibiotic use and stewardship within both the target species and beyond. This includes the potential for reduced overall use of antibiotics but also potential reductions in the use of High Priority Critically Important Antibiotics (HP CIAs). This, thereafter, leads into the One Health agenda and the potential for significant benefits in wider antibiotic stewardship and human health management. These benefits cannot be ignored	Comments related to the risk benefit analysis are noted. Comments about the applicability of the Guideline to all potential ways in which bacteriophages can be used, particularly on the salmon/fish farming sector, are also noted. The scope of the guideline is restricted to bacteriophages used as veterinary medicinal products under Regulation (EC) 2019/6. Other uses such as biocides are out of the framework of this guideline.

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	when appraising alternatives to antibiotics, or those products that may potentiate existing antibiotics. The guidelines do not appear to acknowledge all potential ways in which bacteriophages will be used. This is particularly the case for our sector (salmon / fish farming). Whilst phage therapy may be used to target specific infections in fish, it is more likely that bacteriophages will be used to reduce the bacterial loading of the water in which fish reside, in particular during key husbandry events when fish need to be "handled" (in that they are not in their normal enclosure environment, for example during grading, transfer between farms in wellboats, vaccination). During such events fish can become more sensitive to bacterial infection and thus bacteriophages are likely to be used to control any (harmful) bacterial loading in the water. Thus, bacteriophages are likely to be used in a preventative manner (comparable to vaccines) rather than responsively, to treat infection. Bacteria which cause infection in fish are often found in natural water bodies, but they are not necessarily an issue for farmed fish unless the balance between environment / pathogen / host immune function is shifted. Being able to manage the bacterial loading in water is therefore a key preventative action, that may occur in the absence of clinical infection in the fish. As well as not explicitly referencing the likely way in which bacteriophages will be used within our sector, the guidelines, as currently written, include statements that may hinder the development and use of bacteriophages in fish (i.e., references to the treatment of bacterial infections, as well as broad statements	

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	and controls around metaphylaxis and prophylaxis (p18, lines 591 to 605)). There may be other use scenarios, for other species, that warrant consideration. The guidelines need to include a level of flexibility for any new / novel / future approaches to using phages. But, again, the likely treatment scenario for farmed fish certainly seems to have been overlooked in the guidelines. The guidelines acknowledge that phage therapy is a new approach and thus in certain areas of the licencing process there may need to be "case by case" assessments. This is to be expected with a novel therapy and it can lead to a more flexible approach. However, we wish to note that in the absence of clear guidelines, licencing agencies will (from experience) tend towards over precaution, rather than pragmatism. This could lead to unnecessary delays in licencing, unnecessary added costs and may, in more extreme scenarios, prevent the licensing of a VMP that might otherwise be a valuable addition to veterinary health management and One Health. We fully acknowledged the need for robust licencing controls but would urge guidance to be as thorough as possible, with, if possible, further guidance to support how case by case assessments should be made.	

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12	Interconnection between the text of the EMA and the EDQM (European pharmacopeia) on VMPs is not always clear In Belgium the "cascade rule" (articles 230-231-232 of the Royal decree of the 14.12.2006) allows the use of some VMPs as magistral preparation in some exceptional situations. Is it possible to apply this rule in parallel with the EMA text in the Belgium context?	Cascade rules of VMPs are clearly stated in Regulation (EC) 2019/6.
13	The introduction of the fit-to-purpose flexibility (based on the multi-strain dossiers concept and simplified post-authorisation changes) are promising approaches for phage products. Nevertheless, to open the perspective for a return on investment in drug product development, there has to be a high efficacy of the product. This in turn requires a rather large number of phages to be added on the authorised dossier list - higher than the 3 phages per cocktail presented in the scenarios at the EMA meeting in Amsterdam. 10-30 or more phages per pathogen and phage biobank would be more realistic numbers , . Generating large phage biobanks is mostly limited by cGMP production costs. With current production requirements on cGMP phages, it is not economical to produce libraries of the size needed to ensure a high efficacy. Therefore, elaboration of the production requirements should be considered as a crucial factor for enabling Phage Therapy and we wish to see more guidance on that matter.	GMPs are not under the remit of the CVMP. The corresponding group (inspector´s working group, IWG) is currently updating Annex 4 and 5.

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14	FVE welcomes the ambitions to regulate the use of bacteriophages in veterinary medicine, as they may reduce the need for or be an alternative to antibiotic treatments for certain indications. It is recognised that there is a substantial body of evidence on bacteriophage application against bacteria of veterinary importance in *vitro* and that commercialised *in vivo* application will require detailed guidance. FVE welcomes particularly the flexible composition approach of phage products. This offers the opportunity to establish phagebanks to reduce the cost of development. In addition, the risk-based safety assessment approach of the bacterial host will be beneficial.	Noted.

2. Specific comments on text

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180	1	Comment: "Current scientific knowledge": In our opinion, it would be opportune to better detail the type of publications to be considered and whether or not to include those produced in Eastern Europe Proposed change (if any):	Not accepted. No specific scientific publications can be mentioned here. It would depend on the state of the art in a particular moment.
296-297	1	Comment: "Antibiotics are not expected to be used during production, and toxic chemicals traditionally used for phage purification should be avoided (e.g. chloroform)": why exclude treatment with chloroform, which is the only one that can clean the phages from LPS residues? Proposed change (if any): We suggest not excluding the possibility of purifying bacteriophages with chloroform	Accepted. The GL is modified as follows: Antibiotics are not expected to be used during production, and toxic chemicals traditionally used for phage purification should be avoided ~~(e.g. chloroform)~~. If this is not possible, these substances should be quantified and controlled in the final product.
422-424	1	Comment: "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": Parallelism could be drawn with other drugs in that they all have the same criticism	Noted.

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		Proposed change (if any):	
276-282	2	I would like to share with you an important comment regarding lines 276-282 of the guidelines in relation to paragraph V.1.5.4.4 of Annex II concerning the absence of genes coding for known virulence factors. The "degre of mandatory use" of production strains without such genes should be considered with great care. For example, in Pseudomonas aeruginosa, the endotoxin (lipopolysaccharide or LPS) is a receptor for many phages and it is therefore impossible to produce these phages on strains lacking LPS, which is also a major virulence factor. The same is true for certain membrane proteins that are antibiotic efflux pumps and phage receptors. Perhaps this type of exception should be explicitly stated in the document so as not to limit possibilities. Similarly, it is possible that a therapeutical phage could only be produced on the pathogenic strain of the patient, what is your position on this.	Accepted. The Guideline has been modified as follows: 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 product. If freedom from ~~prophages~~ these elements is not possible, it should be justified that this has no negative ~~detrimental~~ effects on the safety and efficacy of the bacteriophage product. An adequate threshold of the maximal amount of ~~prophages~~ these elements in the ~~final product~~ host bacteria should be set. ~~The maximal amount of excised prophages should be close to the detection limit using PCR-based technics.~~
425-433	3	Comment: The risk of developing/spreading phage resistance should be also carefully considered. There are two ways to use phages:	Not accepted. The key point of the guideline is precisely to give advice on how to register a flexible composition medicinal product. The guideline advices to register a pool of bacteriophages that

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		- Fixed phage cocktail - Bank of phages and the cocktail is evolved according to the bacteria and the resistance. The phenomenon of phage resistance needs to be studied seriously, regardless of the method used. Using a phage cocktail reduces the risk of resistance but the risk is not nul. The most suitable way to reduce phage resistance is the use of a phage bank to be able to change the phage cocktail according to the bacterial strain. This is the principle of master preparation. However, reproducing a number of phages to create a custom phage cocktail is quite cumbersome if each phage has to be checked and controlled. When the applicant follows this approach, the guideline does not provide clear requirements on the data required in terms of efficacy/safety/toxicity for each monophage. If resistance occurred, the phage cocktails must be updated (line 659) and a VRA application has to be submitted to the Agency. The guideline gives information lines 734 to 750 and foresees an evaluation on a case-by-case basis (line 743). It should be stressed that in the case of resistance, the phage cocktail must be adapted very quickly,	could eventually be mixed to be adapted to the epidemiological situation. If new bacteriophage(s) are needed to overcome a resistance phenomenon, the requirements would depend on the comparability of the new bacteriophage and the already authorised one(s). The use of post-approval change management protocols (EMA/CHMP/CVMP/QWP/586330/2010) is also recommended in order to implement changes as fast as possible.

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		therefore a reduced timetable should be considered for the variation process. Proposed change (if any):	
529-535	3	Comment: The large-scale use of a phage cocktail in livestock farming could result in a significant release of phages into the environment. For the moment, we do not have enough time to measure the impact. Proposed change (if any): The guideline could require the monitoring of phage resistance by bacteria for 2-5 years or more after commercial launch. This could be a number of samples from farms after several treatments at different locations to isolate the bacteria and retest the resistance of the bacteria via the initial phage cocktail.	Not accepted. Each applicant will propose a post-authorisation monitoring plan for phage resistance by bacteria, adapted to their specific product. The proposed plan will be assessed during the marketing authorisation application procedure.
187 and 308-313	3	Phages manufacturing process is a key aspect for the development of phage therapy VMPs and also for the final cost of the therapy. Variations between batches should be accepted due to biological complexity. The most important thing is that the phage solution	Noted.

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		respects the requirements in term of purity/toxicity/efficacy.	
149-150 and 606 - 616	3	We welcome the possibility of concomitant use of bacteriophages with antibiotics. This is an interesting way to reduce resistance both to antibiotics and to phages. Synergy was demonstrated by scientific studies.	Noted.
272-282	4	Comment: The requirement that bacterial hosts should be free of nucleic acid sequences coding for (i) toxins, (ii) elements conferring antibiotic resistance, (iii) prophages, and (iv) any other genetic elements might be difficult to be accomplished. Most bacteriophage products aim at pathogen species used as host production strains for phage lysate production. The specificity of phages regarding their host strains prevents using others unless belonging to the same species and that might meet the requirements exposed. Proposed change (if any):	Accepted. Proposed drafting: 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 product. If freedom from ~~prophages~~ these elements is not possible, it should be justified that this has no negative ~~detrimental~~ effects on the safety and efficacy of the bacteriophage product. An adequate threshold of the maximal amount of ~~prophages~~ these elements in the ~~final product~~ host bacteria should be set. ~~The maximal amount of excised prophages should be close to the detection limit using PCR-based technics.~~
302	4	Comment: The requirement for bacterial hosts to be free of pyrogen content: Content of gram-negative endotoxin and/or gram-positive pyrogens, depending	Not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		on bacterial host(s) used for phage propagation. *In vivo* pyrogen tests should be avoided. Proposed change (if any): Indeed, it would be important to specify that the requirement depends on the route of phage application. In the case of applying phages in drinking water or feed, where there is no risk of endotoxin release and causing shock to the animals, this requirement may not be necessary. Therefore, it is advisable to consider and specify this requirement according to the specific route of application.	This information is too specific to be included in the GL. The requirements are well described in Ph. Eur.
315-318	4	Comment: A minimum period of stability could be specified to be accomplished by the product to be approved. Proposed change (if any):	Not accepted. General requirements for stability studies of veterinary medicinal products should be followed.
441-447	4	OECD 216 refers to a test for determining the impact of chemical products on Soil Microorganisms: the nitrogen Transformation Test. As occurs with other guidelines as referred to chemical products by ECHA guidelines for biocide products used in veterinary facilities, the characteristics inherent to bacteriophages as specificity are not the same as a chemical product. The suitability of the application of this guideline to determine the impact of	Accepted. The next change is proposed: "The performance of studies in accordance with or based on OECD tests guidelines might be required, such as OECD 216"

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		bacteriophages on the soil environment would be questionable. Proposed change (if any):	
530-533	4	Comment: The risk development of phage resistance can be calculated during the evolution of preclinical and clinical trials. However, elucidating the coevolution of bacteriophages and bacteria, determining the mechanisms involved and their molecular genetic basis, and their risk of dissemination is a complex issue and involves long-lasting research. Phage defence mechanisms are not always the same in vitro and in vivo conditions. Depends on the route of administration and the phage/host or phage/host/animal combination. Moreover, gene/s involved in phage defence mechanisms, or their function are mainly unknown. Proposed change (if any):	Noted. The information regarding resistance would be the one available at the time of authorisation, obtained from studies performed along the development of the product and/or available in bibliography.
41-42	5	Comment: The risk of a development of bacterial resistance against bacteriophages is being overestimated in our view. Bacteriophage-resistant pathogen-mutants have not been isolated so far in nature, despite the fact that they are constantly exposed to bacteriophage predation. This is due to the general "loss of function"-effect of a mutation of pathogens. Phage-resistant	Noted. However, as there is no previous experience on the release of bacteriophages in significant amounts, it is preferable to be conservative.

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		bacteria may occur in the laboratory, but they rapidly disappear again in nature. Proposed change (if any):	
110-111	5	Comment: We find that this text does not indicate clearly enough that the EU GMP Guideline as published in Euralex 2010, Vol. 4, is not relevant for the purposes of the marketing authorisation of phage products for vet. use, and that it is overruled by the more specific vet. GMP Guideline. We should therefore like to suggest to add the following: Proposed change (if any): 1. Give a definition of "formula magistralis" 2. eventually add: "…, extemporaneous and individual manufacturing of named-patient-products, pharmaceutical manufacturing processes undertaken in vet. clinics as well as phage products manufactured in small quantities (not exceeding 100 doses of the same product), and phage products manufactured for compassionate use are outside the scope of this guideline."	Not accepted. 1. Formula magistralis are already defined in Regulation (EU) 2019/6. 2. The scope of the Guideline on bacteriophages is also clearly defined in the corresponding section. 3. Compassionate use of medicines is restricted to their use in humans. It doesn´t apply to veterinary medicinal products.
113-114	5	Comment: Should the reference to EU Regulations 2019/6 and 2021/508 mean, that the EU GMP Guideline as	Not accepted. Regulation (EC) 805/2021 states in Section I General Principles and requirements I.1.4 the next: "the

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		published in Euralex 2010, Vol. 4, is not considered as applicable, this should be made clear expressly, since national authorities may choose to apply it additionally. It might also be referred to in References at a later occasion. There is a Good Legislatory Practice to prevent any uncertainties on behalf of competitive settings by addressing this issue explicitly. Proposed change (if any): Add "For purposes of the marketing authorization, the rules of the EU GMP Guideline as published in Euralex 2010, Vol. 4. are considered to be substantiated by this Guideline to the extent that this guideline is concluding, and that in cases of divergence, it prevails."	manufacturing processes for the active substance(s) and finished product shall comply with Good Manufacturing Practice (GMP)".
149-153	5	Comment: Phage therapy is sometimes admitted adjunctively to antibiotics and often both treatments act synergistically. Therefore, in many of the phage therapy cases (human) phages were added complementary to SoC chemical antibiotics. Since direct or indirect *negative* interactions are not to be expected and have not been reported so far, demanding complementary data for the combination of phages with all antibiotics will be very difficult to achieve and will not add significantly to the safety of	Not accepted. If a synergistic use of bacteriophages with antibiotics is claimed in the SPC, this claim should be based on data.

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		the product. Notwithstanding the limited effect on the safety of the product, it will have a negative impact on the costs. Proposed change (if any): omit	
159	5	Comment: We entirely agree: Phages are largely recognized as safe which has been largely documented in literature. Proposed change (if any):	Noted.
207-214	5	Comment: We totally agree: It would be a major step forward if such a flexible composition would be allowed. Proposed change (if any):	Noted.
237-239	5	Comment: We entirely agree: Purification and formulation processes that are independent of the bacteriophage (host pathogen) are about to be established. However, they are still very costly and we are doubtful if industrial animal farms will choose to purchase such products deliberately. Proposed change (if any):	Noted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
261-262	5	Comment: A phenotypic characterization is no scientific standard in phage isolation. Whether a bacteriophage is suitable for a practical application or not does not depend on its phenotype. Proposed change (if any): Omit the entire bullet point	Not accepted. Phenotypic characterisation is considered relevant.
263	5	Comment: "Host range" should be characterized further: Proposed change (if any): "Host range (i.e., the ability of a bacteriophage to form plaques on a set of bacterial hosts)"	Accepted.
264	5	Comment: The absence of lysogenic activity is impossible to prove (negative characteristics can generally not be proved scientifically). Obviously, the authors of this draft wanted to exclude temperate bacteriophages, i.e., phages which have the ability to integrate their genomes into the genome of the bacterial host. Hence, bacteriophages are either lytic (=do not have the ability to integrate) or temperate (=do have the ability to integrate or establish themselves as an extrachromosomal unit within the host). The way to distinguish between the two is by genome sequencing	Not accepted. The absence of lysogenic activity should be addressed during the characterisation.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		and bioinformatics and thus the question, in principle, has already been sufficiently addressed to in line 260. Proposed change (if any): Either skip the bullet point or add: Proof of lytic activity is sufficient to exclude lysogenic activity; genome sequencing is sufficient to identify lytic character and thus sufficient in order to exclude the existence of lysogenic activity	
265	5	Comment: We are not quite sure what the authors mean by "potency" in the contest of bacteriophages. The ability of a bacteriophage to form plaques on a lawn of a relevant bacterial pathogen? If so, the analysis for potency is included in the host range analyses above (line 264) Proposed change (if any): skip the entire bullet point	Partially accepted. Potency could be understood as the ability of a bacteriophage to form plaques. The potency or infectious phage titre is determined by a plaque assay or other suitable method.
276-278	5	Comment: Total freedom of nucleic acids coding for (i) toxins … cannot be achieved without reducing the efficacy noticeably. Bacterial strains which are naturally devoid of toxin genes are very rare, if not impossible to find for some	Partially accepted. The paragraph has been modified as follows: 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

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		species such as P. aeruginosa. Absence of these means that one needs to engineer the production strains to remove such genes, which is already very difficult. Moreover, and as instance, the endotoxin (LPS) is a major virulence factor in P. aeruginosa and the receptor of many therapeutic phages. Accordingly, removing it means the phage(s) cannot bind anymore, and therefore they cannot be produced on the production strain devoid of LPS anymore. Similarly, antibiotic efflux pumps could be receptors of important therapeutic phage(s) and removing them could lead to the incapacity of the phage(s) to replicate on the production strain devoir of the efflux pump. This is particularly important for particular phages that can be produced only on the patient strain for instance, which usually harbour such genetic determinants. Proposed change (if any): The level of nucleic acid sequences coding for (i) toxins… should be kept at a scientifically recommended level regarding the prevalence of the efficacy and concentration of the phages (PFU). s. general annotations –	for detrimental effects on safety or efficacy of product. If freedom from ~~prophages~~ these elements is not possible, it should be justified that this has no negative ~~detrimental~~ effects on the safety and efficacy of the bacteriophage product. An adequate threshold of the maximal amount of ~~prophages~~ these elements in the ~~final product~~ host bacteria should be set. ~~The maximal amount of excised prophages should be close to the detection limit using PCR-based technics.~~
293-294	5	Comment:	Not accepted.

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		Difficult in emergency cases Proposed change (if any): provide an exemption for the case where a therapeutic phage should be rapidly produced on a new production strain such as the strain that had infected the patient / the animal	The Guideline has not been drafted for emergency cases.
300	5	Comment: We entirely agree that the proposals shall not be binding. We suggest however, that the table should be given an entire touch-over on a realistic basis, since it will serve the GMP-inspectors as a model for their inspection as long as they haven't got anything else at hands. Proposed change (if any): Add "not (yet) scientifically verified"	Not accepted. The table is given as an example, as clearly stated in the table header.
365-368	5	Comment: We found that the reference to the requirements of animal studies is too uncertain all over. This includes the provisions for pre-clinical studies. It should be put much clearer under which circumstances exactly they are needed or not, since they are extremely time consuming and costly. On the other hand, clinical trials are to be avoided and restricted to a minimum for ethical reasons.	Not accepted. The GL gives flexibility on the fulfilments of the requirements.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): Replace phrase 2 by: "Clinical studies are to be avoided if there are scientific publications which enlighten the mechanism of action and pharmacodynamics of the respective phage(s), and if a deviation from the mechanism of these phages in other species than the target animal species is not to be expected and/or if the respective phage(s) is(are) not expected to act differently from other phages the mechanism of which have already been published."	
390-399	5	Comment: We understand that endotoxins and exotoxins are a major concern of the entire phage therapy for the committee. On the other hand, phages live on bacteria, and if the applicable medicinal product is void of them, the concentration of the phages (PFU/ml) will decrease quickly. On the other hand, endo- and exotoxins occur in any case of a bacterial infection by natural decomposition of billions of bacteria due to the anti-infection defence system of the animal. This means that the entire body of the animal will be flooded with endotoxins and exotoxins in case of a bacterial infection anyway. This will occur with chemical antibiotics as well. It will, however, trigger a merely temporary reaction of the body, like fever, and has been considered safe enough with chemical antibiotics already. Should the phage	Not accepted. It is clearly stated in the guideline that no concern has been identified on the release of exo/endotoxins in treated animals. On the other hand, acceptable levels for exo/endotoxins in the medicinal product should follow the requirements established in Ph.Eur. for veterinary medicinal products.

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		solution add to this natural quantity of endotoxins, which they certainly will do to a certain – if minor - degree, this will certainly not affect the animal's health status significantly. The level of endo- and exotoxins which is tolerated by animals, has already been tested by experts (like e.g., 5 PFU/ml per Kg body weight). There is therefore no need for the safety aspect to be proved in each and any of the future phage products again. Target animal safety studies should therefore be avoided for ethical reasons and in order to avoid significant additional costs. The Guideline should therefore just fix the level of endo- and exotoxins which shall not be exceeded. Proposed change (if any): Replace lines 390-399 by "Endotoxins and exotoxins may be considered as stressful to the animal and should therefore be addressed. However, to the benefit of a better stability of the product, a certain level of endo- and exotoxins seems to be unavoidable. Considered that they occur due the natural anti-infection defence action of the animal anyway, and that the reaction to them is only transitory, a certain level of endotoxins and exotoxins will not add significantly to the burden. Based on scientific recommendations, it has to be	

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		proved that the level of bacterial endo- and exotoxins shall not exceed X PFU/ml per kg body weight (number to be determinated by experts)	
549-550	5	Comment: We assume that these provisions are kind of contradictory in itself: It is not comprehensible why a TAS study should become "necessary" when the safety profile of phages is – correctly - recognized as very favourable (lines 559-561)? Proposed change (if any): Replace line 549-550 as follows: "The implementation of a Target Animal Safety (TAS) study is considered as not necessary since the phages are qualified as entirely safe for animals and humans." Omit lines 551-556	Not accepted. This study is a relevant source of safety information in target animals under controlled conditions that could ensure a safety use in clinical trials. This study will also provide relevant information to determine the safety for users, consumers (if applicable) and environment.
57-59	6	Comment: This background information should not hinder evolution of the scientific knowledge. Example of the three morphotypes might be too restrictive since other morphotypes might prove useful in the future. Proposed change: Please amend as follows: Bacteriophages of **current** interest in phage therapy	Accepted.

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		predominantly belong to three morphotypes: myo-, podo- and siphoviruses (Monribot A et al. 2021)~~.~~ **although other morphotypes could be of interest in the future.**	
95-96	6	Comment: Given the potential for the therapeutic are a to develop, the sentence may rapidly become out of date, although the second part of the sentence is supported. Proposed change: Please amend as follows: ~~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), t~~The advice given in …….	Not accepted. The guideline should be read considering the time it was written.
159-161	6	Comment: This specific veterinary guideline is the opportunity to develop guidance for development of such products specifically for the veterinary field. Whilst we understand the need to learn from experience on the human side, the specific inclusion of references to ICH guidelines in the text may lead to assessors applying them to the veterinary sector. Proposed change: Please delete references to ICH.	Not accepted. It is clearly stated in the guideline that only the principles of the guidelines are applicable, as these ICH guidelines are not applicable to the veterinary medicinal products.
246-247	6	Comment: CPP and CQA are definitions coming from human quality referentials. Proposed change: Please use specific veterinary terms "Specifications" and "in process controls".	Not accepted. CPP and CQA terms are used in the risk management guidelines whose principles are advised to be followed.

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276-278	6	Comment: It may be not possible to obtain a "clean" bacterial host for a given phage, and cultivation of phages lysing selected pathogens on different, non-pathogenicity factor containing bacteria may lead to a shift in specificity and efficacy of the cultivated, expanded, but ultimately adapted-to-the-host-bacterium phage. Appropriate purification methods of the cultivated phages may anyway be applied. Safety tests run in the target species will ultimately confirm safety. Proposed change: Please insert the following sentence: <u>If under certain circumstances, the phage is produced on bacteria with genes/plasmids encoding some or all elements (i)-(iv), testing for sufficiently low levels of these genetic factors or their products in the final phage preparation would be an acceptable alternative.</u>	Partially accepted. This paragraph has been updated as follows: 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 product. If freedom from ~~prophages~~ these elements is not possible, it should be justified that this has no negative ~~detrimental~~ effects on the safety and efficacy of the bacteriophage product. An adequate threshold of the maximal amount of ~~prophages~~ these elements in the ~~final product~~ host bacteria should be set. ~~The maximal amount of excised prophages should be close to the detection limit using PCR-based technics.~~
302	8	Comment: Content of gram-negative endotoxin and/or gram-positive pyrogens, depending on bacterial host(s) used for phage propagation. *In vivo* pyrogen tests should be avoided. Proposed change (if any): Indeed, it would be important to specify that the requirement depends on the route of phage application. In the case of applying phages in drinking water or feed, where there is no risk of endotoxin release and causing shock to the	Not accepted. This information is too specific to be included in the GL. These requirements are well described in Ph. Eur.

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		animals, this requirement may not be necessary. Therefore, it is advisable to consider and specify this requirement according to the specific route of application.	
272-282	8	The requirement that bacterial hosts should be free of nucleic acid sequences coding for (i) toxins, (ii) elements conferring antibiotic resistance, (iii) prophages, and (iv) any other genetic elements is difficult to be accomplished in the majority of bacteria species used for phage lysates production. The specificity of phages regarding their host strains prevents using others unless belonging to the same species that might meet the requirements exposed.	Accepted. The text has been reworded: 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 product. If freedom from ~~prophages~~ these elements is not possible, it should be justified that this has no negative ~~detrimental~~ effects on the safety and efficacy of the bacteriophage product. An adequate threshold of the maximal amount of ~~prophages~~ these elements in the ~~final product~~ host bacteria should be set. ~~The maximal amount of excised prophages should be close to the detection limit using PCR-based technics.~~
315-318	8	Comment: A minimum period of stability could be specified to be accomplished by the product to be approved. Proposed change (if any):	Not accepted. General requirements for veterinary medicinal products should be followed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
530-533	8	Comment: The risk development of phage resistance can be calculated during the evolution of preclinical and clinical trials. However, elucidating the coevolution of bacteriophages and bacteria, determining the mechanisms involved and their molecular genetic basis, and their risk of dissemination is a complex issue and involves long-lasting research. Phage defence mechanisms are not always the same in vitro and in vivo conditions. Depends on the route of administration and the phage/host or phage/host/animal combination. Moreover, gene/s involved in phage defence mechanisms, or their function are mainly unknown. Proposed change (if any):	Noted. The information regarding resistance would be the one available at the time of authorisation obtained from the different studies performed while developing the product and bibliography available.
56	9	Comment: The vast majority of bacteriophages have not yet been characterized. Proposed change (if any): Add the word 'known' to the sentence: "The vast majority (96%) of known bacteriophages belong to…"	Accepted.
281-282	9	Comment: The detection limit of PCR-based technics as a threshold for amount of excised prophages has no	Not accepted. However, the Guideline has been drafted as follows:

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		biological relevance for the quality, safety and efficacy of the phage product. If such technical threshold is set, genetic modification of production host to inactivate prophage induction must be considered. Proposed change (if any): It would be better if the guideline stated that the maximum amount of excised prophages needs to be justified on a case-to-case basis.	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 product. If freedom from ~~prophages~~ these elements is not possible, it should be justified that this has no negative ~~detrimental~~ effects on the safety and efficacy of the bacteriophage product. An adequate threshold of the maximal amount of ~~prophages~~ these elements in the ~~final product~~ host bacteria should be set. ~~The maximal amount of excised prophages should be close to the detection limit using PCR-based technics.~~
657-661	9	"…all these issues requiring scientific assessment by the Agency" Comment: Very time-effective assessment will be required.	Noted.
727-728	9	Comment: "Additional data, e.g. data showing comparable stability, biodistribution and immune clearance may be required." It is important that the word "may" is kept in the final version of the document.	Noted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Lines 101 - 102	10	Comment: The phrase "one or more bacterial infection(s) or infectious disease(s) caused by bacteria, or dysbiotic conditions" does not necessarily includes zoonoses in livestock that may be asymptomatic in animals. Proposed change (if any): one or more symptomatic or asymptomatic bacterial infection(s) or infectious disease(s) caused by bacteria, or dysbiotic conditions.	Not accepted. The key point is already stated in the text.
Line 180	10	Comment: "Current scientific knowledge" may be understood as the state of the art for any phage or as the up-to-date evidence for a specific phage strain. Proposed change (if any):	Noted.
Line 204	10	Comment: A recommendation should be made regarding the kind of evidence that should be provided. For example, if *in vitro* susceptibility is enough to justify the presence of a bacteriophage. Proposed change (if any): Justification, based on in vitro evidence such as host range, should be provided for the inclusion of each monophage components.	Not accepted. The applicant decides the evidence to be provided.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Lines 205 - 206	10	Comment: "Levels of bacteriophage" may be understood as potency (double layer plaque assay); as phage enumeration (qPCR, dynamic light scattering, microscopy, etc.) It depends on the manufacturer wich one is chosen; or as both. Proposed change (if any):	Noted.
Line 276	10	Comment: Due to the specificity of the lytic activity of bacteriophages and the nature of certain infections and infectious diseases associated with the intrinsic toxicity of certain species (such as E. coli LPS) or the existence of particularly virulent pathotypes, it is often very difficult to have access to hosts that do not present toxins, elements that confer resistance to antibiotics or other potentially detrimental elements different from the prophages. Therefore, it would be very positive to allow the use of strains that carry risky elements if the manufacturer demonstrates that the downstream processing and the quality control of the product are robust and/or if safety thresholds are met. Proposed change (if any):	Accepted. The text has been re-drafted as follows: 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 product. If freedom from ~~prophages~~ these elements is not possible, it should be justified that this has no negative ~~detrimental~~ effects on the safety and efficacy of the bacteriophage product. An adequate threshold of the maximal amount of ~~prophages~~ these elements in the ~~final product~~ host bacteria should be set. ~~The maximal amount of excised prophages should be close to the detection limit using PCR-based technics.~~

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Line 302, Potency of individual bacteriophage active substance(s)	10	Comment: The phrase "(...) potency should be determined for each monophage component. This is expected to be technically possible in the majority of the cases. For monophage components where (...)" could be problematic, since it is often impossible to determine the potency of monophages with redundant host ranges. The inclusion of that phrase could make it difficult to justify to any particular local authority. Proposed change (if any): "(...) potency should be determined for each monophage component. For monophage components where (...)"	Not accepted. The text proposed to be deleted gives flexibility and it is applicable to a wider range of medicinal products.
Line 302, Pyrogen content	10	Comment: It would be very useful if the guide could provide guidance on how to establish acceptable pyrogen concentration levels in the finished product and/or the elements that determine that calculation (route of administration, target species, etc.). Proposed change (if any):	Not accepted. This is clearly stated in Ph. Eur.
Lines 398-399	10	Comment: If the phrase "not directly relevant for the specific phages" is understood as "not referred to the same phage strain", it would be very rare to obtain	Not accepted. The relevance of the data should not be understood as not referred to the same phage strain. This issue should be

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		flexibility based on the current state of science. A more precise wording is needed. Proposed change (if any):	addressed in a case by case basis. Applicants are invited to consult the authorities in a scientific advice procedure.
Line 470	10	Comment: In accordance with the phrase "(...) replacement by studies conducted in non-target animal species (...)" it seems possible to justify the use of small species (mice, rats, rabbits, guinea pigs) for different types of studies. It would be very useful if the guideline clarifies whether this is indeed acceptable a priori. Proposed change (if any):	Not accepted. As stated in the guideline, this is not acceptable a priori. However, if it is scientifically justified other approaches could be valid.
Line 470	10	Comment: It would be very useful if the guideline refers to the use of evidence obtained in related animal species (for example, evidence in chicken for products indicated in duck, pheasant, turkey, etc.), or in the same species but in different physiological states (for example, evidence in cows for use in calves, or in breeders for use in broilers, etc.). Proposed change (if any):	Not accepted. The guideline has been drafted at high level.
Line 511	10	Comment: More detail is needed in the definition of "relevant data from literature". It is not clear if the data should	Not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		come from the same bacteriophage or similar ones, and what degree of similarity is needed. Proposed change (if any):	The relevance of data from literature is self-explaining and it would be determined "case by case". The guideline intends to allow flexibility at the time of authorisation.
Line 539	10	Comment: More detail is needed in the definition of "target bacterium", as bacteriophages can have broad or narrow host ranges. If it must be detailed up to a specific "Sequence Type" , it would be necessary to perform a large number of efficacy studies to cover some of the variability of the etiological agents. On the other hand, if only species is required, it could be that some strains are not affected by the product. Proposed change (if any): This should be documented for each target bacterium, up to a serotype level, in each target animal…	Not accepted. As the targeted bacteria are specified in the indications of the product and could range from a genus to a subgroup of a species, it will be a case-by-case issue.
Lines 557 - 558	10	Comment: The omission of post mortem examinations seems quite reasonable. Similarly, it would also be reasonable to apply the same criteria for Clinical Pathology Tests (Haematology, Blood Chemistry, Urinalysis). If this is not acceptable, it would be very useful to state explicitly that these will continue to be required. Proposed change (if any):	Not accepted. The text in the Guideline should be understood as the omission of these tests could be possible when scientifically justified.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		"Normally, post-mortem examinations and clinical pathology tests could be omitted if scientifically justified. In case unexpected or severe adverse events occur, these are to be clarified by other means, e.g. specific clinical or laboratory examinations."	
Line 562 - 564	10	Comment: Considering what has been stated in these lines, it would be important to encourage the combination of Dose Evaluation or Dose Confirmation Studies with Target Animal Safety, to reduce the amount of *in vivo* experimentation. Proposed change (if any): Addition after line 564: It is recommended to perform the TAS study simultaneously with other efficacy studies.	Not accepted. This flexibility is already stated in the text (*safety data derived from use of bacteriophages in diseased animals is generally expected).* And the development plan of the product is under the remit of the applicant.
Line 680	10	Comment: It would be important to include if new target species can be added post-approval, providing sufficient safety and efficacy information. Proposed change (if any): What is the expected nature of future product updates? (exchange of individual monophage components with similar substitute components with	Not accepted. Addition of a target species should follow the rules applicable to any other VMP. The addition of new target species is not specifically related to the use of bacteriophages. Thus, it is not under the remit of this guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		higher activity without affecting total number of monophage components in product, introduction of new monophage components thus increasing the number of monophage components in product, addition of new target species etc.).	
709 to 714	11	Comment: The sentence states that "*the potency of substitute monophage components to the resistant bacteria should be comparable to the potency of the parental monophage components against the susceptible bacteria*". Whilst on first reading this might seem appropriate, it ignores the fact that the VMP is based on a biological agent and that there will be inherent variability in potency across variants of that biological agent – but this should not be a reason to exclude a potential substitute phage which could provide significant veterinary value. If a phage-based (parent) VMP has reached the stage where resistance in the target bacteria has reduced overall potency, it seems inappropriate to assume or to require that any replacement phage would or should have to be demonstrably at least as potent as the parental phage, given likely biological variation in potency. There could be any number of comparable (biologically) phages, which may have different natural potencies. There is no reason to suggest that a substitute phage might not be of veterinary value	Not accepted. Comparable levels of potency are needed to replace phages by new ones.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		nor to suggest that it might not be comparable to the parent phage, but with a lower potency. There is also no reason to suggest it might lead to any greater likelihood of resistance developing in the future. To prevent use of a substitute based on a lower natural potency seems illogical. We do, however, appreciate that this argument does not follow indefinitely and that a level of appropriate potency and veterinary value must be demonstrated. We agree that there should be guidance and controls in this area and that some variability limits may need to be proposed, to ensure that any substitute phage provides suitable veterinary benefits. Proposed change (if any): Suggest refining the language to focus on veterinary value, rather than comparability of potency.	
Line 446 - 447	12	Comment: "Genetically modified bacteriophages need to be additionally assessed like genetically modified organisms according to IIIa.3A6.2." IIIa.3A6.2. only refers to the replication competent genetically engineered medicines that would be used in the context of a "deliberate release" referred to Directive 2001/18/EC. Proposed change (if any):	Partially accepted. The guideline has been modified to complete the reference to IIIa.3A6.2: 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.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Should there be an explicit reference to the possibility of a "contained use" as referred to in Directive 2009/41/ECEN?	No further references are considered needed.
Line 803 - 806	12	Comment: "Engineered bacteriophages (genetically modified 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. Proposed change (if any): Is it really necessary to craft a new definition for engineered bacteriophages for this context when there are already relevant definitions that exist in European legislation that will apply and supersede for all Genetically Modified Medicinal Products (Directive 2009/41/ECEN and Directive 2001/18/EC)?	Not accepted. The Directive mentioned does not give a definition of engineered bacteriophages. The GMO classification under a regulatory point of view is decided by national authorities.
Lines 276-282	13	Comment: For clarity, what is considered as a prophage nucleic acid sequence should be defined further. Are only intact temperate phages capable of excising considered as prophage or are also any other cryptic or non-inducible prophages which can be detected *in silico* considered under this term? See next comment.	Not accepted. See next comment.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change 277 (if any): (iii) inducible prophages	
Line 279	13	Comment: As finding bacteria free from any prophage genetic material *in silico* is very unlikely [1], the definition of what a prophage free host bacteria is should be redefined as: inducible-prophage free host bacteria, where evidence of possibility of phage induction is confirmable experimentally *in vitro* with appropriate methods (e.g. Mitomycin C induction method) and in appropriate conditions. Proposed change (if any): If freedom from inducible prophages is not possible,	Not accepted. The term "prophages" is replaced in the text by "these elements".
Lines 583-587	13	Comment: The probability of phage resistance development during the efficacy trials is considerably high[2],[3]. Therefore possibilities of introducing substitute monophage components, based on phage adaptations on emerging resistant strains, or using suitable phages from the dossier during the efficacy trials should be possible and elaborated in more details: 1. It should be possible to respond to the emergence of a resistance while the study is ongoing in real-time by exchanging or adding phages already listed in the	Not accepted. The efficacy trial could be done a parental preparation instead a representative preparation in case adaptations along the clinical trial are expected to occur.

[1] Feiner, Ron, et al. "A new perspective on lysogeny: prophages as active regulatory switches of bacteria." Nature Reviews Microbiology 13.10 (2015): 641-650.

[2] Schooley, Robert T., et al. "Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection." *Antimicrobial agents and chemotherapy* 61.10 (2017): e00954-17.

[3] Rohde, Christine, et al. "Expert opinion on three phage therapy related topics: bacterial phage resistance, phage training and prophages in bacterial production strains." *Viruses* 10.4 (2018): 178.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		dossier (and pre-manufactured under GMP conditions). 2. It should also be possible to respond in real-time in the study upon resistance occurrence by doing *in vitro* adaptation on the phage used in the efficacy study. Since a real-time GMP manufacturing to introduce such an adapted phage into the study is unlikely, surrogate endpoints such as *in vitro* evidence should be considered. In general, the criteria for efficacy proof and flexibility to respond to resistance occurrence prior approval should be equal to the ones for post approval changes and not more restrictive, as it hampers the chance of a successful approval in the first place, before being able to use the more liberal rules for post approval changes. This should also be considered from the practical time intervals between manufacturing and application of phages and the likelihood of resistance occurrence in the meantime. Proposed change (if any):	
175	14	Comment: Characterisation and specification should be done regularly on pheno- and genotypic level, with a frequency that is appropriate to the risk. In regard to prophages maximum amounts should rather be justified on a case-to case basis than limited by technical thresholdschange (if any):	Not accepted. The current text states: • The defined and controlled quality of the starting materials, including characterisation and specification of

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			phage and bacteria banks and the characterisation of the active substances. It is preferable to keep the text as open as possible.
257	14	Comment: The Phage´s lytic nature must be confirmed by the means of state-of-the-art bio-informatics tools, which are able to determine the whole genome.. The tools, criteria and genes to be determined must follow to the most recent scientific advances and justified on a case-to-case basis. A reference database (that should be maintained over time) would be an important investment in future-proof systems. change (if any):	Not accepted. Applicants decide the methodology to apply to ensure the lytic nature of the bacteriophages.
842	14	Comment: It is nearly impossible to demonstrate the absence of an ability, that counts for both transduction and temperate behaviour. Likely nearly all phages will do transduction to some degree although some transduce at rates many orders of magnitude higher than others. Definitions of the transduction ability below the detection limit under specific conditions would be valuable. Proposed change (if any):	Not accepted. It should be defined by the applicant.
1113	14	Comment:	Accepted.

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		FVE agrees that the absence of resistance genes is an imperative. However, the pure and overall absence of virulence genes in master seeds may be challenging and will rather require a weighted risk-based approach For example, morphological necessary factors such as motility are virulence factors. A risk-benefit evaluation shall be performed in respect to virulence factors and antibiotic resistance traits, that species-specific, weighted for their importance and impact for the intended purpose and depending on the latest scientific advances. Phages should be resequenced with a frequency that is appropriate to the risk to verify their genetic setup and exclude mutations. Proposed change (if any):	The text has been modified, as stated in previous comments.